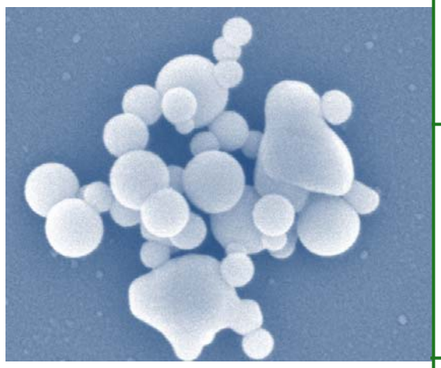


GRIBOI 2013

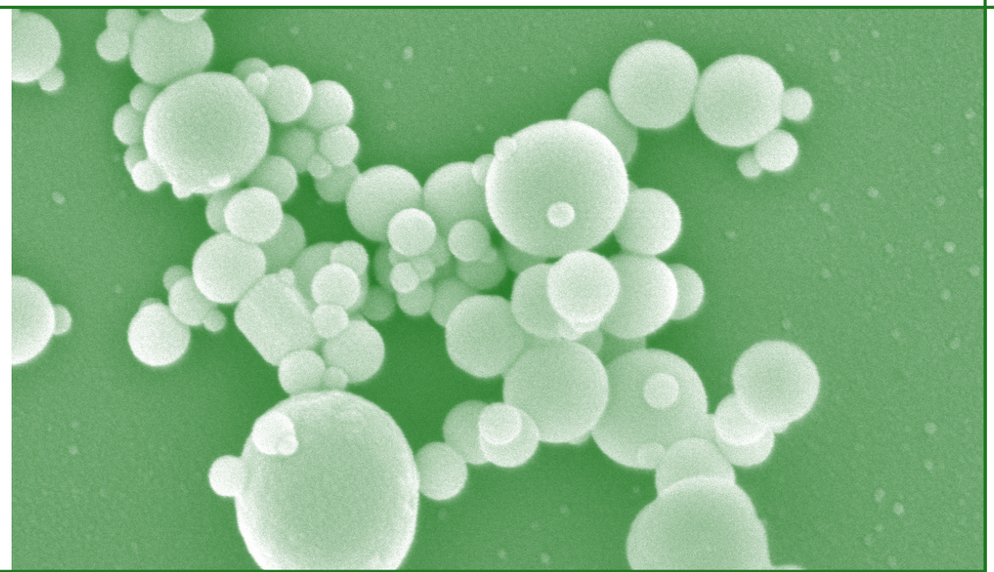
Actes de conférence de la

23rd Interdisciplinary Research Conference on Injectable Osteoarticular Biomaterials in Bone Augmentation Procedures

Groupe de recherche interdisciplinaire sur les biomatériaux ostéo-articulaires injectable (GRIBOI)

G. Baroud, J. Hirsch, M. Bohner

The 23rd Interdisciplinary Research Conference on Injectable Osteoarticular Biomaterials in Bone Augmentation Procedures



у-мл April 20мо, Boston, USA

GRIBOI

G. Baroud
J. Hirsch
M. Bohner

23rd Conference of the Interdisciplinary Research Group on Injectable Osteoarticular Biomaterials

23èmes Journées du Groupe de Recherche Interdisciplinaire sur les Biomatériaux Ostéoarticulaires Injectables

GRIBOI 2013

Boston, 8-10 April 2013

Welcome to the GRIBOI 2013 – Injectable Osteoarticular Biomaterials and Bone Augmentation Procedures Conference.

Building on recent successes in **Uppsala** (2012), **Boston** (2011), **Torino** (2010), **Fort-de-France** (2009), **Montreal** (2008) it is our honor and pleasure to welcome you to Boston.

Expect GRIBOI 2013 to be an interactive meeting where clinicians, scientists and engineers discuss cutting-edge topics related to the clinical use of bone substitutes and injectable biomaterials in bone augmentation procedures.

GRIBOI, the acronym for "Groupe de Recherche Interdisciplinaire sur les Biomatériaux Ostéo-articulaires Injectables", began in 1989 when a group of French physicians met informally to discuss injectable biomaterials. Now, 24 years later, GRIBOI comes to Boston with strong international representation while retaining the intimate, interdisciplinary collegiality intended by its founders.

The unique strength of the GRIBOI is its ability to gather international experts from apparently disparate clinical and laboratory disciplines to advance the field of bone augmentation by mutual inspiration. The conference format consists of lectures and oral presentations.

This year there will be nine plenary sessions:

(**1**) Skeletal repair and biomaterials platforms
(**2**) Hip prophylaxis
(**3**) Under extremities augmentation
(**4**) Scaffold for bone defects
(**5**) Bone augmentation procedures
(**6**) Tumor management and augmentation
(**7**) Procedures and innovation
(**8**) Traumatic vertebral fractures
(**9**) Biomechanics
(**10**) Beyond PMMA and CPC
(**11**) Hydrogels and Biologics
(**12**) Calcium-phosphate platform

We welcome you and wish you a successful GRIBOI 2013.

Prof. **Gamal Baroud**
Chair, GRIBOI 2011

Canada Research Chair
Université de Sherbrooke
Sherbrooke, Qc Canada

Prof. **Joshua Hirsch**
Vice-Chair

Massachusetts General Hospital
Boston, MA, USA

Prof. **Marc Bohner**
Vice-Chair

RMS Foundation
Bettlach
Switzerland

Bienvenue à GRIBOI 2013 – Conférence sur les biomatériaux ostéoarticulaires injectables.

Après les succès de **Uppsala** (2012), **Boston** (2011), **Torino** (2010), **Fort-de-France** (2009), ou **Montréal** (2008), c'est un honneur et un plaisir de vous inviter à nouveau à Boston. Cette année, GRIBOI 2013 vous offre des rencontres interactives où cliniciens, scientifiques et ingénieurs discuterons de sujets avant-gardistes portant sur l'utilisation clinique de substituts osseux et de biomatériaux injectables.

GRIBOI, acronyme de "Groupe de Recherche Interdisciplinaire sur les Biomatériaux Ostéo-articulaires Injectables", a été fondé en 1989 par un groupe de chirurgiens français lors d'une rencontre informelle qui portait sur les biomatériaux injectables. Aujourd'hui, 24 ans plus tard, c'est à Boston que se tiendra cette réunion d'envergure internationale, où règne généralement une très grande collégialité.

La force exceptionnelle et unique de GRIBOI est sa capacité à réunir des experts internationaux travaillant dans des disciplines distinctes et de leur permettre de profiter des connaissances d'autrui afin de progresser dans le domaine de la régénération osseuse. Le congrès consistera de conférences pléniaires, et de présentations orales.

Cette année, neuf séances sont prévues :

(**1**) Réparation du squelette et biomatériaux
(**2**) Prophylaxie dans le traitement de la hanche
(**3**) Augmentation des membres bas
(**4**) Matériau de comblement pour défaut osseux
(**5**) Méthodes d'augmentation osseuse
(**6**) Tumor management and augmentation
(**7**) Techniques et innovation
(**8**) Fractures vertébrales accidentelles
(**9**) Biomécanique
(**10**) Au delà du PMMA et des ciments phosphocalciques
(**11**) Hydrogels et thérapies biologiques
(**12**) Phosphates de calcium

Nous vous souhaitons la bienvenue et beaucoup de succès à GRIBOI 2013

Prof. **Gamal Baroud**
Organisateur, GRIBOI 2013
Chair de recherche du Canada
Université de Sherbrooke
Sherbrooke, Qc Canada

Prof. **Joshua Hirsch**
Co-organisateur

Massachusetts General Hospital
Boston, MA, USA

Prof. **Marc Bohner**
Co-organisateur

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Acknowledgement

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1st Day	08-Apr-13

8:00am-8:15am	**Opening**	

Session 1.1	**Skeletal repair and biomaterials platforms** Chairmen: J. Lane, S. Hollister	
8:15am-8:40am	Plenary No 1 David J MOONEY, USA **Approaches to musculoskeletal tissue repair**	2
8:40am-8:55am	Keynote No 1 Michael RAUSCHMANN, Germany **Development and clinical experience with augmented pedicle screws**	2
8:55am-9:10am	Keynote No 2 Khaled KEBAISH, USA **Spinal deformity surgery in osteoporotic patients. Is a good outcome possible?**	3
9:10am-9:20am	Q&A	
9:20-10:00am	Breakfast (served)	

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10:30am-10:45am	Keynote No 5 Giancarlo ANSELMETTI, Italy **Managing insufficiency fractures after transplant surgery**	6
10:45-11:00am	Q&A	
11:00-11:05am	Break	

Session 1.3	**Skeletal repair and biomaterials platforms** Chairmen: P. Sharrock, A. Kruerger	
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11:20am-11:35am	Keynote No 7 Osamu SUZUKI, Japan **Biodegradable octacalcium phosphate-hyaluronic acid composites as bone substitute materials**	9
11:35am-11:50am	Keynote No 8 Cristele COMBES, France **Osteo-articular pathological calcifications: a physico-chemical point of view**	10
11:50am-12:00pm	Q&A	
12:00pm-13:10pm	Lunch	

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13:35pm-13:50pm	Keynote No 9 Jean-Michel BOULER, France **Bisphosphonates-combined matrices to prevent osteoporotic fracture**		13
13:50pm-14:05pm	Keynote No 10 Jöns HILBORN, Sweden **Injectable BMP-2 containing hydrogels for bone regeneration**		14
14:05pm-14:15pm	Selected Abstract No 1 Olivier GAUTHIER, France **Bone reconstruction with a bupivacaine-loaded injectable calcium phosphate cement can reduce postoperative pain after iliac bone graft harvesting procedure in a canine model**		15
14:15pm-14:25pm	Selected Abstract No 2 Edgar MONTUFAR, Spain **Discerning the role of the setting reaction on the injection behaviour of tricalcium phosphate paste**		16
14:25pm-14:35pm	Selected Abstract No 3 Elise VERRON, France **Injectable BP-loaded cement to reinforce sheep osteoporotic vertebral bodies**		17
14:35pm-14:50pm	Q&A		

Session 1.5	Under extremities	Chairmen: M. Rauschmann, S. Tutton	
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16:25pm-16:55pm	Coffeebreak		

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17:25pm-17:40pm	Keynote No 16 Michael LIEBSCHNER, USA **Development of an intra-operative artificial tissue fabrication approach for reconstruction of large defects**	28
17:40pm-17:50pm	Selected Abstract No 7 Marco Antonio LOPEZ HEREDIA, Germany **Dynamic cell culture on glassy crystalline calcium alkali orthophosphates scaffolds**	29
17:50pm-18:00pm	Short Abstract No 1 Mohamed HABIB, Egypt **Wet-chemical processto synthesisthe biphasiccalcium-phosphate powder**	30
18:00pm-18:10pm	Q&A	

2nd Day	09-Apr-13

7:00am-8:00am	Breakfast

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8:45am-8:55am	Selected Abstract No 8 Matti SCHOLZ, Germany **Cement augmented anterior screw fixation of dens type II osteoporotic fracture**		36
8:55am-9:05am	Selected Abstract No 9 Jose María TRIGUEROS LARREA, Spain **Rescue from the cut-out: cement augmentation of a gamma nail**		37
9:05am-9:15am	Selected Abstract No 10 Georgios VASTARDIS, USA **Cement distribution after percutaneous unilateral transpedicular balloon kyphoplasty for the mid & upper thoracic spine.**		38
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11:35am-11:45am	Robert POSER, USA **Initial clinical exeperience using novel radiofrequency systems for targeted ablation and augmentation of spinal tumors**		51
11:45am-11:55am	Kieran MURPHY, Canada **A Novel Device for Radiofrequency Ablation of Bone Metastases**		52
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12:00pm-12:30pm	GRIBOI Assembly		
12:30pm-13:30pm	Lunch		

Session 2.4	Traumatic Vertebral Fractures	Chairmen: P. Jarzem, B. Georgy	
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13:45pm-14:00pm	Keynote No 24 Natale FANCAVIGLIA, Italy **10-year experience in precutaneous management of traumatic vertebral fractures**		56
14:00pm-14:20pm	Keynote No 25 David NORIEGA, Spain **Cranio-Caudal expandable implant for fracture management**		57
14:20pm-14:35pm	Keynote No 26 Stephan BECKER, Austria **Changed focus in spine surgery: from fusion models/biomaterials to disc models/biomaterials**		58
14:35pm-14:45pm	Selected Abstract No 13 Paul DUFORT, Canada **An automated algorithm for measuring 3D vertebral height restoration**		59
14:45pm-14:55pm	Selected Abstract No 14 Anand AGARWAL, USA **Biodegradable cement to replace PMMA cement in management of VCF**		60
14:55pm-15:05pm	Selected Abstract No 15 Danielle MILES, UK **Injectable peptide/glycosaminoglycan hydrogels and their potential use for minimally invasive nucleus pulposus augmentation**		63
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15:55pm-16:05pm	Selected Abstract No 17 Ondrej HOLUB, UK **Is morphology alone an effective indicator when planning prophylactic vertebroplasty in multiple myeloma patients?**	68
16:05pm-16:15pm	Selected Abstract No 18 Sami TARSUSLUGI, UK **Investigation of facet joint properties for the assessment of interventions in the functional spinal unit**	69
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17:30pm-17:40pm	Selected Abstract No 21 Ingrid AJAXON, Sweden **Compressive fatigue properties of acrylic bone cement for vertebroplasty**	74
17:40pm-17:50pm	Selected Abstract No 22 Ondrej HOLUB, UK **Augmentation of cadaveric vertebrae with an α–TCP/ α–CSH ceramic cement**	75
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3rd Day	10-Apr-13

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8:40am-9:00am	Keynote No 32 Scott GUELCHER, USA **Injectable, settable lysine-derived polyurethane grafts for bone regeneration**		79
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9:10am-9:20am	Selected Abstract No 24 Anne TALLEY, USA **In vivo rhBMP-2 release from degradable polyurethane biocomposites**		81
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9:40am-9:50am	Selected Abstract No 27 Jeroen JJP van den BEUCKEN, Netherlands **Effects of an osteoporotic condition on the biological performance of injectable CP/PLGA cements**		85
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10:55am-11:05am	Selected Abstract No 29 Jim TRIFFITT, UK **Stem cells update**	91
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11:15am-11:25am	Selected Abstract No 31 Andrew HARMATA, USA **Effects of surface modification of 45S5 bioactive glass on dynamic compressive fatigue mechanical**	93
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Session 3.3	Calcium-phosphate platform Chairmen: J. Chang, P. Sharrock	
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11:55am-12:05pm	Selected Abstract No 32 Mei WEI, USA **Influence of iron oxidation state on magnetic properties of iron substituted hydroxyapatite**	97
12:05pm-12:15pm	Selected Abstract No 33 Ross ORMSBY, UK **Development of ion substitutued calcium phosphate cement for spinal repair**	98
12:15pm-12:25pm	Selected Abstract No 34 Rory O'NEILL, UK **Experimental and analytical investigation into the mechanism of filter pressing of calcium phosphate pastes during injection**	100
12:25pm-12:35pm	Selected Abstract No 35 Seunghwan LEE, Denmark **Injectable lubricants for prosthetic articular joints**	101
12:35pm-12:45pm	Selected Abstract No 36 Johanna ENGSTRAND, Sweden **Optimization of an acidic calcium phosphate cement with enhanced radiopacity**	102
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Session 1.1

Skeletal repair and biomaterials platforms

Chairmen: J. Lane, S. Hollister

Plenary No 1

Approaches to musculoskeletal tissue repair

David J MOONEY

MIT, MA, USA

Keynote No 1

Development and clinical experience with augmented pedicle screws

Michael RAUSCHMANN

Orthopaedische Universitaetsklinik Friedrichsheim gGmbH, Frankfurt, Germany

Keynote No 2

Spinal deformity surgery in osteoporotic patients. Is a good outcome possible?

Khaled KEBAISH

Department of Orthopedic Surgery, Johns Hopkins University, Baltimore, MD, USA

Session 1.2

Skeletal repair and biomaterials platforms

Chairmen: J. Hirsch, P. Munk

Tumor; where good ideas come from, innovation in intervention

Kieran Murphy

Joint Department of Medical Imaging, UHN Mt Sinai WCH, University of Toronto, Canada

Metastatic tumors are the most common tumors of bone affecting 10-30% of all cancer patients. Spinal mets are found in 36% of patients who die of cancer. The tumors that most commonly spread to bone are prostate, breast lung kidney and thyroid. These account for 80% of boney mets. The 5 year survival after diagnosis of lung mets is 2% and thyroid is 44%. Pain is the most common presenting complaint and it is usually progressive, unrelenting and poorly controlled. Radiation therapy has been shown to be effective in 75-90% of patients but the benefit is not felt for 2 to 12 weeks after the treatment.

Cord compression from tumor was first described by Elsberg in 1916 in his work "Diseases of the spinal cord and its membranes". 1 in 12700 cancer patients will develop cord compresion. Globally most of these patients will be treated with steroids and radiation. Vast Rad Onc studies of up to 5400 patients have studied the role of IGRT SBRT SABR or HIGRT and other tightly columated spinal radiation therapy to treat spinal mets and cord compresion. The rigour of these NCI NIH CIHR and international studies is admirable. It is clear the role of surgey is limited to the relief of cord compresion causing myelopathy in patients with greater than 1 month life expectancy. Do we as interventionalist have a role in this patient population. Do we have the technology that would allow us have a lower risk minimalist impact on these patients quality of life. Multiple small studies of between 30 and 2 patients would say that we do have s something to offer. Our studies are Cochran level 3 or lower in most cases however. Our interventions are currently paliative and our role is niche. This is unlike our role in Liver primary or secindray disease where RFA TACE Y90 is changing survival, and allowing patients become eligible for liver transplant.

