The 18th International Research Conference on Injectable Biomaterials/Biomechanics for Minimally Invasive Clinical Applications

GRIBOI 2008 CONFERENCE

May 5th and 6th, 2008
Montreal, Canada

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WELCOME TO GRIBOI 2008

On the behalf of the international scientific committee and the local organizing committee, it is our pleasure to welcome you to Montreal for the 18th Interdisciplinary Research Conference on Injectable Biomaterials/Biomechanics for Minimally Invasive Clinical Applications. This event follows the last conference at Oxford, UK in 2007; at Berne in 2006; at Shanghai in 2005; at Limoges in 2004, and so on. This geographical mobility underlines the international character of the conference, initially started by a group of French physicians interested in bone augmentation and bone substitutes in 1989. It is our pleasure and honor to enhance the international vision of Griboi by organizing the conference in Montreal for the first time.

The focus of Griboi 2008 is on Injectable Biomaterials for Bone Augmentation. The conference provides a forum for discussing the latest research and clinical results towards innovations for improved bone augmentations. The conference consists of lectures, oral and poster presentations, and social events. There will be nine sessions:

- Vertebral Body Augmentation
- Osteoinductive Biomaterials
- Biomaterials Functionalization
- Biomechanics of Vertebral Body Augmentation
- IP and Regulatory Environment in Medical Device and in Bone Augmentation
- Biologics and Tissue Engineering
- Plasma Technology and Applications in Biomaterials
- Injectable Biomaterials and Delivery
- Characterization of Injectable and Implantable Biomaterials

The scope of the conference ranges from clinical experiences to research advances in biomaterials and biomechanics for minimally invasive bone augmentation. The strength of the conference is its ability to gather international experts, from apparently disparate disciplines, to focus on one common goal. Scientists will be inspired by the clinical research and needs, while physicians will be aware of advances in biomaterials and biomechanics. It is our wish that the conference will enhance collaboration and promote advances in the field of bone augmentation.

The success of the conference will depend directly on the enthusiasm of the participants, which has been already exemplified by the high quality of the research abstracts submitted and clinicians attending the conference. For this, we thank our lecturers who accepted our invitation.

Finally, we would like to express our thanks to Dr. Fergus McKiernan, his role was instrumental in the organization of the clinical session.

Gamal Baroud, PhD
Chair, Griboi 2008
Associate Professor
Canada Research Chair in Skeletal Reconstruction
Director, Biomechanics Laboratory
Université de Sherbrooke

François Gitzhofer, PhD
Vice-Chair, Griboi 2008
Professor
Director, Research Center for Energy, Plasma and Electrochemistry

Université de Sherbrooke

BIENVENUE À GRIBOI 2008

C'est avec grand plaisir que le comité scientifique international et le comité organisateur local vous invitent à la 18e édition de la conférence Griboi (Groupe de recherche interdisciplinaire sur les biomatériaux ostéo-articulaires injectables) qui aura lieu à Montréal les 5 et 6 Mai 2008. Cette conférence s'inscrit dans la tradition d'excellence et de qualité des conférences Griboi, comme celles d'Oxford en 2007, Berne en 2006, Shangai en 2005, Limoges en 2004, etc., qui ont toutes été un très grand succès. Cette mobilité géographique souligne le caractère international de Griboi qui a été fondé en 1989 par un groupe de cliniciens français dédiés à cette discipline. 19 ans plus tard, les défis de recherche de Griboi sont toujours présents et de nombreuses équipes relèvent ces défis et veulent présenter et partager leur expertise lors de cette conférence Griboi qui est à Montréal pour la première fois de son histoire.

La conférence Griboi 2008 est plus particulièrement centrée sur les biomatériaux injectables pour la régénération osseuse. La conférence offre aux participants un forum de discussion sur les derniers résultats de recherche et cliniques en régénération osseuse. La conférence consiste en un équilibre de communications orales et d'affiches agrémentées d'un banquet et d'une visite de Montréal. Neuf sessions sont prévues:

- Régénération vertébrale
- Biomatériaux ostéoinductifs
- Fonctionalisation des biomatériaux
- Biomécanique de la régénération vertébrale
- Propriété intellectuelle et règlements pour les équipements médicaux et pour la régénération osseuse
- Ingénierie biologique et des tissus
- Technologie des plasmas et ses applications en biomatériaux
- Biomatériaux injectables
- Caractérisation des biomatériaux injectables et implantables

La conférence concerne les chercheurs qui sont aussi bien intéressés par les expériences cliniques que dans la recherche de pointe en biomatériaux et biomécanique pour la régénération osseuse minimalement invasive. Une des forces de la conférence est de rassembler des experts internationaux de disciplines très diversifiées qui profitent de cette occasion d'échanger autour d'un objectif commun. Les chercheurs en laboratoire seront inspirés par les recherches cliniques et leurs besoins et les cliniciens seront informés des dernières avancées en biomatériaux et biomécanique. Le comité organisateur souhaite que cette conférence améliorera les collaborations et permettra des avancées dans le domaine de la régénération osseuse.

Le succès de la conférence va dépendre directement de l'enthousiasme des participants, qui se mesure déjà par le grand nombre et la grande qualité des résumés soumis et par la qualité des chercheurs et des cliniciens qui participent à la conférence. Nous remercions les conférenciers invités qui ont accepté de partager leur expérience et leurs recherches.

Pour terminer, nous souhaitons remercier plus particulièrement le Dr. Fergus McKiernan, qui a été une personne clef pour l'organisation de la session clinique.

Gamal Baroud, ing. PhD
Organisateur, Griboi 2008

Professeur associé
Chaire de recherche du Canada en reconstruction du squelette
Directeur, Laboratoire de biomécanique

Université de Sherbrooke

François Gitzhofer, ing. PhD
Co-organisateur, Griboi 2008

Professeur
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ACKNOWLEDGEMENTS

Organizers would like to express gratitude to the following organizations for the support of the Griboi 2008.

- *Canadian Institute of Health Research (CIHR)*
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LIST OF SPEAKERS

[1]*INSERM UMR 791 LIOAD, University of Nantes, France* [2]*CNRS UMR 6513 LSO, University of Nantes, France* [3]*National Veterinary School of Nantes, France*

LISTING OF POSTERS

Monday: 3.05 PM to 3.50 PM

Tuesday: 9.30 Am to 10.00 AM and 1.30 PM to 2.00 PM

Session 1

VERTEBRAL BODY AUGMENTATION

Chairman: F. McKiernan

Session 2

OSTEOINDUCTIVE BIOMATERIALS

Chairmen: M. Bohner & S. Becker

Calcium Phosphates: Blocks Granules Pastes and Cements

Jake Barralet
Faculty of Dentistry, McGill University, Montreal, Quebec, Canada

This talk will discuss current progress on the evolution of bioceramics for bone repair. Phosphates were first injected 70 years ago in an attempt to accelerate bone healing and since then a variety of osteoconductive materials have been formulated to act as synthetic bone graft materials. In oral surgery applications restoration of sufficient bone volume for implant fixation is a common procedure, yet restoration of bone height remains challenging due to constraint of the soft tissue. The introduction of gradually soluble synthetic graft materials means that constituent ions are released into the wound site during dissolution. This in turn could enable simultaneous sustained and localized release of bioactive compounds. The original rational for use of calcium phosphates was that they were similar chemically to bone mineral, however their non-toxicity and osteoconductivity are not unique and now many compounds such as calcium silicates, pyrophosphates as well as insoluble magnesium salts are being applied. As regenerative products are now becoming available in dentistry it seems probable that inorganic materials will find use as delivery matrices in addition to being passive scaffolds and this in turn may bring us closer to eliminating the need for bone autografts.

Injectable calcium phosphate-based drug combined device : proximal femur reinforcement : a sheep study

E. Verron[1], J.-M. Bouler[1], P. Janvier[2], J. Lesoeur[1], J. Guicheux[1], B. Bujoli[2] and O. Gauthier[1,3]

[1]*INSERM UMR 791 LIOAD, University of Nantes, France*
[2]*CNRS UMR 6513 LSO, University of Nantes, France*
[3]*National Veterinary School of Nantes, France*

☒ Biomaterials	☐ Biomechanics	☐ Clinical	☒ Innovation

INTRODUCTION: Osteoporosis has been defined as "a systemic disease characterized by low bone mass and micro architectural deterioration of bone tissue, with consequent increase in bone fragility and susceptibility to fracture"[1]. Resorbable calcium phosphate (CaP) biomaterials have proved a noticeable efficacy in bone reconstruction surgery. Furthermore bisphosphonates (BPs) are well known antiresorptive agents largely used in systemic clinical treatments of osteoporosis. An injectable BP-combined CaP matrix has been developed in order to reinforce locally osteoporotic bone by increasing bone mineral density and improving bone micro architecture [2-4]. The purpose of this study was to implant such a combined device in ewes' osteoporotic proximal femurs and to quantify bone structure modifications. The properties of bone reinforcement after implantation of our combined BP-CaP materials were investigated by three-dimensional microtomography (3D-µCT) that was first developed for nondestructive analysis of trabecular bone architecture [5]

MATERIALS AND METHODS: Calcium deficient apatite was loaded with zoledronate (7%w/w) according to a method previously described [3]. The resulting powder was gamma sterilized and peroperatively mixed (50%w/w) with a sterile cellulosic-derived hydrogel that made it injectable. Eight ewes were ovariectomized in order to induce osteoporosis. Five cubic centimeters of the tested biomaterial were implanted monolaterally for 12 weeks in the proximal femur of 6 mature osteoporotic ewes. Osteoporosis was induced 6 month earlier by ovariectomy. 3D-µCT analysis was conducted on all implanted and control (non implanted) femurs. Bone specimens were collected immediately after sacrifice and conserved frozen. Proximal femurs were sectioned with respect to the implanted area to fit into the analysis chamber of the µ-CT device. For the analysed samples, bone or newly formed bone volume density (BV/TV), trabecular thickness (TbTh), space between trabeculae (TbSp) and number of trabeculae (TbN) were measured. Osteoporosis was validated by comparing, before and 6 month after ovariectomy, BV/TV measurements performed on iliac crest of 2 animals. As control and treated femurs were paired, a non-parametric Wilcoxon test (=O.O5) was applied for statistic analysis.

RESULTS AND DISCUSSION: osteoporosis induction is confirmed (see figure) by a 40.0% decrease of BV/TV (iliac crest). After 12 weeks of implantation most of BP-loaded CDA particles have been resorbed and significant modifications of the bone density and micro architecture are observed inside all the treated proximal femurs. Those modifications have been quantified by measuring conventional histomorphometric parameters [5]. Comparing treated versus control femurs for µ-CT histomorphometric measurements show significant increases ($p<0.05$) for bone volume density (+32,3%), trabecular thickness (+15,8%) and trabecular number (+16,8%) and a significant decrease for trabecular space (-12,8%). For the first time to our knowledge, a local combined effect of calcium phosphate particles and bisphosphonate is evidenced on sheep osteoporotic proximal femurs. Those preliminary results can be considered as a first step for a local approach that aims in delaying or even preventing osteoporotic fractures. Reinforcing specific bone sites like proximal femurs, vertebral bodies or wrists by implanting calcium phosphate materials that can promote bone ingrowth and release controlled quantities of bisphosphonates could be considered, in the near future, as an alternative to current systemic injections. Indeed, this combined device allows using small quantities of BPs that present a pure local effect because of the high affinity of BPs for bone apatite. This way of delivery could then decrease described side-effects (e.g. jaw osteonecrosis), due to regular and long-term BPs treatments. Obviously complementary in vivo experiments (mechanical tests, undecalcified histology) have to be conducted in order to better characterize the potential efficacy and eventual limits of such a local approach.

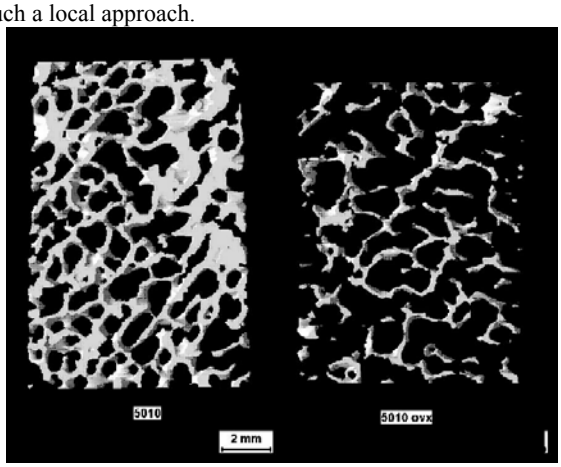

Figure : 3D µCT scan image of bone (iliac crest) before and 6 month after ovariectomy

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2. Josse, S.,... and Bouler, J.M. Adv. Mater. , 16: 1423-27, 2004.
3. Josse, S.,.. and Bouler, J.M. Biomaterials, 26: 2073-80, 2005
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Minimally invasive vertical bone augmentation with injectable brushite cement

F. Tamimi[1], J. Torres[2] , P. Habibovic[1], E. Cabarcos[2] J. Barralet[1]
[1] Faculty of Dentistry, McGill University, Canada. [2] Department of Physical Chemistry II, University Complutense of Madrid, Spain.

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INTRODUCTION: Injectable bone cements are of great interest in oral and orthopaedic surgery due to their exceptional handling properties. Among these biomaterials, brushite cements are unique in their osteoconduction, osteointegration and bioresorption capacity. However, brushite based biomaterials present biocompatibility problems due to their low pH [1]. In the present study we describe the use of two types brushite cement in a vertical bone augmentation procedure.

METHODS: Two cement powders were prepared in aseptic conditions. Cement A was prepared by mixing 1.428 g β-tricalcium phosphate (β-TCP) with 0.8 g monocalcium phosphate anhydrate (MCPA), while cement B consisted of 1.928 g β-TCP that was mixed with 0.8 g MCPA. The cement liquid phase was a 500 mM citric acid solution. The cement powders were mixed with the liquid phase intraoperatively in a powder to liquid ratio of 1.7 in an aseptic environment.
The animal study was approved by the ethical committee for animal experiments of the Complutense University of Madrid (UCM). 4 New Zealand White rabbits were anaesthetized, and two incisions of 0.5 cm were made bilaterally through the periosteum of the calvaria. The perisoteum was elevated through the incisions without increasing their size. Subsequently, cements A and B were injected under the periosteum on the respectively left and the right side of the calvaria.
The cement was left to set *in vivo*, and the animals were sacrificed 8 weeks after the intervention. Histological examinations were performed on dehydrated and resin embedded non decalcified sections that were stained with methylene blue and basic fuchsine.

RESULTS: Upon histological observation, all cement samples showed scarce sings of resorption. In all 4 rabbits fibrous encapsulation was observed around the cement A, while cement B (with excess β-TCP) was well osteointegrated. Moreover, vertical bone augmentation was observed between cement B and the original bone surface (See Fig 1).

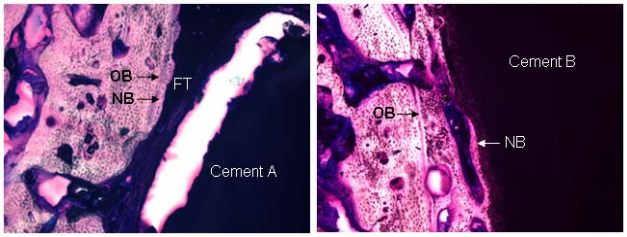

Fig. 1: Histological sections showing Cement A (left) and Cement B (right).
FT: fibrous tissue, OB: original bone, NB new bone.

CONCLUSIONS: Brushite cement can be used in vertical bone regeneration applying minimally invasive surgical techniques. An excess amount of β-TCP in the cement is necessary for achieving the cement bioactivity.

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[1] M. Bohner, U. Gbureck and J.E. Barralet *Biomaterials* 2005; *26(33)*: *6423-6429*

ACKNOWLEDGEMENTS: We thank the "Alfonso Martin Escudero" Foundation for sponsoring Dr Faleh Tamimi with a Post-Doc Grant. This work was financially supported by DGI, Spanish Science and Education Ministry.

Bone reconstruction in irradiated bone sequels: comparison between mesenchymal stem cells and total bone marrow associated to calcium phosphate scaffold

F. Espitalier[1,2], C. Vinatier[1], E. Lerouxel[1], J. Guicheux[1], P. Weiss[1], O. Malard[1,2].
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INTRODUCTION:

Treatment of carcinomas of the upper aero-digestive tract requires large surgical bone removal and radiation therapy. Side-effects of the treatments are aesthetic and functional disorders and increased risk of failure of vascularised flaps used for reconstruction. Since few years, the use of biomaterials was studied and appeared to be an option to autogenous bone graft in non-irradiated areas. In normal bone defects, the mixture of mesenchymal stem cells (MSCs) and biomaterial showed to allow better bone reconstruction than biomaterial alone, or when associated with total bone marrow (TBM). Studies in irradiated bone showed that biphasic calcium phosphate (BCP) and TBM provide better bone reconstruction that TBM or BCP alone. Thus, this study was the first to evaluate bone reconstruction of BCP associated with MSC in an animal model of irradiated one, and to compare it with BCP associated with TBM.

METHODS: Twenty-three inbreeded rats were used, to allow allograft without risk of reject. Calcium phosphate scaffold were biphasic calcium phosphate (BCP) granules. Three weeks after an external irradiation (equivalent of 60 gray by conventional therapeutic multi-fractionated delivery) on the hind limbs, four bone defects were created per rat. The defects were filled with either BCP alone, or a mixture of BCP and TBM, or a mixture of BCP and MSCs (either adipose-derived MSCs or bone marrow-derived MSCs). Three weeks after implantations, new bone formation was qualitatively and quantitatively assessed.

RESULTS AND DISCUSSION:

Histological examination showed osteo conductive and osteointegrative properties of BCP in irradiated tissue. The BCP-TBM mixture significantly improved bone ingrowth ($p<0.05$). However, the BCP-MSC mixture did not provide better new bone formation than BCP alone (p=ns).

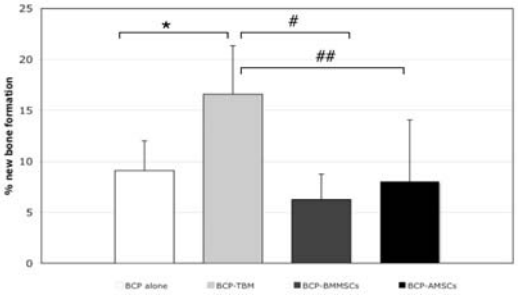

Fig. 1 Bone ingrowth in osseous defects (#p=0.0025 compared with BCP-BMMSCs, ∗p=0.0264 with BCP alone, ##p=0.0111 with BCP-AMSCs).

It can be suspected a link between this results and the cell and vascular weakness observed in irradiated bone. The BCP-TBM mixture allowed an increased vascula- risation of bone and thus balanced after-effects of irradiation. This can be explained by the presence of all components in bone marrow that missed when MSCs were implanted in association to BCP.

CONCLUSIONS: BCP associated with TBM appears to be the most efficient material for bone substitution in irradiated areas.

REFERENCES:

1- Malard O et al. Calcium phosphate scaffold and bone marrow for bone reconstruction in irradiated area: a dog study. *Bone* 2005; 36(2): 323-330.
2- Yuan et al. Repair of canine mandibular bone defects with bone marrow stromal cells and porous β-tricalcium phosphate. *Biomaterials* 2007; 28(6): 1005-1013.
3- Petite et al. Tissue-engineered bone regeneration. *Nature Biotechnology* 2000; 18(9): 959-963.

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Biomatlante™, France for the BCP supply. This work was supported by GEFLUC and “les gueules cassées” association.

Chemical and microstructural aspects on the contact zone establishment and integration of Ca-aluminate based biomaterials onto hard tissue

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[1] *Doxa AB, Sweden.* [2]*Department of Engineering Sciences, Uppsala University, Sweden.*

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INTRODUCTION: Injectable bioceramics may be based on phosphates, aluminates, silicates or sulphates. This study deals with chemical and microstructural aspects on the contact zone developed and integration of Ca-aluminate cement (CAC) onto hard tissue. The applications for CAC include possibilities within odontology and ortho-pedics. The priority field for CAC is to offer *in vivo* developed biomaterials. This is primarily obtained by hydration reactions. The implication of this and the microstructure developed and the resulting integration with hard tissue is discussed.

MATERIALS AND METHODS: CAC-materials comprise double oxides of CaO (C) and Al_2O_3 (A). Several different phases exist, but in this work only $CaAl_2O_4$ (CA) was used. The reaction products, hydrates, and the microstructure obtained, were examined by SEM, HRTEM, STEM and ED, respectivily, more details in [1].

RESULTS: Chemical aspects. In water CA reacts in an acid-base reaction to form hydrates. The reaction involves the following steps; 1) dissolution of CAC into the liquid, 2) formation of ions, and 3) repeated precipitation of the hydrates katoite, $3CaO{\bullet}Al_2O_3{\bullet}6H_2O$ and gibbsite $Al(OH)_3$ until the CA is consumed.
In body liquid complementary reactions occur including both early apatite, $Ca_5(PO_4)_3OH$, formation just after injection as well as possible transition reaction of katoite into apatite and gibbsite with time. During setting and hydration due to the presence of Ca^{2+} and OH^- ions, the hydrogen phosphate ions in body liquid are neutralized to phosphate, PO_4^{3-}, and as a consequence apatite formation occurs. Long-time contact with body liquid induces a transition of katoite into the somewhat more stable phases apatite and gibbsite.
Microstructural aspects. The amount of water involved in the hydration reactions is very high, and all water necessary for good handling is consumed in the curing reactions, yielding the final material low porosity, < 10 v/o. HRTEM microscopy reveals the hydrates to be in the size range 20-50 nm, and nano-size pore channels 1-2 nm in width [2]. The reactions occur upon the biomaterial periphery towards tissue. The apatite is as well found to be in the nano-size range.

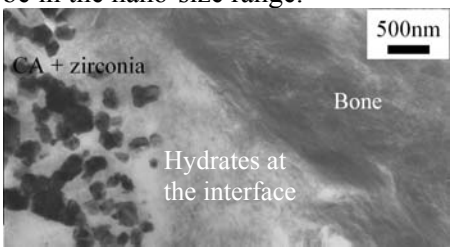

Fig. 1: Closing of gaps between injected CA-material and bone (to the right) - example from sheep vertebrae. Black particles in the bulk material is zirconia, added to increase the radiopacity.

CONCLUSIONS: Hydration and precipitation of nanosize crystals on tissue walls, and the earlier demonstrated small expansion during curing [3], result in complete gap filling. The apatite formation in the contact zone to hard tissue also implies a possible bioactivity. Nevertheless an integration on nanolevel, described as either chemical or mechanical, occurs.

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2. H Engqvist and L Hermansson, RILEM Symposium, Quebec 2006
3. L Kraft. PhD Thesis, Uppsala University, 2002

Session 3

BIOMATERIAL FUNCTIONALIZATION

Chairmen: G. Daculsi & H. Serhan

EFFECT OF ADHESION PEPTIDES ON PREOSTEOBLAST RESPONSES TO BMP

E. Bergeron, M.E. Marquis, E. Lord, H. Park, M. Tremblay, O. Drevelle and N. Faucheux

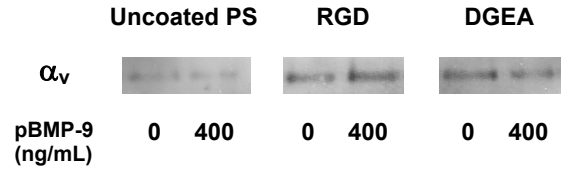
Laboratory of Cell-Biomaterial Biohybrid Systems, Chemical Engineering Department, Université de Sherbrooke, J1K 2R1, Québec, Canada

☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Adhesion peptides are currently used to enhance the ability of biomaterials to interact with osteoblasts using selective integrins. However, little is known about the influence of these adhesion peptides on cell responses to growth factors, especially the bone morphogenetic proteins (BMPs). BMP-2 is the most commonly used in clinical bone healing studies [1]. Nevertheless, BMP-9 was recently shown to induce greater differentiation of osteoblasts than BMP-2 [2]. Here, we first analyzed murine MC3T3-E1 preosteoblast behaviors induced by BMP-2, BMP-9 and a peptide derived from human BMP-9 (pBMP-9). We then determined the effects of adhesion peptides Ac-CGGNGERPRGDTYRAY-NH_2 (pRGD) and Ac-CGGDGEA-NH_2 (pDGEA) on the responses of cells to pBMP-9 in serum-free medium.

METHODS: Western blotting of the phosphorylated Smad1/5/8 was carried out on cell extracts after incubation between 0 and 4h with or without pBMP-9, BMP-2 or BMP-9. Effect of pBMP-9 on alphav and beta1 integrin subunits at cell membrane was investigated using immunolabeling and western blotting. Phosphorylated FAK (Y397) and total FAK were also analyzed by western blotting. Early cell differentiation was monitored by quantification of both alkaline phosphatase (ALP) activity and mRNA encoding procollagen type I alpha1 chain at 24h.

RESULTS: After 1h incubation, pBMP-9 activated Smad pathway through phosphorylation cascade similarly to BMP-2 and BMP-9. After 24h incubation, pBMP-9 promoted ALP activity similar to that generated by BMP-2 or BMP-9. Then, we analyzed the effects of pRGD or pDGEA on cell responses to pBMP-9. After 1h incubation, cells attached to peptides-coated polystyrene (PS) spread out and organized their cytoskeleton. Furthermore, pRGD- and pDGEA-coated PS respectively bound to alphav and beta1 integrin subunits. Only on pRGD-coated PS, pBMP-9 increased the amount of alphav integrin subunits in cell membranes (Figure), but had no significant effect on the beta1 integrin subunits.



After 24h incubation with or without pBMP-9, cells on pRGD-coated PS expressed the highest collagen type I mRNA level and they organized beta1 integrin subunits at their focal adhesion points. ALP activity measurements also showed that pBMP-9 only promoted early cell differentiation on pRGD-coated PS. This ALP activity decreased using DGEA pretreatment therefore demonstrating the involvement of these integrins in early cell differentiation.

CONCLUSIONS : Increase in ALP activity was only observed on pRGD-coated PS in the presence of pBMP-9. This phenomenon seems to depend on both alphav and beta1 integrin subunits. Therefore, restricted selection of integrins in biomimetic materials may be inappropriate for promoting optimal cell responses to growth factors.

REFERENCES: 1. Burkus, J.K *et al.* Orthopedics, 27, 723, 2004. **2.** Kang, Q. *et al.* Gene Therapy, 11, 1312, 2004.

ACKNOWLEDGMENT: This work was supported through funds granted by Canada Research Chair.

DEVELOPMENT AND CHARACTERIZATION OF DICLOFENAC SODIUM RELEASING NANOSCAFFOLD

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Abstract. Research of nanotechnology has recently taken place also in biomaterials science. Biological environments are full of nano size constructs, hence making biomimetic materials should comprise structures within nano range. To produce fibrous nanostructures, self-assembly or electrospinning can be used. Adding drug release function to such material may advance applications further for use in controlled tissue repair. The resulting device can be seen as multifunctional fibrous structure with desirable porosity to support cells and drug releasing properties to control tissue reactions.

Keywords: Electrospinning, nanoscaffold, drug release, biodegradable

MATERIALS AND METHODS: A bioabsorbable poly(D,L lactic-co-glycolide) 80/20 (PLGA80/20) was dissolved to chloroform to form dilute solution. 20w-% of test drug was added. Nano-fibers were made by electrospinning onto substrate. Microstructure of the resulting nanomat was studied using SEM and drug release profiles with UV/VIS spectroscopy.

RESULTS

Microstructure

Thickness of electrospun nanomat was about 1 mm. SEM analysis showed that polymeric nano-fibers containing drug particles form very interconnected porous nano structure (Fig 1). The average diamete of nano-fibers was 500nm.

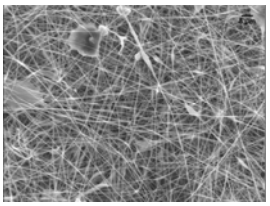

FIGURE 1. SEM micrograph of electrospun DS releasing PLGA nano scaffold. Magnification is x 1000.

Drug Release

After the high start peak of drug release the rate was decreased during 11 days considerably. During drug release tests the material was degraded quickly and vanished. Drug release tests lasted for about 60 days (Fig 2).

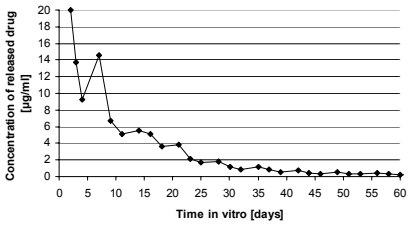

FIGURE 2. Concentration of released drug from nanoscaffold.

CONCLUSION: The nano-fibrous porous structure made of bioabsorbable polymer loaded test drug is feasible to develop and hoped to improve biomaterial properties for controlled tissue repair and regeneration.

ACKNOWLEDGMENTS: This study is done under the scope of EU project EXPERTISSUES (NMP3-CT-2004-500283). The research funds from Technology Development Center in Finland (TEKES) are appreciated.

Influence of TCP sintering process on osteoblast viability

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INTRODUCTION: Osteoblasts are cells that support the synthesis, secretion and mineralization of extracellular bone matrix. Therefore, the investigation of their behavior in the presence of bioceramics is important to evaluate biocompatibility. Bioceramics composition, crystallinity, and porosity are characteristics directly related to cell physiology [1], while ionic products from bioceramics dissolution are responsible for alterations in osteoblast proliferation [2]. Recently, TCP has been extensively considered as a promising injectable component for curing bone traumas. However, much effort is largely directed to synthetic TCP. Our team believes that biomaterials and particularly injectable bioceramics made by the Mother Nature herself must have superior performance in bone healing than synthetic ones. In the present study we have investigated viability and proliferation of osteoblasts in the presence of 5 different TCP (tri-calcium phosphate) powders, obtained from 5 different natural sources, prepared using ultrasonic and hotplate as pre-synthesis and preheating processes.

METHODS: Medium containing each TCP powder was put in contact with osteoblasts that have been plated at $1x10^5$ cell density. The cell / particle ratio was 10/1 and controlled visually by optical microscopy. The experiments were performed 72 hours after incubation. Morphological changes were investigated under light microscope. Viability was assayed by MTT method that is based on the capacity of viable cells to metabolize tetrazolium to formazan crystals, a purple dye that can be solubilized and measured by optical density.

RESULTS AND DISCUSSION: The experimental results of the biocompatibility of TCP extracted from different natural sources were very interesting. The ultrasonic pre-synthesis process seems to enhance viability/proliferation since all investigated samples increased the absorbance rate by more than 20% (Fig. 1). Among them, Ostrea Edulis derived TCP seems to be the best choice for further investigation.

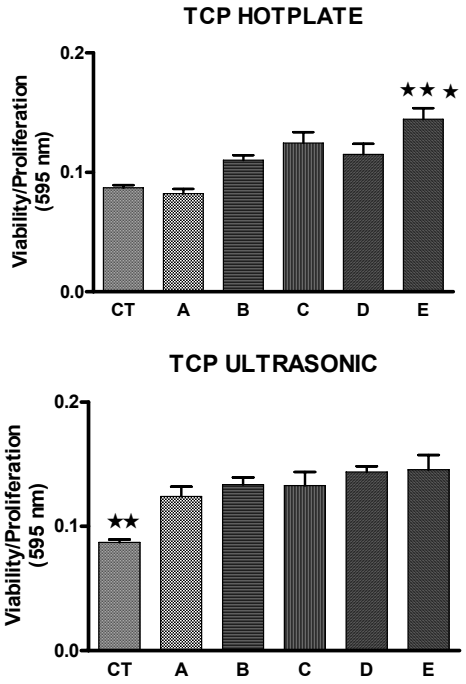

Fig. 1: (A). (B) All tested samples showed good biocompatibility and most of them an increasing viability/proliferation rate. A: Chineese sweet water pearl powder. B: bone of Cuttlefish. C: Crasostrea skamea. D: Venus Verucosa. E: Ostrea Edulis (Results reflect mean ± SD of triplicates from 3 different experiments P<0,01 ** P<0,001 ***).

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ACKNOWLEDGEMENTS: CNPq, CAPES, Brazil. Turkish Republic Government Planning Organization framework: "Manufacturing and Characterization of Electro-Conductive Bioceramics" with project number 2003 K120810. ENTER project 04EP26, Greece. The authors also acknowledge the support of Dr. L.S. Ozyegin.

Chitosan/Gelatin Hydrogel as Immunoisolative Material for Injectable Bioartificial Pancreas

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: The chitosan/gelatin solution added with glycerol 2-phosphate disodium salt hydrate is liquid phase in room temperature and will gelation as hydrogel in situ at 37 °C which can be utilized as injectable cell deliver substrate to human body.[1] We hope the chitosan/gelatin hydrogel can protect the insulinoma/agarose microspheres when xenogenetic transplantation is performed.[2]

METHODS: Agarose was used to encapsulate insulinoma as microspheres and suspended in chitosan/gelatin/β-GP solution. The chitosan/gelatin/β-GP solution contained insulinoma/agarose microspheres was injected into culture plate, gelation and evaluated *in vitro* first. The activity and insulin releasing profiles of insulinoma/agarose microspheres macroencapsulated in chitosan/gelatin hydrogel were evaluated. The insulinoma/agarose microspheres suspended in chitosan/gelatin/β-GP solution was also injected into the subcutaneous of diabetic rats. The non-fasting blood glucose concentration of diabetic rats were measured and recorded. After predetermined intervals, the chitosan/gelatin hydrogel contained insulinoma/agarose microspheres were retrieved for histological examination.

RESULTS: Results showed that the insulinomas/microspheres macroencapsulated in chitosan/gelatin hydrogel can have normal activity and secreted insulin continually. The diabetic rats injected with chitosan/gelatin hydrogel contained insulinoma/agarose microspheres can restore normalglycemia and continue for 45 days.

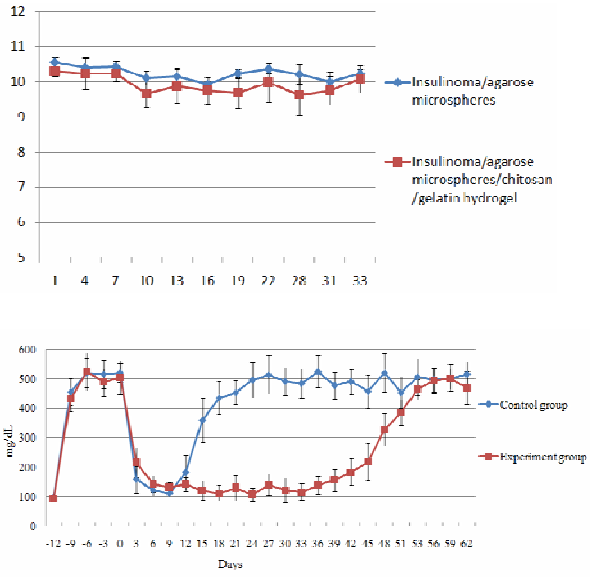

Fig. 1: (A) Insulin secreting profiles. (B)Diabetic rats injected with chitosan/gelatin hydrogel contained islets.

CONCLUSIONS: This study indicated that the chitosan/gelatin hydrogel can be utilized as cell deliver substrate for injectable bioartificial pancreas, the hydrogel can also protect the cell encapsulated in agarose microspheres when xenogenetic transplantation is performed.

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A DELIVERY SYSTEM WITH BMP AND BIOACTIVE GLASS MICROSPHERES IN A COLLAGEN GEL

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Growth factor delivery systems constitute an alternative to bone autografting in a variety of clinical situations such as spine degenerative disease. Osteoblasts play a crucial role in bone repair by secreting collagen type I which is the most important component of the extracellular bone matrix. Both bone morphogenetic proteins (BMPs) and bioactive glass (BG) microspheres also promote osteoblast differentiation [1]. Currently, BMP-2 is the most used in commercial delivery systems. Since BMP-9 seems to exert a more potent osteogenic activity than BMP-2 [2], we decided to compare the effect of a peptide derived from BMP-9 vs BMP-2. For this purpose, a delivery system using these BMPs and BG in a collagen gel was developed. We first analyzed each component of this delivery system. Then, we determined the influence of the delivery system on MC3T3-E1 preosteoblast differentiation using ALP activity measurement and the ability of these cells to synthesize matrix metalloproteinases (MMPs) which degrade collagen.

METHODS: Murine preosteoblasts MC3T3-E1 ($4x10^4/cm^2$) were grown at 37°C in α-MEM with 10% fetal bovine serum. *Delivery system:* BMP (100ng/mL) and BG (1mg/mL) were added to collagen (1.5mg/mL) before its gelation. This gel was supported by an insert to avoid direct contact with cells. At day 1, 3 and 5, 100 µL medium outside the insert was collected to determine the amount of secreted MMP using zymography assays. *ALP staining:* On day 5, medium was removed and the cells were fixed during 30 seconds using a solution containing 60% acetone and 40% citric acid buffer. ALP was stained with a Fast Blue RR salt and Naphtol AS-MX phosphate solution.

RESULTS: Collagen type I extracted from rat tail tendons was the main component of the matrix. Both BG and silicon oxide microspheres which had a diameter of 25 µm, had no cytotoxic effect on the MC3T3-E1 preosteoblast proliferation after 3 days. Quantitative measurements of the ALP activity demonstrated that peptide derived from BMP-9 induced a similar cell differentiation than other BMPs such as BMP-2 and BMP-9 (Figure). The cells treated by pBMP-9 or BMPs can secrete a protein of about 62 kDa corresponding to the active form of MMP-2. We performed densitometric analysis of the 62 kDa protein band. Using the delivery system, BG and BMP-9 generated a lower MMP-2 content compared to the other conditions.

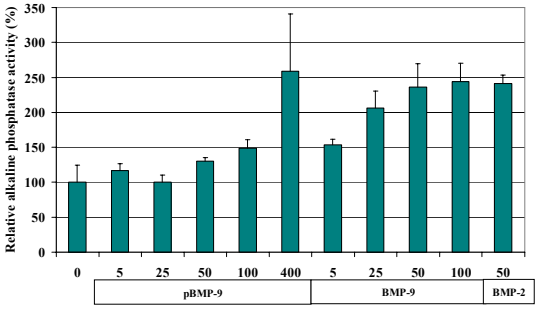

Figure : Relative ALP activity in preosteoblasts after 24 h incubation with or without BMPs

CONCLUSIONS : Development of a delivery system using pBMP-9 instead of BMP-2 seems an excellent choice since pBMP-9 promotes more preosteoblast differentiation. Producing less MMP-2 than BMP-2, pBMP-9 constitutes also a good candidate while using a collagen matrix.

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ACKNOWLEDGMENT: This research was supported through a *Natural Sciences and Engineering Research Council of Canada* program.

Session 4

BIOMECHANICS OF VERTEBRAL BODY AUGMENTATION

Chairmen: M. Liebschner & R. Hall

Adjacent Fracture in Patients with Vertebral Compression Fracture: Related to PMMA Bone Cement or Vertebral Deformity?

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Kyphoplasty and vertebroplasty are minimally invasive methods to treat painful vertebral body compression fractures (VCFs) secondary to osteopaenia. Kyphoplasty seeks to correct vertebral deformity, while vertebroplasty generally does not, but both usually involve PMMA bone cement to stabilise the spine. Adjacent fractures have been observed after both procedures. The high compressive stiffness of PMMA has been invoked to explain this. Limiting bone cement volume, and/or developing less stiff bone cements, have been proposed to address this.

METHODS: Review of biomechanical and clinical literature, in light of mechanical principles.

RESULTS: Among 1425 post-menopausal women, 58% with >1 VCF had adjacent fractures[1]. Vertebral body (VB) deformities increase future VCF risk linearly by number of prior vertebral body (VB) deformities (up to 8)[2] and by VB deformity severity[3], independent of bone mineral density and other known VCF risk factors. VCFs are found most often around the apex of the thoracic (T7-T8) and (T12-L2) lumbar curves[4].

Multiple independent mechanical studies have shown that bone cement augmentation does not increase compressive VB stiffness beyond its pre-fracture state (see, e.g., ref. 5). Compressive stiffness testing reveals the following relationships (in descending order): Cortical bone > PMMA > vertebral endplate > normal cancellous bone > osteoporotic cancellous bone > hypertrophied disc > normal disc.

CONCLUSIONS: Mechanical principles predict the documented natural history. Uncorrected VB deformity shifts the centre of gravity anterior and inferior, increasing VB stresses. The resulting forward bending moment increases with increasing deformity, explaining the increasing effect of VB deformity number and severity. Stresses are greatest at the apex of a curve, predicting the observed common VCF locations. Uncorrected vertebral deformity results in increased or new local curves, predicting adjacent fracture.

The concern that increased PMMA stiffness will cause adjacent VCF is not supported by mechanical testing, because the VB is not stiffer. Nor is this concern supported by mechanical principles. The functional spine unit (FSU), consisting of the vertebra-disc-vertebra, is the mechanical equivalent of three springs in a series, because the bones and disc are stacked and connected, and cannot load independently. For three springs in a series, the response to a compressive load by is driven by the **least** stiff component. In the FSU, the least stiff component is the disc, unaffected by the procedures. In contrast, spinal fusion removes the disc, and spring mechanics predict that adjacent discs will experience greater stresses due to removal of the (less stiff) disc inbetween.

The results of this analysis support the goal of correcting VB deformity. They do not support limiting the volume of bone cement, nor developing a less stiff bone cement, to prevent adjacent VCF.

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Preliminary biomechanical evaluation of vertebroplasty in the management of spine metastases and multiple-myeloma: an in-vitro cadaveric study

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INTRODUCTION: In the spinal column, bone metastases (BM) and lesions arising from multiple myeloma (MM) can cause severe weakening of the vertebral body (VB) leading to an increased risk of fracture[1]. These vertebral fractures may induce severe pain, deformity and increased risk of neurological deficit[2]. At present, however, there is very little is known about the mechanical behaviour either of the infiltrated vertebrae or that following vertebroplasty (VP). The purpose of this preliminary investigation was to evaluate (i) the mechanical behaviour of vertebrae with lesion involvement, and (ii) the effectiveness of VP with coblation.

METHODS: Individual vertebrae from two spines, one with MM (n=13) and one with BM secondary to bladder cancer (n=12) were dissected free of soft tissue with the posterior elements retained. Three MM vertebrae with evidence of previous fracture were excluded. Each vertebrae was fractured under an eccentric flexion load from which fracture strength and stiffness were derived[3]. VBs were then assigned to two groups. In group 1, lesion material was removed by coblation prior to VP (Figure 1) and in group 2, no coblation was performed prior to VP. All vertebrae were fractured post-augmentation under the same loading protocol. At each stage microCT assessments were conducted to investigate lesion morphology and cement volume/distribution.

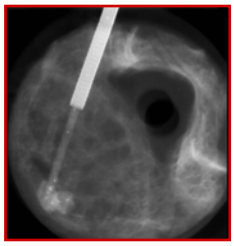

Figure 1: VP following coblation

RESULTS: MM vertebrae were characterised by several small lesions, severe bone degradation and multiple compromise of the cortical wall. In contrast, large focal lesions were present in the BM vertebrae and the cortical wall generally remained intact (Figure 2).

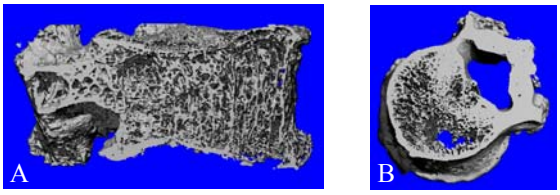

Figure 2: µCT reconstructions of (A) MM vertebra and (B) BM vertebra

The initial failure strength of the MM vertebrae were significantly lower than BM vertebrae (L=2200N vs 950N, P<0.001). A significant improvement in relative fracture strength was found post augmentation for both lesion-types (1.42 ± 0.51, P=0.0006). Coblation provided a marginally significant increase in the same parameter post-augmentation (P=0.08) and, qualitatively, improved the ease of injection.

CONCLUSIONS: Bladder BM and MM vertebral lesions showed significant variations in lesion morphology, bone destruction and the level of cortical wall breach, causing significant changes in the bone fracture behaviour. Account should be taken of these differences to optimise the VP intervention in terms of cement formulation and delivery. Preliminary results suggest the current VP treatment provides significant improvements in failure strength post-fracture.

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ACKNOWLEDGEMENTS: EPSRC and the Yorkshire Children's Spine Foundation. Arthrocare® UK gratefully acknowledged for providing the Coblation® technology, Cavity SpineWand™, delivery systems and cement.

Simulation of cement augmentation for analysis of vertebroplasty

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Finite element (FE) models are increasingly being used to assess the mechanical response of spinal segments treated with vertebroplasty [e.g. 1, 2]. However, assumptions have been made about the behaviour of the cemented region of bone that have not been fully experimentally validated. This study examines the validity of current modelling methods using specimen-specific techniques with direct comparison to corresponding experimental specimens.

METHODS: Eight vertebrae were harvested from two cadaveric spines and four were augmented with cement. All the vertebrae were then imaged using micro CT (μCT80, Scanco Medical, Switzerland) and tested to failure under axial compression in the laboratory. Specimen-specific FE models of the vertebra were generated from the μCT images using a combination of custom-written and proprietary software (ScanFE, Simpleware, UK). Elements representing bone were assigned material properties based on the mean image greyscale over the element using conversion factors that were derived from separate specimens tested previously. In the augmented specimens, elements representing the cement were assigned a uniform elastic modulus throughout. This value was varied to a) represent that of pure cement as has been used in previous FE studies with E = 2040MPa; b) represent the bone-cement region from our previous FE studies where the properties were tuned to match experimental tests on augmented pellets [3], with E = 345MPa. The models were simulated under axial load and the results compared with the corresponding experimental specimens.