If we are to go beyond the palliative intervention described above and move into to the definitive treatment of patients with oligo metastatic disease ie limited metastatic disease in 5 sites or less we need to apply a new level of rigor and total tumor kill, with by definition marginal obliteration that we currently dont deliver. The application of pre procedural radiation theraphy soft ware planing and dose distribution estimation is critical. Ths is the subject of our research for the past 2 year. In this section of this session I will review the state of the literature and discuss our approach to oligo metastatic disease. In addition i will discuss the paucity of innovation in our field since the late 90s and the need for renewed effort.

Keynote No 4

Sacroplasty and augmentation in other weight bearing bones: Practical decision paradigms

Allan BROOK

Interventional Neuroradiology, Montefiore Medical Center, Albert Einstein College of Medicine, NY, USA

Keynote No 5

Managing insufficiency fractures after transplant surgery

Giancarlo ANSELMETTI

Interventional Radiology Section of the Institute for Cancer Research & Treatment of Turin, Italy

Session 1.3

Skeletal repair and biomaterials platforms

Chairmen: P. Sharrock, A. Kruerger

MODULAR SCAFFOLD ENGINEERING FOR SKELETAL RECONSTRUCTION

S. J. Hollister
The University of Michigan, IL, USA

Large skeletal defects present significant challenges to restoring natural tissue, geometry, and load bearing. Tissue engineering approaches combining scaffolds with osteobiologics (i.e. cells and/or growth factors) have long been proposed to repair such defects when conventional bone grafting or implant therapies fail. However, to date there has been limited large pre-clinical model testing of such approaches, let alone clinical implementation. This can be attributed in part to the significant technical challenges faced in reconstructing large defects and in part to the significant regulatory and commercial challenges that face even a technically successful approach. Clearly, there is no silver bullet for skeletal reconstruction.

The combination of no technical silver bullet with the significant regulatory challenges has led us to propose a modular approach to skeletal tissue engineering (Hollister and Murphy, Tissue Eng., 2011). This modular approach not only incorporates integration of multiple structural and biologic components to create a skeletal engineering therapy, but also integrates multiple design and manufacturing processes to achieve these integrated therapies that bridge multiple scales. In this talk, we will address how to design, fabricate, and functionalize skeletal engineering constructs at multiple scales, touching upon how such platform technologies can interface with regulatory requirements.

The ultimate goal is to create an engineering platform that allows design of constructs to reconstruct any complex defect as well as the capability to fabricate such constructs. Furthermore, given that such a platform technology provides a plethora of design choices (e.g. scaffold material, scaffold pore architecture design, scaffold surface modification, osteobiologics) which raises questions as to what choice of modular components will lead to optimal skeletal regeneration, and how do we test such choices for example in vitro, in small animal models or in large pre-clinical animal model. In this talk we will also present results on how choices of material, architecture design and biologic affect bone regeneration *in vivo*. Open questions and issues resulting from the modular approach will finally be presented to forge a path going forward.

Biodegradable property of octacalcium phosphate-hyaluronic acid composites as bone substitute materials

K Suzuki[1,2], T Anada[2], T Miyazaki[2], N Miyatake[2], M Hosaka[1], H Imaizumi[2], E Itoi E[1], O Suzuki[2*]

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INTRODUCTION: Octacalcium phosphate (OCP; $Ca_8H_2(PO_4)_6{\cdot}5H_2O$) enhances in vitro osteoblastic differentiation [1,2] and osteoclast formation [3]. OCP is used as a source material for preparing osteoconductive inorganic-polymer composites, such as OCP/gelatine [4]. It is of interest to learn whether OCP can be used as an injectable material in bone defects. We have reported that hyaluronic acid (HyA) works as an agent to combine OCP without losing the osteoconductivity and provide the injectability [5]. However, the effect of HyA on the biodegradability of OCP and the cellular response in bone formation remains un-clarified. In the present study, bone tissue response and in vitro osteoclastic response to OCP/HyA were examined.

METHODS: OCP was prepared by a direct precipitation previously reported [6]. Three sodium hyaluronic acids with different molecular weights were used: 1) 9 x 10^5, 2) 19 x 10^5 and 3) 60 x 10^5. The granules of OCP (300 to 500μm diameters) and HyAs were mixed at room temperature. The composites were referred to as OCP/HyA90, OCP/HyA190 and OCP/HyA600 (a chemically-modified sodium hyaluronate derivative). These composites or OCP alone were implanted respectively in polytetrafluoroethylene (PTFE) rings (OD 8 mm, ID 6 mm, 1 mm thickness) placed onto eight-week old ICR mice until 6 weeks. The rate of the remaining implants was histomorphometrically estimated after the decalcification and the hematoxylin and eosin staining. The area was calculated using Image J public-domain software. Osteoclastic cells were formed by incubating macrophage RAW264 cells with RANKL in the presence or absence of HyAs and OCP. Tartrate-resistant acid phophatase (TRAP) staining was used to estimate the effect of the HyAs and OCP.

RESULTS: OCP/HyAs exhibited liquidity (Fig.1) thereby the composites were easily able to inject into PTFE ring at the surgical operation. The bone formation was observed primarily around OCP granules in HyAs until 6 weeks. However, it seems likely that the capability of bone formation differs much from the type of HyA used for combining OCP: OCP/HyA90 and OCP/HyA600 could be enhancing the osteoconductivity of OCP more than that of OCP alone. Histomorphometric study revealed that the biodegradation of the three composites was enhanced with time but the rates were similar with OCP alone. In vitro assay suggested that HyAs may have a role stimulating osteoclast formation if co-existed with OCP.

Fig. 1: Image of an OCP/HyA composite.

DISCUSSION & CONCLUSIONS: The present study confirmed that HyAs provide OCP the injectability in handling at the operation and suggests that HyAs modify the osteoconductivity of OCP through increasing the cellular activity in particular bone formation and/or resorption–related cells. Further study is underway to establish the linkage between the osteoconductivity and the biodegradability of OCP/HyAs composites.

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ACKNOWLEDGEMENTS: This study was supported in part by Grants-in-aid (23390450, 2363988, 23650282, and 23106010) from the MEXT and JST FS A-STEP.

Osteo-articular pathological calcifications: a physico-chemical point of view

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INTRODUCTION: Osteoarthritis (OA) is the most common form of rheumatic diseases, leading ultimately to chronic pain and disability for the patient. Aggravating factors identified to date include a family history of OA, mechanical overload, older age, and the presence of calcium-containing microcrystals in the joint. The two main types of calcium-containing crystals found in the joint (synovial fluid and cartilage) are hydrated calcium pyrophosphates (CPP: $Ca_2P_2O_7$, nH_2O) and calcium phosphates crystals (CaP including octacalcium phosphate, carbonated apatite,…) [1]. Although the physico-chemical reactivities of synthetic and biological CaP phases are largely studied in the literature, the formation and dissolution of CPP crystals has been less investigated and not fully understood. Like for CaP crystals, *in vitro* synthesis of CPP phases appears as an alternative route to progress easier and faster in the understanding of the mechanism of their formation *in vivo*. The objective of this study is to synthesise and characterise nanocrystalline apatites and CPP phases of biological interest and to study their properties in aqueous medium.

METHODS: Nanocrystalline apatites and various hydrated CPP phases have been prepared by precipitation in aqueous solution at controlled pH and temperature. Some precipitates have been left to mature in the mother solution during various periods of time. The as-synthesised and matured precipitates have been characterised by using powder X-Ray diffraction, FTIR and Raman spectroscopies, thermogravimetry (ATG), scanning electron microscopy (SEM) and chemical analysis. Dissolution tests have also been performed.

RESULTS: The main structural characteristic of biomimetic nanocrystalline apatites, as demonstrated especially by spectroscopic analyses is an association of rather stable apatite domains forming the core of the nanocrystals associated with a surface hydrated layer containing mainly bivalent ions such as Ca^{2+}, HPO_4^{2-} or CO_3^{2-} in "non-apatitic" environments. These structural characteristics determine the chemical and biological properties of these compounds: maturation, ionic exchange and adsorption properties [2].

In the case of CPP phases, we showed that monoclinic and triclinic CPP dihydrates ($Ca_2P_2O_7$, $2H_2O$), the most common polymorphs encountered *in vivo*, are formed only at high temperature and in acidic media with the synthesis system implemented *in vitro*. An amorphous phase was found to form easily in a wide range of pH and temperature. This phase is very stable and do not crystallize even after several years at 37°C. Such a stability of amorphous CPP was also pointed out by Slater et al. in other conditions of synthesis [3].

DISCUSSION & CONCLUSIONS: Several articles have suggested that amorphous phase precursor route was the most common process of minerals formation *in vivo* [4]. Amorphous CaP or CPP phases can be easily obtained *in vitro*, whereas the low supersaturation ratio *in vivo* should not favour the formation of amorphous phases. However, other components in biological fluids can promote their formation and/or stabilise these phases. Physical-chemical studies on calcium phosphate and pyrophosphate phases formation should be developed for explaining the dynamic phenomena involved in crystal induced osteoarthritis and also for exploring new methods allowing an improvement of the diagnostic.

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Session 1.4

Hip prophylaxis

Chairmen: K. Kebaish, O. Suzuki

Hip repair and prophylaxis

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Osteoporosis is noted for the increased fracture risk – particularly following a low energy fragility fracture. Unfortunately, less than 25% of patients sustaining a low energy fracture actually undergo drug therapy. As a result, for the majority of individuals with fragility fracture there is a five-fold increased risk of vertebral fracture and a two-fold increased risk of hip fracture.

Currently, treatment strategies are primarily directed at systemic interventions including calcium, vitamin D and drugs. The initiation of treatment at the time of the fragility fracture is not only appropriate but clearly understandable to the patient ("perfect storm" concept). An alternative approach would be to utilize local secondary fracture prevention.

The primary target for local prevention would be hip fracture. Fourteen percent of patients undergoing a fractured hip are at risk for the contralateral hip fracture within 5 years. A hip fracture profoundly compromise the patient with 70% losing mobility skills and 24% dying within one year.

To prevent hip fracture the goals could include prophylactic implant fixation, biomechanical enhancement and biological stabilization.

Cannulated screw insertion would only prevent femoral neck fractures and not influence intertrochanteric fractures. Conversely, the insertion of materials to enhance bone mass and improve bone biology throughout the peritrochanteric region would address all forms of hip fracture.

Candidates for rapid bone mass augmentation are centered on injectable ceramics, glasses and polymers. The advantage of ceramics rests in their ability to be integrated and remodeled compared to polymers (PMMA) and glass.

Drugs and biologics include anti-resorptives such as the bisphosphonates, anabolics such as PTH's, and growth factors such as BMPs.

The local insertion of an agent that prevent a secondary fracture is feasible and attractive. It could easily be performed under the same anesthesia as the fracture hip repair. By the time the patient has recovered from the primary hip repair, the contralateral hip would be protected. Systemic drugs require 6+months to work and only decrease the hip fracture rate by 40%. Local treatment would work more rapidly and possibly with greater efficacy. Now is the time to test this avenue of intervention.

Bisphosphonates-Combined Matrices to Prevent Osteoporotic fracture

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The integration of drugs and devices is a growing force in the medical industry. The incorporation of pharmaceutical products not only promises to expand the therapeutic scope of device technology but to design a new generation of true combination products whose therapeutic value stem equally from both the structural attributes of the device and the intrinsic therapy of the drug. In this context, a new calcium phosphate cement-based (CPC) medical device was developed, capable of providing mechanical reinforcement and delivering a bisphosphonate (BP) antiresorptive drug locally. While BPs behave as setting retardants, a novel and efficient route was designed for introducing a bisphosphonate in a CPC while keeping the main properties of the cement suitable for practical application as a drug delivery system, implantable by minimally invasive surgery. This BP-combined CPC was implanted in proximal femurs of sheep presenting an induced osteopenia. This in vivo investigation showed a promising ability of an alendronate-loaded cement to resorb while promoting new bone formation and improving bone micro-architecture.

Injectable BMP-2 containing hydrogels for bone regeneration

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INTRODUCTION: The gold standard procedure for the treatment of an alveolar cleft is autologous bone transplantation. Although the technique where cancellous bone from the anterior iliac crest is used, gives bone healing in approximately 90% in follow-up studies (1) and is considered safe with low complication rates, the procedure is accompanied by donor-site morbidity including pain, scarring, risk of bleeding, infection and nerve injury. (2) To avoid these complications, and to shorten surgery time and hospital stay, bone substitutes including bone morphogenetic protein-2 (BMP-2) have been studied. The BMP-2 efficacy in a clinical setting is however remarkably low and superfysiological milligram doses of the growth factor are required to obtain therapeutic results. Interestingly, the carrier used for BMP-2 delivery has been shown to affect efficassy significantly.

For a number of years, our laboratory has been working with hyaluronic acid (HA)-based hydrogels. These are extremely biocompatible and degraded enzymatically by the endogenous enzyme hyaluronidase. We have successfully evaluated these as carriers of BMP-2 to obtain ectopic and orthotopic bone at the site of injection (3-5) as well as to heal critical-sized cranial defects (6) in large animal models. In addition, tailored versions of these hydrogels may engineer significant amounts of mandibular bone upon subperiosteal injection in rats (4). The material acts as an expander that defines the space to be substituted by newly formed bone.

Despite the the lowered concentration of BMP-2 and successful outcome in animal trials, trials in humans resulted in unacceptable swelling although adquate bone formation resulted (7). We have therefore optimized the source of BMP-2, (8) the stability of the hydrogel crosslinks, the handelling of the gel, incoporation of extracellular cell adhesive fragments (9) and used precomplexation with heparin to result in improved bone fomation at lower BMP-2 doses. Using these strategies we are convinced that significant increase in BMP-2 stabilization and release profiles can be achieved.

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Bone reconstruction with a bupivacaine-loaded injectable calcium phosphate cement can reduce postoperative pain after iliac bone graft harvesting procedure in a canine model

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INTRODUCTION: The aims of our study were:
- To establish an animal model that would reproduce the human iliac crest bone graft harvesting procedure;
- To evaluate the induced pain after such a harvesting procedure;
- To evaluate the reconstruction ability of an injectable calcium phosphate (CaP) bone cement to fill the iliac crest bone defect;
- To evaluate the benefit in pain relief obtained with a CaP cement loaded with bupivacaine compared to the same cement without any analgesic agent.

MATERIALS AND METHODS: A 2 x 2 cm square bone defect was created with an orthopaedic bur on the iliac wing, mimicking a unicortical posterior iliac bone graft, on 12 adult female beagle dogs, according to European Community guidelines for the care and use of laboratory animals (2010/63/UE) after approval of the Local Animal Welfare Committee.
Briefly, with the animal in lateral recumbency, a dorsal approach of the left iliac crest was performed. The middle gluteal muscle attachements were incised dorsally and cranially on the iliac crest, elevated and reflected ventrally together with the deep gluteal muscle to expose the whole iliac wing. The contours of the iliac bone defect were stamped with the bur, starting on the dorsal cortex and extending on the lateral one that was detached with an osteotome. The inner iliac trabecular bone was removed with the bur to expose the medial iliac cortex. Each dog was implanted unilaterally with either the bupivacaine-loaded CaP cement[1] or the unloaded control one (Graftys® QUICKSET cement). A few minutes after cement injection, the muscle flap was repositioned over the defect and the middle gluteal muscle attachements were sutured dorsally with absorbable sutures. Subcutaneous and skin sutures were routinely performed.

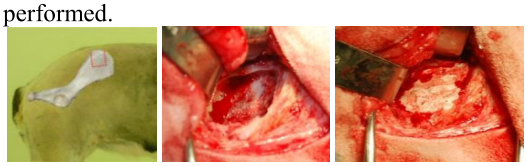

Fig.1. Standardized iliac crest bone defect location, before and after injection of the CaP bone cement.

Postoperative evaluations were performed 6h, 12h, 24h after cement injection and every 24h until day 7. They included physical and orthopedic examinations that defined a lameness score, a visual analogic scale pain score and a postoperative pain score using the 4A-Vet pain scale. Sensitivity measurements with a Von Frey sensor were carried out by application of progressive pressure with a plastic cone on predetermined locations until the animal reacted (between the two first lumbar vertebras, laterally to the tibio-patellar ligament, medially to the iliac crest and on the iliac insertion of the middle gluteal muscle just over the surgical site). All measurements were done in triplicate. Rescue analgesia with morphine was available.
Six months after the first surgery, the 6 animals that had received the control unloaded cement underwent the same surgical procedure on the contralateral iliac crest where the created bone defect was left unfilled to provide negative control sites. Osteointegration of the CaP cement was investigated with CT, microCT and histology.

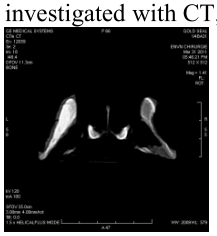

Fig. 2. CT image of the dog pelvis with both cement filled and unfilled iliac crest bone defects

RESULTS: Pain relief after cement bone filling was highly significant regardless the cement used, compared to unfilled surgical site conditions where significant postoperative pain was observed during the first 4 postoperative days. Bupivacaine-loaded CaP cement provided very local and short-term better pain relief compared to the unloaded cement, during the first 6 postoperative hours. CT imaging confirmed the bone reconstruction of the iliac crest by the injected cement that remained in place and showed very good osteointegration on both microCT and histological analysis.
DISCUSSION & CONCLUSIONS: Bone filling of the iliac crest harvesting site with a CaP cement significantly reduced postoperative pain and allowed restoration of the iliac crest bone morphology. The injection of CaP cement may be an effective bone augmentation method to decrease permanent induced local pain and thus long-term morbidity after iliac crest harvesting procedure.
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Discerning the role of the setting reaction on the injection behaviour of tricalcium phosphate pastes

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INTRODUCTION: Understanding the mechanisms underlying injectability of calcium phosphate pastes is of paramount interest both for the delivery of calcium phosphate cements via minimallly invasive surgical techniques and for tissue engineering scaffold fabrication by robocasting. In the past, several research articles focusing on injectability of non-self-setting calcium phosphates were published [1]. The aim of the present work is to gain further insight in the role of the setting reaction on the injectability of tricalcium phosphate (TCP) pastes. Pastes prepared with both polymorphs of TCP, alpha (α; self-setting) and beta (β; non-self-setting) were studied. Reaction kinetics was modified either by thermal treatment of the powders or by the addition of nanocrystal seeds or accelerant solution, and their effect on injectability was studied [2].