RESULTS: The agreement between the FE-predicted stiffness and the corresponding experimental values are shown in Fig 1. For the non-augmented vertebrae, high levels of agreement were found for both stiffness and strength, indicating that the specimen specific models can predict the vertebral behaviour relatively accurately. For the augmented specimens, models representing the cement with E = 2040MPa overestimated the vertebral stiffness (mean error = 50%) and strength (mean error = 63%). Models representing the cement with E = 345MPa provided better estimates of stiffness (mean error = 21%) but still overestimated the vertebral strength (mean error = 40%)

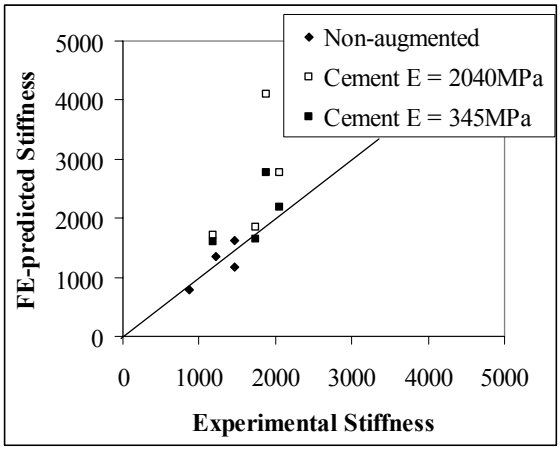

Fig. 1: Experimental vs FE stiffness

CONCLUSIONS: Representing the cement region using the bulk properties of cement considerably overestimates the vertebral stiffness and strength. A lower modulus for this region can more accurately match the vertebral stiffness, although there was still a bias towards overestimation. Further work is needed to fully understand the cement-bone interactions and failure mechanisms in augmented vertebra in order to simulate the response more accurately.

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ACKNOWLEDGEMENTS: This study was funded by the EPSRC.

The Effect of Cement Augmentaion on the Structural Response of Recovered Osteopenic Vertebrae: An Anterior-Wedge Fracture Model

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Age related vertebral fractures are associated with significant functional impairments. Vertebroplasty is efficacious in alleviating pain associated with these conditions. However, little is known on the biomechanical response of the augmented vertebra under complex loads.The aim of this study was to investigate the effect of cement augmentation on the post-failure structural behaviour of single thoracolumbar osteopenic vertebrae in response to combined compression-flexion loads was investigated. The objective of this study is to quantify the effect of Polymethylmethacrylate (PMMA) cement on the restoration of three-dimensional load carrying capacity of failed osteopenic vertebrae following a recovery period under combined flexion and compression loads.

METHODS: Nineteen thoracic and lumbar human vertebrae were radiographically evaluated for morphometry and bone density and tested to failure under combined flexion and compression loads. Following 30 minutes recovery, the vertebrae were tested, augmented with PMMA, allowed to rest for two hours, and then re-tested. The mechanical response of the vertebrae was measured using a 6 Degree of Freedom load cell.

RESULTS: Augmentation caused a highly significant change in the structural response of the recovered vertebrae showing an increase of up to 228% in compressive and

118% in flexion strength. Bone Mineral Density and vertebral body geometry were not correlated with the increase in structural competence of the augmented vertebrae. However, bone density was moderately correlated with the amount of cement injected and the occurrence of cement leakage.

Fig 1: A intact vertebral specimen undergoing test

DISCUSSION: The current study has shown the augmented vertebrae to exhibit significantly higher compressive, anterior-shear and flexion stiffness, when compared to the response of the recovered vertebrae (195%, 69% and 25% respectively) and to a lesser extent when compared to that of the intact vertebrae (40%, 30% and 5% respectively). Several factors may have contributed to this finding. The volume of injected cement, depending on the type and composition used, was reported to significantly effect the compressive failure load and strongly effect the recovery of the stiffness of the vertebral body with a 100% increase in injected volume projected to cause a 50% increase in estimated stiffness. In addition, the flexion-compression injury

model, employed in this study, has clear differences compared to the crush fracture model largely used by previous studies. The latter injury model often presents considerable damage to the middle and anterior columns of the vertebrae with the recovered vertebrae exhibiting significant loss of height at the posterior portion of the vertebral body. By contrast, the current anterior wedge injury model largely retained the structural integrity of the posterior portion of the vertebral body with the recovered vertebrae anterior and posterior heights showing a mean decrease of 17.5% and 7.1% respectively, compared to that of the intact vertebrae. It is therefore that both the amount of cement used and the maintenance of the middle vertebral column were likely to have been instrumental in achieving the increased levels of stiffness observed in this study.

CONCLUSIONS: This study demonstrated that augmentation significantly alters the structural response of the recovered vertebrae. Neither BMD nor the change in vertebral geometry predicted the load response of the recovered or the augmented vertebrae. However, BMD was found to affect the occurrence of cement leakage and may therefore provide additional information in the clinical planning of this procedure.

This study investigated whether cement augmentation of failed osteopenic fresh human-cadaver vertebrae following recovery restores their structural response and geometrical properties. The role of vertebral geometry and bone density in affecting the outcome of augmentation was similarly assessed. The augmentation of the failed vertebrae significantly enhanced their load carrying capacity and caused a moderate restoration in the geometry of the vertebral body. However, under the applied loads, this procedure lead to significant alteration in the overall response of the vertebrae with the achieved restoration of vertebral geometry largely diminished. Neither the measurement of the failed vertebral geometry or the estimation of its bone density was found to offer a predictive value for pre-operative planning.

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Aggressive Vertebroplasty May Cause Adjacent Bone Fractures

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Clinical studies of vertebroplasty cases have reported that more than 20% of patients with augmented vertebrae are expected to sustain further fractures [1-3] with approximately 67% of the new fractures occurring in the adjacent vertebrae [2]. The elevated risk has been hypothesized to be attributed to the shift in load distribution within the vertebroplasty treated vertebrae causing a "stress-riser" effect along the spinal column [4]. The objective of this study was to investigate to whether vertebroplasty lowered the fracture strength of adjacent untreated vertebrae under physiological loading conditions.

METHODS: Twelve fresh-frozen cadaveric spinal segments (3VB+ 2Discs) from six human spines (six T10-T12 and six L1-L3) were utilized in this study. L1 and L3 vertebrae (treated group) were injected with 10 cc of PMMA using a trans-pedicular-bilateral approach. Specimens were mounted to a 6DOF robotic arm to apply pure compressive load, thereby following the path of minimum resistance to fracture the samples. The compressive load was increased in increments till the segment was fractured or compressed 20% based on its initial height. At each 600 N load increment, plane X-ray radiographs were taken and visually reviewed.

Figure 1: *Simulation of physiological loading through path of least resistance using a 6-DOF robotic arm.*

RESULTS: The average fracture stresses of the vertebroplasty treated specimens was 38% less compared to the specimens in the control group (range 7.7% to 45.5%). Treated segments had biconcave fractures while wedge fractures were mainly seen in the untreated segments. In all twelve samples, changes within the load-displacement curve were more sensitive than inspection of the X-ray films in detecting fractures. In the treatment group the superior and inferior endplates of all L2s were fractured, whereas in untreated group, the segment failures were mostly due to anterior wedge fractures.

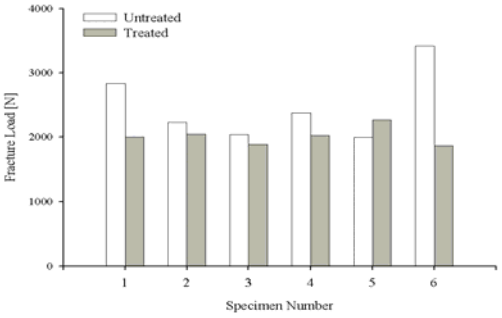

Figure 2: *Paired comparison between predicted fracture loads from untreated lumbar segments and the treated lumbar segments. Strength reduction was observed for all specimen except for Specimen #5 which had a 13.8% increase.*

CONCLUSIONS: The results support our hypothesis that vertebroplasty acts as a "stress-riser" therefore causing the endplates of the adjacent vertebra to collapse [4,5]. This effect may be due to changes in load-transfer between adjacent vertebral bodies after vertebroplasty treatment, which reduces the effective cross sectional area for load bearing. New types of bone cements and modified bone cement distribution pattern may prevent adjacent bone failure.

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Aspiration Techniques enhance Controlling the Filling of low viscous Bone Cement in Leakage Experiments

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: The injection of a progressively curing polymeric material (PMMA bone cement) is time sensitive. The later cement is injected, the higher are the force necessary for injection, until eventually injection is no more possible. On the other hand, the earlier the PMMA is injected, the higher the risk of leakage. Baroud & Bohner [1] showed that the leakage can be controlled by injecting the cement in its high viscous regime. This study is to examine whether or not aspiration techniques with a new cannula enhance the control of cement filling and the removal of displaced bone marrow both of which are important limitation in vertebral augmentation.

METHODS: *Cannula Design:* The tool consists of two concentric tubes forming an internal conduit for cement delivery, and an annular space attachable to a suction system. This new cannula design enables the physician to inject and aspirate the bone marrow from one surgical site, as well as to control the filling pattern. *Leakage model:* The existing experimental leakage model of Baroud & Bohner [1] was adopted with some modifications to ensure higher reproducibility. *Study Design:* The factors examined include the aspirating pressure-time regime at the exit of the cannula and the elapsed time from mixing the cement till injection. A Factorial design (2 factors, 4 and 2 levels) with 4 repetitions per treatment was used.

RESULTS: The cannula design fulfils its task to create a pressure gradient driving the cement to spread more homogeneously. The bone marrow substitute was aspirated to a certain extend, but less than wanted by the experimenter (Fig.1 B). Furthermore, a weak correlation between the amount of aspirated bone marrow substitute and the homogeneity of the filling pattern was revealed.

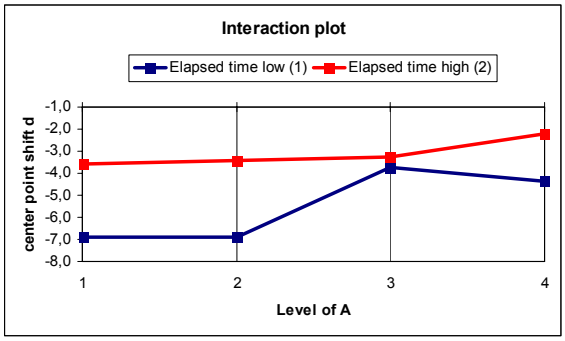

Fig. 1: (A) Leakage model experiment. (B) Cut vertebra model after injection with red highlighted cement distribution using the new cannula design.

CONCLUSIONS: The experiments are giving much evidence that controlled filling of cement is feasible even in its low viscosity regime by using aspiration techniques and thus, the risk of leakage can be reduced. Their application for low elapsed times led to results similar to those for higher elapsed times obtained without any aspiration technique but with the thick cement.
There are two main advantages of controlling the filling with the low viscosity cement: firstly, the required injection pressure is smaller; secondly the aspiration of bone marrow reduces the risk of cardiovascular instabilities. The very best treatment combination was found to be injecting the cement after the higher elapsed time (8 min) combined with “Advanced Aspiration”. The new cannnula design is simple and fits well into extant clinical practice.

REFERENCES: [1] G. Baroud, M. Bohner, Spine 31 (2006) 2562-68

Mechanical efficacy of vertebroplasty

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Vertebroplasty can help restore mechanical function following vertebral fracture, but clinical outcomes are variable, and the risk of adjacent level fracture may increase. This study aimed to characterise the variable influences of bone mineral density (BMD), disc degeneration, fracture severity and type of injected cement on the efficacy of vertebroplasty. A second aim was to determine how vertebroplasty affects vertebral deformations (strains) at injected and adjacent levels.

METHODS: 15 pairs of thoraco-lumbar motion segments (51-91 yrs) were loaded to induce vertebral fracture. One of each pair was augmented with Cortoss, the other with Spineplex. Load distributions on vertebrae were investigated by pulling a pressure-sensitive needle through the intervertebral disc whilst under 1.5kN load. "Stress profiles" indicated the intradiscal pressure (IDP), stress peaks in the posterior annulus (SP_P), and neural arch load-bearing (F_N). BMD was measured using dual photon X-ray absorptiometry, and severity of fracture was quantified from height loss. Sixteen additional motion segments (42–89 yrs) were creep loaded at 1kN for 30 mins while deformations of both vertebral bodies were tracked using an optical MacReflex system. Deformations were remeasured under the same loading conditions after inducing fracture and again after augmenting the fractured vertebra with Spineplex.

RESULTS: Following fracture, IDP fell by 43-62%, depending on posture ($p<0.001$), whereas SP_P and F_N increased ($p<0.001$). Following vertebroplasty, these effects were significantly reversed. However, no differences were observed between PMMA- and Cortoss-injected specimens. After fracture, decreases in IDP, and increases in SP_P and F_N, were greater in specimens with lower BMD or more severe fractures ($p<0.05$). After vertebroplasty, specimens with lower BMD showed greater increases in IDP, and those with more degenerated discs showed greater reductions in SP_P ($p<0.05$). Quasi-continuous "creep" deformations of the vertebral body were observed. These increased after fracture but were reduced following vertebroplasty (Figure 1A). However, at adjacent levels, vertebral creep increased further after vertebroplasty (Figure 1B).

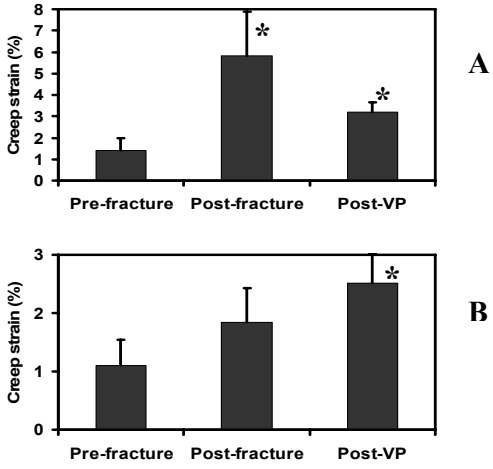

*Fig. 1: Creep deformation of fractured (A) and adjacent (B) vertebrae. *Significantly different from pre-fracture.*

CONCLUSIONS: Restoration of spinal load-sharing following vertebroplasty is independent of cement type but is influenced by BMD and disc degeneration. Vertebroplasty can increase vertebral creep strains at adjacent levels during subsequent loading and this may contribute to an increased risk of adjacent level fracture.

The Ideal Biomaterial for Vertebroplasty

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Since its introduction about 25 years ago, multiple unanswered questions still remain about the biomechanics of vertebroplasty. These questions are related to: 1) optimal volume of augmentation material 2) ideal placement of that material and 3) ideal properties of augmentation material.

While the biomechanical recover after vertebroplasty treatment has been numerously reported [1-3], it is still unclear what criteria define an ideal biomaterial for this procedure. The goal of this study was to identify parameters that most influence the biomechanical efficacy of vertebral augmentation. Specifically, we investigated patient specific, procedural specific and loading specific parameters.

METHODS: A combinatory approach of cadaveric testing and computer modelling was used to determine the effects of patient factors (bone mineral density, fracture severity, intervertebral disc properties, spinal level), procedural properties (augmentation volume, biomaterial strength, biomaterial stiffness, biomaterial distribution) and loading conditions (monotonic, repetitive loading) on the biomechanics of vertebroplasty.

A series of anatomically detailed and experimentally calibrated vertebral body models have been generated. Preventive vertebroplasty as well as vertebroplasty for fracture treatment have been evaluated with regards to the above-mentioned parameters. Human cadaveric experiments have been conducted to support the numerical results.

RESULTS: Greater augmentation effects were observed for low density vertebral bodies compared to vertebrae with higher bone density. However, fracture treated vertebrae failed to achieve low fracture risk. Preventive vertebroplasty was more effective in reducing secondary fracture risk compared to fracture treatment. Cement interdigitation requires less fill to recover biomechanics compared to localized placement. Localized delivery of cement causes a more rapid loss of vertebral biomechanical properties compared to interdigitated distribution pattern.

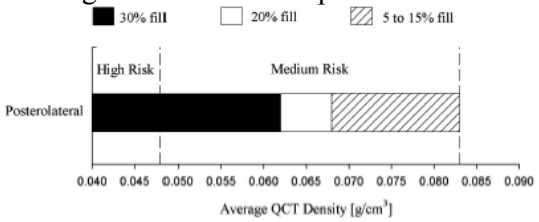

Figure 1: Minimum fill to reinforce vertebral body to low fracture risk.

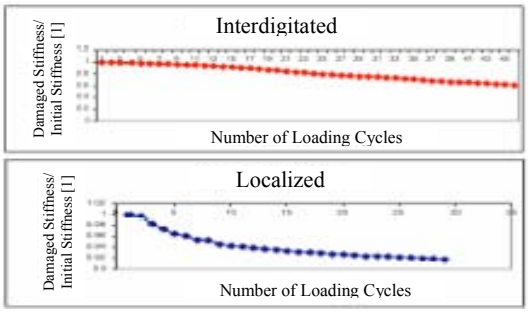

Figure 2: Normalized stiffness reduction due to fatigue loading and material distribution.

CONCLUSIONS: There is a delicate balance between recovery of biomechanical properties after cement augmentation and internal stress distribution near the cement/ bone interface. Our results indicated that interdigitated cement in preventive augmentation achieved the best biomechanical short and long-term outcome.

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ACKNOWLEDGEMENTS: Partial funding was provided by Orthovita Inc.

Session 1 - Suite

VERTEBRAL BODY AUGMENTATION

Chairmen: F. Cabana & K. Murphy

Utility of Combined Radiofrequency Ablation and Cementoplasty in Painful Neoplastic Lesions of the Axial Skeleton

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Purpose: To report our experience in the use of combined radiofrequency ablation and cementoplasty. To assess the safety and efficacy of the combined procedure in the treatment of painful neoplastic lesions of the axial skeleton.

MATERIALS AND METHODS: Between the 1st of June, 2006 and 15th February, 2008 (20 months), 20 combined treatments were completed in 15 patients. 9 vertebrae (8 lumbar and 1 thoracic), 7 acetabulae, 3 sacra and 1 pubic symphysis were treated with a total of 29 radiofrequency ablations. The patient age range was 49-82 (mean 59.8) comprising 5 females and 10 males. All patients had a documented primary malignancy including 6 primary lung cancers (4 NSCLC, 2 SCLC), 3 breast, 1 colonic, 1 prostate, 1 synovial sarcoma, 1 myeloma, 1 TCC and 1 oral SCC. For each patient the location of the primary neoplasm, lesion size, pain pre and post procedure (visual acuity score, McGill pain scale and Oswestry disability questionnaire, number of RF treatments, temperature, RF time, cement volume and estravasation were documented. The vertebral radiofrequency ablations were performed with 15 cm Valleylab radiofrequency electrode kits having 1 cm active tips (Tyco Healthcare USA). The sacral and acetabular radiofrequency ablations were performed with Boston Scientific Radiotherapeutics Probes with 3 cm active tips. In all cases, osteofirm radio opaque bone cement (Cook Bjaeverskov, Denmark) was injected.

RESULTS: A total of 20 combined radiofrequency ablations and cementoplasties were performed. There was 100% technical success rate (20/20) combined radiofrequency ablations/cementoplasties and 29/29 individual radiofrequency treatments). No major complications were encountered. One minor complication of self –limited estravasation into the needle tract (4.8%) occurred. The mean RF time was 9.4 minutes (range 9-12 minutes). The mean cement volume injected was 4.5 ml (range 2-10 ml). The mean pre-procedure pain as measured by the visual analogue scale was 7.9 (range 7.0-9.0) The post combined treatment value being 3.9 (range 2-6)

CONCLUSION: The technique of combined radiofrequency ablation and cementoplasty appears to be safe, practical and effective in the treatment of painful neoplastic lesions involving the axial skeleton.

TricOsTM and Fibrin Sealant Combined for Bone Defect Filling: From Pre-Clinical Tests to Prospective Clinical Study. Preliminary human data

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Keywords: TRICOS, mouldable self-hardening biodegradable bone substitute, tolerance, histology/morphometry, X-rays and CT-scan follow up.

☒ Biomaterials	☐ Biomechanics	☒ Clinical	☒ Innovation

INTRODUCTION: The association of TricOsTM (Macroporous Biphasic Ceramic Phosphate MBCP granules) and the fibrin sealant FS VH S/D 4, has been developed to answer a challenging request of orthopaedic surgeons: a biocompatible, osteogenic, mouldable, and self-hardening bone substitute able to fill randomly shaped bone defects. The aims of this study was the evaluation of the performance and safety of the bioactive bone substitute TricOs™ associated with a fibrin sealant in regeneration of functional bone.

METHODS: The pre-clinical tests were conducted to optimize MBCP granules size and ratio MBCP–FS VH S/D 4 (sheep maxillary sinus grafting, femoral epiphysis defect in rabbits, long bone defects in sheep). A clinical study design was set up as an exploratory prospective French multicentric phase II study sponsored by INSERM (Institut National de la Santé et de la Recherche Médicale). The application was the TOV (Tibial Osteotomy of Valgisation) using osteosynthesis and bone substitute: TricOs™ mixed with the fibrin sealant (FS VH S/D 4) for filling the space created. The follow up is 13 months with safety checks, clinical assessments, highsensitivity X-ray, and CT-scan imaging. A bone sample will be collected from the reconstructed area at 12 months, during the osteosynthesis material removal surgery. The principal criterion is CT-scan imaging performed 12 months after TOV surgery, before material removal, to assess qualitative and quantitative bone reconstruction. Animals' studies demonstrate that the biomaterial is safe to use and shows osteoconductive properties, granules resorption and bone ingrowth at the expenses of the implants.

RESULTS: As for clinical trial, 7 patients are today included in the study: This paper present the first results obtained from X-ray imaging during follow up.

TEMPERATURE IN VIVO MEASUREMENT DURING POLYMERIZATION OF BONE CEMENT IN PERCUTANEOUS VERTEBROPLASTY

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☐ Biomaterials	☐ Biomechanics	☒ Clinical	☐ Innovation

INTRODUCTION:

Percutaneous Vertebroplasty (PV), first introduced by Galibert and Deramond in 1987[1], is gaining acceptance as a first choice treatment for painful osteoporotic compression vertebral fractures and malignancies in the spine when conservative medical therapies failed. Several large clinical studies showed good long-lasting pain relief, rapid rehabilitation and, consequently, improvement of the quality of life [2,3,4,5,6,7,8,9].

The exothermic reaction of the polymethylmethacrylate (PMMA) during polymerization is often believed to be the leading cause of pain regression resulting from thermal necrosis of neural tissues of the vertebral body[10,11]; whereas other studies assert that mechanical restoring of vertebral integrity plays the main role in the clinical outcome[12,13,14].

The purpose of this in-vivo experimental study is to define the role of exothermic reaction, occurring during the polymerization of injectable bone cements, in pain relief .

A further goal was to assess potential bone tissue necrosis and nerve root or spinal chord thermal damage caused by exothermic reaction of bone cement.

METHODS: 22 women (60-80 years mean 75), suffering from painful osteoporotic vertebral collapse, underwent 22 bilateral transpedicular PV using 11 different PMMA (Confidence 1, MendecSpine 2, Osteopal-V 3, Osteofirm 4, Spinefix 5, KyphX-HVR 6, ArthroCareSpine 7, CementoFixx 8, Vertebroplastic 9, AvaTex 10, Cortoss 11). Temperatures of different products were constantly measured after having inserted a thermoablation needle (Starburst XL, RITA Medical System, Mountain View, CA, USA) coaxially trough a previously inserted 10 gauge vertebroplasty needle, while PMMA (3 ml) was being injected on the other side. PV was completed after removal of the RITA needle by omolateral injection of PMMA.

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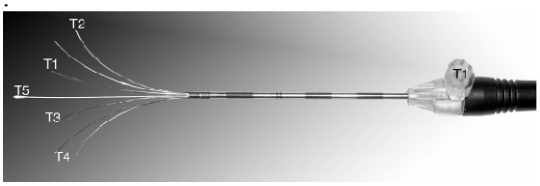

Figure1. *RITA radiofrequency thermoablation needle carrying 5 thermocouples (T) clockwise alternated in odd order.*

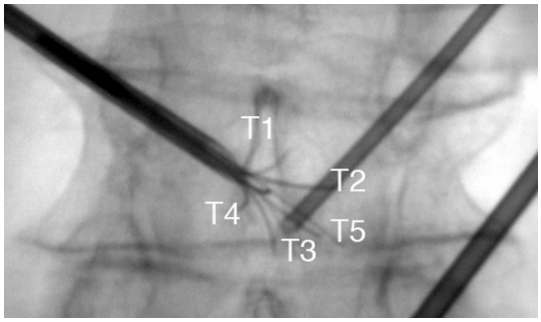

Figure 2.*Bilateral transpedicular approach with RFA needle trough the vertebroplasty needle on the left pedicle. (thermocouples are identified by "T" letters)*

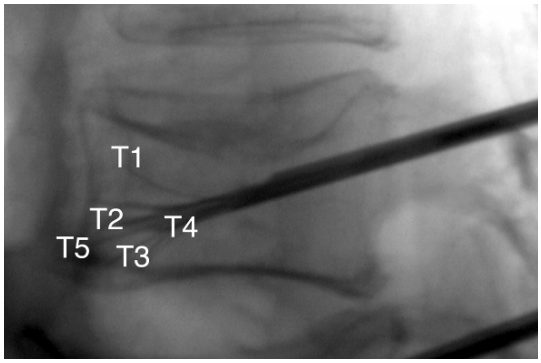

Figure 3 .*Latero-lateral view showing positioning of the needles in the anterior 2/3 of the vertebral body. (thermocouples are identified by "T" letters)*

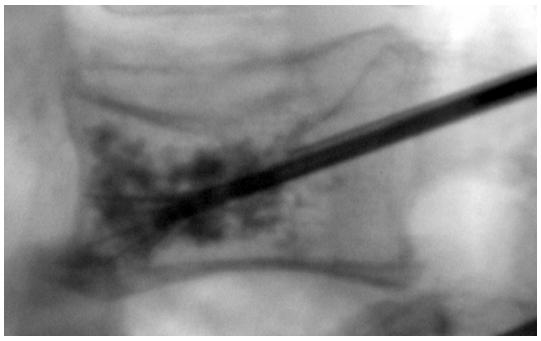

Figure 4.*Latero-lateral view demonstrating the thermocouples complete surrounded by the Bone cement*

RESULTS: Highest temperature (97.0°C) was measured in PMMA (1) which also had the highest viscosity; peak temperature ranged from 78.0 to 56.5°C in most of PMMA (2,3,4,5,6,7,8,9). Two cements (10,11) showed a lower peak temperature (range from 42.0 to 47.5°C). Peak temperature was demonstrated to be between 5 and 9 minutes from the beginning of the injection (avg 6':49" ±1':06") in all PMMA. Complete backpain regression was achieved in all patients within 48 hours from the procedure.

CONCLUSIONS: As complete injury to sensory nerves was demonstrated at temperature over 45°C applied for more than 30 min[15], high temperature developed during PMMA polymerization seems not to play a role in pain regression as it was achieved also with PMMA that showed lower peak temperature from 42.0 to 47.5°C. These data strongly suggest that backpain regression in PV is obtained by mechanical stabilization of the fracture and that spinal cord and nerve roots are not at substantial risk for thermal damage. On the other hand, most of tested cements can cause bone tissue necrosis that normally occurs above 50°C for more than 1 min[16] .

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Étiologie de la calcification des bioprothèses implantées chez l'humain

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L'implantologie humaine de valves porcines traitées pour le remplacement de valves cardiaques défaillantes remonte à l'intervention princeps de Binet en 1965. L'adoption d'un vocabulaire plus recherché, en l'occurrence, « bioprothèse » plutôt que « valve porcine traitée », n'occulte en rien mode de défaillance : la calcification limite leur biodurabilité. Elle s'accompagne fréquemment de déchirures et de rétention lipidique, entraînant fuites et régurgitations.

Soixante-deux bioprothèses Liotta Low Profile furent collectées lors de réopérations pendant une décennie. Elles furent implantées chez 56 patients, 31 femmes (âge moyen 52.0 ± 13.5, extrêmes 22/76) et 22 hommes (âge moyen 52.0 ± 18.0, extrêmes 19/71). Dans 3 cas, le sexe n'était pas précisé. Pour 46 patients, il s'agissait d'une implantation monovalve (15 aortiques, 31 mitrales). Dix patients reçurent 2 valves (aortique et mitrale), 1 patient reçut 3 valves (aortique, mitrale et tricuspide) 3. Les valves aortiques furent majoritairement explantées pour régurgitation (71.43 %). Ce pourcentage était plus faible dans le cas des valves mitrales (55.0 %).

La première cause de défaillance valvulaire fut la détérioration structurale des feuillets valvulaires causée par la calcification. Des déchirures furent fréquemment associées à cette pathologie. Ces minéralisations apparaissaient généralement dès la seconde année d'implantation et progressaient d'autant plus rapidement que le patient était jeune et de sexe féminin. Il s'agissait toujours d'hydroxyapatite, comme les études par rayons-X et en résonance magnétique nucléaire sur le ^{31}P l'ont démontré.

Malgré la faiblesse congénitale que représente la minéralisation, le concept de bioprothèses demeure très séduisant, car l'anticoagulation systémique n'est pas requise et la défaillance n'est pas abrupte contrairement aux valves mécaniques. Les recherches en cours visent à prolonger leur durabilité, car de nouveaux besoins apparaissent pour le déploiement percutané de valves susceptible de supplanter la chirurgie à cœur ouvert.

2008-02-24

The affect of inter-operator variability and experience in vertebroplasty outcomes

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☐ Biomaterials	☐ Biomechanics	☒ Clinical	☐ Innovation

INTRODUCTION: Over the past decade, percutaneous vertebroplasty has become a widely adopted therapy to treat vertebral compression fractures of diverse etiologies. Despite the success of this procedure, no studies to date have addressed whether operator variability or experience play a role in these beneficial clinical outcomes. Accordingly, we sought to determine whether various peri- or post-procedural parameters and outcomes displayed operator variability and whether these had any relation on beneficial clinical outcomes.

METHODS: We performed a retrospective review of all clinical outcome data from 7 of our primary vertebroplasty operators comprising 845 patients culled from our study population since its inception in 1999. Of these 7 operators, 2 were recruited to our institution with previous vertebroplasty experience, whereas the remaining 5 had never performed a vertebroplasty procedure prior to this study. Intra-operative measures such as cement volume, and post-operative measures such as pain, narcotic use, and mobility were tracked over time and operator. These clinical outcome data, including the Roland Morris Disability Questionnaire (RDQ) and analogue pain scale, mobility and narcotic use, were collected pre-operatively, immediately post-vertebroplasty and at 1 wk, 1 mo, 6 mo, and 1 yr following treatment.

RESULTS: As an average, all 5 operators offered similar clinical improvements in post-operative pain, disability, narcotic use, and mobility. However, when tracked over time, 4 of the 5 operators with no prior experience showed reductions in cement volume utilization and post-operative RDQ and rest pain showed significant temporal correlation reduction or improvement.

CONCLUSIONS: A training effect is apparent in the vertebroplasty procedure. Cement volume and immediate post-procedural pain improve with greater experience but, importantly, long term pain or mobility outcomes are not affected.

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Session 5

INTELLECTUAL PROPERTIES AND REGULATORY CONSIDERATIONS IN VERTEBRAL BODY AUGMENTATION

Chairmen: R. Mitchell & L. Renaud

Regulatory Affairs and Innovations in Vertebral Body Augmentation
Emmanuel Montini, BCF Certification, Montreal, Quebec

The talk will focus on several items of interest to the community of injectable biomaterials and bone augmentation, including (1) certification environment in Europe and in North America; (2) underpinning logics for the classification; (3) norms and standards applicable to medical device; (4) review of example devices in the field of vertebral body augmentation.

Strategies Relating to Patent and Other Intellectual Property for Bone Augmentation Systems and Devices
Robert E. Mitchell, Skeltex Inc., Boucherville, Quebec

Patents, in particular, are the both the curse and salvation of any new commercial embodiment of the latest laboratory “gem”. As a curse patents are expensive, time sensitive, geographically discordant and fatally vulnerable in the face of publications. As salvation patents are, although considered to be an intangible property, in most cases the only tangible asset for a start-up company on the basis of which financing can be obtained. In the longer run patents may be the main armour to protect market share for the new device especially against cheaper competitive products. We shall examine some simple steps that may help to minimise the negative aspects of patents in the field of bone augmentation while providing an acceptable level of protection.

Session 6

BIOLOGICS AND TISSUE ENGINEERING

Chairmen: J. Triffitt & K. Dai

Development of scaffolds and bioreactor to grow vascularized tissue substitutes

Patrick Vermette
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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

MICRO-VESSEL FORMATION is the result of a complex process of de novo emergence of endothelial cell progenitors with modification of the initial network, endothelial cell sprouting from a pre-existing vascular network, and micro-vessel adaptation to flow. The vascular formation requires that cells have sequential and distinct micro-environmental interactions with extracellular matrix and growth factors in a synergic and co-ordinated manner.

ENGINEERING of tissue mass or encapsulated cells is limited by the occurrence of hypoxia and acidosis in the central area of growing tissues or cells. With few exceptions, tissues in the body are permeated by vascular and micro-vascular network to supply essential nutrients and regulatory factors. The distance between capillaries generally ranges from 20 to 200 microns. To obtain tissue thickness clinically valuable, dimensionless and other types of analysis tend to point out that diffusive transport will have to be matched with an important convection to bring sufficient oxygen molecular flux to the growing cells located within a tissue mass. Thus, micro-vessel formation has important implication in tissue engineering, as they can serve that purpose. Lack of adequate vascularization to nutritionally support tissues has been shown to be an important drawback in many tissue engineering applications. Survival of cells deeply embedded in engineered tissue constructs is compromised leading to necrosis, which in turn also alters cells present at the periphery of the construct. On the other hand, cultures of engineered tissue devices in bioreactor have not been focused and are therefore poorly explored towards angiogenic response and micro-vessel formation. To address this situation, our Team has developed scaffolds and a bioreactor to direct angiogenic response and micro-vessel formation in tissue culture.

OBJECTIVES: The general objective of this research program is to develop and to use scaffolds and culture processes to guide vascularization within a defined 3-D environment. The main concept consists to use a bioreactor system in which operating conditions can be modulated to guide micro-vessel formation. A well defined orientation of cells/tissues that form capillaries is necessary to achieve adequate blood supply and normal tissue development into 3-D scaffolds.

RESULTS AND SIGNIFICANCE: A report compiling interviews with a number of the leading companies in the tissue engineering and regenerative medicine industry revealed that engineering challenges and the development of scaleable processes are urgently needed to give the tissue engineering industry a chance to meet the clinical and economical expectations. This research program should yield more insights and knowledge in comprehensive regenerative medicine. From a more practical point of view, scaffolds and bioreactor culture conditions that lead to vascularized tissues will have subsequent implications in engineered tissue products and cell encapsulation. This accomplishment could lead to significant advances in fabricating functional and fair-size mammalian tissues (e.g., calcified tissues, liver, kidneys and pancreas) for therapeutic purposes.

ACKNOWLEDGEMENTS: This research is supported by the CFI, NSERC, FQRNT, and by the Université de Sherbrooke.

In Vivo Xenogeneic Models for Assessment of the Regenerative Potentials of Human Mesenchymal Stem Cells

James T. Triffitt and Zhidao Xia

Botnar Research Centre, Nuffield Department of Orthopaedic Surgery, University of Oxford, Nuffield Orthopaedic Centre, Oxford OX3&LD, UK

☐ Biomaterials	☐ Biomechanics	☐ Clinical	☒ Innovation

INTRODUCTION: Experimental evaluation of the specific differentiation potentials of human cells *in vivo* is difficult. However, this is desirable for preclinical assessment before use of mesenchymal stem cells in musculoskeletal tissue regeneration or tissue engineering protocols. In the past the use of diffusion chambers to compartmentalise and physically separate donor and host cells [1], or steroid treatment to diminish immunorejection mechanisms [2], was obligatory. However, currently immunodeficient mouse models are becoming increasingly used as a greater variety of genetic mutations are known that impair immune functions in mice. With specific detection of the human donor cells, such models are very useful for understanding mechanisms supporting allogeneic and xenogeneic tissue growth following cell transplantation[3, 4]. These mice may be used as models of normal human osteogenic differentiation in neophysiological situations, to study the ontogeny of skeletal system development, to investigate the growth of tissues and the influences of biological factors and new healing strategies.

METHODS: To determine the potentials for development of cell populations immunodeficient mice (eg. nude, beige, Scid, NOD/Scid ,Scid/beige) are anaesthetised and receive implants of isolated cell pellets or injections of cell suspensions from allogeneic or xenogeneic animals. These are given into one of the following sites; subcutaneous, intraperitoneal, intramuscular, kidney capsule, juxta-skeletal, intramedullary, intracardiac or intravenous, either as free grafts or enclosed in diffusion chambers or other receptacles, such as collagen gel capsules, and including biomaterial scaffolds as appropriate. After periods up to 3 months cell differentiation and production of musculoskeletal tissue is assessed by using a variety of specific procedures. Detection of the donor cells is by using genetically-marked cells or species-specific identification with antibodies.

RESULTS: Figure 1 shows production of human bone tissue by GFP-marked MSCs as detected by fluorescence (A) or by immunohistochemistry (B) with mouse antibody to human vimentin.

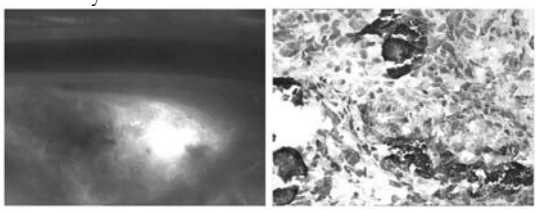

Fig. 1. **A** **B**

CONCLUSIONS: Immuncompromised mouse models yield novel *in vivo* experimental systems that are useful for assessment of the capacities of xenogeneic human mesenchymal stem cells for tissue regeneration. Such models require optimisation to evaluate cytotherapy, gene therapy and skeletal tissue engineering strategies using hMSCs in neo-physiological situations. In particular, the control of any residual osteoclast or macrophage responses of the mouse host towards the human osseous tissue needs to be considered for support of long-term osteogenesis from hMSC in immunocompromised mice

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A Preliminary Biological and Mechanical Investigation of HA Coated Zein Scaffold

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Zein is a natural protein. Both zein and its degraded products show good cell compatibility. In order to evaluate its possible application in tissue engineering, a 3-D zein scaffold has been developed and studied [1]. For satisfactory osseointegration and faster bone regeneration to be achieved,the zein scaffold is considered to be improved by HA coating.

METHODS: Porous zein scaffolds which were fabricated by salt-leaching were coated with calcium phosphate layers by a solution precipitation process. The compressive strength and the Young's modulus was determined by the computer controlled Electronic Universal Material Testing Machine. The osteoblastic differentiation of hBMSC on the surface of HA-coated zein scaffold was analyzed by alkaline phosphatase activity and RT-PCR.

RESULTS: The porosity of HA-coated zein scaffold is around 75% and the pore diameter is 150 μm. The porosity and pore diameter are adjustable. The morphological investigation under scanning electron microscopy showed that between the pores located on the surface and within the scaffold had good interconnectivity. The compressive Young's modulus was 34.36±12.63 MPa, and the compressive strength was 4.238±0.79 MPa which is suitable for repairing different sizes of cancellous bone defect and smaller defect of cortical bone. From the in vitro study with hBMSC cells, the osteoblastic differentiation on the inner surface of the porous HA-coated zein scaffold was increased, as expressed by the alkaline phosphatase activity and RT-PCR analysis for marker genes (Fig.1).

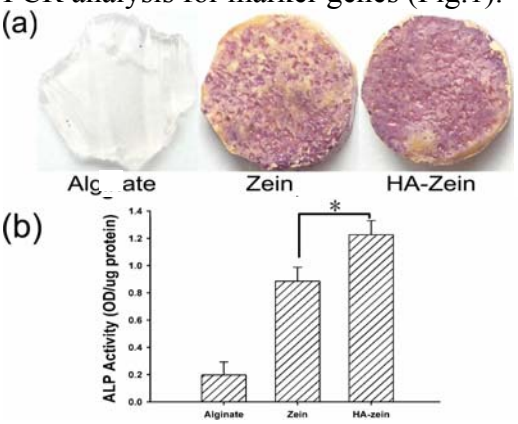

*Fig.1: ALP staining (a) and ALP activity quantification (b) of hBMSCs on zein scaffolds before (middle) and after HA-coating (right). HA-coating increased the ALP activity to a higher level during cultivation (alginate as control group). *p<0.05; n = 6*

CONCLUSIONS: Both the mechanical and biological evaluations showed that the HA-coated zein scaffold had better biocompatibility and was found to be a potential optimal biomaterial for bone tissue engineering.

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A New Method for the Synthesis of Disulfide Crosslinks Injectable Hyaluronan Hydrogel

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Hyaluronan (HA), a major component of the cartilage extracellular matrix (ECM) and an important molecule for wound healing has been modified using varieties of chemical methods and injectable hyaluronan hydrogels synthesis already reported for tissue engineering applications. For example, a redox sensitive sulfhydryl-modified HA using a disulfide containing hydrazide which reacted with the carboxylic groups of the D-glucuronic acid repeating unit on the HA structure in the presence of a carbodiimide was synthesised.[1] In this report, a new one-pot synthesis for the preparation of disulfide crosslinks hyaluronan hydrogel, adapted from De Nooy A.E.J. *et al.* and which does not necessitate the synthesis of hydrazide is explored.[2]

METHODS: In a 2 $mg.ml^{-1}$ hyaluronan (MW ~500 kDa) solution (water/ethanol mixture), L-cystine hydrochloride, formaldehyde and phenylcyanide were successively added with a strong stirring at room temperature for 3 minutes. The reaction was left to proceed for 24 hours and the hydrogel collected, dialyzed with deionised water before being freeze dried for 48 hours and characterized by infrared spectroscopy [FTIR Perkin-Elmer, KBr pellet]. The hydrogel gelation time was recorded as the time at which the hydrogel no longer flows when the vial was inverted (Figure 1). The following experimental conditions variations were explored: water/ ethanol volume ratio, carboxylic function/ reactants (L-cystine:formaldehyde:cyanide) molar ratio and initial pH.

RESULTS: The HA hydrogel is formed using an "Ugi" four component condensation.[2] Carboxylic acid functions on D-glucuronic acid repeating unit, L-cystine hydrochloride two amine functions, formaldehyde and phenylcyanide reacted to yield α-(acylamino)amide bonds creating disulfide crosslinks in between HA molecules.

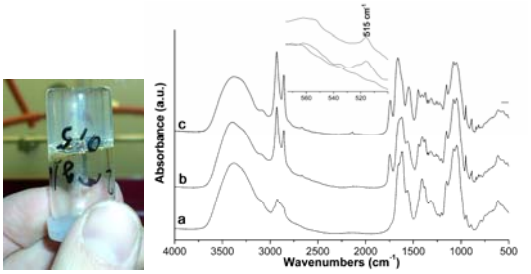

Fig. 1: Representative HA hydrogel image and FTIR spectra of HA (a), HA+L-cystine.HCl (b) and HA Hydrogel (c).

Disulfide crosslinks in HA hydrogel was verified by the presence of S-S stretch IR vibration at 515 cm^{-1} (Figure 1).

Table 1: Experimental conditions effect on HA gel formation and gelation time.

H_2O/ C_2H_6O	COOH/ Reactants	pH Initial	Gel	Gel. Time
1/0	1/0.5:1:1	4.5	✗	-
1/1	1/0.5:1:1	4.5	✔	15 min
1/0.5	1/0.5:1:1	4.5	✔	5 min
1/0.5	1/0.5:1:2	4.5	✔	3 min
1/0.5	1/0.5:1:2	4	✔	1 min

Addition of a reducing agent (TCEP) to a HA hydrogel induced reduction of the disulfide bond. HA/TCEP solution dialysis in oxygenated water allowed the reformation of the HA hydrogel.

CONCLUSIONS:

Further optimization and characterizations are necessary to assess the HA hydrogels. Injectability and biocompatible of HA hydrogel will be studied for its use in cartilage tissue engineering.

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Preparation and properties of injectable polymeric calcium phosphate cements derived from thermosensitive PEG-PLGA-PEG

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Osteoporosis is defined as a disease characterized by microarchitecture deterioration of bone tissue which leading to enhanced bone fragility and a consequent increase in fracture risk. In this study, we fabricated a injectable thermosensitive polymer of poly (ethylene glycol-*b*-[DL-lactide-co-glycolide] -*b*-ethylene glycol) (PEG-PLGA-PEG) which undergoes sol to gel transition. Furthermore, we added the hydroxyapatite (HA) into the triblock copolymers. Hydroxyapatite was widely used as the bioceramic component because of it is the main inorganic component of the hard tissues in bones.

METHODS: The PEG-PLGA-PEG triblock copolymer was synthesized by ring-opening polymerization of d,l-lactide and glycolide with monomethoxy-poly(ethylene glycol) in the presence of stannous octoate, and then coupled using hexamethylene diisocyanate. On the other hand, hydroxyapatite compounds were prepared by solution-precipitation method using $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ as starting materials and ammonia solution as agents for pH adjustment.

RESULTS: The viscosity change of PEG-PLGA-PEG copolymer is shown in Fig.1, which shows the viscosity increased with the temperature was elevated. The viscosity versus temperature was plotted can be obtained the critical gel temperature (CGT).

Fig.2 shows the change in mass of the hydrogels. The mass loss of 15%wt hydrogel was fast in first 20 days. The rate of the degradation would be slow down when added hydroxyapatite.

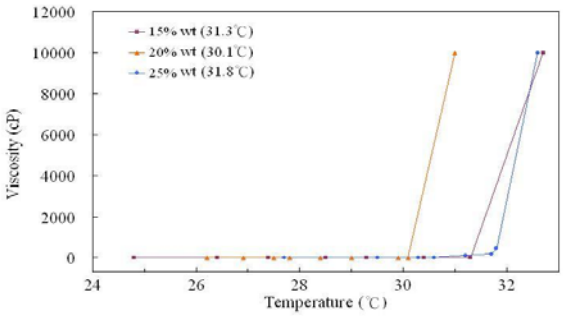

Fig. 1: Viscosity change of the PEG-PLGA-PEG hydrogels at various concentrations.

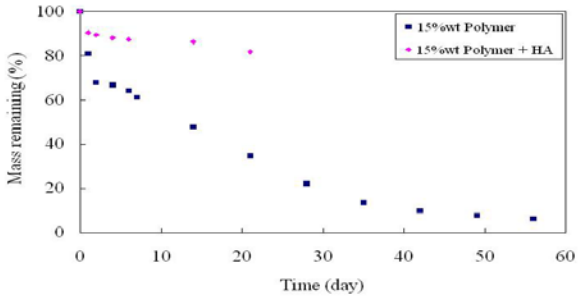

Fig. 2: Mass residue of hydrogels during degradation.

CONCLUSIONS: In this study, thermo-sensitive triblock copolymer was successfully prepared. In the future, we'll research the injectability of the PEG-PLGA-PEG / HA hydrogel, which can apply to the bone repair.