METHODS: α-TCP and β-TCP were synthetized and milled to obtain powders with two different particle size distributions (PSD), fine and coarse. Powders were used as milled or after calcination at 500 °C for 24 h, and mixed with the liquid phase at a L/P ratio of 0.45 ml/g. The injection test was performed at 15 mm/min, to a maximum injection load of 100 N, starting at different times after mixing. The paste was extruded either through the 2 mm syringe aperture or through a 0.84 mm diameter cannula. The injectability percentage, yield load and injection load were determined and correlated with the type of polymorph, setting times, PSD, syringe aperture and the use of additives that accelerate the setting reaction, i.e. Na_2HPO_4 (2.5 wt.% in the liquid phase (water)) and/or 2 wt.% of hydroxyapatite (HA) seeds in the powder.

RESULTS: Non-self-setting β-TCP pastes were more injectable than self-setting α-TCP pastes (Fig. 1). The differences were more marked when small diameter cannulas were used or when the beginning of injection was delayed. Fine powders were more injectable and required smaller injection loads than coarse ones. While powder calcination resulted in an increased injectability due to lower α-TCP reactivity, the addition of setting accelerants, such as HA seeds, tended to reduce the injectability of the α-TCP pastes, especially if adjoined simultaneously with a Na_2HPO_4 solution.

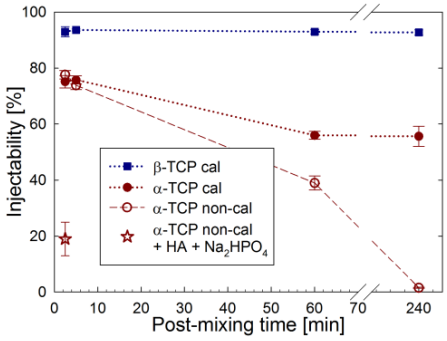

Fig. 1: Injectability as a function of post-mixing time for calcined (cal) and non-calcined (non-cal) TCP fine powders. The pastes were injected through a 2 mm aperture, without additives, unless otherwise stated.

DISCUSSION & CONCLUSIONS: The parameters affecting powder reactivity were shown to play a significant role in the injectability of TCP pastes. α-TCP was in general less injectable than β-TCP and required higher injection loads. Whereas powder calcination resulted in an increased injectability, the addition of setting accelerants tended to reduce the injectability of the TCP pastes. As a general trend faster setting pastes were less injectable, although, some exceptions were found, e.g. in absence of setting accelerants fine TCP powders were more injectable than coarse ones in spite of shorter setting times. When setting accelerants were added the opposite was true.

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Injectable BP-loaded cement to reinforce sheep osteoporotic vertebral bodies

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INTRODUCTION: Reinforcing osteoporotic bone is a clinical challenge for prevention and treatment of bone fractures. In this context of bone regeneration, we developed a local approach based on the use of calcium phosphate (CaP) bone substitutes that can deliver *in situ* bisphosphonate (BP) [1-2], an inhibitor of resorption largely used in the treatment of osteoporosis. Our previous study demonstrated the ability of granules of CaP loaded with BP to form new bone and to reinforce existing trabeculae in femur of osteoporotic ewes [3]. In continuation to this *in vivo* study, we have optimized the formulation of CaP by designing an injectable apatitic cement (CPC) that (i) hardened *in situ* without degrading the BP loaded and (ii) presented immediate mechanical properties more adapted to clinical applications in an osteoporotic environment.

METHODS: CPC was loaded with BP as previously described by Schnitzler et al [4]. Twelve adult female Vendeen sheeps were ovariectomised 9 months earlier to induce osteoporosis. An injection of the CPC loaded or not with BP was performed into lumbar vertebral (L3-L4) to achieve bone reinforcement in the vertebras. After 3 months, ewes were sacrified and vertebras and iliac crests were collected. Bone modifications have been determined by histological analysis, 3D-microtomography and SEM analysis performing in 3 regions of interest according to a growing distance from implant (0.8, 1.2 and 1.8 mm) as showed in figure 1. Calculation of parameters included (i) morphological parameters, mainly bone volume fraction (BV/TV, %), trabecular thickness (TbTh, mm), trabecular separation (TbSp, mm), trabecular number (TbN, mm^{-1}) and total porosity (PO, %), (ii) topological parameters like trabecular bone pattern factor (TbPf, mm^{-1}), structure model index (SMI) and Euler.

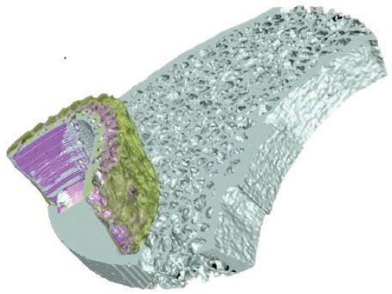

Fig. 1: Reconstruction of 3D image by using CTAnn® software of an implanted vertebra: three regions of interest have been defined according to the distance from implant (0.6, 1.2 and 1.8 mm).

RESULTS: As previously demonstrated [3], osteoporosis has been validated in iliac crest in ovariectomized ewes. CPC loaded with BP induced a distance-dependent effect on bone microarchitecture. Moreover, histological data showed the quality of osteointegration of combined-CPC.

DISCUSSION&CONCLUSIONS:
Ovariectomized ewes have been previously used as a model for post-menopausal osteoporosis [3] and it has been validated as a clinically relevant model of human post-menopausal bone loss. It is the first time that a BP-combined cement has been implanted in vertebrae in ovariectomized ewes. Interestingly, biological effect was distance-dependent that reflects the bone distribution of BP after the release process from cement. Quantitative (micro-architectural parameters) and qualitative (histological staining) analysis demonstrated that implantation of our combined cement in vertebra induced a beneficial impact on microarchitectural properties of trabecular bone.

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Session 1.5

Under extremities

Chairmen: M. Rauschmann, S. Tutton

Nanocrystalline Calcium Phosphate Cements in Tibial Plateau Fractures: Implant Augmentation Designs Derived From Clinical Studies

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Nanocrystalline Calcium Phosphate Cements developed in Cambridge, MA by ETEX, founded by Dr. D. Lee have been extensively studied in animal and human models. A level I prospective randomized multi-center study on tibial plateau fractures comparing Alpha-BSM (ETEX, Cambridge, MA) to autogenous iliac bone graft with standard reduction and internal fixation techniques published in JBJS (A) in 2008 documented a decreased articular subsidence rate with this ceramic cement compared to cancellous graft. The study mirrored the findings by Welch et al reported in JBJS 2004 showing the improved healing of trabecular bone with Alpha-BSM compared to autogenous bone graft.

The technical problems associated with proper placement of ceramics in fracture defects has hindered the widespread clinical adoption of these materials in general practice. The optimal combination of surgical implants with these ceramic materials would permit placement of the ceramics in intimate proximity to the fracture cavity, in adequate volume, without significant dilution by blood and fluids in the fracture cavity. Also, the fracture implant would require modified designs to optimize strength, hydraulic modifications for cement intrusion without excessive force and a method of extraction of the implant after fracture healing without associated damage to the regenerated bone.

The N-Force Fracture Fixation system (InnoVision, Inc., Memphis, TN, USA) received USFDA clearance in the fall of 2011, and was commercially released in the USA in 2013. It is a titanium alloy screw with the initial offering of a 4.0mm headless screw design with strength equivalent to non-fenestrated 4.0 Cannulated screws. It is cleared for cement delivery of ETEX proprietary Beta-BSM and CarriGen materials only at this time due to requirements by the FDA. It is capable of nondestructive removal by hand force after fracture healing. The hurdles faced in obtaining the first clearance in the USA of a combination implant and biomaterial delivery system centered about safety issues raised by the FDA.

Initial clinical studies have begun in fracture surgery and selected cases will be presented. This system opens the door for future combination implants and orthobiologic materials delivered by the implant to optimize function and placement of both materials.

Designing augmentation systems –from cement to application a hard road

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G. Insley Managing Director, PBC ltd., Ireland
A. Greter, Director Technology, Medmix Systems AG, Switzerland

Whilst the idea of using cements to augment device fixation in poor bone is far from new its clinical adoption has remained slow and the authors discuss a number of issues that may account for this. Brent Constanz's Norian Corp seeded many clinical studies in the 1990's and these showed us for the first time that not all CaP indications would prove added clinical value. Despite this the indications for use provided by cement makers continue to suggest that all voids in the body are equally valid targets.

The Norian clinical pioneers had to improvise their own specialty cannula sets to enable effective access in each indication and even today the lack of appropriate cannulas is a major limiting factor in application development. Nowhere is this better demonstrated than is cement augmentation in hip fractures where there is a very long access path (> 30cm typically) and it may be that both the fixation device and the operative technique may need modification to ensure clinical safety and efficacy. The authors present a case study to illustrate how an application specific approach is needed in hip fractures. Additionally typical "Do's" and "Don'ts" in delivery system design, for both cements and granules, are suggested by to an experienced system designer (Medmix).

The necessary cement properties have still to be properly established for each application. Some manufacturers have chosen to use PMMA (or like) material for bone augmentation in the osteoporotic hip whilst others suggest that CaP may provide sufficient augmentation in osteopenic bone. The local delivery of Bisphosphonates is starting to be clinically tested combined either with a CaP cement or even delivered via the screw in a planned in an RSA study of hip screw mobility in-vivo. The authors speculate that the combination of drug delivery via the screw together with local cement injection may be an even more attractive

Keynote No 13

Controlling the orientation of Ca-derived elements within structural composite

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Developments in CaP Injectable Cement for Screw Augmentation

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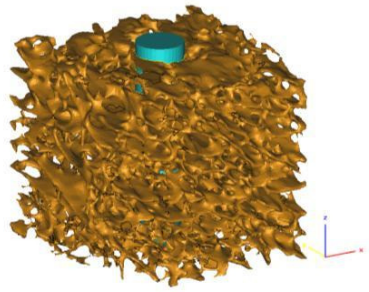
INTRODUCTION: The use of CaP injectable cement for screw augmentation has been described at GRIBOI[1]. The current paper presents some developments in the modelling of cements using finite elements. Techniques that have proved successful, along with some others that are more problematic, are outlined. Further results from the parallel test series are presented.

METHODS: The experiments were carried out on two densities of Sawbones® open-pored bone substitute materials. HydroSet® calcium phosphate bone cement was used to fill pre-drilled holes, and 4mm bone screws inserted. Pull-out tests were undertaken, and the specimens cut to examine the distribution of cement that had been achieved. A series of tests with and without gelatine, and with or without cement has been carried out. The gelatine is used to provide a resistance to the injectable cement representing fluids in the pore space.

Finite element models of cement-augmented cancellous bone with and without cortical layers have been developed using ANSYS commercially available software. The use of real bone geometry has been abstracted and developed from CT scans; the CT scans have been processed using MIMICS software. Subsequent methods for processing models have proved to be important.

RESULTS: The results of pull-out tests (Fig. 1) would indicate that the use of cement always increases the pull-out force required for screws in Sawbones® material. The use of gelatine as a fluid filler in the pores of the polymer bone substitute means that cement penetration is limited to more practical amounts (Fig. 2). Nevertheless, the amounts of cement injected with the use of a bone substitute – even with the gelatine - are considerable.



Finite element models[2] (e.g. left) show the variability of pull-out force with position of the screw in the bone.

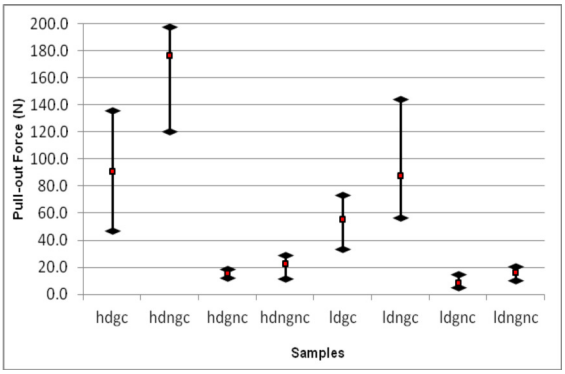


Fig. 1: Variation of pull-out force with cement (c) or no cement (nc), gelatine filled (g) or not gelatine filled (ng), for low density (ld) and high density (hd) Sawbones material.

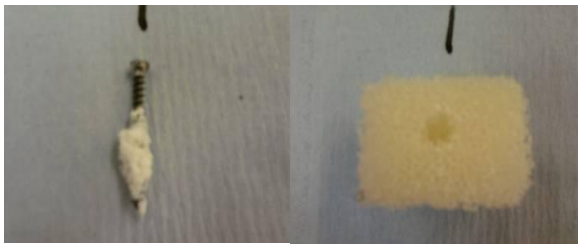


Fig. 2: (left) Screw and attached cement after pullout from sample shown (right).

DISCUSSION & CONCLUSIONS:
The variation in pull-out force from position alone can be significant, but the cement may have the effect of removing some of this variability. The use of gelatine to restrict the radial flow of cement is demonstrated in Sawbones material.

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ACKNOWLEDGEMENTS: Some work presented has been funded by studentships from the Stryker Corporation.

macroporous ceramics of carbonated hydroxyapatite for bone grafting applications

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INTRODUCTION: Calcium phosphate based materials, specifically hydroxyapatite (HA) and tricalcium phosphate (TCP), have been extensively used as bone graft substitutes for various applications (e.g. bone augmentation procedure). They temporarily substitute for bone while simultaneously supporting its regeneration. However, they present inappropriate resorption rate. Indeed, ideally a bone substitute should degrade only after the regenerated tissue has been remodelled at least once in the natural remodelling cycle [1]. Thus, the solubility of HA is too slow and that of TCP too fast to foster successful bone re-growth. To further improve the properties of calcium phosphate ceramics, ionic substitutions could be used. One way to modulate their resorption rate would be to substitute carbonate ions (CO_3^{2-}) for phosphate ones (PO_4^{3-}) into the HA structure [2]. Thus, the aim of the present work was to elaborate interconnected macroporous scaffold of pure carbonated hydroxyapatite (CHA).

METHODS: CHA powders were synthesised by an aqueous precipitation method using calcium, phosphate and carbonate salts solutions. The amount of reagents was calculated according to the general formula: $Ca_{9.2}(PO_4)_{5.2}(CO_3)_{0.8}(OH)_{1.2}$. Green materials with controlled macroporous architecture were then shaped *via* a template casting process [3]. The ceramics scaffolds were finally obtained after sintering at 1100°C under controlled atmosphere. To assess the phase composition of the materials, X-Ray Diffraction (XRD) and Fourier Transformed Infra-Red (FTIR) analysis were performed. Calcium, phosphorous and carbonate content of the scaffolds were determined by elemental analyses (e.g. inductively coupled plasma atomic emission spectroscopy). The architecture of the ceramics was also observed by means of Scanning Electron Microscopy (SEM).

RESULTS: The XRD pattern of the sintered material shows only hydroxyapatite diffraction peaks; no secondary crystalline phase is observed (data not shown). The FTIR spectrum of the CHA ceramic is in accordance with the hydroxyapatite structure (data not shown). Moreover, this spectrum exhibits characteristics bands of carbonate groups from both A and B sites. The carbonate content of the material was evaluated at 4.9 wt. %, amount which is very close to the theoretical value of 5.2 wt. %. The architecture of the sintered material (fig. 1) reveals a macroporous interconnected structure with macropores of 400 ± 50 µm and interconnections of 150 ± 30 µm.

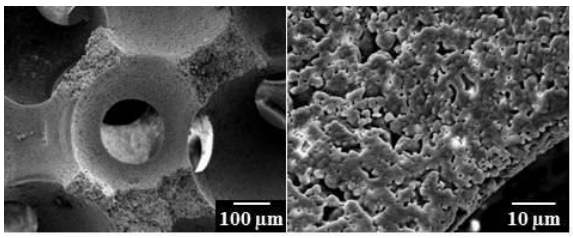

Fig. 1: Macroporous architecture of CHA ceramics obtained via a template casting process (left) and detail of the microstructure (right)

DISCUSSION & CONCLUSIONS: The physic-chemical analysis of the sintered ceramic (XRD, FTIR and elemental analysis) confirmed the incorporation of carbonate into the HA structure in B and A sites during, respectively, the precipitation and the heat-treatment. The thermal treatment under controlled atmosphere (p_{CO2} = 1 bar) allowed to prevent undesired decarbonatation of the material that occurs around 700°C under air atmosphere. With the template casting process adapted to CHA powders, it was possible to control the architecture of the final ceramic. To conclude, interconnected macroporous materials made of carbonated hydroxyapatite free of secondary phase has been successfully manufactured. To fulfil the characterization of this scaffold, solubility product measurements are under progress as well as *in vitro* and *in vivo* biological assays.

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PMMA-hydroxyapatite composite material increases lifetime of augmented bone and facilitates bone apposition to PMMA: Biomechanical and histological investigation using a sheep model

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INTRODUCTION: The most commonly used filler material for vertebral body augmentation is polymethylmethacrylate (PMMA), whose mechanical properties differ from that of cancellous bone that might result in fracture in the adjacent bone. The purpose of this study was to investigate whether the approximation of mechanical properties of the composite material to cancellous bone by addition of nanocrystalline hydroxyapatite (HA) (Nanostim®, Medtronic) to PMMA is able to prolong the lifetime and retard fatigue failure of surrounding bone tissue under cyclic compression and to investigate the histological reaction of bone tissue to the composite material.