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ACKNOWLEDGEMENTS: The financial support of this research by the National Science Council (subsidy no. 95-2627-M-033-001) is gratefully acknowledged.

A cellulose-based self-setting hydrogel for articular cartilage repair

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☒ Innovation

INTRODUCTION: Spontaneous healing of articular cartilage lesions is limited due to its avascular and aneural nature. To promote the repair of this tissue, transfer of autologous chondrocytes with a three dimensional matrix appears promising. In this attempt, we developed a self-setting silanized cellulose derivative (Si-HPMC)[1]. In previous works, we have shown that Si-HPMC enabled the proliferation ofchondrocytes and the expression of the major chondrocyte markers during a three-dimensional culture *in vitro*[2]. We also demonstrated that our Si-HPMC hydrogel containing human chondrocytes allowed the production of a cartilage-like tissue in subcutaneous site in *nude* mice[3]. The aim of the present work is to evaluate the preclinical interest of autologous nasal chondrocyte (RNC) associated to Si-HPMC for the treatment of rabbit articular cartilage defects.

METHODS: RNC were phenotypically characterized after 3D culture in Si-HPMC by Real-time PCR. RNC were then amplified *in vitro* during 4 weeks before transplantation with Si-HPMC in critical-size defects created in rabbit articular cartilage for 6 weeks. Implants were histologically characterized by Alcian blue and Masson's trichrome stainings as well as type II collagen immunostaining.

RESULTS: Real-time PCR indicated that dedifferentiated RNC recovered expression of chondrocytic markers during in vitro 3D culture within Si-HPMC. Histological analysis of autologous RNC transplanted in an articular cartilage defect revealed the formation of a repair tissue. The repair tissue exhibited positive Alcian blue and Masson's trichrome stainings, indicating the presence of sulphated glycosaminoglycans and Collagen respectively. In addition, this repair tissue was positive for type II collagen immunostaining. This repair tissue also exhibited a well-defined structural organization quite similar to that observed in native articular cartilage.

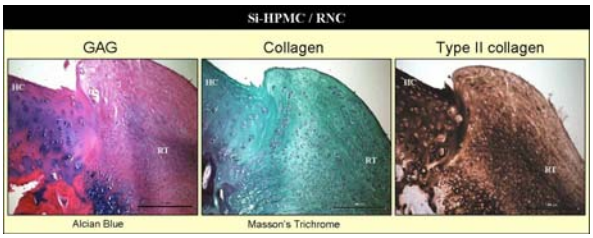

Figure 1: Representative sections of defects filled with Si-HPMC containing RNC after 6 weeks in vivo. Sections were stained with Alcian blue and Masson's trichrome and immunostained for type II collagen. HC: healthy cartilage. RC: repaired cartilage. Bar: 200 µm

CONCLUSIONS: The transplantation of Si-HPMC hydrogel containing autologous nasal chondrocytes led to the successful repair of an articular cartilage defect in rabbit. This study therefore indicates that Si-HPMC hydrogel is a potential scaffold for the cellular therapy of articular cartilage.

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ACKNOWLEDGEMENTS: This study was supported by grants from "Fondation Arthritis", "Société Française de Rhumatologie", "ANR-young researcher" and GRAFTYS S.A.S.

Hyaluronic acid/Chitosan nanoparticles as a new carrier for gene delivery

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: The present work shows hyaluronic acid/chitosan nanoparticles as a new carrier for gene delivery. Polyelectrolyte complexes (PEC) formed using biocompatible materials represent a promising way to inject and delivery DNA in-vivo [1]. Chitosan (Ch) is a natural cationic polysaccharide, which has been well studied as a carrier for DNA transfection in-vivo and in-vitro [2]. Hyaluronic acid (HA) is an anionic polysaccharide present in the extracellular matrix and known to link the CD44 receptor, which is implicated in tumor metastasis [3]. In this work, we took advantage of the properties of these polymers and developed biocompatible HA/Ch nanoparticles with improved stability and increased cell transfection efficiency. **METHODS:** HA17, 35 and 64kD (Lifecore Biomedical, USA), and chitosan 5 kD (Medipol SA) were used in the preparation of nanoparticles. Zeta PALS (Brookhaven Instruments, USA) and low angle dynamic light scattering (DLS) (HPPS, Malvern instruments, GB) were employed to measure nanoparticle charge and size. Atomic force microscope (AFM) (Nanoscope III a, Digital Instruments) served to determine the shape of nanoparticles and to confirm there size. Proliferation and toxicity of HA/Ch nanoparticles on HEK-293T cells were evaluated with Vybrant MTT Cell Proliferation Assay Kit (Invitrogen, CA). Transfection efficiency of pEGFP-C3 plasmid in HEK-293T was measured with flow cytometry (FACScalibur, BD Biosciences, CA). Data was then analyzed with Flowjo software. **RESULTS:** The imaging of nanoparticles by AFM in dry conditions revealed that particles have a spherical shape. The HA/Ch ratio and the molecular weights of the two polysaccharides were varied with the aim of obtaining small nanoparticles with an Z-average size of 146 ± 1 nm particles. MTT assays demonstrated that the nanoparticles are not toxic and the proliferation rate was similar in treated and non-treated cells. The optimal parameters for transfection are a pH ranging from 6.4 to 6.8, 0.25 ug of EGFP plasmid / well and a nanoparticle/cell incubation period of 4 hours. Using these optimized parameters, HA/Ch/DNA nanoparticles successfully transfected 25% of the 293T cells with pEGFP, compared to 0.7 % obtained for Ch/DNA in the same conditions.

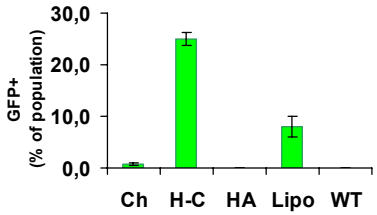

Fig. 1: (A) Comparison of transfection efficiency measured with GFP expression in-vitro when using chitosan (Ch), Hyaluronic acid / Chitosan (H-C), Hyaluronic acid (HA) and lipofectamine (Lipo) with HEK-293T cells.

CONCLUSIONS: Our results have shown that the combination of hyaluronic acid with chitosan generates stable and small nanoparticles with a narrow size distribution and high transfection efficiency. The ongoing work aims at using HA/Ch nanoparticles with cells which have a high occurrence of CD44 receptors, such as cancer cells [3]. This will provide us a great opportunity for developing cancer targeting nanoparticles for gene therapy.

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Inter-penetration network of an injectable tissue engineering hydrogel

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: In the last years biopolymers have been investigated for potential applications in the biomedical field, mainly as materials for tissue engineering scaffolds [1]. Silated hydroxypropylmethylcellulose (Si-HPMC) appeared as a potential scaffold for three-dimensional amplification and transfer of chondrocytes in cartilage tissue engineering [2]. The Si-HPMC viscous solution (pH 12.8) cross-links by decreasing the pH using an acid buffer, since Si-HPMC solution transforms into an elastic state (pH 7.4). The self-hardening principle of the Si-HPMC hydrogel is based on the silanes grafted by an epoxy function along the HPMC chains. The kinetics of cross-linking and final elastic properties is influenced by several parameters such as polymer concentration, pH and temperature [1].
The aim of this study was to characterize the rheological and the mechanical properties of the three-dimensional network of the Si-HPMC hydrogel after modification by inter-penetration network (IPN) using a viscous phase (HPMC). This association can simulate the extracellular matrix production within the hydrogel.

METHODS: The HPMC was grafted with 3-glycidoxypropyltrimethoxysilane. 3% (w/w) of Si-HPMC powder was solubilized in NaOH (0.2M) solution. This Si-HPMC solution was then dialyzed and sterilized. To create a viscous phase on the inside of Si-HPMC network, HPMC was solubilized at different ratios in an acid buffer (pH 3.2) and used to decrease pH of Si-HPMC from 12.8 to 7.4.
Three weeks after crosslinking, rheological (frequency sweep) and mechanical (stress relaxation) measurements (n=3) were realized at 25°C. RS 300 rheometer (ThermoHaake®, Germany) and TA-HD-plus texture analyzer (Stable Micro Systems™, UK) were used, respectively.

RESULTS: The rheological data qualitatively shows the elastic behavior of all preparations (0, 0.47 and 0.95% of HPMC). Si-HPMC hydrogels without added viscous phase behave like an elastic medium with a storage modulus G' which is frequency independent and a loss modulus G" which decreases with decreasing frequency. Results indicate the dependence of the viscous phase concentration on the storage (G') and the loss (G") moduli in the frequency range, since G' decreases slightly with HPMC concentration and G" increases strongly with HPMC concentration.
The modeling of the stress relaxation curves reveals the dependence of the added HPMC concentration on the viscosity of the formed Si-HPMC hydrogel.

CONCLUSIONS: Mechanical results confirm those obtained by rheology. The network modification is possible, and the extracellular matrix production within the three dimensional networks could be controlled by rheology and/or mechanics.

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Session 7

PLASMA TECHNOLOGY AND CHARACTERIZATION

Chairman: M. Moisan

Gaseous cold plasmas: basic properties and application to surface treatment

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Plasma is by far the most common form of matter (99% of the Universe). It is a gaseous state (often called the 4th state of matter with respect to energy gradation from solid to liquid and then (neutral) gas) that contains electrons, ions and photons. Strictly speaking, a plasma is a hot gas consisting of approximately equal numbers of positively charged ions and negatively charged electrons. For surface processing, we tend to use a medium of much lower temperature than plasma, namely an ionized gas, which contains, in addition to charged particles, electrically neutral particles such as molecules, fragments of molecules and atoms, all these particles being in an internal energy state that is minimum (ground state) or higher (excited states). As a rule, the term cold plasma is used as an equivalent to ionized gas. Various types of surface processing can be made utilizing ionized gases: thin film deposition (e.g. for hardening purposes), etching (e.g. in chip manufacturing), cleaning, disinfection and sterilization. Surface modifications are obtained in various ways, including ion bombardment, chemical reaction (e.g. functionalization), photon activation.

First, we briefly review the basic properties of plasmas [1]. Second, we discuss the essential characteristics of different types of plasma sources that can be efficiently used for surface processing.

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Medical Applications of Non-Thermal Plasma

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☐ Biomaterials	☐ Biomechanics	☐ Clinical	☒ Innovation

The focus of this work is direct application of non-thermal atmospheric pressure plasma to human tissue, where this tissue serves as a second active electrode (fig. 1). Such use was shown to be advantageous over the existing technologies. Ultra Violet (UV) radiation, especially high energy Vacuum UV (VUV), does not have good penetration depth in air and VUV exists only in the discharge area. Short-lived active species and radicals (like OH, NO, $O_2(1\Delta_g^+)$ and other electronically excited species, atomic Oxygen, etc.) do not, generally, exist outside of the discharge gap, either. Electron and ion bombardment of the surface has been linked to very efficient sterilization rates and that also only exists in plasma. We observe experimentally a difference of a few times up to a few orders of magnitude improvement in bacteria inactivation rates under direct plasma treatment as compared to a "jet" where we blow out plasma and only treat the surface with the afterglow. Direct plasmas were not yet used in medicine for fear of application of 35,000 Volts directly to a human body. However, in the presented setup no voltage is applied directly to the body and most of the power is deposited in the discharge itself, leaving the living tissue undergoing treatment unharmed.

Floating Electrode Dielectric Barrier Discharge (FE-DBD) was shown to rapidly *coagulate blood* (<15 seconds), efficiently *sterilize living animal tissue* (~7 log reduction in 5 seconds), and *inactivate various skin diseases* (Melanoma, Leishmaniasis, etc) without causing any visible or microscopic damage to the treated tissue. This experimental evidence will be presented and discussed.

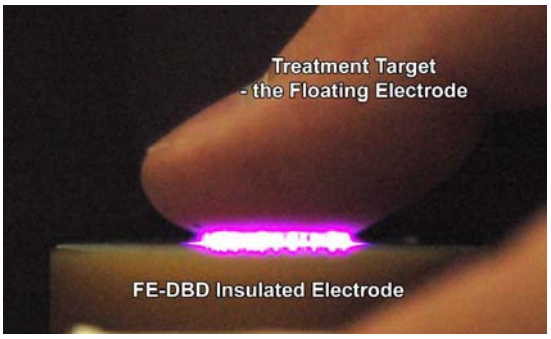

Fig. 1. Direct Application of FE-DBD electric plasma to living tissue. Here tissue serves as an active electrode.

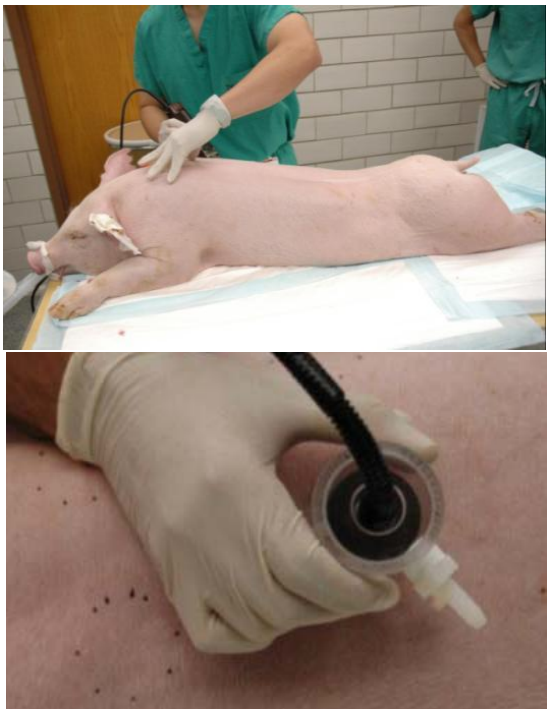

Fig. 2. Treatment of a living animal by floating electrode dielectric barrier discharge plasma. No damage is observed to animal skin in 5 minutes of treatment.

Plasma- and Photo-chemically Deposited Organic Coatings for Biomedical Applications: the Role of Primary Amines

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Abstract: Nitrogen (N)-containing polymeric surfaces are attractive in numerous technological contexts, particularly for biomedical applications. Our laboratory has developed several methods for depositing N-containing "polyethylene"-like thin film coatings, designated "PE:N", namely by atmospheric- and low-pressure plasma CVD ("H-PPE:N" and "L-PPE:N", respectively), and using vacuum ultra-violet (VUV) photochemistry ("UV-PE:N"). Chemical and structural characterisations of these materials where performed using X-ray photoelectron (XPS), Near-edge X-ray absorption fine structure (NEXAFS), and Fourier-transform infrared (FTIR) spectroscopies. Chemical derivatisation with 4-trifluoromethylbenzaldehyde (TFBA) enabled estimations of primary amine concentrations, the most relevant functionality here. While the same chemical functionalities were observed in all films, their relative abundances varied greatly. We then investigated the adhesion of U-937 monocytes to PE:N, cells that do not adhere on commercial cell-culture surfaces. In all cases we observed their adhesion and proliferation above certain minimum "critical concentrations" of primary amines, $[NH_2]_{crit}$. Values of $[NH_2]_{crit}$ were practically the same for all three PE:N types, independent of other surface characteristics such as total nitrogen concentrations, [N].

Keywords: Plasma polymerisation, VUV photo-chemistry, surface chemistry, cell culture

Plasma inactivation of biofilms within narrow-bore dielectric tubes

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Plasma sterilization is an alternative to conventional sterilization techniques. Plasma biocide species (UV photons, ions, radicals (e.g. O, OH)) can be used to inactivate microorganisms and sterilize surfaces. We have recently designed a plasma sterilization system to process narrow-bore thermosensitive dielectric tubes [1, 2]. A possible application is the sterilization of catheters (inner diameter tubes: 0.5-5 mm, length up to 1,5 m), these tubes being presently hard to sterilize (thus of single use only), since they do not resist to conventional treatments based on heating (both wet and dry heat). The process that we have developed allows to create plasma directly within the channels [1] and thus sterilize their inner surface (6 log of bacterial spores inactivated in less than 10 min). Within these narrow-bore tubes, microorganisms can not only sediment (a case for which we have already achieved sterility [2]), but also can grow as biofilms by developing a protecting envelop (sugar matrix), thus implying a more demanding inactivation process.

First, we review different methods of plasma sterilization [3] and their principal characteristics. Second, we discuss the problematic of biofilm sterilization. Finally, we present our most recent results on biofilm sterilization of *S. aureus* within Teflon tubes.

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2. Pollak, J., et al., *Plasma sterilization within long and narrow-bore dielectric tubes contaminated with stacked bacterial spores*. Plasma processes and polymers, 2008. **5**: p. 14-25.
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Suspension Induction-Plasma Spraying of α-TCP

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INTRODUCTION: Alpha Tricalcium Phosphate [α-TCP - $Ca_3(PO_4)_2$] is the main constituent of most apatitic calcium phosphate bone cements (CPBCs). Indeed, its hydrolysis leads to calcium-deficient hydroxyapatite [$Ca_9(HPO_4)(PO_4)_5OH$], which is a cement. The current standard fabrication process is a high-temperature solid/solid reaction between dicalcium phosphate anhydrate [$CaHPO_4$] and calcium carbonate [$CaCO_3$].[1] However, injectability of CPBCs is currently one of the major limitations of this elaborate process. Spherical particles would be an important asset for cement injection[2] but can not be fabricated by current synthesis. To obtain this particle shape, we set up suspension induction-plasma spraying (SIPS). This new method of synthesis is based on the allotropy of TCP. The alpha high-temperature phase (α-TCP) is obtained by SIPS of the low-temperature phase, β-TCP. The aim of our study is to prove that SIPS is able to produce spherical α-TCP.

METHODS: Powders were characterized before and after plasma spraying in order to specify their composition and their shape. X-ray diffraction (XRD) and Fourrier transformation infra-red (FTIR) were used to investigate the composition and the crystallinity. Particle size distribution (PSD) was measured both by scanning electron microscopy (SEM) and by laser particle size analysis. The characterization of particle surfaces was also completed by X-ray photo-electronic spectroscopy (XPS).

RESULTS: XRD spectra (Fig. 1) proves that a phase transformation occurs during the process. Indeed, before the projection, the powder consists of β-TCP, whereas after, α-TCP is the main constituent. Furthermore, the initial particles have randomly distributed shapes. After plasma treatment, they all are spherical, and two populations were found, with diameters of the order of 10 nm and 10 μm, respectively. Moreover, the smaller ones are predominantly agglomerated on the biggest ones (Fig. 1). Finally, the XPS data show that nano- and microparticles do not have the same surface composition. The nanoparticles are deficient in calcium in comparison to the microparticles.

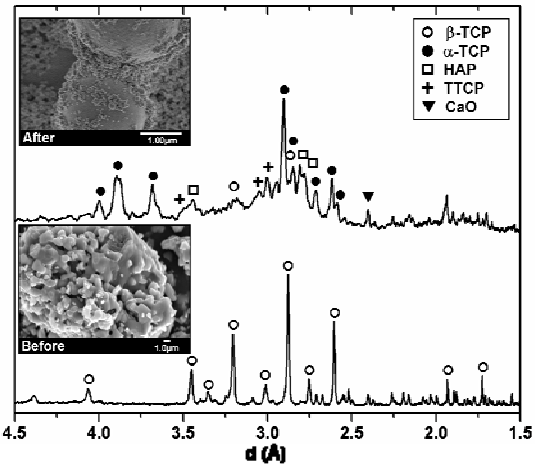

Fig. 1: XRD spectra and SEM of powders before and after the SIPS process.

CONCLUSIONS: In addition to minimizing the number of steps in the process, SIPS process is able to produce spherical α-TCP using β-TCP as raw material. Moreover, the particles produced feature a large distribution that is very likely to enhance the injectability of CPBCs. Nevertheless, the parameters of plasma spraying are currently being studied in order to limit the formation of unwanted phases and to promote α-TCP.

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Session 8

INJECTABLE BIOMATERIALS AND DELIVERY

Chairmen: P. Weiss & J. Barralet

What is injectability? A new injectability method for hydraulic cements developed for minimally invasive surgery

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Injectability is often addressed as one of the most important properties of materials to be used in minimally invasive surgery. But what is injectability and how is it measured?
Several methods have been described[1-3] but none of them gives satisfactory results since they, by their own, do not give all information needed to fully understand the actual system. There are two main phenomena that affect the injectability of hydraulic cements, i) the setting and ii) the phase filtration (filter pressing). In this work a new method is presented, where both phenomena are taken into account, which may be an important tool in the development of new injectable materials.

METHODS: To simulate injection in thin duct systems e.g. cancellous bone, the following method has been developed. The material is mixed and the paste is transferred to four 1 ml syringes with attached cannulae of minimal size. At 3 min first extrusion (0.1ml) is started with syringe no. 1. Smallest cannula possible to extrude from is searched. First extrusion is made with a cannula of 0.3 mm in outer diameter. If the extrusion is not possible with this size, it is replaced by a cannula of larger diameter. Larger and larger cannulae are tested until extrusion is possible. The cannula size where it is possible to extrude the paste is noted in the test protocol (see Fig 1). New extrusion trial is performed every minute. At 6 min first extrusion is started with syringe no. 2, at 9 min first extrusion is started with syringe no. 3, and at 12 min first extrusion is started with syringe no 4, according to the principle explained above. The test continues until there is no more paste in the syringe or until the material is too thick to be extruded, even without cannula.

Material:									
Time point (min)									
3	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	1	1	1 2	1 2	1 2	1 2 3	1 2 3	1 2 3	1 2 3 4

Fig. 1: Test protocol for injectability test with four syringes. The cannula size where extrusion is possible is noted for each syringe (1-4) at each corresponding time point.

RESULTS AND DISCUSSION: This novel method allows both phase filtration and the influence of the setting process to be observed simultaneously. Testing the same syringe repeatedly during time gives information about how the injectability is affected by the filter pressing effect. Leaving untested syringes for different time points gives information about how the injectability is affected by the setting of the material. Rheology measurements should also be a part of investigational work.

CONCLUSIONS: The present method describes a way of testing injectability that simultaneously gives information about both how the setting process and possible filter pressing affect the flowability in narrow ducts over time. This is a powerful tool in the development of injectable materials.

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Rheological characterization of calcium carbonate injectable cement

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: With the development of minimally invasive surgical techniques, there is a growing interest in the research and development of injectable biomaterials. Fast-setting calcium phosphate (CaP) bone cements have developed considerably in the last few years due to their excellent biocompatibility and bioactivity properties and also ease of use especially as an injectable paste [1]. An injectable calcium carbonate cement has been recently presented as promising biomaterials for bone filling and/or reconstruction [2].

We present herein the preliminary results on the rheological properties of this calcium carbonate injectable cement characterized under dynamic measurements (oscillations of small amplitude).

METHODS: The cement paste was prepared by mixing the appropriate amount of liquid phase (deionised water) with a powder mixture of metastable calcium carbonate phases (amorphous $CaCO_3$ and vaterite).

The rheological tests were conducted using a CARRI-Med CSL^2 100 rheometer (TA Instruments) equipped with a plate-and-plate geometry. A specific chamber allowed to maintain an atmosphere saturated with water around the sample tested throughout the measurements. The rheological characterization was performed at two temperatures 25°C and 35°C. Two experimental procedures have been set up to study the structuring kinetics of the cement subjected to one (procedure 1) or several flows (procedure 2) at constant shear stress rate (30 s^{-1} during 10 s) imposed during setting. Experimental procedures included a pre-shear of the paste (to start the measurements from the same pre-existing paste state) followed by low amplitude (i.e.< 0.05%) oscillatory shear to allow the paste to set and harden. Cement hardened during rheological measurements was characterized using FTIR spectroscopy, X-ray diffraction and scanning electron microscopy (SEM) techniques.