METHODS: Composite material (30% volume fraction HA) was chosen for implantation in one medial condyle of sheep femur and plain PMMA in the other as control. 18 adult female sheep were divided in two groups, one group for a follow-up period of three months and the other group for a follow-up period of six months. After the follow-up period the animals were killed and specimens containing the implant material and surrounding bone were cut out. Samples from 3 animals were assigned for histological examination and from 6 animals for mechanical testing. For evaluation with light microscopy, the sections were stained with hematoxylin and eosin as well as with toluidine blue. The mechanical testing was performed under cyclic compressive loading. Failure was defined as a ten percent reduction of the initial modulus E_0. The applied stresses (σ/E_0) were plotted as a function of the cycle numbers (N_f), at which the failure criterion was reached and the lifetime curves were calculated. The significance of differences between the lifetime curves of groups and the follow-up periods was calculated by analysis of covariance ANCOVA.

RESULTS: Composite implants exhibited direct surface contact with the bone tissue throughout the whole circumference, whereas PMMA implants were completely covered by a layer of fibrous connective tissue and thus separated from surrounding bone. In both, three and six months follow up groups, the bone-composite specimens showed a significant higher lifetime curve than the respective bone-PMMA specimens ($p = 0.0031$ and $p = 0.04$, respectively). Considering the follow up time after the implantation, a significant increase of specimen lifetime was observed for PMMA ($p = 0.0013$) and composite ($p = 0.0015$) implant groups from three to six months follow up.

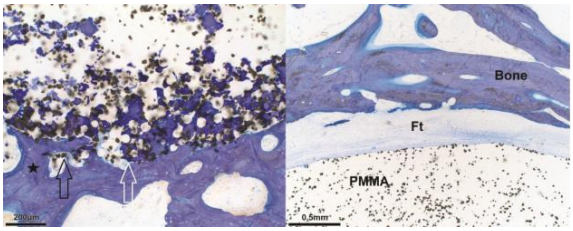

Fig. 1: Histological section of distal femur containing composite material (left) and PMMA (right) six months after implantation.

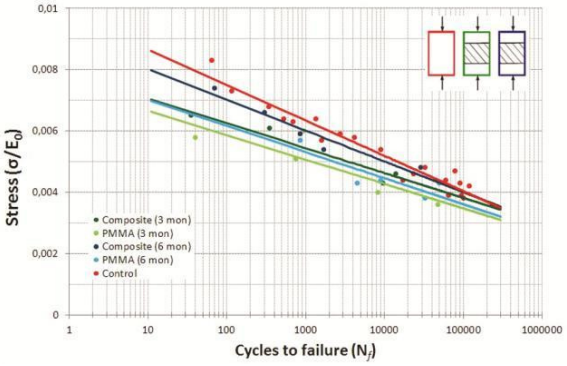

Fig. 2: Applied stresses (σ/E_0) to native bone, augmented bone with PMMA and composite material as a function of cycle number until the failure criterion was reached.

DISCUSSION & CONCLUSION: The histological results in this study suggest that the addition of nanocrystalline HA to PMMA enabled bone apposition to the implant material, whereas PMMA alone became encapsulated by fibrotic connective tissue. However, a significant bone ingrowth into the porous structure of the composite material was not observed. Specimens augmented with composite material with compressive elastic property close to cancellous bone exhibited better mechanical performance and lifetimes compared to the respective plain PMMA groups. Addition of nanocrystalline HA to PMMA is able to retard fatigue failure of augmented bone. This is of particular interest for augmentation of osteoporotic vertebral body compression fracture where adjacent vertebral body is at high fracture risk.

Session 1.6

Scaffold for bone defects

Chairmen: J.M. Bouler, M. Bohner

Architecture control and characterization in injectable bone graft substitutes

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Synthetic materials emerge as an alternative to bone grafts, since in recent years it has been shown that they can effectively foster bone regeneration while simultaneously avoiding some of the limitations associated with bone grafts such as limited availability, morbidity or disease transmission.

Bone graft substitutes must satisfy various requirements to act as a guide for new bone formation, stimulating tissue repair at the site of implantation. Besides the prescriptive biocompatibility, bioactivity and adequate resorbability, porosity appears as one of the key requirements for the material to act as a substrate for bone regeneration.

Moreover, nowadays, when the use of minimally invasive surgical techniques represents a major achievement in orthopaedic surgery, there is a growing need for injectable biomaterials. We have shown that it is possible to obtain injectable calcium phosphate foams, which are able to set *in vivo* and retain macroporosity after injection[1,2]. Different surfactants can be used, either from natural or synthetic origin, to control not only the amount and size of macropores introduced in the material, but also the level of interconnectivity of these macropores. In these materials, macroporosity is superimposed to the intrinsic nano/microporosity of calcium phosphate cements, which can in turn be tailored by adjusting specific processing parameters. Therefore, a complex architecture is obtained, with interconnected pores covering from the nano to the macroscale, which can be of interest also when designing tissue engineering scaffolds or drug delivery matrices for the musculoskeletal system[3].

Porosity is a multifaceted property that cannot be characterized with a single parameter. Apart from total porosity, other features like pore architecture, pore size distribution, pore shape, pore interconnectivity and tortuosity, are also aspects that affect the interaction with the living organism at different levels and therefore condition material performance in multiple scenarios. The spatial configuration provided by macroporosity plays an important role in guiding new tissue ingrowth within the material, so that cell colonization and angiogenesis events can take place. Moreover, not only macroporosity but also microporosity will influence the kinetics of resorption of the material. In addition, micro/nanoporosity results in larger surface area that contributes to higher protein adsorption and ionic exchange; and pore architecture at this level can also lead to protein concentration/entrapment, creating osteogenic microenvironments. For all these reasons, a careful characterization of material's architecture and textural properties, from the nano- to the macroscale, is required to understand the mechanisms of interaction between the material and the biological environment, and their influence on the events leading to bone formation[4].

At present there is no single technique that allows a comprehensive characterization of architectural features of this type of materials, at all the levels that are relevant for the biological response. For this reason it is important to combine the complementary information provided by imaging techniques like scanning electron microscopy or microcomputed tomography, and physicochemical techniques like mercury intrusion porosimetry, gas adsorption or thermoporometry.

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Multiscale cell seeding in CaP scaffolds containing controlled macro and microporosity

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INTRODUCTION: The most severe bone defects, complex and/or critical size defects, do not heal with current treatments of allo-, auto-, or synthetic graft. Research in scaffold-based bone repair focuses on the use of CaPs and other materials to repair these defects that can cause disfigurement and loss of function. One major challenge in the repair of critical size bone defects is ensuring efficient and rapid cellular ingress into the scaffold. Forced cell seeding in porous ceramic scaffolds has been shown to enhance bone regeneration[1]. This study demonstrates a cell localization and *self*-seeding mechanism driven by capillary forces in porous, 2D CaP substrates *in vitro*. The approach shows promise for populating scaffolds with cells *in vivo* in a tunable manner.

METHODS: 2D CaP substrates were fabricated as in previous work. PMMA beads in the colloidal suspension serve as sacrificial porogens and allow for precise control over the micropore size and fraction. Cell localization on 2D substrates in the presence of capillary forces was measured for substrates with and without porosity (MP, NMP), for dry substrates, and for PBS-soaked substrates (wet). Cells stained with Cell-Tracker Green were placed on top of the substrates and cell density measured using a fluorescent microscope. For cell penetration experiments, the cell suspension lined a glass slide, and then was brought into contact with a dry MP substrate (Fig. 2a). Cell penetration distance was measured for MC3T3-E1, MSC, and D1 cells.

RESULTS: The pore fraction in MP samples was 46% and fully interconnected[2]. Pore size, measured by MIP[2], was nominally 5μm. Dry, MP substrates showed the greatest cell localization and the other conditions were all statistically similar (Fig. 1). NMP samples had negligible porosity and therefore low capillary pressure (Fig. 1a). In wet samples, PBS filled pores and eliminated capillary forces (Fig. 1b,c). These factors explain the differences observed. Cell penetration distance (Fig. 2b,c) depended on cell type (i.e. size and stiffness) and their ability to deform and squeeze through the pores and interconnections.

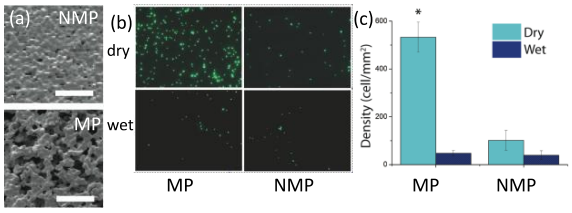

Fig. 1: (a) SEM images of MP, NMP microstructures. Scale bar: 50μm. (b) Images show cell localization on top of substrates. (c) Cell density for MP/dry was significantly higher than all others.

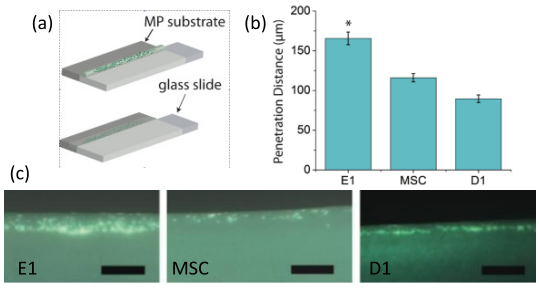

*Fig. 2: (a) Schematic of cell penetration experiments. (b) MC3T3-E1 cells penetrated significantly further than MSCs or the D1s. (c) Fluorescent images show cell penetration. Scale bar: 500*μm.

DISCUSSION & CONCLUSIONS: This paper shows a novel, simple mechanism to *self*-seed CaP scaffolds and substrates. Cell localization and self-seeding depends both on the presence of capillary forces, determined by the microstructure (MP, NMP) and the state of the pores (wet, dry), and the cell type. Optimization by tuning micropore and pore interconnection size and pore fraction size could lead to efficient and deep cell penetration into 3D scaffolds. This will improve their efficacy for healing and repair of large bone defects.

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ACKNOWLEDGEMENTS: The work was supported by NSF DMR (No. 1106165).

Development of an intra-operative artificial tissue fabrication approach for reconstruction of large tissue defects

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INTRODUCTION: Current technologies for large tissue defect reconstruction are imperfect solutions that come with heavy burdens for patients and an enormous economical cost [1]. Engineered biological tissue that is customizable and immune-compatible can potentially make a significant difference in the lives of patients. Furthermore, on-demand scaffold and tissue fabrication has the promise for broad applicability towards many medical maladies and to allow fabrication of complex *in-vitro* tissue models, thereby facilitating discovery of novel drugs and diagnostic tests [2]. We are developing a concept for intra-operative fabrication of custom implants utilized in reconstructive surgery. The main application will be for the treatment of non-unions after tumor resection or injury, an unsolved clinical problem.

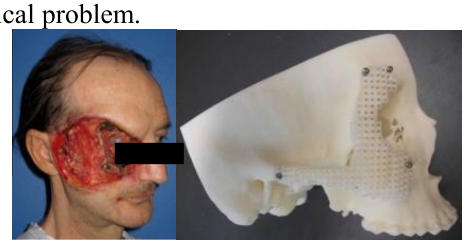

Fig. 1: Patient requiring complex 3D reconstruction after tumor resecction.

In this phase of development we focused on 1) building block manufacturability; 2) developing a robotic platform for concept validation; and 3) integrating tissue engineering concepts into blocks.

MATERIALS and METHODS: Unit block polyhedral were generated using Rhinoceros 3D with a bounding box of 3x3x3mm. A common interface between building blocks was designed. Several different architectures representing the in-homogeneity found within human bone tissue were designed and subsequently fabricated using additive fabrication. Dimensional accuracy and mechanical properties were evaluated. We built a robotic test platform for our block assembly system that is based on the Adept Quattro high-speed parallel robot. A test setup was generated to investigate robot path optimization. We examined 6 different strategies for fluid flow perfusion through an array of building blocks. An analytical model following Hagen-Poiseuille equation was established for comparison of the different flow perfusion strategies.

RESULTS: Geometric errors between designed and fabricated building blocks were less then 1.5% for overall dimensions and around 10% for the smallest of all features. The stiffness of the building blocks was not significantly affected by the removal of the fluid pathways, 44.9 ± 7.7 MPa w/o fluid channels versus 48.8 ± 8.7 MPa with fluid channels. The overall strength of the building blocks was higher for the building blocks with fluid pathways (41.4 ± 1.9 MPa) compared to building blocks that were empty (26.5 ± 3.1 MPa).

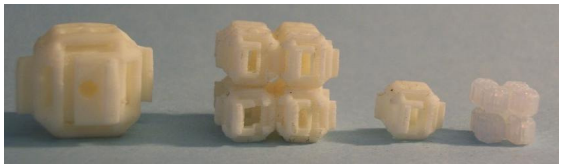

Fig. 2: Building blocks at 4 different scales

The six different flow strategies exhibited vastly different flow conditions. There was a 10-fold difference in magnitude between the construct with the least and the highest pressure loss. As expected, adding transverse flow channels did not improve pressure loss. However, it is expected that during *de-novo* tissue growth transverse channels provide redundant pathways for flow perfusion.

DISCUSSION & CONCLUSIONS: We successfully developed a viable concept for the evaluation of robotic intra-operative scaffold fabrication. The micro-architecture of building blocks can be engineered to balance mechanical properties and fluid perfusion parameters independently. Engineering artificial flow channels into a scaffold construct may allow perfusion at the time of surgery.

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Dynamic cell culture on glassy crystalline calcium alkali orthophosphates scaffolds

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INTRODUCTION: Calcium phosphate (CaP) materials are well documented synthetic bone substitutes. The standard CaP materials include hydroxyapatite and/or α/β-tricalcium phosphates as main components [1]. A new kind of CaP was developed in the mid-90's; these CaPs are composed of glassy crystalline calcium alkali orthophosphates (CAOPs) [2]. The aim of the present work was to study the feasibility and outcome of dynamic cell culture on CAOP scaffolds.

METHODS: The properties and preparation of the CAOP used, *i.e.* GB14, have been described previously [2]. This material is commercially available as Osseolive ® (Curasan, Germany) and has CE and FDA approval. Cylindrical scaffolds (Fig 1) were obtained by rapid prototyping (RP) and by investment casting (IC) methods. Briefly, RP scaffolds, with controlled architecture and porosity, were built by injecting a CAOP slurry via a printing machine (RX Series, Prometal, Germany). IC scaffolds were obtained by embedding a polyurethane foam with a CAOP slurry and burning out the polyurethane. MC3T3-E1 cells (ATCC, USA) were used. Seeding media consisted of DMEM (PAA, Germany) supplemented with 10% FBS (PAA, Germany), 2*mM* L-Glutamine (Gibco, Germany), 5*mM* β Glycerophosphate (SigmaAldrich, Germany) and 50µg/ml of Penicillin-Streptomycin (Gibco, Germany). Culture media was seeding media with additional 50µg/ml of ascorbic acid (SigmaAldrich, Germany). Cell densities of 1.5 and 3.0 E6 cells/ml were used. Dynamic cell seeding and culture was performed in a Bioreactor (TEB100; Ebers, Spain). Scaffolds were seeded under dynamic revesible perfusion for 24 h at 0.25 ml/min. Dynamic culture was performed for 7 days at 0.5 ml/min. After 7 days, samples were fixed in Histochoice™ (Amresco, USA), dehydrated and embedded in PMMA/BMA for histological observation [3]. 50 µm-thick sections were cut using a sawing microtome (SP1600; Leica, Germany). Sections were Giemsa stained and analyzed for histology.

RESULTS: Histological analysis demonstrated that, under dynamic seeding and culture, cell attachment and invasion was possible in both scaffolds. However, cell presence was more prominent at 3.0 E6 cell/ml than 1.5 E6 cell/ml, for the RP scaffolds, as compared to the IC ones. After 7 days of culture a change of the scaffold behaviour was observed.

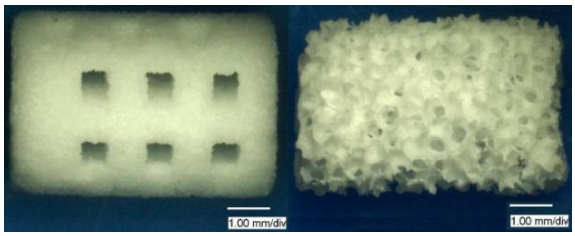

Fig. 1 Images of representative rapid prototyping (left) and investment casting (right) scaffolds.

DISCUSSION & CONCLUSIONS: Depending on the scaffold architecture and cell density, a more advantageous cellular interaction could be observed with the RP scaffolds. This behavior is related the initial cell availability and to the fluid dynamics created within the scaffold, which affect nutrient supply to the cells [4-5]. However, further tests and quantification are needed to confirm this observation. After 7 days, both scaffolds presented a squeezable behavior when handled with tweezers. Without cells, the CAOP becomes fragile due to its dissolution. Hence, this behavior is due to the cell-material interaction. This study opens the possibility for further study of these scaffolds as elements for tissue engineered interbody fusion cages.

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Wet-chemical process to synthesis the biphasic calcium-phosphate powder

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☒ Biomaterials	☐ Biomechanics	☐ Clinical	☐ Innovation

INTRODUCTION: Wet-process is mainly used for the synthesis of calcium deficient HA. However and recently, it can be used in direct processing of biphasic calcium-phosphate ceramics HA/TCP. When compared to both α- and β-TCP, HA is a more stable phase under the physiological conditions, as it has a lower solubility and, thus, slower resorption kinetics. Therefore, the biphasic calcium-phosphate BCP concept is determined by the optimum balance of a more stable phase of HA and a more soluble TCP [2]. Due to a higher biodegradability of the α- or β-TCP component, the reactivity of BCP increases with increasing TCP/HA ratio. Therefore, in vivo bioresorbability of BCP can be controlled through the phase composition. This study examined the use of wet-chemical process to synthesis the (BCP) powders and the effect of the aging on the phase composition.

METHODS: $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$, as precursors, were mixed according to an initial Ca/P ratio = 1.5 and chemical reactions take place between calcium and phosphorus ions under a controlled pH and temperature of the solution. The alkalinity and reaction temperature were fixed at pH 8 and 60˚C for the preparation of BCP respectively. A part of the precipitated powder was then got dried and the other part was aged overnight. All precipitated powders were then calcined for 2hr at 1000 °C, and heating and cooling rates were 20 °C/min.

RESULTS:

The chosen pH value was optimal in order to produce BCP powders. In particular pH less than 9 favors the synthesis of TCP powders while pH value of a bout 9-10 favours the production of straight fibrous HA. That is due to the increase of Ca/P molar ratio of the product with the increase of the pH value of starting solution. Moreover, increasing the pH induced the substitution of PO_4 groups with CO_3 groups.

The effect of aging was clearly demonstrated with XRD. The draying stops the evolution of the burshite phase and hence the as-dried and calcined powders showed higher β-TCP/HA in the formed BCP than that of the non-dried and calcined powders.

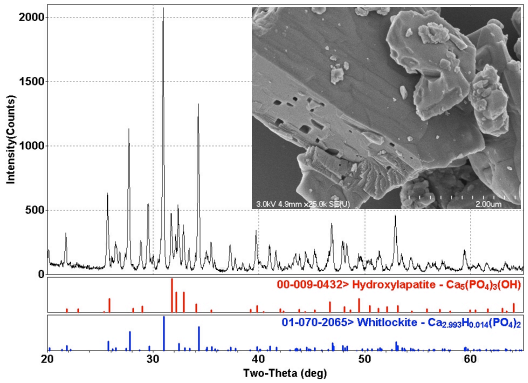

Fig.1 XRD and SEM of as-dried and calcined BCP powder.

CONCLUSIONS:

Wet process is a low cost fabrication process to synthesis HA/β-TCP via wet chemical reaction. It is also suitable for an industrial production, as it requires inexpensive reagents and BCP products with variable phase composition can be easily obtained. Yet, very few studies exist here and different compositions were investigated. In such compounds, significant differences in the characteristics may exist between materials of very close chemical compositions and consequently their usage in the different applications. Within these applications, the particle size, presence of substituting elements and the crystallinity strongly affect resorbability. Thus, the ability to prepare biphasic mixtures with controlled crystallinity, size and phase composition will be of our future studies.

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Session 2.1

Bone augmentation

Chairmen: D. Noreiga, T. Russell

Keynote No 17

Screw augmentation and image guidance

Sean TUTTON

Medical College of Wisconsin. Milwaukee, WI, US

Prophylactic Vertebral Augmentation

Luigi Manfrè

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Prophylactic vertebral augmentation (PVA) has became the new challenge for spinal interventionist, particularly after the increasing number of papers claiming increase in adjacent vertebral fractures (AVF) after PMMA vertebroplasty for vertebral compression fracture (VCF).

The conservative option, that is, not to treat patients with a VCF with vertebral augmentation (VA) cannot be accepted nowadays, considering the increasing risk of bed-rest complications, the cardiovascular distress related to increasing kyphosis, worsening of Altzheimer symptoms and muscles and bone loss in patients forced to stay in bed.

Rehabilitation of osteoporosis Program Exercise (ROPE) can be a valid tool in prophylaxis, according to the fact that patients undergone VA + ROPE treatment showed a new AVF only 20.4 months in comparison to simple VA, who showed new fractures after 4.5 months only.

The rationale of doing PVA is strictly dependent on the fact that a VCF is responsible not only for local but whole spine changes, involving ligaments, muscles, disc, joints and even sagittal balance: so a need for stiffness restoration is mandatory to prevent new AVF.

The first question is: "how we can predict a fracture?". There are several factors that can be take in account about. Besides global factor such as the degree of osteoporosis, Bone Mineral Density (BMD), other concurrent diseases, local factors seems to be more and more important for generating an AVF. Local condition that can increase the risk of an AFV are the position of a vertebra into the spine architecture, the degeneration of adjacent disc, and endplates fracture & deformation. There are imaging tools that will help us in finding out vertebra-at-risk for fracture, such as spectroscopy, perfusion MRI, High resolution MRI scanning and Diffusion MRI.

The disc however seems to play a master role in increasing AVF. In case of VCF with regularly hydrated disc, the axial load is mainly distributed on the anterior 2/3 of the vertebra, the posterior arch being compressed for 8% and 2% only in the erect position and forward bending respectively. However, in case of VCF with degenerated disc, despite dramatic increase of axial stress load on the posterior 2/3 or a vertebra, there is evident increase up to 59% in anterior endplates portion on forward bending. This could explain the typical involvement of bone under endplates in case of AVF, with new AVF clustered at the endplates closest to the previously treated vertebra.

Using software for Finite Element analysis (FEA) on CT scans helps us to better understand the area-at-risk for fracture. By calculating FEA before VA, one can predict local area of vertebral bone weakness, and area supposed to fracture in the adjacent vertebra. In particular, a protective effect of VA has been demonstrated on adjacent vertebra: in fact, in case of AVF, new fractures were depicted only in area above adjacent endplates without PMMA under, while no fracture where depicted when endplates below were above PMMA. Moreover, in contrast to the decrease in bulge of the augmented vertebra endplates, the bulge of the adjacent endplates substantially increased, by as much as 17%. According to Trout (2006) the number of incident fractures is not as many as the that of prevalent fractures after bone augmentation in osteoporotic patients: consequently, VA do not increase the real risk of fracture, but the risk of AVF only, as 54% incident fractures in 934 patients after PMMA were AVF. When performing VA, one should take more attention in kind of distribution of PMMA, as augmentation under the endplates seems to be the key to prevent AVF. And PVA can be nowadays justified by the fact that the eight loss of intact vertebra adjacent to a 2-level augmented vertebra was smaller than the one adjacent a 1-level augmentation, according to Chang (2009).

Recent studies calculating distance across the disc variation under compressive stress demonstrated that by restoring normal load sharing, vertebroplasty has the potential to decrease the risk of recurrent and adjacent level fractures (Dolan 2010). Besides the distribution of PMMA, changing the modulus of injected material (i.e. a material more similar to human bone) could be the solution for preventing AVF. In the past, morphological changes of injected calcium

phosphate cement (CPC) in osteoporotic compressed vertebral bodies have been studied, demonstrating that CPC only is not sufficient for increasing the stiffness of a fractured vertebra.

Recently, osteo-conduction with 60% α-CalciumSulphate (α-CaS) and 40% Hydroxyapatite (CaP), the so-called Cerament ®, showed good capability in treating a VCF. New silicons as the VK100, fully biocompatible and radiopaque, is able to adheres to bone, and the elastic modulus acts as a shock absorber, with excellent adhesion to bone. Moreover, osteo-induction with recombinant human Bone Morphogenetic Protein-2 Calcium Phosphate Cement Nanoscale (4.6 m particles) showed CaP degradation happening simultaneously to new bone regrowth, with no gaps, no inflammatory reaction, no fibrous capsule, no connective tissue or scars, with fibrocartilagineous regrowth of the new bone, as for normal human bone.

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Does balloon kyphoplasty deliver more cement safely into osteoporotic vertebraes with posterior wall defects compared to vertebroplasty?

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INTRODUCTION: Kyphoplasty and Vertebroplasty are commonly used tools for providing pain relief in fractures due to osteoporosis or cancer. However, cement leakage during these procedures, especially in the cases of posterior vertebral wall defects, can be a major source of morbidity and degraded outcome.

METHODS: Forty artificial vertebral analogues made of polyurethane with osteoporotic cancellous matrix representing the L3 vertebrae were used for this study that were divided into 4 groups of 10 vertebrae each. The 4 groups tested were: Low viscosity cement injected using vertebroplasty, High viscosity cement injected using vertebroplasty, Low viscosity cement injected using balloon kyphoplasty, and High viscosity cement injected using balloon kyphoplasty. The procedures were carried out under fluoroscopic guidance. Injection was stopped when the cement started protruding from the posterior wall defect. The main outcome measured was the volume of cement injected safely into a vertebra before leakage through the posterior wall defect.

RESULTS: The highest volume of cement injected was in the vertebroplasty group using high viscosity cement which was almost 2 twice that injected in the other 3 groups. One-way ANOVA comparing the 4 groups showed a statistically significant difference ($P < 0.0001$). Post-hoc analysis showed a statistically significant difference in the volumes when comparing the high viscosity vertebroplasty groups with all the other 3 groups respectively. However, there was no statistically significant different in the volume of cement injected between the other 3 groups.

DISCUSSION & CONCLUSIONS: High viscosity cement injected using vertebroplasty delivers significantly more cement and fills the vertebral body more uniformly when compared to balloon kyphoplasty in osteoporotic vertebrae with a posterior wall defect.

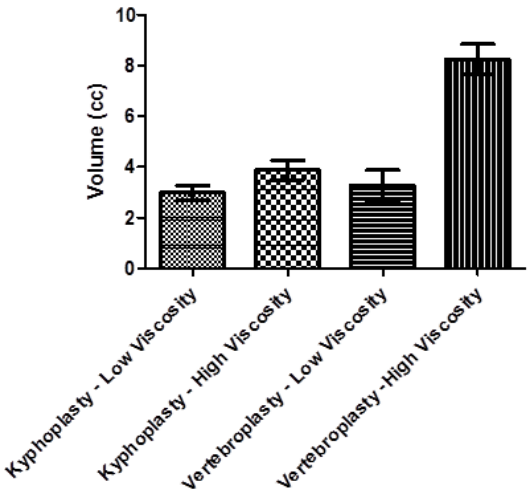

Fig. 1: Volume of cemented injected.

Table 1. Results for volume of cement injected into the vertebrae.

	Cement volume injected (ml) – Calculated from vertebral weight pre- and post- filling	Cement volume Injected (ml) – Calculated from Length of bone filler devices used
Vertebroplasty – Low viscosity (V1)	3.3 (SE: 0.6, Range: 0.7 – 6.0)	3.4 (SE: 0.6, Range: 1.1 – 5.9)
Vertebroplasty – High viscosity (V2)	8.2 (SE: 0.6, Range: 3.7 – 10.2)	8.0 (SE: 0.6, Range: 4.1 – 10.5)
Balloon Kyphoplasty – Low viscosity (K1)	3.0 (SE: 0.3, Range: 1.0 – 4.3)	3.2 (SE: 0.2, Range: 1.8 – 4.3)
Balloon Kyphoplasty – High viscosity (K2)	3.9 (SE: 0.4, Range: 2.5 – 6.0)	4.3 (SE: 0.3, Range: 2.9 – 6.3)
P Value	< 0.0001	< 0.0001

SE indicates Standard Error of Measurement

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Cement augmented anterior screw fixation of dens type II fracture and presence of osteoporosis

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INTRODUCTION:
Anterior screw fixation is the "gold standard" of surgical treatment of an uncomplicated Anderson D´Alonzo type II odontoid fracture. Insufficient bony screw hold can cause severe procedure-related complications and result in screw breakouts with secondary fracture dislocation. Therefore a posterior atlantoaxial fusion is recommended in case of severe osteoporosis. However, the posterior approach is associated with a higher morbidity and a significant loss of cervical spine mobility. The aim of this study was to evaluate whether an additive cement augmentation of the C2 body will increase the screw hold and lead to a safe fracture after anterior screw fixation of Anderson type II fracture and osteoporosis.

METHODS:
Between 2009 and 2010 5 patients (Ø 87 years) with a displaced odontoid Anderson type II fracture and osteoporosis were treated using anterior odontoid screw fixation. After closed reduction patients received a standard anterior screw fixation using two screws. Thereafter an additional anterior vertebroplasty of the C-2 corpus with an average amount of 2.5 cc PMMA bone cement was performed using a conventional bone filler. Care was taken that the bone cement completely surrounds the screws. Postoperatively, patients were advised to carry a soft collar within six weeks. Patients were followed clinically and radiologically using plain x-rays and computed tomography.

RESULTS:
All patients survived the operation and the postoperative course was uneventful. Due to the additional vertebroplasty, operation time was increased by 11 minutes on average. The postoperative CT scan showed in all cases a cement layer embracing the screws. In two patients there was a slight leakage of bone cement laterally across the fracture line without clinical relevance.
A 90 years old patient died 4 months after the operation by cardiac cause and was therefore not available for follow-up. 4 out of 5 patients were available for a follow-up with an average follow-up time of 23 months. These patients were free of neck pain and showed adequate rotation of the upper cervical spine. Computed tomography was able to detect in 3 out 4 patients (one patient denied a radiological examination on follow-up) solid bony fusion without implant complications.

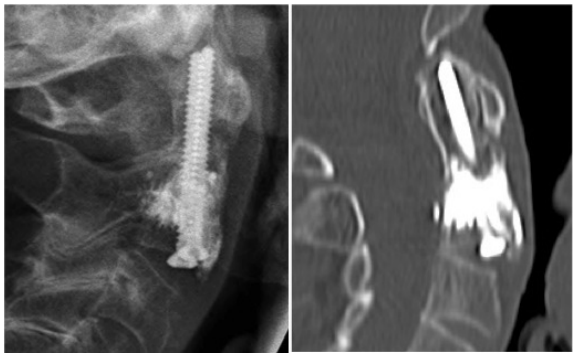

Fig. 1 Patient example 32 months after the operation: X-ray and 2D-CT reconstruction after cement augmented anterior screw fixations of the dens axis showing bony fusion.

DISCUSSION & CONCLUSIONS:
In these patients with traumatic unstable odontoid fracture Anderson D´Alonzo type II and prevalence severe osteoporosis a cement augmented anterior screw fixation leads safe bony healing of the dens. This procedure - compared to the standard treatment of osteoporotic fractures type Anderson D´Alonzo II odontoid with atlantoaxial spondylodesis - might lead to a lower morbidity and maintenance of the upper cervical spine mobility.

Rescue from the cut-out: Cement Augmentation of a Gamma Nail

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INTRODUCTION: Rates as high as 16% of Cut-out has been reported as a major complication after intramedullary nailing stabilization of a pertrochanteric fracture. Recent studies of cement augmentation of the cephalic screw has shown no cases of cut-out, but there is no reports on rescue of a cut-out with a new cement augmentation.

The aim of this paper is to report a case of a rescue from a cut-out with an augmentation of a gamma nail and ultra-high density cement COHEsION (VEXIM©) for filling the gap.

METHODS: A 87 women suffered a pertrochanteric fracture 31A2 after a casual fall. She was operated in the first 48 hours with a Gamma Nail and she was discharged 8 days latter to a residence. 1 month after the operation the patient joined again in our institution with a cut.out of the Gamma Nail.

We planned the surgery for avoiding previous mistakes (lack of reduction, tip-apex distance > 2cm). We remove the previous nail and improve the reduction of the fracture using a reduction clamp or a Hoffman retractor place on the anterior side of the femoral neck and implanting a new Gamma Nail and cementing the bone gap with COHESION through a trocar as in the fashion of kyphoplasties, whose density allowed us to control the cement distribution without intra-articular penetration

RESULTS: The patient was allowed for full weight bearing with two crutches after 6 weeks and radiographic consolidation was

At last revision 10 months after surgery no other complications have been found and the patient recovered her previous walking status

DISCUSSION & CONCLUSIONS:

Cut-out is a major complication after aintramedular nailing of a perthrocanteric fracture and in many cases a hip arthroplasty is needed to solve the problem, as in many cases there is no bone purchase of another cephalic screw. Otherwise augmentation with other kind of cements cannot be used in case of intraarticular penetration as leads to cement leakage due to the lack of control of the cement distribution.

With COHESION cement through a trocar and controlling the implant, manipulation and stabilization times we can direct the cement wherever we need to fill the bone loss, improving bone purchase with no additional risk of intra-articular cement.

This technique can be considered as a new option for the treatment of the cut-out with severe bone loss and an alternative to the arthoplasty

Cement distribution after percutaneous unilateral transpedicular balloon kyphoplasty for the mid & upper thoracic spine.

[1,2]Vastardis G, MD; [1]Dial B, BS; [1]Stojanovich M, BS; [1]Marjan A, BS; [2]Potluri T, MS; [2]Carandang G, MS; [3]Hadjipavlou A, MD, PhD, FACS; [1,2]Voronov L, MD, PhD; [1]Zindrick M, MD; [1,2]Patwardhan A, PhD.
[1]Loyola University Chicago, Maywood, IL; [2]Edward Hines Jr. VA Hospital, Hines, IL;
[3]University of Crete, Greece

INTRODUCTION: The most common method to perform transpedicular kyphoplasty is using a bilateral approach to ensure maximum cement distribution. Augmenting only a single half of the vertebral body could lead to medial/lateral angular deformation of the vertebral body. With the advent of balloons that fit down an 11-guage trocar (outer diameter of 3.0mm) and the curved tipped cement applicator, AVAflex (CareFusion, McGaw Park, IL), one can augment both the ipsilateral and contralateral regions of the mid to upper thoracic vertebral bodies using a unilateral approach. Using this approach with a small gauge needle decreases both the surgical risk and surgical time of the kyphoplasty procedure. The purpose of the study was to (1) define the general morphology of the T1-T12 thoracic pedicles, (2) perform unilateral transpedicular kyphoplasty from T1-T12, and (3) assess the placement of cement in the various compartments of the vertebral bodies.