RESULTS: Figure 1 showed the influence of the temperature on the cement setting/structuration. For example, the time needed to reach a level of G" of 3.10^6 Pa is 38 min at 35°C and 96 min at 25°C. The setting and hardening properties of calcium carbonate cement are due to the progressive dissolution of calcium carbonate and formation of an entangled network of aragonite crystals which can be enhanced by an increase of temperature. Physical-chemical characterization of the cement showed that these processes occurred as expected indicating that the experimental procedures set up involving low mechanical stress on the paste all through the rheological characterization did not disturb/prevent the setting reaction and hardening of the cement.

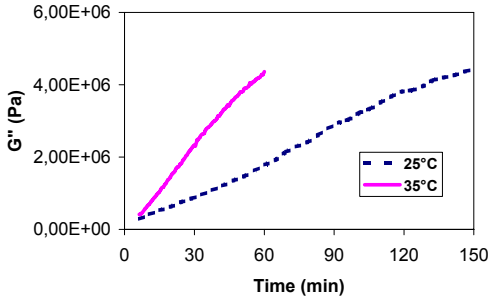

Fig. 1: Evolution of the loss modulus G" measured at 25°C and 35°C (experimental procedure 1).

The dynamic rheological measurements following the other experimental procedure (paste subjected to several flows imposed at about 2, 22 and 42 minutes after the preparation of the paste) showed that the paste had a shear thining behaviour and that the thixotropy property of this cement at 35°C is interestingly still "visible" 40 minutes after paste preparation. However, it seems that this property is more affected when tests are conducted at 25°C probably due to competition between two different properties: the cement setting (irreversible) and thixotropy (reversible).

CONCLUSIONS: Even if it appeared difficult to distinguish the cement setting which is an irreversible process from the cement thixotropy which is a reversible one, the experimental procedures set up in this study based on dynamic rheological characterization (flow and oscillatory shear) of $CaCO_3$ bone cement bring us important data in a view to improve and control its rheological properties during its preparation at room temperature and its injection *in vivo*.

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Processing and Characterization of HAp-based Biocomposite Pastes

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Repair of skeleton defects and filling fracture gaps requires bioactive, biocompatible, biodegradable and easy-to-handle bone grafts. Hydroxyapatite (HAp) paste satisfying such requirements is one of the best candidates for bone grafting applications. HAp paste pre-mixed in a ready-to-use syringe allows easy injection without conversion reaction (unlike calcium phosphate cements). In the present study, a custom-developed viscometer was designed for practically assessing flow of HAp paste contained in a syringe. Based on these data, a process for improving injectability of HAp-based biocomposite pastes through surface modification of HAp with surfactants, is proposed.

METHODS: Four types of pastes were prepared by mixing HAp powder with the liquid media of de-ionized water (D·I), binary solution of D·I and ethylene glycol (EG), and poly(dimethyl siloxane) (PDMS), in an intensive planetary ball mill. Particle dispersant (tri-sodium citrate, TSC) was dissolved in the D·I containing media. The practical viscometer was setup by mounting a syringe with a standard opening needle in an Instron 3360 series Universal Testing System. The applied injection force was collected by Series 9® software, and apparent viscosity calculated knowing the force and system geometry. The response surface method was used to statistically analyze the combined (interactive) effects of EG and TSC on paste injectability. SEM, particle size distribution analyzer, zeta-potential analyzer and FT-IR were used for the characterization of the HAp-based biocompostie pastes.

RESULTS: EG is effective in decreasing the overall surface tension of the water-EG solutions, improves wettability of the liquid on the HAp and lowers apparent viscosity of the EG-HAp pastes as shown in Fig. 1a. TSC prevents particles from agglomeration resulting in improved flowability of the TSC-HAp pastes, Fig. 1b. The optimized composition of EG-TSC-HAp paste was at 44.3 vol% EG and 0.45 wt% TSC. The viscosity of the paste was 9.38 ± 0.233 Pa.s. PDMS-HAp pastes were found to be far more viscous than the other liquid medium-based pastes.

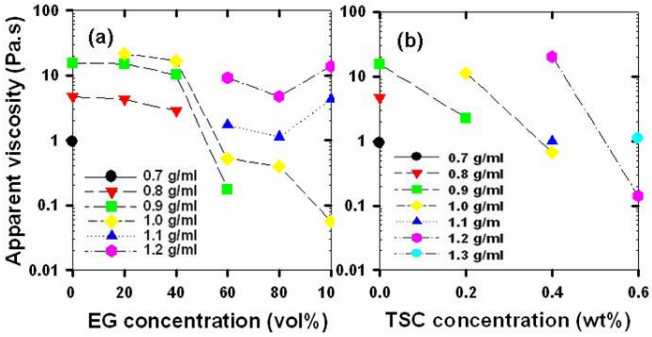

Fig 1. Apparent viscosity change vs. EG vol% in EG-HAp pastes (a) and vs. TSC wt% in TSC-HAp pastes (b).

CONCLUSIONS: EG and TSC surfactants provide good injectability of the water-based HAp pastes. The low-cost and practical, syringe-based injection viscometer can be used to assess the flowability and shelf-life of pre-mixed pastes. The proposed simple methodology can be used for optimization of any paste compatible with off-shelf syringe.

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Iron Oxide Nanoparticles Significantly Enhances the Injectability of Apatitic Bone Cement for Vertebroplasty

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Vertebroplasty and kyphoplasty are efficient procedures for the treatment of painful vertebral compression fractures. Nowadays, calcium phosphate cements are used to treat these fractures mainly due to the similar bone apatitic phase formed after setting. However, clinicians have reported great difficulties in filling the vertebral bodies due to the high pressures needed to inject these materials. Thus, new approaches are needed to improve the initial flowing properties of these cements without affecting or even improving their short-term mechanical stability and their long- term *in vivo* cement transformation into bone tissue. The objective of this research was to investigate the setting, flowing and biocompatibility properties of new iron-modified calcium phosphate bone cements.

METHODS: Cement setting times were measured by the *Gillmore* needles method. The evolution of the compressive strength accounted for the cement hardening process. Scanning Electron Microscopy followed the evolution of the cement microstructure with hardening. X-ray diffraction analysis confirmed the evolution of the crystalline phases underlying the setting and the hardening processes. Injectability tests were performed by using syringes filled with bone cement and recording the evolution of the injection force needed to empty the syringe. Finally, the cytocompatibility was analysed by culturing human ephitelial cells onto the cements and evaluating both the relative cell viability and the adhesion cell density.

RESULTS: The modification of the powder phase of an alpha-tricalcium phosphate cement with iron oxide nanoparticles significantly enhanced, at constant liquid to powder cement mixing ratio, the resulting cement injectability by lowering the extrusion force required for cement delivery. For example, 24 *wt%* iron oxide addition resulted in 83% of cement injected with an extrusion force lower than 25 N. In fact, the setting and the working times of the cement pastes increased with iron oxide addition. Moreover, the new cement pastes showed improved compressive strength in agreement with the crystalline microstructure evolved during hardening. On the other hand, iron modification did not produced cytotoxic cements as compare to non-modified cements.

CONCLUSIONS: It has been shown that the addition of iron oxide nanoparticles into the powder phase of an alpha-tricalcium phosphate based cement improved both, the initial injectability and maximum compressive strength of the cement without affecting their physico-chemical setting reactions and their cytocompatibility. These results could be further exploited by designing improved injectable apatitic cements with suitable mechanical properties and *in vivo* cement transformation ratios into bone tissue by incorporating phases creating porosity.

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Session 9

CHARACTERIZATION OF INJECTABLE AND IMPLANTABLE BIOMATERIALS

Chairmen: J.M. Bouler & N. Sahraoui

Study of porosity of a calcium phosphate bone cement

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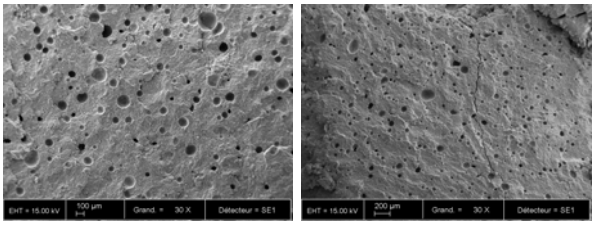
☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Calcium phosphates cements (CPC) are used as bone substitutes in orthopaedic surgery, stomatology and dental applications because of their bioactive properties and similarity to natural bone. Conventional CPC remain dense after implantation due to the lack in macroporosity [1]. However, clinicians have a preference for using their products macroporous in order to speed up the osteointegration and the bone ingrowth. The macroporosity in a biomaterial allows the colonisation of bone cells into the material and hence provide a rapid bone substitution. Another reason for having porosity in cements may be to have the possibility to incorporate drugs into their structure which can be released after implantation. For such purposes the newly developed calcium phosphate cements are even more attractive. The aim of this study was to induce porosity in the cement by organic and inorganic phases [2].

MATERIALS AND METHODS:

Different cement formulations were studied. The powder phases were composed of α-TCP, DCPD, MCPM and HPMC (hydroxypropylmethylcellulose). DCPD and MCPM were added to α-TCP and HPMC separately of combined. HPMC at different molecular weight M_w and degree of substation SD were used. The cement liquid in all cases was a solution saline (0.9 % NaCl). Cylinders with a height of 12 mm and a diameter of 6 mm were prepared and soaking was carried out during 24 hours in saline solution at 37°C prior to determination of the compressive strength. Porosity data were performed by mercury porosimetry. Scanning electron microscopy SEM were used for microstructural analysis.

RESULTS: All cements after setting they transformed to poorly crystalline apatite. The cement formulations prepared with α-TCP, DCPD, HPMC and α-TCP, MCPM, HPMC showed respectively a visible macroporosity after 24h of setting. The macropores ranged from 105 to 200 µm and 50 to 170 µm respectively. The compressive strength was 11 MPa and 13 MPa respectively. The use of an HPMC with a high SD seemed to favourite the induction of macopores.



Cement prepared with DCPD Cement prepared with MCPM

Figure1. SEM pictures showing the morphology of both cements after 24h setting.

CONCLUSIONS: This study showed that the cements prepared with DCPD and an HPMC with high SD seemed to induce the formation of macropores up to 200 microns. Those prepared with MCPM had more pores but with a mean diameter of about 100 microns

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Heterogeneous vs homogeneous hydroxylapatite composites

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: There is continued interest in constructing bone replacement materials with physico-chemical properties closely matching those of real bone. We have elaborated a range of composite biomaterials which show chemical contents and mechanical properties similar to those of bone.

METHODS: Hydroxyapatite (HA) ceramics (cylinders of 12mm diameter) were prepared with a range of porosities and fired at 1200°C. Solutions of polylactic acid (PLA) or hydrolysed pig skin collagen (type A gelatine) were prepared with 10% and 20% concentrations and infiltrated into the HA ceramics using vacuum variations. After drying in air, they were weighed to evaluate the uptake of polymer, and tested in compression with a Zwick/Roell mechanical testing machine.

RESULTS: The compressive strength of non-porous HA cylinders increased with sintering temperature as follows (temperature in °C-strength in MPa): 900-1.17; 1000-2.57; 1100-5.36; 1200-8.27. Using the strongest and most porous samples (78% porosity) the compressive strength levelled at 0.57 MPa.

With PLA infiltration, the compressive strength increased to 1.81 MPa at 11.4% loading and to 3.31 MPa at 30.3% loading (a three to six fold increase).

With gelatine, the strengths and loading were as follows: 5.15 (6.8%); 11.3 (18.2%) and 14.7 (22.6%). In this case, the compressive strength increased by a factor of up to 25! Compared to PLA, gelatine was at least five times more effective in increasing compressive strength.

The outstanding properties of natural bone are clearly related to the high affinity of the mineral phase for the organic matrix. To simplify, collagen provides continuity and hydroxylapatite brings stiffness.

In previous work we reported so-called homogeneous composites formulated with type A gelatine and micron-sized HA particles. The composites were elaborated by introducing the solid phase into a warm solution of gelatine, followed by cooling, drying then heating depending on the crosslinking agent used. Resistant composites were thus obtained which could be shaped, drilled or cut by sawing. However such composites reabsorb water and loose rigidity with time.

Another class of "homogeneous" composites was made by dissolving polylactic acid (PLA) in chloroform and mixing in various amounts of HA powders. Sodium chloride crystals could also be included before molding and drying. Water extraction then produced PLA-HA materials with various degrees of porosity. The compressive strength was found to decrease when the porosity increased, and the ultimate strength was lower than that of bone specimens of equivalent densities.

CONCLUSIONS: The heterogeneous composites described here provide a wider choice of composites for bone replacement. The mechanical properties (including Young's modulus) are closer to those of real bone.

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New drug device combination system for preventing osteoporosis fractures

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☒ Innovation

INTRODUCTION: Metabolic bone disorders are widely treated by the systemic administration of bisphosphonates (BP) because of their potent inhibitory effect on osteoclastic bone resorption. In this context, our project is to develop a drug device combination approach for the treatment of osteoporotic fractures based on the chemical association of a BP with a calcium phosphate (CaP) that could be injected on main potential osteoporosis-induced fracture sites, using minimally invasive surgery [1, 2].

METHODS: 500 mg of β-TCP (β-$Ca_3(PO_4)_2$) is placed in 2.5 mL of a BP solution (e.g. Zoledronate, 0.07mol.L^{-1}). After 48H of stirring, the solid is washed 4 times with 5mL of water and allowed to dry. β-TCP-A is obtained. 500mg of β-TCP-A is placed 1H in 10 mL of water. After filtration, the isolated solid was equilibrated four additional times in 10 mL of water. After final filtration, the β-TCP-B product is allowed to dry.

RESULTS: From SEM and ^{31}P MAS NMR (1D, 2D) experiments, the presence of various types of association modes was obvious[3], depending on the nature of the CaP support. In some cases (e.g. calcium deficient apatites) a strong chemisorption of the BP takes place on the surface of the support driven by a PO_3/PO_4 exchange process. On the contrary, in other cases (e.g. β-TCP-A), a crystalline BP complex forms onto the surface of the CaP providing a rapid BP release profile. This metastable complex lead to β-TCP-B with a pure BP calcium complex (as with Alendronate) onto the surface (Fig. 1). In this latter case[4], Zoledronate is released at very low

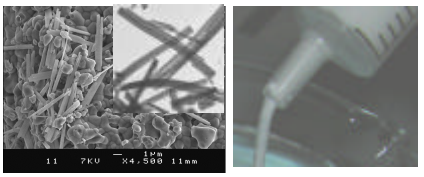

Fig.1 Left, β-TCP-B, id. with β-TCP and Alendronate. Right, as injectable cement.

concentration, directly depending of the phosphate concentration in the medium and making possible the evaluation of the biological activity. β-TCP-B diluted in pure β-TCP (1/1000) shows the same inhibition of osteoclastic resorption that the most effective in vitro concentration of Zoledronate (10^{-6}mol.L^{-1}). In vivo evaluation is currently in progress using animal osteoporosis models (rats, ewes).

CONCLUSIONS: We have shown that a modified calcium phosphate β-TCP-B allows the release of low doses of BP in phosphate media. The same methodology has been extended to the preparation of an injectable cement. After implantation in bone tissues, we can expect that the BP will be released in greater concentration the more the bone is being resorbing, acting as a local very potent antiresorption agent.

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Novel composite based on thermosensitive hydrogel and BCP granules: *in vivo* rabbit experiments

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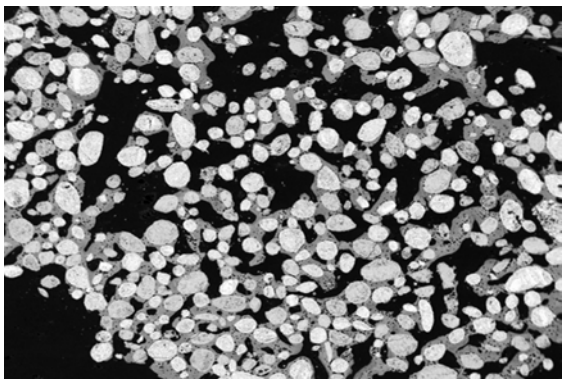
☒ Biomaterials	☐ Biomechanics	☐ Clinical	☒ Innovation

Introduction: Calcium phosphates like hydroxyapatite (HA), beta-tricalcium phosphate (beta-TCP), and their mixtures (biphasic calcium phosphates; BCP) are used clinically to repair bone defects. Specific granules concept developed by Daculsi [1] were able to realize various suspensions or composite associating polymer of biological origin (as Tricos® with Tisseel® Fibrin glue) or synthetic polymer as HPMC (MBCP Gel ®), or resorbable calcium phosphate cement (MCPC®). The aim of this study was to develop and to test *in vivo* handling, setting and osteogenic properties of injectable bone substitute materials by combining specific granules of BCP with or without radiopaque elements with a thermo sensitive resorbable inert carrier such as Pluronic F-127 (Pluronic, BASF, Mt. Olive, NJ) [2]. The composite is liquid at ambient temperature and set as hydrogel at 37°C.

Methods: Three different rounded BCP granules bioceramics were prepared in the range of 80-200µm. Rounded granules were prepared from calcium deficient apatite (CDA) with or without Barium sulphate radio-opaque. The sintering involved crystallization of BCP with different HA/TCP ratio (60/40 and 20/80 Biomatlante SAS France). 60% in weight of BCP granules were mixed with Pluronic F127 (A: 60/40 BCP, B 20/80, C: 60/40 with Barium). 15 New Zealand rabbits were used. Bilateral 6 mm intra-femoral epiphysis bone defects were totally filled by composite. Lumbar muscular implantation (1cc) were bilaterally implanted. After 3 and 6 weeks, the explants were fixed in a solution of neutral formalin solution, dehydrated and embedded in GMA. Micro CT was realized for 3D reconstruction. Thin sections were stained using Movat's pentachrome. Thicker sections realized with a diamond saw microtome were examined in SEM using BSE and image analysis. Muscular implants were prepared for histology, after paraffin embedding, sections were stained by hematoxylin eosin. Bone regeneration and BCP resorption were evaluated quantitatively by histomorphometry Statistics were performed using Student's t-test.

Results: Bone regeneration was similar over the time period studied. From 3 to 6 weeks lamellar bone trabeculae appeared at the expense of both composites. BCP granules play the role of scaffolds for osteoconduction. Radio opaque composite have same bone ingrowth than the two others samples BCP. Newly-formed bone was



observed mainly in deep zones of defects from the surface to the core. Thermosensitive polymer increased the handling and the moldability without compromising BCP osteoconductivity. Intramuscular implantations confirm the biocompatibility.

Conclusion: These results suggest that Pluronic can be used to enhance handling and moldability without any negative effects on the osteogenicity of BCP specific granules.

1:Daculsi G. (2006) Biphasic CaP Granules concept for Injectable and Mouldable Bone Substitute. Adv. Sci. Technol. vol 49:9-13

2: Reverse phase osteoconductive composition patent WO/2007/107012 ; International application PCT/CA2007/000476

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POSTER PRESENTATIONS

Enhancing Non-destructive Characterization of Porous Bone Substitutes

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☐ Biomaterials	☒ Biomechanics	☐ Clinical	☒ Innovation

INTRODUCTION: The scaffold structure has been considered to be essential for the biological response. Therefore, characterizing scaffolds has received more attention to find optimum structure for bone substitute. Combining micro-computed tomography (μCT) and advanced image processing provides an accurate tool for geometric analysis. The goal of this study is to present the effect of subvoxel process, threshold values and additional conditions in computing structural parameters of the different β-TCP scaffolds.

METHODS: four groups of scaffolds with variable geometric features were scanned by μCT with 30 μm resolution. To gain sharper and rounder boundary of pores especially for small pore size samples (A and B) an artificial subvoxel process algorithm called "single-pass subvoxel processing" (SPSP) [1] was applied to reduce the voxel size. According to SPSP algorithm the allotted intensity of each subvoxel is determined by local neighbouring criteria and strict conservation principles. Due to similarity of grey level distribution before and after subvoxel process, the same threshold values were used in both cases. Then for geometric analysis; the images are fuzzified by sigmoidal function, 3D fuzzy distance transform map (FDT) of pore structure voxels was determined [2] and pore size was computed based on FDT values of the local maximum points in FDT map. Further conditions were applied to exclude the maximums corresponding to remaining part of pores at the border of samples.

RESULTS: sensitivity analysis of porosity to threshold values (Fig.1) indicates rapid change in porosity of sample A and B by threshold values. Porosity of samples except sample A, at ±20% of average grey value (AGV) comes close to porosity computed by visual inspection threshold values. By applying subvoxel process, the pores achieve sharper and more spherical boundary that leads to accurate measurement of structural parameters. Also the linear fuzziness index decreased 8% for samples A and B and 5-6% for samples D and C respectively that means SPSP algorithm provides less fuzzy images specially for samples A and B. the average pore size by effect of excluding condition and subvoxel process summarized in table1. As illustrated in table 1 average pore size decreased after subvoxel process, the pattern of change increase from sample D (4%) to sample A (15%). It can be described by detecting small struts in images provided by SPSP algorithm which result in smaller pores among structure and so decreasing the average pore size. Also, the considerable effect of excluding condition on pore size in samples C and D is explained by increasing the ratio of pore size to sample size.

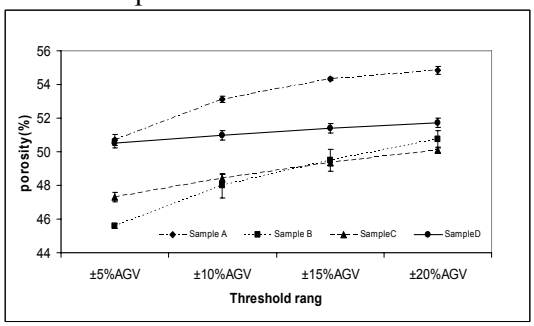

Fig.1- Porosity versus different threshold values

Sample	Ave. PS (μm)	Ave PS after excluding(μm)	Ave PS after excluding and SPSP algorithm(μm)
Sample A	125.2±3.4	127.9±3.5	111.2±2.6
Sample B	188.2±2.1	193.2±2.1	170.6±1.7
Sample C	337.9±2.8	360.3±3.6	323.5±3.6
Sample D	770.8±9.2	870.6±9.2	838.6±12.0

Table 1- Average Pore Size (PS) and standard deviation

CONCLUSION: Limited spatial resolution may reduce the characterization accuracy. Advanced image processing tool algorithms can lead to a more accurate characterization of structural parameters of scaffolds.

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Dielectric properties of the calcium phosphate paste

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INTRODUCTION: Separation between the liquid and solid phases of calcium phosphate (CaP) pastes occurs when pressure is applied to inject the paste through a cannula. The separation affects the homogeneity of the paste extruded and the quality of the cement (1,2). Monitoring the electric permittivity of CaP paste might be a promising method of Liquid to Powder Ratio (LPR) detection. The permittivity of the pastes was assumed to be strongly correlated with the water content, since it is determined by the polarizability of the free water. The aim of this study is to present a new avenue that can lead to CaP paste water content monitoring during paste delivery.