METHODS: Four fresh frozen human osteoporotic thoracic specimens (74±1.8 years) with intact rib cages and skin were screened to exclude pre-existing fractures and then scanned using Computed Tomography (CT) at a 0.6mm slice thickness. The CT images were uploaded into the Mimics software package (Materialize Inc, Ann Arbor, MI) where the following parameters were measured: pedicle height, width, and angulation in sagittal and axial planes. Measurements were made for all specimens by 4 observers in an inter- and intra-observation scheme separated by 1 week's time. The kyphoplasty procedure was performed at T1-T12 levels on the same specimens using the 11-gauge trocar (3mm diameter) for pedicle insertion, followed by the use of the 11-gauge AVAflex curved applicator and 11-gauge AVAmax balloon for vertebral body cavitation, and 11-gauge AVAflex curved applicator for cement injection. For all specimens the transpedicular approach was conducted through the right pedicle under C-arm fluoroscopy to facilitate proper alignment of the trocar with the pedicle axis. Qualitative observations of cortical wall encroachment and fracture of the pedicle were made by observing post-op CT scans. Postoperative CT scans and 3D reconstructions of T1-T12 vertebral bodies were obtained for each specimen using the Mimics Software package.The 3D reconstructions were imported into MatLab for volumetric analysis.Each vertebral body was divided into 12 zones to assess the distribution of the injected cement. The percent augmentation in each of the twelve zones was determined and a percentage was calculated comparing the volume of cement and the total volume of the vertebral body in each zone.

RESULTS: The anatomically-limiting parameter for height (average of 4 specimens) was in the upper levels (T1-T4) at 5.38±1.08mm, highlighting a linear trend from T1 to T12. The width was most limiting in the middle levels (T5-T8) at 2.46±0.32mm, which emphasizes a parabolic trend from T1-T12. The absolute minimum for height was at T1 with left and right pedicle measurements of 3.80 and 3.87mm. Similarly, this minimum for width was at the left pedicle of T4 (2.10mm) and right pedicle of T5 (2.00mm).Kyphoplasty was successfully performed in T1 in one specimen, T2 in three specimens, and T3-T12 in all four specimens.The curved needle applicator allowed cement augmentation in all 12 zones of the vertebral body. On average, 43.22% of the vertebral body volume was augmented with cement at T2-T12 levels.

DISCUSSION & CONCLUSIONS: The 11-gauge trocar has an outer diameter of 3.0 mm. The trocar's 3.00mm diameter exceeds the isthmus in the upper and middle levels whose average widths were 2.68±0.50 and 2.46±0.32mm respectively. Post operative CT scans indicated no fracturing of the pedicles in the upper and middle thoracic levels or any other vertebral levels.This unexpected finding indicates the potential for elastic properties inherent to cortical bone and/or angular leeway allowing for trocar penetration without fracture. We could augment both the ipsilateral and contralateral regions of the mid to upper thoracic vertebral bodies with a unilateral approach using the curved tipped cement applicator. It is critical to target cement placement at the desired location. The 11-gauge curved needle cement applicator allowed for cement augmentation in all twelve zones of the vertebral body. The instrumentation was shown to be effective in percutaneous transpedicular balloon kyphoplasty of the thoracic spine using a unipedicular approach.

ACKNOWLEDGEMENTS: US Department of Veterans Affairs; CareFusion McGaw Park, IL.

Beyond the beginning -- Cervial kyphoplasty

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INTRODUCTION: Cervical osteolytic metastasis is a problem for every spine surgeon. It leaves us to either large procedures with ventral or dorsal stabilisations or conservative treatment with immobilisation of the cervical spine for a prolonged time. In our clinic we do approx. 60 cases of kyphoplasty on the thoracic or lumbar spine per year and since being comfortable with ventral approaches to the cervical spine with started doing cervical kyphoplasty as a palliative procedure for those suffering from cervical osteolytic metastasis.

METHODS: A literature research came up with only a few reported cases[1, 2] and one case report[3].We started to establish our own protocol. Indications were strict: destabilising osteolysis of the cercical spine (mostly c2) without a defect of the corticalis and no prior operations on the cercical spine. We used a standard ventral approach to the cervical spine over a small incision positioning the patient in backrest and reclined cervical spine. Blunt preparation to the spine and access of the vertebral body with a shard guide wire. Followed by a regular kyphoplasty using a 10mm ballon under continous fluoroscopic control.

RESULTS: From September 2011 to December 2012 we treated a total of 15 patients with osteolyic metastasis of the cervical spine. 12 patients were treated on C2, 2 patientes on C3 and 1 patient on C4. No neurological complications postoperative, one patient suffered from mild difficulties while swallowing for a few days. Average discharge from the hospital was 3 days after the procedure. All patients reported a significant decrease of neck pain.

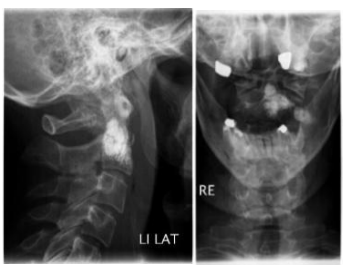

Fig. 1: postOP X-Rays (C2 Kyphoplasty)

DISCUSSION & CONCLUSIONS: Kyphoplasty of the cervical spine is a easy, quick and safe procedure for the treatment of osteolytic metastasis of cervical vertebral bodies, especially C2. It should only performed by an experienced surgeon doing ventral cases of the cervical spine on a regular basis.

Compared to a dorsal stabilisation is has a significantly lower rate of complications, especially wound infections, neurological complications etc)

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ACKNOWLEDGEMENTS: This template was modified with kind permission from eCM Journal.

Session 2.2

Tumor management

Chairmen: GC. Anselmetti, K. Murphy

Osteoplasty of the Pelvis

P.L. Munk , P.I. Mallinson
Vancouver General Hospital, University of British Columbia, Canada

A variety of disease of the osseous pelvis have proven very challenging to treat by conventional methods. Amongst the most common of these is metastatic disease. Traditionally most cases of bone metastases at this site have been treated with radiotherapy often in combination with chemotherapy. Although very useful treatment failure often occurs and onset of pain relief can at times take weeks. Very occasionally, especially in the case of isolated disease surgery is performed, but can involve considerable morbidity and expense. The introduction of Cementoplasty has provided a new useful palliative treatment which provides rapid onset of pain relief with a single treatment. It may be combined with thermo ablative techniques (radiofrequency ablation or cryoablation). Addition of thermoablation extends the region of tumor treatment beyond the region reached by cement in many instances allowing destruction of more tumor and potentially improves the durability of treatment (pain relief and non-recurrence of tumor locally)

Over the past several years new technical advancements have appeared to facilitate treatment of tumors at this anatomic site. This includes cryoablation with small bore probes as well as streerable curved needles which allow previously difficult to reach lesions to be injected. Examples of tumors treated using these techniques will be shown and discussed.

Keynote No 21

Tumor management

Bassem GEORGY

University of California San Diego, San Diego, CA

Keynote No 22

Tumour management

Alexis KELEKIS

University of Athens, Greece

Vertebroplasty with chemotherapeutic agents. An experimental study in pigs

M Alfonso[1], A Silva[2], R Llombart[1], C Villas[1]

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INTRODUCTION: The possibility of performing vertebroplasty with the use of cement containing antineoplastic agents implies the potential to perform local metastasis control together with stabilization of the fracture. In vitro studies have shown that cement containing methotrexate, doxorubicin, or cisplatin maintains its mechanical characteristics, allows diffusion of the active form of these agents from the cement, and is able to inhibit growth of breast carcinoma cells, particularly in the first 24 h and up to 15 days following exposure.

The aims of this study were:

1. To investigate the feasibility of performing percutaneous vertebroplasty with methotrexate-loaded and cisplatin-loaded cement in a porcine model.
2. To determine the concentration of methotrexate and cisplatin in blood following vertebroplasty.
3. To study the clinical outcome and histological changes in mieloradicular structures and perivertebral muscles alter vertebroplasty with polymethyl-metacrilate (PMMA) loaded with antiblastic drugs in pigs.

METHODS: In the Control group in 5 pigs we performed vertebroplasty of two vertebrae using vertebroplasty cement, provoking an anterior leak to the psoas and another leak to the vertebral canal.

In the Methotrexate Group, vertebroplasty of two vertebrae was performed in ten female pigs using vertebroplasty cement to which 1 g of powdered methotrexate had been added. After creating the mixture, the monomer was added and cement was injected in two vertebrae, provoking an anterior leak to the psoas and another leak to the vertebral canal. Methotrexate concentration in blood following cement administration was measured in serial determinations during 1 week.

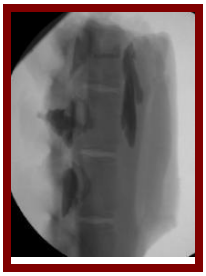

Fig. 1: Lateral x-ray showing anterior and intracanal leakage.

In the second group the same procedure than group 1 was performed on 11 female pigs using vertebroplasty cement to which 0.5 g of powdered cisplatin had been added. Cisplatin blood concentration were measured during one week too.

Three weeks later the pigs were put to death. We did a histological study of the soft tissue that came into contact with the cement.

Fig. 2: Images of PMMA with cisplatin surrounding the spinal cord.

RESULTS: In the Control group there were no cord lesions. In the group Methotrexate there were no major incidents associated with the technique and none of the animals had cord lesions following cement leakage. Diffusion to the blood was detectable at 3 days, in some cases up to 7 days.In the group Cisplatin four animals presented paraparesis (1 immediate and 3 later). Mean cisplatin values in blood were found up to 3 days. Histological results: Control and Metotrexate group: Normal spinal cord. In duramater inflammatory infiltrate and synovial metaplasia were found. Sligth muscle necrosis was found.

Cysplatin group: Spinal cord necrosis was observed in 7 pigs. Wide areas of muscular necrosis were observed.

DISCUSSION & CONCLUSIONS: The use of methotrexate-loaded cement is feasible in a porcine model. Despite massive epidural leakage, no neurological compromise was observed in MTX group. Also no changes in the spinal cord were observed; it is likely that the dura and the cerebrospinal fluid are enough to isolate the neural structures from the cement.

In Cisplatin group we observed extensive areas of cord and muscle necrosis associated with slight inflammatory reaction. The use of cisplatin-loaded cement in this porcine model did not yield favorable results in presence of massive leakage because of the incidence of late paraparesis, probably due to the neurotoxic potential of this agent.

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Session 2.3

Procedures and innovations

Chairmen: S. Becker, M. Lorio

New Augmentation devices and regulatory hurdles

Eric W. Gilbert

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Since 2007, regulatory hurdles have increased requiring randomized clinical data for innovative products. Benvenue Medical's flagship product is the Kiva VCF Treatment System which features a proprietary flexible implant made from PEEK-OPTIMA®. The implant is designed to function as a mechanical support structure and a reservoir to contain and direct the flow of bone cement. It is currently engaged in its FDA clearance trial, KAST, which is a randomized, controlled trial of Kiva versus the current gold standard of care, balloon vertebral augmentation.

Additionally, In the February edition of Spine, an independent, prospective, randomized study of patients with vertebral compression fractures (VCFs) comparing the effectiveness of balloon kyphoplasty with the Kiva VCF Treatment System found that only the Kiva system significantly restored vertebral body wedge deformity, Gardner angle. The Kiva system also resulted in significantly lower rates of extravasation and cement volume than balloon kyphoplasty.

Percutaneous vertebral augmentation assisted by peek implant in osteolytic vertebral metastasis: experience on 40 patients

GC. Anselmetti

Vertebral metastases are associated with significant pain, disability, and morbidity. Open surgery for fracture stabilization is often inappropriate in this population due to a poor risk-benefit profile, particularly if life expectancy is short. Vertebroplasty and kyphoplasty are appealing adjunctive procedures in patients with malignancy for alleviation of intractable pain. However, these patients have higher risk of serious complications, notably cement extravasation.

We prospectively evaluated clinical results of PEEK implant assisted vertebroplasty (KIVA, Benvenue Medical) performed in malignant painful osteolytic lesions at risk for cement extravasation due to vertebral wall involvement.

KIVA was performed in 40 consecutive patients suffering from back pain due to malignant vertebral involvement failing conservative therapies and without surgical indications. Follow-up was prospectively evaluated in all patients after KIVA with clinical interviews.

Methods: 40 patients (22 females; mean age 66.8±12.4), suffering from a painful spine malignancy with vertebral wall involvement not responding to conventional therapies and without surgical indications, underwent to KIVA for pain palliation. Procedure was performed in local anesthesia under combined digital fluoroscopy and computed tomography guidance. After the coil-shaped polyetheretherketone implant was deployed within the vertebral lesion, bone cement was injected under continuous digital fluoroscopic control. Patients were discharged from the Hospital the next procedural day. The Visual Analog Scale (VAS) for pain, Oswestry Disability Index (ODI), analgesic requirement and use of external brace support evaluated efficacy. The main end-point was safety and efficacy at 1 month after the procedure. Furthermore, all the patients were scheduled to be followed-up at month 3, 6, and every 6 months thereafter.

Results: Median pre-treatment VAS of 10 (range 6-10) significantly ($p<0.001$) dropped to 1 (range 0-3), with all patients achieving a clinically relevant benefit on pain at 1 month. Differences in pre- and post-treatment analgesic therapy were significant ($p<0.001$). All patients no longer use external brace after KIVA. In 7 out of 43 (16.3%) treated vertebrae a bone cement leakage was detected.

Conclusion: The Kiva System represents a novel and effective minimally invasive treatment option for patients suffering from severe pain due to osteolytic vertebral metastases.

Anatomical reconstruction technology

Anthonio KRUEGER

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SJ joint technology and pain management

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One year results from a US IDE trial evaluating the OsseoFix implant for treatment of vertebral compression fractures

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INTRODUCTION: Vertebral compression fractures (VCF) are a burgeoning problem for the aging spine. Previous kyphoplasty type treatments have been shown effective for pain relief but have also reported endplate fractures and cement leakage. An expandable titanium mesh device has been designed to provide surgeon directed control in the reduction of the VCF and to facilitate cement delivery (Osseofix®, Alphatec Spine, Carlsbad, CA). The implant was designed to treat symptomatic patients suffering from VCF between T6-L5 by providing internal structural fixation prior to cement delivery (unlike a balloon type device). This minimally invasive (one or two level) procedure takes about 30 minutes per vertebra.

OBJECTIVES: The purpose of this study is to present data of a prospective multi center clinical trial from three sites treating patients presenting with one or two VCFs that were treated with the implant and PMMA.

METHODS: This study was a prospective multi center clinical trial. Patients enrolled were limited to those exhibiting painful vertebral compression fractures between T6-L5 which required surgical treatment after failing conservative care. The outcome variables in the study were changes in pain (VAS), Oswestry Disability Index (ODI), and adverse events.

These data are the one year results from the prospective, multi-centered clinical study. Pain (VAS) and function scores (ODI) were collected starting pre-operatively with follow-up visits at four weeks, three months, six months and one year. Data were pooled from three surgical sites involved in the ongoing study. All available data are presented with 13/15 patients with a twelve month end point analysis.

RESULTS: Fifteen patients (11 females, 73.3% and 4 males, 26.7%) with an average age of 80.5±8.0 years were treated for one level (14/15 or 93.3%) or two level (1/15 or 6.7%) VCF. At 12 months, improvement of VAS exceeded more than 51 points on average, demonstrating a dramatic and sustained relief in pain. At 12 months, improvement of ODI exceeded more than 40% change on average, demonstrating a dramatic and sustained improvement in function. A one-way ANOVA with a Tukey's post hoc test found a statistically significant improvement in pain (VAS $p<0.0001$) and function (ODI $p<0.0001$) at 4 weeks compared to pre-op which was maintained out to one year with no statistical difference between 4 week and subsequent time points. There were no device related adverse events demonstrating a risk profile lower than that previously reported.

CONCLUSIONS: Although these results are promising--we are not implying a definitive difference rather simply presenting a limited analysis for a small incomplete cohort. There were no device related complications that required intervention, nor were there observed endplate fractures. There was one instance of an asymptomatic cement leakage. Preliminary analysis shows OsseoFix augmentation for VCF decreased pain and improved function. The one or two level minimally invasive surgical procedure is a new option for the aging population that most often suffer from VCF. The surgical technique allows the surgeon to place and expand the device where desired as opposed to other pneumatic systems that do not allow for surgeon directed control. Further study and completion of the full enrollment is necessary prior to definitive confirmation of success.

REGULATORY STATUS: Not approved in US, CE mark clearance in Europe

Initial clinical experience using novel radiofrequency systems for targeted ablation and augmentation of spinal tumors

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INTRODUCTION: It is estimated that 10% to 20% of the 1.64 M patients annually diagnosed with cancer will develop symptomatic spinal metastasis.[1,2] Less than10% of patients with painful spinal metastatic lesions are referred for vertebral fracture repair. Use of radiofrequency ablation (RFA), first described by Rosenthal et al. [3], has been limited in spinal metastatic disease due to requirement of targeted ablation in close proximity to neural elements and challenges navigating the unique anatomy of the spine. Treatment of painful spinal lesions using novel RF technology is described.

METHODS: Image guided targeted RF ablation (t-RFA) was performed with the STAR Tumor Ablation System (DFINE Inc.), which includes a robust articulating, navigational, bipolar electrode containing two active thermocouples (TC) along length of electrode to permit real time monitoring of peripheral edge of ablation zone. See Figure 1.

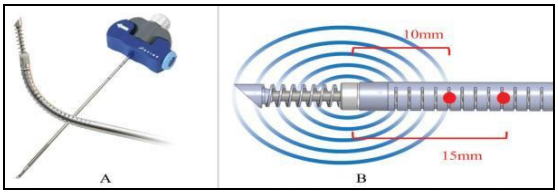

Fig. 1: A. SpineSTAR Instrument, 10 g, articulated, extendable bipolar electrode. B. Distal end of SpineSTAR containing 2 thermocouples (red dots), located 10 & 15 mm from center of ablation zone.

Over 100 lesions in 76 minimally invasive t-RFA procedures were performed. Pre-procedural planning was based on cross-sectional imaging to determine number of targeted ablations based on lesion size & thermal distribution curves. Treatment was controlled by adjusting power while monitoring TC temperature in-situ. Augmentation using high viscosity RF warmed high viscosity cement (StabiliT System) was performed via same guiding cannula in vertebrae with pathologic fractures or where structural integrity was significantly compromised. In select cases, post-procedural contrast enhanced magnetic resonance imaging (MRI) was performed to assess ablative zone and post-op metastatic lesion status. In a subset of patients at one institution, pain was assessed by Visual Analogue Scale (VAS).

RESULTS: t-RFA procedures were successfully performed in all 76 procedures. Cement augmentation was performed in majority of cases. Lesion etiology included wide variety of metastatic lesions involving T2 to S2, ilium and sternum. Post-ablation MRIs demonstrated discrete ablation zones consistent with thermal monitoring by TCs during the ablation. Ablation zone morphology was typically 3:2 length to width aspect ratio. TCs were used to confirm re-establishment of physiologic temperature in-situ prior to cement augmentation. Cement augmentation following RF ablation was efficient and resulted in predictable cement filling. Average VAS improved from 7.0 pre to 3.3 post t-RFA (53% improvement). No device related adverse events were observed.

DISCUSSION & CONCLUSIONS: The STAR Tumor Ablation System, a bipolar RF device, purpose built for targeted ablation of spinal malignant lesions, was successfully used to navigate and treat spinal malignant lesions. Post-ablation MRI's confirmed lesion necrosis. Ablation size was accurately determined by monitoring TCs on articulating electrode and morphology was similar to that extrapolated from thermal distribution curves. The navigational ability allowed for easy access to posterior vertebral body lesions, previously difficult to access with other ablation devices. Further, use of RF energy to modulate cement viscosity permitting delivery of ultra-high viscosity cement over an extended period of time proved useful in vertebrae with large cortical destruction .

REFERENCES: [1]Noone, et al., SEER Cancer Statistic Review, 1975-2009, Table 1.1; National Cancer Institute, April 2012. [2]K. D. Harrington, Cancer, vol. 80, supplement 8, pp. 1614–1627, 1997. [20]. [3]Rosenthal and Callstrom. Radiology: 262: No 3, March 2012.

A Novel Device for Radiofrequency Ablation of Bone Metastases

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[1]University of Washington, [2]University of Toronto, [3]Baylis Medical Company

INTRODUCTION: More than 50% of cancer patients suffer from skeletal metastases that may cause significant pain, fractures, and spinal cord and nerve compression, leading to increased morbidity and mortality. Radiofrequency ablation (RFA) is a minimally invasive technique based on high frequency oscillating electrical current which generates heat in the tissue causing protein denaturation. RFA devices have been developed and optimized for tumor ablation in soft tissue (e.g. liver). However, it has been challenging to apply existing technologies for ablation of spinal metastases because of unique electrical properties of osseous structures. Bone is less thermally and electrically conductive in comparison to soft tissue. Moreover, proximity to the neighboring vital structures (i.e. neuroaxial space) as well as large size and high vascularity of tumors make it difficult to generate sufficient heating in vertebrae and safely achieve sufficient ablation that are anatomically relevant in size. This work investigates the physics and validates performance of a novel RF ablation system that is custom designed to treat spinal metastases. First technical clinical experience is also presented

METHODS: The OsteoCool™ RF Ablation System contains a 17G internally cooled coaxial bipolar probe which is designed for optimal performance in bone. It has a bipolar cooled RF design for localized and optimal RF energy delivery in bone, direct tissue temperature monitoring for controlled tissue heating, coaxial design to minimize procedure invasiveness by creating lesions with a single insertion, and an active tip geometry and treatment settings customized to vertebral body tissue characteristics and anatomy for effective heating. See Figure 1.

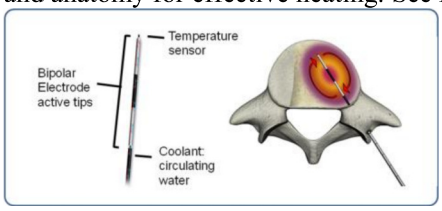

Fig 1. Biopolar design and device placement

The efficacy of the OsteoCool™ RF Ablation System was evaluated in 6 New Zealand rabbits whereby VX-2 tumor cells were directly injected into the femoral canal. RF treatment was administered 2 weeks later (65˚C cooled temperature, 15 minutes). The effect of RF ablation on tumor cells was determined by histology analysis. Treatment area of effect was characterized by MRI analysis immediately after treatment. Animals were euthanized 0 days (N=4) or 2 weeks (N=2) post-treatment. The safety of the OsteoCool™ RF Ablation System was evaluated when RF was performed in 5 healthy pigs with vertebral dimensions more relevant for human application, using a transpedicular approach with a 13G bone access needle under fluoroscopic guidance (65˚C cooled temperature, 15 minutes). A temperature monitoring probe was placed adjacent to vertebrae MRI scans of the resulting treatment area were analyzed at 0 day and 2 weeks following treatment. Neurological examination was conducted before and after treatment, and prior to euthanasia (0 days: N=2, 2 weeks: N=3).

RESULTS: For efficacy, all RF procedures produced the desired controlled temperature response. MRI analysis showed uniform ellipsoid lesions of approximately 3cm x 2cm, while histology revealed corresponding tumor cell death (figure 2).

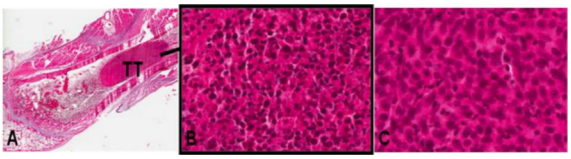

Figure 2. H&E staining showing treated tumor (TT) region in the femur (A). RF ablated tumor cells in the treated tumor (B) is shown in contrast to untreated tumor cells (C).

For safety, the RF system consistently elicited the desired tissue heating response. Tissue temperature immediately outside the cortex remained normal. Post-treatment MRI at 0 day and 2 weeks post-treatment revealed clinically and anatomically relevant lesions spanning half the vertebrae (average 25mm x 13mm, projecting to the inner wall of the vertebral cortex) (Figure 3 C,D). All animals demonstrated normal behaviour during neurological assessment up to 2 weeks following treatment.

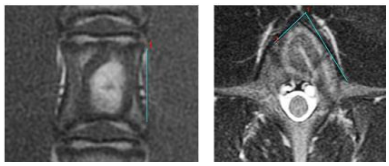

Figure3. MRI performed two weeks after procedure demonstrated bone marrow signal consistent with controlled damage.

For clinical experience, RF was performed with or without supplemental cementoplasty under FDCT guidance. Vertebral and non-vertebral (rib, pelvis, femur) tumors were treated (Figure 4). 1 case was performed under general anesthesia and 7 cases under local anesthesia and sedation. Due to non-experimental conditions, post-procedure follow up and imaging was performed by oncology or palliative care team according to medical necessity.

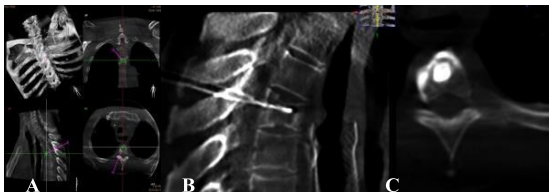

Figure4. Metastatic lesion of T4 vertebra. Stereotactic planning (A) and the device placement (B). Upon completion of RF 2mL of PMMA were injected (C)

DISCUSSION & CONCLUSIONS: The internally cooled bipolar OsteoCool^{TM} RF Ablation System, designed for ablation in bone, was proven to be consistent in creating lesions in diseased and healthy bone that are anatomically relevant in size and shape. Bipolar localization of RF energy and heat shielding by the vertebral cortex resulted in preservation of sensitive neural structures adjacent to the spine. The OsteoCool^{TM} RF Ablation System may represent a suitable option for vertebral tumor ablation and pain palliation.

Session 2.4

Traumatic Vertebral Fractures

Chairmen: P. Jarzem, B. Georgy

SURGICAL MANAGEMENT OF VERTEBRAL TRAUMATIC FRACTURES

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Abstract

While low-energy mechanism, osteoporotic compression fractures can be effectively treated with vertebral augmentation if nonoperative treatment has failed, traumatic fractures of the thoracic and lumbar spine require a different set of treatment principles. In general, surgical management is targeted towards achieving three primary goals: stabilization, neurological decompression, and realignment of deformity. There is a wide array of injury patterns that can arise following high-energy trauma, each with unique treatment requirements. While there remains controversy and ongoing discussion about the ideal thoracolumbar fracture classification system, there a few widely recognized injury patterns that can help formulate a surgical treatment plan: burst fractures, seat-belt injuries, and fracture-dislocations.

There is a large body of literature regarding treatment of thoracolumbar burst fractures. In general, surgery is indicated in patients who have disruption of the posterior ligamentous complex (PLC) or neurological deficit. With PLC disruption without neurological deficit, posterior instrumented fusion is the most common method of surgical treatment. For cases in which deficit is associated with canal compromise, an anterior corpectomy is the most reliable method of decompression. This is followed by reconstruction of the anterior column with a strut graft or cage and instrumentation. Supplemental posterior instrumentation and fusion can also be performed. Seat-belt injuries occur from a flexion-distraction mechanism and thus inherently disrupt the PLC. They are rarely associated with canal compromise from vertebral comminution and are thus usually treated with a short-posterior instrumented fusion. Fracture-dislocations occur from very-high energy mechanism and are usually associated with severe neurological deficits, particularly in the thoracic spine. Canal compromise is usually the result of misalignment of the spine. These injuries are uniformly unstable and are best treated by an open reduction followed by long posterior instrumented fusion.

Keynote No 24

10-year experience in precutaneous management of traumatic vertebral fractures

Natale FANCAVIGLIA

Palermo Neurosurgical Department, Italy

Clinical outcome after the use of a new cranio-caudal expandable implant for vertebral compression fracture treatment. 1-year results from a prospective multicenter study

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[1,2]Spine-Unit, University hospital Valladolid, Spain; [3,4]University Medical Center Hamburg-Eppendorf, Hamburg, Germany; [5]Loretto-Krankenhaus, Freiburg, Germany; [6]Interventional Neuroradiology Department CHU, Bordeaux, France

INTRODUCTION: This study aims to evaluate the use of a cranio-caudal expandable intravertebral implant in combination with a new high viscosity PMMA cement as well as to determine whether this treatment provides good clinical results for vertebral compression fractures.

METHODS: A prospective international observational multicenter study was designed and set up. In the reported four study centers a total of 28 patients were enrolled (26 female, 2 male; mean age 70,2 years (SD = 10,7)). 22 osteoporotic and 6 traumatic fractures were treated. The clinical parameters pain (VAS), functional capacity (Oswestry Global score, ODI), quality of life (EQ-5D) and analgesic intake were assessed, along with radiological parameters. All data was collected prior to surgery, after surgery, at 6 months and at 12 months postoperatively. The rate of cement extravasation was analyzed using postoperative CT scans in half of the cases.

RESULTS: The results for patients followed up until 12 months postoperatively (n = 21) showed a 12-month mean reduction in VAS pain score of 6,0 from mean 7,2 (SD = 2,5) pre-op to 1,3 (SD = 1,9) at the 12 months follow up visit. The ODI score also decreased from 68,0% at baseline to 8,6% after 1 year. The EQ-VAS, measuring the self-rated health state, was 35,2 (22.3) preoperatively and increased to 76,9 (12.5). The number of patients requiring moderate to strong analgesics dropped from 63.6% at pre op to 9.0% at 12 months. All changes in clinical parameters (VAS, ODI, EQ-VAS) were statistically significant ($p<0.001$). Cement leakage occurred in 28.6% of cases, all were asymptomatic. 50% of these leakages were detected in post operative CT scan. Adverse events were documented during the whole study period. None of the seven serious adverse events reported (including 2 deaths (metastatic disease and heart disease), 1 degenerative lumbar syndrome, 1 paralysis of the diaphragm, 1 hypophysis adenoma, 1 fall in blood pressure and 1 cerebral infarction) were device or procedure related. 6 technical incidents occurred, none of which impacted the safety of patients.

Fig. 1: SpineJack® procedure. Controlled fracture reduction with SpineJack® 5.0 implant followed by stabilization with high viscosity PMMA cement.

DISCUSSION & CONCLUSIONS: These results demonstrate that all clinical outcome scores (patients' pain, functional capacity, quality of life) were significantly improved one year after patients were treated using this new surgical procedure. The cement leakage rate of 28.6% is comparable to the 27% showed in the FREE study [1], especially considering that CT scans were used to identify cement leakages as opposed to less sensitive radiographs in the FREE study, since earlier publications have shown that more leakages are identified by CT scans than in radiographs by a factor of 1,5 [2].

REFERENCES: [1] D. Wardlaw, et al (2009) *Efficacy and safety of balloon kyphoplasty compared with non-surgical care for vertebral compression fracture (FREE): a randomised controlled trial. Lancet.* 2009; 373: 1016-24. [2] J.S. Yeom, et al. (2003) *Leakage of cement in percutaneous transpedicular vertebroplasty for painful OP compression fractures. J Bone Joint Surg* 85:83-89, 2003

ACKNOWLEDGEMENTS: This template was modified with kind permission from eCM Journal.

Keynote No 26

Changed focus in spine surgery: from fusion models/biomaterials to disc models/biomaterials

Stephan BECKER

IMSART, Vienna, Austria

A fully automated algorithm for measuring 3D anatomical restoration of fractured vertebrae across longitudinal CT scans

PA Dufort

Joint Department of Medical Imaging, University Health Network, Toronto, Canada

INTRODUCTION: We describe a new technique for performing longitudinal comparisons of vertebral shape from CT scans taken before and after surgery. Following a single click to locate the target vertebrae in pre-op and post-op scans, the program automatically computes a full 3D reconstruction for each vertebra, as well as a pre-trauma reconstruction predicting the shape of the vertebra prior to injury. The three reconstructions are aligned and used to compute shape measurements and 3D visualizations of the vertebra at each stage (Figure 1). The program is being used to measure 3D anatomical restoration following surgery to repair vertebral compression fractures using the Vexim SA SpineJack™ device. The details of the algorithm are described, as well as the results of a validation study comparing the program's measurements against trained experts.

METHODS: The user clicks on the approximate centre of the target vertebra and the two adjacent vertebrae in the pre-op scan, and on the target vertebra in the post-op scan. The software then proceeds in four stages: (i) vertebral models in the form of point clouds of oriented features are deformably registered with each of the selected vertebrae to segment them. This is achieved by optimizing a dense correspondence field between the oriented features from the model and a corresponding set on the outer surface of the vertebrae in the CT scan; (ii) the segmentations are used to isolate the posterior, uninjured region of the target vertebra in both the pre-op and post-op scans and to rigidly register them; (iii) a deformable interpolation is performed between the two adjacent uninjured pre-op vertebrae to compute the pre-trauma reconstruction; and (iv) a set of regional height change measurements over the upper and lower endplates are computed and displayed, along with 3D visualizations of the reconstructions.

In order to validate the method, a set of 24 vertebrae each scanned at 4 longitudinal stages were selected, and height measurements using the standard six-point method were performed by 8 trained experts. The software was then used to produce the same measurements from the computed correspondence fields.

RESULTS: For each of the 288 individual expert measurements (24 x 4 x 3 heights), the mean and standard deviation were computed. These were used to convert each software measurement into a Z-score. Over 288 measurements, the average Z-score was 0.03 ± 0.07 (mean ± SEM). In physical units, this corresponded in most cases to errors well under 1 mm.

DISCUSSION & CONCLUSIONS: We describe a novel deformable template registration and segmentation algorithm for measuring vertebral anatomical restoration across longitudinal CT scans. The fully automated software produces 3D reconstructions of the vertebra from scans before and after surgery, and also computes a predicted reconstruction approximating the target vertebra prior to injury. It has been validated against human experts and is in production use at Vexim SA, where it has been used to successfully process several hundred vertebrae.

ACKNOWLEDGEMENTS: This work was sponsored in part by Vexim SA, Toulouse, France.

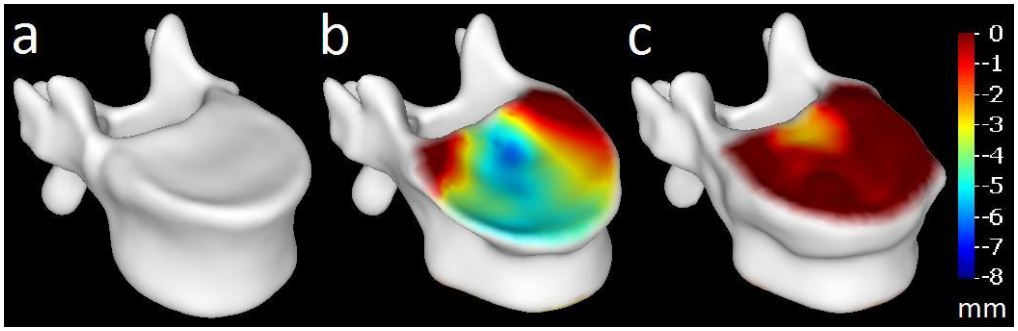

Figure 1: 3D reconstructions of injured T12 vertebra. a: pre-trauma; b: pre-op; c: post-op. Colour: height difference from pre-trauma.