METHODS: Mixtures of β-Tricalcium phosphate powder (β-TCP Fluka, Switzerland) and different distilled water content were used to investigate the dielectric properties of calcium phosphate (CaP) pastes. Permittivity was measured in capacitor-type cells to subject the material to an alternating electric field. Basically, the cell is comprised of a pair of parallel plate electrodes which sandwich the material under measurement to form a capacitor. The two brass electrodes installed in the acrylic mold containing the paste under study were 50*50 mm and 5 mm apart. The capacitance (Cp) and resistance (Rp) of the materials were measured using the Fluke PM6304 LCR meter. An experiment was designed to observe the frequency response of the capacitor sensor of eight water contents pastes. For each test; Cp and Rp values of the sensor output were measured at 5 frequencies. The real and effective imaginary permittivities were obtained. These data were used in a quantitative calibration model through a regression procedure.

RESULTS: Permittivity of CaP paste can be conveniently expressed in terms of LPR since it is proportional to the number of dipole moments per unit weight. The real and effective imaginary permittivities of test pastes as a function of LPR at different frequencies are shown in Fig. 1. As illustrated in Fig. 1a, the real permittivity of the paste increased with increasing LPR and decreasing frequency. The real permittivity of CaP paste is determined primarily by the polarizability of the free water. An increase in the water content means an increase in the number of the permanent electric dipoles which are responsible for orientational polarization. Therefore, the real permittivity of the paste is significantly affected by the amount of water. In Fig. 1b, the effective imaginary permittivity of the paste increases with increasing the LPR and decreasing frequency. Increases in the effective imaginary permittivity of pastes with the LPR can be explained by the increase in conduction loss.

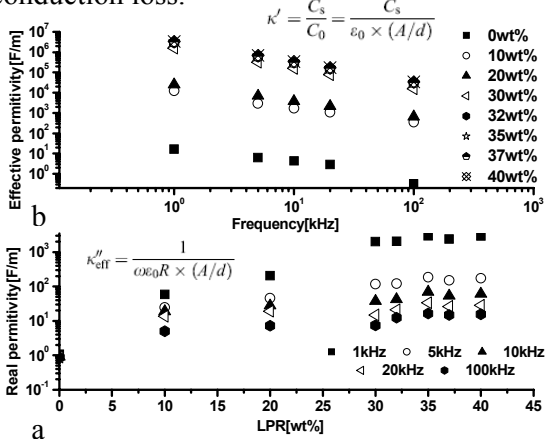

Fig. 1: Relationship between complex permittivity and LPR for the CaP paste.

CONCLUSIONS: The non-homogeneity of the injected CaP paste could lead to un-expected mechanical properties and setting time. The capacitance technique is a promising method that can lead to a good monitoring of the extruded paste quality.

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Towards periodic unit cell reconstruction of trabecular bone structure

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INTRODUCTION: Trabecular bones possess a complex internal structure which is difficult to quantify. However, it would be desirable to quantify its local geometry by an idealized periodic unit cell (PUC) whose characteristic parameters would be directly computed on three dimensional digital images obtained from microcomputed tomography (μCT). Insight into the morphology of an idealized PUC would be helpful for understanding the microphysical basis behind transport phenomena, such as the control of the kinematics of cement infiltration for vertebroplasty applications.

METHODS: The reconstruction of a PUC necessitates two steps: (i) the acquisition of the representative cell size parameters (pore and window diameters, strut thickness, strut length) on digital images by computer routines followed by the generation of PUCs having in average the same purely geometrical macroscopic parameters than the real trabecular bone samples the are assumed to mimic (porosity, specific surface) [1,2], (ii) and the resolution of the local field equations governing the transport phenomena under interest such as the viscous permeability [3]. The representativity of the reconstructed PUC is assessed through comparisons of independent estimations with either experimental measurements, or more interestingly rapid numerical computations performed directly on the three-dimensional digital μCT images of the entire scanned sample. In our case, permeability estimations of vertebral trabecular bones have been recently obtained using lattice Boltzmann simulations [4].

RESULTS AND CONCLUSIONS: Let us now consider the flow of an incompressible Newtonian fluid at low Reynolds number

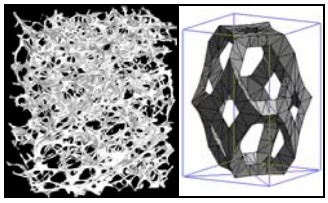

Fig. 1: (A) μCT *of trabecular bone structure, (B) and corresponding reconstructed PUC.*

which is governed by the steady Stokes equations with the non slip condition at the wall [3]. The seepage velocity $\langle \mathbf{v}_i \rangle$ is a linear function of the macroscopic pressure gradient $\nabla \langle p \rangle$, $\Phi \langle \mathbf{v} \rangle = -[\mathbf{K}/\eta] \cdot \nabla \langle p \rangle$, where Φ is the open porosity, and η is the viscosity of the fluid. Here only the component $K_{xx} = 3.5 \times 10^{-8}$ m^2 of the permeability tensor **K** has been computed, corresponding to a 0.9 mm inner pore radius with a 1.5 degree of anisotropy and a 111 μm ligament radius. This value of K can be directly compared with the lattice Boltzmann virtual experiment data $2.98 \times 10^{-8} < K_{exp} < 5.05 \times 10^{-8}$ m^2 depending on the flow direction for the entire scanned sample. The agreement is reasonable. Future work should include a sensitivity study of the local characteristic dimensions on the permeability tensor components.

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Rheokinetic Characterization of PMMA Cement

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INTRODUCTION: Rheological properties and their variations play a key role in the outcome of cement delivery and more importantly for controlling the intra-vertebral cement filling.

The aim of the present contribution is to develop an understanding of the mutual interaction between rheology of acrylic bone cement and the polymerization kinetics. Since the viscosity is dependent not only on the shear rate and temperature, but also on the degree of conversion. Ultimately, to accurately model the constitutive behaviour of PMMA cement.

METHODS: Oscillatory rheometry, as well as pressure driven tube flow viscometry are used to characterize the acrylic bone cement rheokinetics. Oscillatory tests were done, using the concentric cylinders with cone geometry, with frequencies ranging from 0.01 Hz to 100 Hz. The tube flow tests are done using flow rates in the ranges applicable in vertebroplasty.

The viscosity is believed to be a function of shear rate, degree of conversion β which in turn is a function of time, and temperature, and could be represented in a separable form as a first approximation.

$$\eta(\beta, T, \dot{\gamma}) = \eta_0(\dot{\gamma}) e^{\left(\frac{E}{RT}\right)} f(\beta)$$

The degree of conversion is assumed to be 1st order reaction [1].

$$\frac{d\beta}{dt} = k(1-\beta)e^{-U/RT}$$

RESULTS: Figure 1 shows the pressure drop vs. time using a tube of diameter 1.92 mm, and length 4.5 mm for three flow rates of 1 cc/min, 5 cc/min and 10 cc/min.

Figure 2 shows the oscillatory tests for three different frequencies, 0.1 Hz, 1 Hz, and 10 Hz.

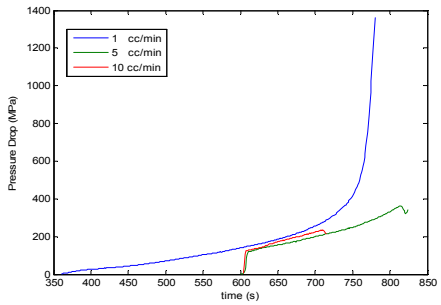

Fig. 1: The pressure drop through tube for three different flow rates.

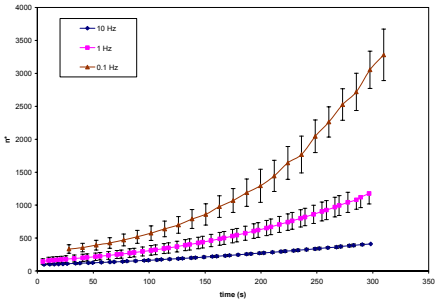

Fig. 2: The viscosity profile from oscillatory tests.

CONCLUSIONS: Complementing the oscillatory and tube flow tests with squeeze flow rheometry will provide a deeper understanding of the rheokinetics of bone cement, especially the low shear rate range. The rheological data will be extracted from the squeeze flow tests using lubrication approximation for generalized Newtonian fluid [2, 3]. Solving the formulation equations coupled with the conservation laws from continuum mechanics, in the respective geometry, is expected to give a good prediction of the constitutive behaviour.

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Nanocristalline calcium deficient apatite-based composition presenting analgesic properties

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INTRODUCTION: Synthetic calcium-deficient apatites (CDA) are structurally close to biological apatites. They are well-known as chemical precursor for biphasic calcium phosphates (BCP) consisting in mixtures of hydroxyapatite and β-tricalcium phosphate that are widely used as bone substitutes in human surgery. The purpose of the study was to define *in vitro* release profiles of an analgesic which has been previously loaded on CDA using isostatic compaction and to study the in vivo performance of such a local release on pain.

MATERIALS & METHODS: CDA powder presenting a Ca/P=1.6 was obtained from alkaline hydrolysis of dicalcium phosphate dihydrate[1] and loaded with 1%, 4% and 16% of bupivacaïn (sigma®) using an isostatic compaction process [2]. The so obtained blocks are crushed in a mortar made of alumina to an approximate mean granule size of 40-80 µm. 200mg of those granules were incubated in 15mL of distilled water. After an incubation time of 30 min, 2h30, 5h, 24h, 48h, 2mL liquid were removed, filtered and assayed by UV spectrophotometry (270nm). Wistar male rats were implanted in distal femur with 50mg of CDA associated respectively with 0, 1%, 4% and 16% of bupivacain (N=10). Analgesia was measured using electronic Von Frey monofilament electronic version [3], inflammatory response and neurological score.

RESULTS: The associated bupivacaïn was totally released after 48 hours of incubation.
During the first post-operative day, we observed a dose dependant analgesic effect with the bupivacaïn adsorbed on resorbable implant (figure). Both inflammatory response and neurological score confirmed this result.

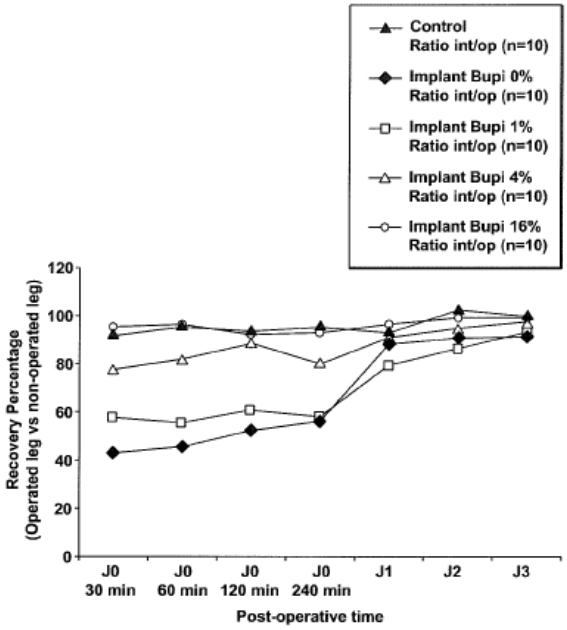

Figure : comparative pain measurements using electronic Von Frey monofilament

DISCUSSION:
This combined device system has been shown to provide a release of local anesthetic adapted to prevent or limit post operative pain relative to bone surgery. This innovative approach could be integrated in the global management of pain after specific prosthetic (hip or knee) surgery where bone reconstruction is needed.
However the rat model seems not to be optimal to measure mid-term post op pain as it disappears in all measured groups after only 24h. Furthermore bone substitution conducted by CDA has to be verified. Therefore additional *in vivo* experiments are under progress with rabbits and dogs.

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Rheology and injectability modeling of an injectable calcium phosphates ceramics suspension

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INTRODUCTION: Calcium phosphate (CaP) ceramics are the main raw materials used to elaborate granules for bone substitutes. These ceramics are being increasingly used in orthopedic surgery and dental applications. More than 10 years ago, a CaP aqueous suspension was developed [1] to obtain an injectable biomaterial. The first generation of this injectable calcium phosphates ceramics suspension (ICPCS) associates biphasic calcium phosphate (BCP) particles (40-80 or 80-200µm in diameter) with a cellulosic biopolymer (hydroxypropylmethylcellulose (HPMC)). The BCP granules are an association of hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP). The suitable proportions of HA (60%) and β-TCP (40%) has provided BCP ceramics with controlled bioactivity and biocompatibility [1].
The aim of this study is to characterize the rheological behavior of an injectable calcium phosphate and investigate the injectability through a syringe.

METHODS: 3% w/w of HPMC solution was prepared in deionized water. The granules of BCP were obtained by precipitation of calcium-deficient apatite (CDA) and sintering. Different preparations of ICPCS were made by mixing BCP granules (40-80 or 80-200µm in diameter) with HPMC polymer solution at different ratios (35, 40, 45 and 50% w/w).
Rheological measurements were performed using RS 300 rheometer (ThermoHaake®, Germany) with parallel plates geometry. The injectability simulations were performed using a syringe with needle and the necessary stress to inject the biomaterial was determined using TA-HD-plus texture analyzer (Stable Micro Systems™, UK).

RESULTS: The flow curves of ICPCS preparations could be well described using the power law equation of Ostwald-de-Waele. The results showed that consistency of ICPCS increased with weight ratio and particles size of BCP. The injectability study confirmed the rheological results. In particular, an increase of the ratio and/or particles size of BCP results in an increase of necessary stress to inject the biomaterial. To predict the extrusion stress from rheological behavior, four compositions were tested; and the particles size of BCP was 40-80µm.
On the basis of the Rabinowitsch equation, a theoretical model was developed to predict the extrusion stress from the rheological parameters (consistency and flow index). Experimentally, an effect of rheological behavior was observed.

CONCLUSIONS: This present study show that ratio and particles size of BCP influence rheological properties of ICPCS. The dependence in formulation and injectability was also obtained. The results obtained show that there is a correlation between the experimental data and theoretical model.

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ACKNOWLEDGEMENTS: This work was supported by the regional program "Biomatériaux S3, Région Pays de la Loire, 2004-2008".

MODULATION OF PREADIPOCYTE RESPONSES TO pBMP-9 BY INTEGRINS

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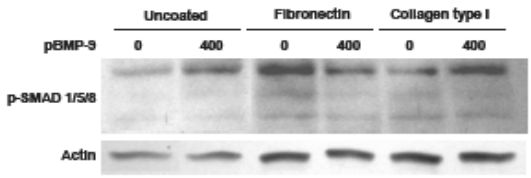
☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Specific selection of integrins has been shown to be essential in directing bone morphogenetic proteins (BMP)-2 differentiation of osteoblasts [1]. Preadipocytes respond to BMP by activating the SMAD pathway and can be differentiated into osteoblasts [2]. However, little is known about the crosstalk between specific cell-biomaterial interactions and BMP signaling in these cells. We recently described the osteogenic properties of a small peptide derived from human BMP-9 (pBMP-9) [3]. In this study, we first investigated the specific integrin involved in human and murine preadipocytes attachment to adhesive protein coated surfaces. We investigated the effects of these different coatings on the BMP signaling and cell differentiation with pBMP-9.

METHODS: Human preadipocytes (HWP) and murine 3T3-L1 in serum-free medium were seeded on uncoated polystyrene (PS), 10 µg/mL fibronectin or type I collagen-coated PS or self assembled monolayers (CH_3, NH_2, COOH) and incubated for 2 h at 37°C. Cells were fixed and immunostained with specific integrin antibodies and visualized with FITC-conjugated immunoglobulins. Proliferation of HWP and 3T3-L1 treated with pBMP-9 (0 to 400 ng/mL) on the different adhesive proteins was determined using cell counting. SMAD pathway activation was also analyzed in cell lysates using blots probed with specific antibodies directed against phosphorylated SMAD 1/5/8.

RESULTS: After incubation for 2h, HWP were able to spread on both fibronectin and type I collagen coated-PS, while murine 3T3-L1 preadipocytes remained round on collagen. At focal adhesion points, HWP organized alpha2beta1 integrins on type I collagen and alpha5beta1 on fibronectin. After 5 days, proliferation of HWP in 5% foetal bovine serum was not influenced by fibronectin or type I collagen coating. pBMP-9 dose dependently decreased proliferation on adhesive proteins. By contrast, murine 3T3-L1 preadipocyte proliferation was not inhibited by pBMP-9 on both fibronectin and collagen-coated PS. In 3T3-L1 preadipocytes and HWP attached to PS, pBMP-9 activated SMAD pathway through phosphorylation cascade. However, fibronectin prevented the SMAD pathway activation induced by pBMP-9 (Figure).



CONCLUSIONS: pBMP-9 activated the SMAD pathway in human preadipocytes. However, fibronectin which was recognized by alpha5beta1 integrins, prevented SMAD 1/5/8 phosphorylation induced by the peptide. Thus, selection of specific adhesive proteins to functionalize biomaterials might ultimately be used in modulating the differentiation of preadipocytes.

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Influence of tension actives molecules on a calcium phosphate cement

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION:

Some previous studies have shown that the use of hydrophobic and surface active compounds [1, 2] trapped in calcium phosphate cement allowed the increasing of the porosity and the improvement of the injectability [3, 4]. In our work, we found that surface active derivatives more especially derived from carbohydrates promote the development of a new interesting property that was not pointed out before. Actually, some of these additives enhance the adhesion properties of the cement toward various substrates, including bone. Thus, the different properties exhibited by well-known calcium phosphate cement in the presence of two class of sugar surfactant, namely, AlkyPolyGlucosides (APG) and Sucrose Esters (SE), have been investigated in detail. Our study is related to the following cements, already available on the market: Cementek® and Cementek LV® [5; 6].

MATERIALS & METHODS:

The properties followed were: porosity, injectability, setting time and adhesion.

The setting time was evaluated by a home-built Gilmore needle test. The injectability was tested by measuring first the mass expelled from the syringe or second the necessary force to expel it. Finally, the adhesion of the cement to various substrates was determined by tack-tests by monitoring force-displacement curves during mobile withdrawal (figure 1).

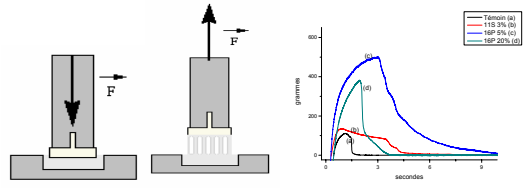

Figure 1: Adhesion paste test protocol and sample of curves.

Two characteristic values were determined: the adhesion strength (N/mm^2) corresponding to the force peak of the curve and the adhesion energy (kJ/m^2) which is proportional to the area under the curve.

RESULTS & DISCUSSION:

For the standard formulation we observed that more the additive is hydrophilic and more the setting time is high. However both the porosity and the injectability increased. Concerning the low viscosity formulation, the presence of silicone makes the cement "hydrophobic". So despite the addition of sugar surfactants, the porosity and the injectability were not so advantageous.

The test performed using 3% of 11S, or 5% of 16P and a nylon substrate showed that the adhesion energies are respectively $2.3.10^{-3}$ kJ/m^2 and $9.3.10^{-3}$ kJ/m^2 i.e. 3.8 times greater and 15.5 times greater than the adhesion energy of a surfactant-free control cement. These tests were also performed on several substrates like bone, stainless steel with similar results. The adhesion energy can be enhancing by a coefficient of up to 20 times the adjuvant-free mixture value.

CONCLUSIONS & PERSPECTIVES:

The addition of sugar surfactants in bone cements, in addition to the modification of porosity and injectability, allowed too the development of adhesion properties. This property should not be imputed to the sugar part only, since non-amphiphilic polysaccharides or monosaccharides, disaccharides or oligosaccharides did not displayed such a phenomenon. Probably, the combination of viscoelastic properties of sugar surfactants, in addition to their ability to form hydrogen bonds would be responsible for these effects. The satisfactory adherence of the paste on the operating site will enable the formation of an improved bone-filling material interface, and very easy application. Biological and biocompatibility are currently performed on these cements.

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5) Lacout et al, French Patent FR2776282

6) Lacout et al, French Patent FR2805748

A standardised comparison of CAC and PMMA bone cements

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Usually, a comparison of different biomaterials intended for the same application/indication tends to be very hard to interpret since different types of measurements have been performed to obtain the material properties. A specific such case is a comparison between ceramic and polymer materials as bone cements. Current standards for bone cement evaluation only consider PMMA chemistries[1-3]. In the present study Xeraspine® (XS) [calcium aluminate cement (CAC)] was compared to Vertebroplastic® (VP) (PMMA) both indicated for percutaneous vertebroplasty and kyphoplasty treatments. They were evaluated with respect to their extrusion properties[2], setting time[1], exotherm[1], compression strength[1] and bending strength[1].

METHODS: The characterisation of the materials was conducted according to refs 1 and 2. The materials were mixed according to manufacturer's instructions for use. The setting time, exothermic and mechanical property measurements were partly performed in an *in vitro* environment with Phosphate Buffered Saline (PBS) at 37°C to simulate the intended use of the bone cements[1]. The extrusion properties[2] were evaluated using a rheometer built from a Merit Medallion 6ml Syringe connected to a Hamilton Needle Gauge 11 and mounted in Zwick Roell Z005 mechanical testing equipment at a flow rate of 1.5ml/min. 4-point bending strength[1] was measured with an Instron 6025 machine.

RESULTS: Extrusion properties of XS and VP were measured on three separate kits of each material and are shown in Fig. 1. The viscosity measurement was started at 2:30 min and the initial viscosities were ~70 Pa*s and ~25 Pa*s, respectively. The measurement was stopped when the syringe was empty or the viscosity exceeded 500 Pa*s. Xeraspine's viscosity curve was close to linear whereas VP's curve had an exponential appearance. The other properties are presented in Table 1.

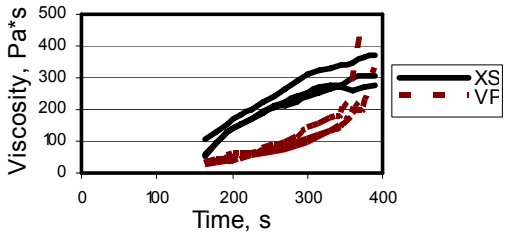

Fig. 1: Apparent viscosity of XS and VP.

	Xeraspine	Vertebroplastic
Setting time (min:sek)	11:25	11:05
Exotherm (°C)	69	78
Compressive strength (MPa)	118	82
4-point bending strength (MPa)	30	45

Table 1: Properties measured according to ISO5833

CONCLUSIONS: A direct comparison between the two different materials shows that they have similar mechanical properties, which is reflected in their biomechanical properties[4]. The exotherm that is considered as a reason for necrosis is slightly lower for XS than for VP. The viscosity for Xeraspine is suitable for injection directly after mixing and has a predictable evolvement with time.

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Histomorphometry analysis of osseointegration in a novel PMMA-HA bone cement for kyphoplasty

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Injection of polymethylmethacrylate (PMMA) bone cement to augment vertebral bodies during kyphoplasty treatment of vertebral compression fractures may result in formation of a fibrous encapsulation around the hardened cement. Osseointegration (direct apposition between cement and surrounding bone without an intervening fibrous layer) would be considered to be a desirable property. Hydroxyapatite (HA) is known to be osteoconductive, so a composite PMMA-HA bone cement is hypothesized to exhibit enhanced osseointegration when injected into a vertebral body. The purpose was to use histomorphometry to determine the amount of osseointegration exhibited by PMMA-HA vs. PMMA bone cement.

METHODS: Either PMMA (KyphX® HV-R™ Bone Cement, Kyphon, Sunnyvale, CA, USA) or PMMA-HA (ActivOs™ Bone Cement with Hydroxyapatite, Kyphon, Sunnyvale, CA, USA) was implanted into three lumbar vertebrae of each of 6 skeletally mature sheep[1]. Vertebrae were harvested at 1, 3 and 6 months and histologically processed into stained slides. The entire perimeter of the cement on each slide was visually inspected under a microscope to identify arcs exhibiting either osseointegration or fibrous tissue. Image processing software was used to calculate the length of each arc, and the amount of osseointegration was expressed as a percentage of the total perimeter[2]. The average percentage osseointegration for each bone cement was analyzed for significant ($p<0.05$) differences between PMMA-HA and PMMA. Seven (7) slides from three (3) sheep implanted with PMMA-HA, and seven (7) slides from another three (3) sheep implanted with PMMA were inspected. Statistical analysis indicates this sample size has a power of 64% to detect a 25% difference at an alpha level of 0.05.

RESULTS: Direct lamellar bone apposition on the PMMA-HA cement surface was observed on a mean 40.5% (range: 10.7-97.6%), seen as a nearly-continuous bony capsule around the cement (Fig. 1, *left*), with a thin (10-100 μm) fibrous layer in other areas. Direct bone apposition adjacent to the PMMA cement was observed infrequently (mean: 8.1%, range: 2.1-12.2%), with a thick (>200μm) fibrous capsule surrounding the cement and new bone formation parallel to the orientation of this layer (Fig. 1, *right*). The difference was statistically significant ($p<0.05$).

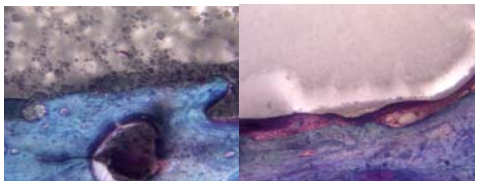

Fig. 1: (*left*) Direct apposition of new bone (blue) and PMMA-HA (grey). (*right*) Intervening fibrous tissue (red) seen between PMMA (white) and new bone (blue).

CONCLUSIONS: The PMMA-HA bone cement used in this study exhibited five times more osseointegration than a PMMA control group in a sheep spine model.

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Surrogate-bone vertebral models for the characterisation of percutaneous vertebroplasty in-vitro

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INTRODUCTION: Surrogate bones are regularly used in the pre-clinical testing of medical implants due to their low variability and ready availability[1]. It is important, however, that synthetic bone provides an appropriate representation of the structural and mechanical behaviour of human cadaveric bone. The objective of this study was to evaluate the use of surrogate-bone vertebral models for the characterisation of vertebroplasty in-vitro.

METHODS: Nine surrogate-bone whole vertebral models with an open cell trabeculae configuration within the vertebral body (VB) were used for testing (Sawbone®). Initial µCT scans were performed and a bone marrow substitute with appropriate rheological properties[2] was injected into the trabeculae. The cranial and caudal surfaces were mounted in bone cement and quasi-static loading was performed to determine the initial stiffness and fracture strength in a manner previously used with human cadaveric VB[3]. Specimens were subject to an eccentric axial load, 25% from the anterior margin of the cranial endplate in the mid-sagittal plane with the endpoint of compression defined as a 25% reduction of the original VB height (Fig. 1). Following fracture, vertebroplasty was performed using a bi-pedicular approach with an estimated 20% volume fill of the trabeculae space.

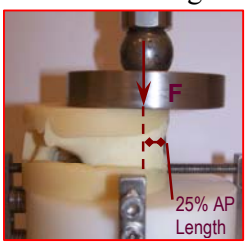

Fig. 1 VB Loading

µCT imaging was performed to assess the cement volume. The augmented VBs were then axially compressed to failure using the same loading protocol.

RESULTS: The surrogate models had a thicker cortex in comparison with human osteoporotic VB. µCT scans showed an average cement fill of 19.87% ± 2% S.D.. Interdigitation of the cement in the trabeculae was noted (Fig. 2).

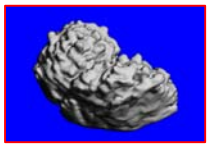
Fig. 2: µCT cement

During compression, the surrogate-bone did not exhibit the characteristic 'toe-region' in the force-deformation profile that is commonly observed with cadaveric vertebrae. The mean initial failure strength of the surrogate vertebrae was 1.35kN ± 0.15kN S.D. with post-fracture and augmentation failure strength of 1.90kN ± 0.68kN S.D.. This equates to a significant post-vertebroplasty increase by a factor of 1.38 ($t = 3.3$, $P = 0.006$; paired t-test). No significant difference in the relative stiffness was found post-augmentation.

In comparison with human osteoporotic bone[3], no significant difference was noted in the relative increase in fracture strength ($t = 1.5$, $P = 0.151$; unpaired t-test) or change in relative stiffness ($t = -1.5$, $P = 0.140$; unpaired t-test) between the artificial and human VB following augmentation.

CONCLUSIONS:

Following augmentation, the relative change in strength and stiffness of the surrogate-vertebral models was similar to those observed in recent investigations with osteoporotic vertebrae. The substantial differences in the morphology and shape of the load-deformation curves for artificial and human VB, however, implies a difference in the behaviour of the construct under compressive load. These differences should be considered when undertaking studies with these surrogate vertebrae.

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ACKNOWLEDGEMENTS: Apatech Ltd are gratefully acknowledged for funding this study.

Sintering Effect on Mechanical Properties of Composites of Bovine Hydroxyapatite (BHA) and Boroxide Containing Bioactive Bioglass (BBB)

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INTRODUCTION: Hydroxyapatite (HA) is very popular for hard tissue restorations because it accelerates bone growth [1]. Although HA materials feature great biocompatibility with the human body, their poor mechanical properties limit the applications of purely HA materials to non load-bearing devices. With a second phase HA can be reinforced with other materials such as ceramic oxides or glasses. Recently, HA and derivatives have been also extensively considered as a promising injectable component for curing bone traumas like commercially injectable commercial biomaterials like Norian SRS and other brands.

METHODS: The lead author has also presented in an earlier study the production of some B_2O_3 containing bioglasses, which were melted between 950-1050°C (these low temperatures also aim at the economic production of the glasses) [2]. Composites of calcined bovine bone derived hydroxyapatite (BHA) doped 5 and 10 wt% with boroxide containing bioactive (BBB) bioglass were prepared by sintering. The production of BHA from natural sources is preferred for economic and time saving reasons. In this study, the investigated glass was BBB B5-3.5 % B_2O_3, which was produced in the Oktar study (BBB B2-31.5 % B_2O_3, BBB B3-12.48 % B_2O_3, BBB B4-6.5 % B_2O_3, BBB B5-3.5 % B_2O_3, and BBB B6-2.5 % B_2O_3.) [2]. These glasses have been recently tested with cell cultures. Pressed pellets were sintered between 1000-1300°C. Compression strength, density measurements, SEM and X-ray analyses were carried out.

RESULTS AND DISCUSSION: There are very few studies on the contribution of boron to biomaterials. Some of them were very negative and some of them quite promising. The experimental results indicated that the compression strength of the composites increase when sintering temperature increases. The best compression strength was achieved after sintering at 1200°C 5 % BBB addition. The results are in agreement with densification measurements and microstructure analysis.

Table 1: Compression test results depending on various sintering temperatures.

Sintering °C	MPa 5 %	MPa 10 %
1000	36.65	26.74
1100	32.18	34.44
1200	46.49	38.42
1300	32.98	8.78

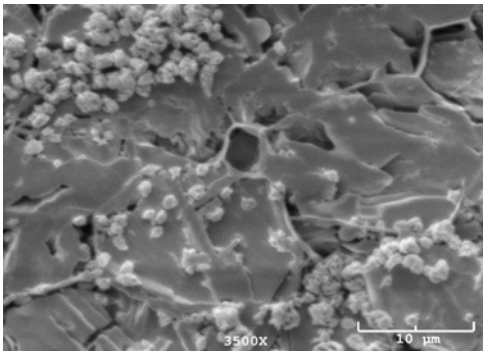

Fig. 1. Typical microstructure of the BHA-BBB produced composites. (5 % BBB 1300°C)

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ACKNOWLEDGEMENTS: Turkish Republic Government Planning Organization framework: "Manufacturing and Characterization of Electro-Conductive Bioceramics" with project number 2003 K120810. ENTER project 04EP26, Greece. S. Salman acknowledges also to R.L. Snyder from Georgia Institute of Technology.

In Vitro and In Vivo studies of Premixed, Injectable and Biodegradable Filler: Poly-(propylene fumarate)(PPF), α-tricalcium phosphate(α-TCP) and Hydroxyapatite Particle (HAP) Composite for Vertebroplasty and Kyphoplasty

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Lots of filler materials have been applied in vertebroplasty and kyphoplasty for vertebral fractures, but no current material is perfect in all biomechanical and biological characteristics.The most common injectable fillers is poly methylmethacrylate (PMMA) with shortages including exothermic reaction damaging adjacent soft tissues, non-biodegradability, adverse effects of MMA monomer and abnormally high mechanical strength. Other literature reported deaths are caused mainly by reactions of extravasated PMMA during procedures. Many novel injectable bone fillers introduced and commercialized in clinical uses are claimed to solve the current problems of PMMA. Although these cements do improve temperature, bio-degradability and biocompatibility problems, the mechanical strength are relatively low with subsequent problems including fixation collapse, early resorption and poor handling properties. The current study is meant to evaluate in vitro properties of a novel composite of biphasic CPC, **α**-TCP/HAP and PPF in compare with PMMA and previously reported HAP and PPF composite cements for possible future applications. In order to evaluate the cements in vivo, porcine vertebral model was used to compare this degradable cement with PMMA. We also evaluated another composites made of PPF with tetracalcium phosphate (TtCP) and dicalcium phosphate (DCP) simultaneously.

METHODS:PPF was fabricated by heating diethyl fumarate and propylene glycol and biphasic **α**-TCP/HAP powders were synthesized by phosphoric acid and calcium hydroxide. PPF was cross-linked by N-Vinyl pyrrolidinone (NVP) and was mixed with **α**-TCP/HAP powder in different ratios (50%, 60% and 70%) with benzoyl peroxide (BP) as an initiator and N,N-dimethyl-p-toluidine (DMT) as a catalyst. The setting temperatures were measured every 20 seconds while the cements solidified in a cylinder shape model with 5mm in diameter and 10mm in length. We also compared the study groups with 50% HAP and PMMA (Osteobond, Zimmer). Radio-opacities of different materials with the same block figurations were assessed by a X-ray machine and computed tomography and average Hounsfield units (HU) of 10 mm^2 circle area were calculated and compared. The degradation of the blocks releases fumaric acid and acidifies the immersed solution and loss of block weight. All solidified blocks were preserved in 2 ml phosphate buffer solution (PBS) in different wells inside an incubator with temperature set at 37°C and PBS were collected and changed every 2 days. The pH values of the PBS were recorded by an electric pH meter eight weeks. Other group of immersed blocks were for weight losses and mechanical compression test. After immersion of 1, 3, 7, 14, 28, 42, 56 days in PBS, the blocks were retrieved and dried in vacuum for 2 days. Percentage of weight losses was counted with an analytical balance. The mechanical compression test of dried blocks by a biomechanical tester was performed. The collected fractured surfaces were analysed by scanning electron microscope test. The biocompatibility tests including Water-soluble tetrazolium salts test (WST-1 test) for cell proliferation and lactate dehydrogenase tests. (LDH test) for cytotoxicity of the PBS collected immediately after cements curing and the same tests of the immersion solution after 1, 3 and 7 days' incubation with immortal human osteogenic sarcoma cell line (U2-OS).

The fabricated material compositions of PPF, **α**-TCP/HAP and TtCP/DCP had been determined

by NMR or XRD tests and the materials had been packed with proper proportions (CPC 70% weight proposed by in vitro results) and sterilized for intra-operative uses. Twelve Lanyu miniature pigs (Sus barbatus sumatranus) of 4 months old had been enrolled in this study. The pigs were placed in semi-lateral position under intravenous anesthesia by continuous infusion of Thiamylal Sodium. The lumbar vertebral bodies were exposed retroperitoneally after dividing psoas muscle and ligation of segmental vessels. Holes (5mm in diameter and 10mm in length) were drilled by a drill bit at the center of lateral cortex of vertebral bodies of lumbar spine. The PPF were cross-linked by NVP and mixed with powder of **α**-TCP/HAP (70% weight) in one group and with powder of TtCP / DCP (also 70% weight) in other, with BP as an initiator and N,N-dimethyl-p-toluidine (DMT) as a catalyst were injected into the holes after randomization. The PMMA cement was also injected at different level and one control hole was drilled with no filler. The setting time and temperature were recorded. Six pigs were sacrificed at 3 months and 6 months. The retrieved spines specimens were examined with X-ray and CT scan and histological studies with and without decalcification were performed. The different appearance of the injected cements and the interaction zones between cement and bone were analyzed and compared to the histological pictures.

RESULTS: The curing temperatures of 39.7~44°C in composite groups were significantly lower than PMMA group (74.2°C). The radio-opacity checked by CT of 70% composite (1456HU) is lower than PMMA group (1461HU) with no significance (p=0.45). The HU of 50% HAP composite were higher than those of 50% **α**-TCP/HAP with significance (p<0.0001) and were higher than 60% **α**-TCP/HAP with no significance (p=0.055). 3. The compression strength of 70% composite was 58~60MPa more close to that of bone and did not decrease after emersion for 2 months. The pH value changed more significantly in 50% **α**-TCP/HAP and 50% HAP composite and not significantly in 70% composite group. Significant weight losses were observed in 50% **α**- TCP /HAP (15.3%) and 50% HAP composite (33%), but scanty loss in 70% composite (9.1%) at 8th week. Lots of interdigitalized laminar crystals were observed at fractured surfaces of **α**-TCP /HAP groups in SEM pictures and no such appearance had been found at the scanning pictures of blocks surface and fractures surfaces of HAP composite. The cell proliferations (WST-1 test) of all studied groups were lower than that of cell culture only group with significance (all p< 0.0001). The values of PMMA group were significantly higher than those of composite groups in first, third and seventh days. The cytotoxicities of all the studied groups are higher significantly than those of cell only culture group. The cytotoxicities of PMMA immediately after cement curing were significantly higher than those of other groups (all p<0.0001), but those of PMMA were lower than the other 4 groups at day 1, 3 and 7. Higher cytotoxicities of 50% and 60% **α**-TCP /HAP and HAP groups than 70% composite were observed only at day 1 and 3 with significances. Significant higher setting temperature of PMMA than the other 2 groups (p<0.0001) Two leakages of cement in canal were without significant neurological complication. It's hard to tell differences with plain X-ray and Ct scans provided morphologic comparison between groups. Fibrous tissues were observed from histological studies of the radio-lucent junctions and new bone formation along the border in the groups without radiolucent zone, even in PMMA group. New bone substitution was observed in the composite cement groups and new cortex formation along the cement (not in PMMA group) Good bone formation was found in control group (complete union in one pig, only one without any bone growth at 6 months group)

Fig. 1: Cylinder blocks for tests

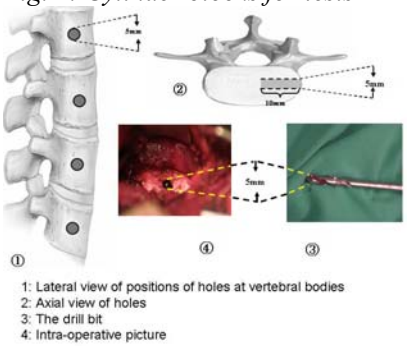

Fig. 2: In vivo procedures

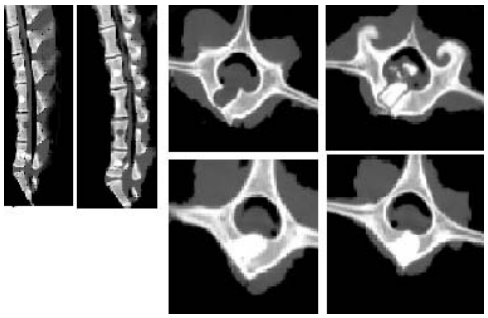

Fig. 3: CT scans

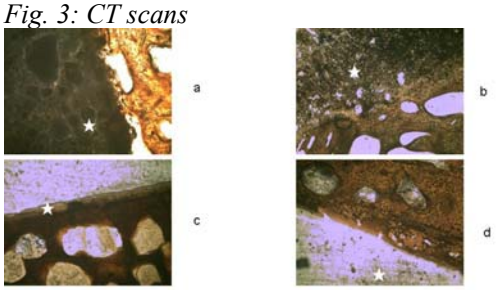

Fig.4: Non-decalcified histology

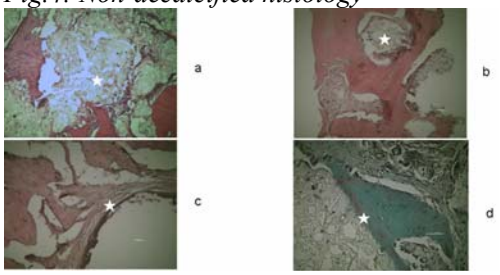

Fig.5: Decalcified histology

CONCLUSIONS: PPF, α-TCP/HAP composite is a promising biodegradable filler and 70% α-TCP/HAP composite revealed superior properties in vitro tests.

In this porcine in vivo model, although the results of experimental groups were not better than the control groups, there are still lots of adjustments and corrections cloud be done to improve this novel filler.

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Synthesis of silicon containing calcium phosphate by a solid state route

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Recent studies showed that silicon clearly influences the process of mineralisation of bone [1]. Consequently, it seems interesting to examine the use of silicon-containing apatite (HA-Si) as a bioceramic for bone substitution or prosthesis coating. To that end, it is of particular relevance to study the synthesis of HA-Si and their thermal stability. The HA-Si expected chemical formula is $Ca_{10}(PO_4)_{6-x}(SiO_4)_x(OH)_{2-x}$, x varying in the range [0 , 2].

METHODS: The aim of this study is the preparation of HA-Si by a solid state reaction method. Several initial mixtures with different amounts of silicon were tested. The compound corresponding to the expected formula was obtained starting from calcium carbonate, calcium phosphate and silicon oxide or calcium silicate. The heating cycle was carried out at different temperatures between 1100°C and 1400°C, with a heating rate of 5°C/min and various plateau times. The reaction products were characterised by X-Ray diffraction and Fourier Transformed Infrared spectroscopy.

RESULTS: A first result is related to the temperature above 1300°C. Whatever the nature and the ratio of the initial reactants used, the stable phase $Ca_{10}(PO_4)_4(SiO_4)_2$, silicocarnotite, was formed. For x=2, the final compound was a pure phase of silicocarnotite. For x< 2, other phases are present: apatite, tricalcium phosphate and calcium oxide, the quantity of which depends on the value of x. For a heating temperature between 1100 and 1300°C, the final compound depends on the source and the amount of silicon. Infrared spectroscopy confirms that silicate enters the apatite or silicocarnotite structure. Silicocarnotite was often observed whatever the temperature. Nevertheless, for x =1 (fig 1) it is possible to obtain a pure apatitic phase, when the silicon is provided in the initial mixture by silicon oxide. One can propose that in those conditions, the synthesis rate of silicocarnotite is low and the apatitic phase can be formed.

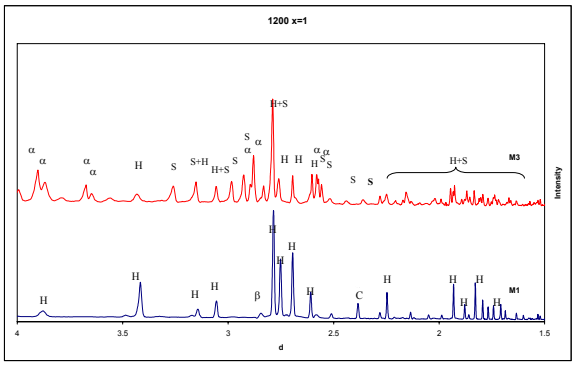

Fig 1: XRD patterns of different mixtures with x=1 1200°C, 3hours; M1 with SiO2; M3 with CaSiO3

The preliminary results of biological tests show, in the case of the pure apatitic phase, the non-toxicity of the materials, and the proliferation study shows the interest of the silicon substitutions.

CONCLUSIONS: A silicon-containing apatite $Ca_{10}(PO_4)_6(SiO_4)(OH)$ was synthesised by a solid state reaction at 1200°C, starting with a mixture of tricalcium phosphate, calcium oxide and silicon oxide. Nevertheless, it appears that the thermodynamically stable phase at high temperature is the silicocarnotite. Also, encouraging preliminary biological assessments were obtained.

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Influence of the precursors composition on Plasma-Sprayed Hydroxyapatites

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

Cyto-toxic CaO can be formed when HA coatings are produced using plasma technology. Potentially this residual CaO content can reduce the bio compatibility of those coatings. HA nanocrystals were prepared by a wet chemical precipitation reaction following the reaction of Tagai and Aoki:

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

The temperature during the precipitation reaction was maintained at 80 C°. The addition of the 0.3 mole/1 H_3PO_4 solution was stopped when the pH of the suspension became of 8. This colloidal suspension had to undergo centrifugation to increase its solid/liquid weight ratio. This final ratio of 20%. H_3PO_4 was added to three of our four suspensions. The H_3PO_4 excesses were of 1%, 5% and 11.6%. The purpose of these excesses was to determine the influence of the suspension's Ca: P ratio on the CaO percentage.

Method: The HA nanocrystals are deposited on sandblasted titanium substrates by using a plasma spraying device (HF60-ICP). This deposition technique gives to the coatings a good adherence and a high degree of porosity. The HA suspension is pumped into a High Frequency-ICP (Inductive Coupled Plasma) torch, where the HA crystals are spheroidized melted and eventually partially vaporized under the extreme temperature of the plasma (≈ 10 000 C°).

Spraying Parameters: The depositions obtained were about 300µm thick and shown a good adherence to the substrates.

Device: HF-ICP PL 50	
Sheath : (Ar) 20 mm (O_2) 100 mm	Ip = 5.4 A
	Vp = 7.5 kV
Central : (Ar) 70 mm	Ig = -- A
Powder : (Ar) 40 mm	Ng = --
Pressure : 100 Torr	Power = 40 kW

XRD Results: In order to determine the percentage of CaO in our coatings, we made X-rays analysis of our samples. The results of the XRD analysis show that the Ca: P ratio of the HA suspension has a significant influence on the materials properties.

H_3PO_4 excess	CaO
—	5%
1%	4%
5%	2%
11,6%	0%
Before plasma	0%

We can notice from these results that the reduction of the CaO percentage is almost proportional to the H_3PO_4 excess. Also, we can see that a large excess of H_3PO_4 can lead to the production of HA/β-TCP coatings. Finally, we can notice that the HA does not contain any CaO before plasma.

Conclusion: The HA suspension's Ca: P ratio has a significant influence on the CaO percentage in the HA. A large excess of H_3PO_4 results in the production of HA/β-TCP coatings. Thus, pH-metric measures of the reagents should be used jointly with quantitative Ca/P measurements.