Proceedings of the 23rd Interdisciplinary Research Conference on Injectable Biomaterials and Bone Augmentation Procedures
GRIBOI2013
April 8-10, Boston, MA, USA

EVALUATION OF POLYMERIZED BIODEGRADABLE CEMENT TO REPLACE PMMA CEMENT IN MANAGEMENT OF VERETEBRAL COMPRESSION FRACTURE

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INTRODUCTION

Kyphoplasty/vertebroplasty procedures traditionally use PMMA to treat the fractured vertebrae due to its mechanical properties. However, with time the bone erodes around the cement due to osteoporosis and inhibited bone remodeling due to the cytotoxicity of PMMA (1). The exothermic reaction of PMMA is also responsible for thermal necrosis and can cause complications in cases of extravasation (1; 2; 3). Lastly, PMMA is not bioactive and will not be reabsorbed (1; 2; 3). Thus, alternative cements with similar mechanical strengths are being explored. Calcium phosphate cements (CaP) have been explored due to their bioactive and non-thermal properties (3; 4). Despite these advantages, there are reservations of traditional CaP cements due to deficiencies in mechanical properties (1; 4). We evaluated new polymerized calcium phosphate (pCaP) cement which is not brittle like traditional CaP cements. Mechanical properties pCaP alone, and of vertebral bodies augmented with either PMMA or pCaP after fracture were determined in vitro. The biologic response to pCaP and Norian SRS were determined in a bilateral tibial metaphyseal defect model in New Zealand White rabbits.

METHODS

In vitro:

7 spines were scanned with DEXA. 23 vertebral levels were classified as osteoporotic based on their T-scores. Spines were carefully dissected leaving the posterior elements intact and vertebrae were cleaned, making sure no damage was done to the endplates. Vertebral body height was measured as well as the mid sagittal plane distance and medial lateral distance of the superior and inferior endplates. The medial lateral distance was measured at the halfway point of the mid-sagittal plane distance of the superior and inferior endplates. The inferior endplate wrapped with plastic-wrap and then potted using bondo. The plastic-wrap prevented the bondo from infiltrating the vertebral body.

All specimens were fractured before being restored with either PMMA or pCaP. For fracture creation, a transverse slit was made with a hand saw with a blade width of 1.5mm in the middle of the vertebral body. Samples were secured in a vice on an MTS load cell. The vice was fixed to an XY table and 3 axes vice, allowing the endplates to be leveled and aligned before compression. A fixture on the MTS machine applied a compressive force 10mm anterior to the geometric center of the superior endplate. The MTS was operated under displacement control and loads were applied at a rate of 5mm/min for a total displacement of 50% of intact sample height. Loads were recorded for each vertebral failure. These failure loads were used to calculate the maximum cyclic load for the dynamic testing.

Height restoration of the fractured samples was performed using Osseoplasty by Osseon ballon. Access to the vertebrae was provided by hammering a pin, or in some cases, drilling a hole through one pedicle. A channel for the balloon was created using the Osseoflex tool provided by Osseon. The balloon was inserted into the channel and adjusted so it would inflate in the desired location. Fluoroscopic images were taken during the procedure to ensure proper location of the balloon in the vertebrae. Once proper balloon position was obtained, the balloon was inflated with contrast solution until satisfactory height restoration was achieved. Cement was injected into the specimens until signs of extravasation were present. Samples were cured for 24 hours in a water bath at 37ºC. Height measurements were made before and after restoration.

Static testing was performed on 10osteoporotic specimens. These specimens were divided into two equal groups of 5 for PMMA and pCaP augmentation. The procedure for the static testing was identical to the fracture creation protocol.

Samples designated for dynamic testing had the superior endplate potted to provide a flat and uniform surface. Compressive loads of 25, 50, and 75% of the mean fracture load were applied at a rate of 5Hz for 100K cycles. Augmentation variables used for dynamic testing included pCaP and PMMA in osteoporotic samples. 2 specimens were tested for each augmentation group and loading condition for a total of 12 samples. Displacement was recorded for the dynamic testing and potted superior and inferior endplate height was measured pre- and post-fatigue. As the data was collected at a frequency much different than the loading frequency, a 4th order Butterworth Filter with a cutoff frequency of 6Hz was used to analyze the displacement data.

Physical properties of pCaP were determined *in vitro* according to ASTM standards[5,6]. Pre-set pCaP test articles were loaded to failure in tension and compression with a uni-axial MTS machine to determine biomechanical metrics (yield load, peak load, strain, ultimate compressive strength, ultimate tensile strength, elastic modulus, and break location), and measured for exothermic behavior during setting.

In vivo:
A bilateral rabbit tibial defect model as described by Stubbs et. Al. [7-9] was performed using (22) 6 month-old female New Zealand White rabbits. Defects were treated with pCaP, Norian SRS (Synthes, positive control) or left empty (negative control). Animal were euthanized at 0, 12, and 26 weeks (Table 1) for macroscopic inspections, radiographic grading, biomechanical evaluation, paraffin histology, Immunohistochemistry for MMP1 and, IL-1, IL-6 , Cathepsin K, PMMA histology of the implant site and distal organs, histomorphometry, and haematology. The peak torque and stiffness were evaluated using a 3- way analysis of variance. Histomorphometry data was analyzed using a two way general linear model of analysis of variance followed by a Games Howell post hoc test.

Treatment Group	0 weeks	12 weeks	26 weeks
pCaP	2	8	8
Norian SRS	2	8	8
Empty	0	4	4
TOTAL	4	20	20

Table 1: Animal Study Design

RESULTS

In Vitro
Osteoporotic intact samples had a mean failure load at 2816 ± 1451N and pCaP increased the failure load to 5042 ± 2710N (p=0.008) while PMMA increased the limit to 7274 ± 1303N (p=0.049).

Dynamic test results measuring the displacement under loading revealed that for the 25% mean failure loading condition, osteoporotic PMMA and pCaP samples performed similarly with a mean displacement of 6.86mm and 7.62mm respectively (p=0.739). For the 50% loading condition, PMMA had a mean displacement of 8.60mm compared to the pCaP group that had a mean displacement of 16.46mm (p=0.070). For the 75% loading condition, the PMMA group had a mean displacement of 13.81mm compared to the pCaP group mean displacement of 17.12mm. Statistical comparison between the two osteoporotic groups for the 75% loading condition could not be obtained due to premature failure of one of the PMMA augmented samples.
There was no statistical difference in deformation during dynamic testing between osteoporotic and normal specimens augmented with pCaP. For the 25% loading condition, normal vertebrae had an average displacement of 10.99mm while osteoporotic vertebrae had an average displacement of 7.62mm (p = 0.2989). For the 50% loading condition, normal vertebrae had an average displacement of 13.28mm while osteoporotic vertebrae had an average displacement of 16.46mm (p = 0.5512). There were no normal vertebrae augmented with PMMA for the cyclic loading testing.

Physical Properties of pCaP were evaluated *in vitro* according to ASTM standards. [5,6] Ultimate compressive strength (UCS) of 70 MPa with an elastic modulus of 0.54 GPa, ultimate tensile strength (UTS) of 36.33 MPa and Exotherm of 41°C were measured.

In Vivo
Haemtology and Biochemistry revealed no abnormal findings in any animal compared to controls. Radiographs did not demonstrate any adverse reactions to either material in terms of any osteolysis or bone resorption. Radiographs revealed both treatment groups present in the defects at 26 weeks, and the cortex of the empty defects had not healed by 26 weeks. Radiographic grading data demonstrated a statistically equivalent resorption over time for both groups at 12 and 26 weeks. Cortical bridging grading data did not differ with time or treatment group.

Biomechanical Testing demonstrated a progression in healing between 12 and 26 weeks for all groups. No differences were detected. The mean torque values did not reach the values of an intact tibia [8].
Paraffin and PMMA histology demonstrated new bone formation on all material surfaces at 12 and 26 weeks. No adverse reactions were found with either implant at 12 or 26 weeks. Normal bone remodeling and marrow space development were observed. Bone ingrowth was noted in the pCaP material while this was not observed with the Norian SRS material. Empty defects were not healed at 12 or 26 weeks, although a progression in maturity of the cortex was noted. The medullary canal appeared normal. Paraffin histology of the heart, kidney, liver, lung and spleen were unremarkable.

Histomorphometric data analysis revealed an increase over time in the percent bone for both implants as the anteromedial cortex healed. More bone was observed with the pCaP material than the Norian SRS material by post hoc analysis at 12 weeks (P=0.041). Mean implant material differences we not observed between treatment groups at 12 or 26 weeks in post hoc analysis.

Immunohistochemistry demonstrated expression of Cathepsin K in all groups at 12 and 26 weeks. Cathepsin K expression appeared linked with the presence of bone on the surface of the materials and reflected the increased amount of new bone formation associated with the pCaP material at 12 and 26 weeks. No differences were noted in IL-1ß or MMP-1 staining intensity/distribution, and no TNFα or Mouse IgG expression was noted at 12 or 26 weeks in the empty or treatment groups.

DISCUSSION

Paired T-Test revealed that PMMA and pCaP both statistically improved the mechanical compressive strength of osteoporotic bodies with no significant difference between pCaP and PMMA augmentation (p=0.327).

Dynamic testing showed that there was no significant difference between osteoporotic specimens augmented with either PMMA or pCaP after 100K loading cycles up to 50% of intact failure load. Statistical significant could not be determined for the 75% of intact failure load case.

The similar performance between normal and osteoporotic vertebrae under cyclic loading conditions could be due to part in the difference of value of the maximum load between the two vertebral conditions. For example, normal samples in the 25% fracture loading case were subjected to a max load of 2300N while osteoporotic samples in the same loading group were subjected to a max load of 1400N. It should also be noted that for the 50% and 75% pCaP and 75% PMMA test groups, displacements as large as 7mm occurred within the first 200 cycles before data collection began. This could be due in part to deterioration of samples over time as samples had been unfrozen for multiple days before dynamic testing began, although the time that the specimens were thawed was the same for all test groups and conditions.

Quasi-static strength data and cyclic loading data suggests that pCaP could be a replacement for PMMA for use in kyphoplasty.

The pCaP material demonstrated *in situ* setting, osteoconduction, resorption, and bone healing in a bilateral tibial metaphyseal defect model in New Zealand White rabbits.

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Injectable peptide/glycosaminoglycan hydrogels and their potential use for minimally invasive nucleus pulposus augmentation

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INTRODUCTION: Low back pain is strongly associated with degeneration of the intervertebral discs. Current surgical treatments for low back pain are highly invasive and have relatively low long term success rates. The present work aims to develop a novel, minimally invasive therapy for nucleus replacement without the need for surgical incision.

SUMMARY OF METHODS AND RESULTS: A versatile class of synthetic tape-forming, self-assembling peptides based on natural amino acids were examined and the design criteria for a suitable peptide hydrogel estabilished. These patented materials harness the intrinsic ability of peptides to self-assemble into micron-sized aggregates, which establish a nanostructured network and cause gelation of physiological solutions. The peptides were analysed using a series of complementary analytical techniques (NMR, FTIR, CDUV & TEM) to determine their behaviour at the molecular and nanoscale levels. Systematic changes in peptide structure led to aggregates with different morphologies, self-assembly profiles and mechanical properties (Fig.1).

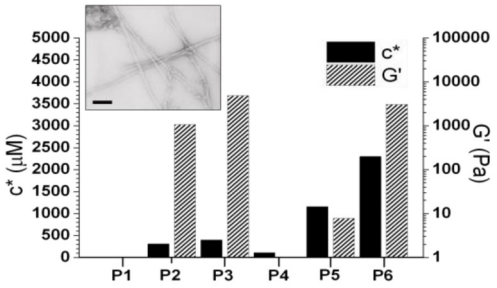

Fig.1 – Critical concentration for self-assembly and elastic modulus for six peptides with differing primary structures. Inlay: TEM image of 20 mg/ml P3 fibrils in PBS, scale bar = 100 nm.

The peptide material properties were further optimised by mixing with GAGs that are naturally found in the disc to mimic the vital biological osmotic pumping action and aid in disc swelling pressure. These solutions can be switched from fluid to gel inside the body, offering the opportunity to develop an injectable treatment. Another exciting finding was strong evidence that the mechanical properties of the gels can be controlled by peptide design and GAG ratio, allowing up to a 10,000 fold variation in the stiffness.

A range of systematically varying peptide solutions were then injected in a bovine caudal disc model to assess the potential to remain at the treatment site and to restore disc mechanics. The presence of the peptide greatly reduced the leakage of injected GAG and a denucleated disc repaired with a peptide:GAG gel was found to restore the mechanical behaviour to that of a disc with a healthy nucleus intact (*Fig.2*).

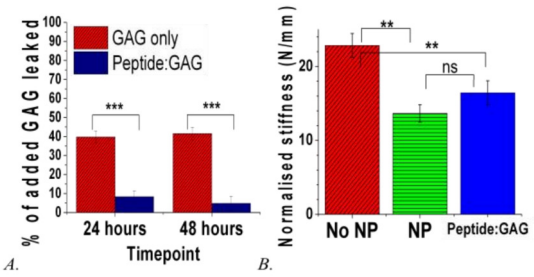

Fig.2 – A. Comparison of injected GAG leaked with and without injected peptide. B. Plot of normalized stiffness for each sample type under static compressive loading.

DISCUSSION & CONCLUSIONS: In summary although further optimisation is still required a gel material has been developed that has triggerable gelation and therefore is injectable and minimally invasive as a treatment. It forms a stable hydrogel with mechanical properties similar to that of the natural tissue and contains a high GAG content to aid in maintaining the swelling pressure of the disc. The work presented here is the first step to the development of an improved nucleus augmentation treatment. If successful, it could improve the quality of life for patients and reduce the economic burden of disc degeneration.

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Session 2.5

Biomechanics 1

Chairmen: M. Bohner, M. Ginebra

Potential of fibre reinforced calcium phosphate cements for load bearing orthopaedic applications

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INTRODUCTION:

Unfortunately CPC systems demonstrate relatively poor tensile and shear properties due to their inherent brittleness, therefore limiting their application to non-load bearing defects. Typical applications include the treatment of maxilla-facial defects and augmentation of the spine following a compression fracture. A successful improvement of the mechanical properties could significantly broaden the applicability of CPC, e.g. potential treatment of burst fractures. Reinforcement of CPC with natural fibres could potentially provide this improvement; therefore providing the opportunity to make possible the use of CPC systems in load bearing applications. With this in mind, this study aimed to incorporate collagen from different sources into a CPC system to compare their physical, mechanical and biological properties.

METHODS:

The CPC used contained 100% alpha tricalcium phosphate and di-sodium hydrogen phosphate solution (Na_2HPO_4) using a liquid to powder ratio of 0.35mL/g[1]. Three types of collagen were then incorporated into the CPC system being, type I bovine collagen fibres (BF)[2], marine collagen fibres (MF) and marine collagen particles (MP) which were added at 1 wt.% using unreinforced samples for comparison (C). A number of techniques were employed to understand the *in vitro* and *in vivo* material properties. Routine tests to determine the static mechanical properties, injectability and setting properties were used[1-2]. Biological (*in vitro*) evaluation of the collagen-CPC composites was conducted using human bone marrow stromal cells (hBMSCs) seeded at a density of 5 x10^4 cells/cm^2, a commercially available PMMA cement was used as a control Outcome measures were cyto-toxicity, proliferation and differentiation, determined using lactate deghydrogenase (LDH), PicoGreen® and alkaline phosphatase (ALP) activity assays. Biological (*in vivo*) evaluation of the different collagen-CPC composites was conducted using a NZW rabbit model (female/28 weeks old). Each conical implant (Ø=4mm; L=8mm) was press-fitted into a defect in the distal femoral condyle. All defects were randomly assigned an implant type or no implant. Femora were retrieved in two groups; 5 and 10 weeks post-implantation. Histological analysis was also conducted to determine the biocompatibility and bioactivity of each implant. Outcome measures were: bone apposition assessed following toluidine blue staining; mineral apposition rate (MAR) determined via the use of fluorochromes and osteoclast activity quantified using tartrate resistant acid phosphatase (TRAP) staining.

RESULTS & DISCUSSION:

Addition of MF at 1 wt.% significantly increased ($p<0.01$) the compressive strength to 37.8±6.4MPa, which is within the range stated for vertebroplasty (30MPa)[3]. Both BF (13.6±2.3MPa) and MP (19.6±3.6MPa) significantly reduced compressive strength ($p<0.01$). Adding MF showed no significant effect on injectability (55.9±9.0%), both BF (10.4±1.5%) and MP (20.2±4.4%) significantly reduced the injectability ($p<0.01$). The reduction in injection capability of MP could be due to a high tendency for the particles to agglomerate and BF could easily restrict the flow through the syringe thereby reducing cement extrusion. Introduction of all forms of collagen reduced the setting times, with the comparable reductions achieved for 1 wt.% MP (13.5±1.6min) and MF (15.5±0.5min). The recommend setting time for vertebroplasty is ≤15min[3].The level of cyto-toxicity measured for all cements was similar and considered not to be clinically relevant. SEM analysis showed cells adhering and proliferating onto all cement types. The dsDNA levels suggested that the cells were proliferating equally well on all the cements up to the final time point. Cell differentiation increased with time; but differentiation was higher for the CPC sets. No significant difference ($p>0.05$) in differentiation was observed between the CPC, MP-CPC and MF-CPC sets. Add BF to the CPC significantly lowered ($p<0.05$) the level of differentiation. All implants were well tolerated *in vivo* with little inflammatory response observed. Bone apposition onto the CPC implants was higher than on the PMMA control cement; bone grew very close, but was not apposed to the PMMA control implant. The effect of collagen incorporation on bone apposition is not thought to be significant within the first 10 weeks with no significant difference in bone apposition observed between any of the CPC at either time point. No significant differences ($p>0.05$) in MAR between any of the implant sets or time points were observed. The presence of positive TRAP staining within the CPC suggested that active osteoclasts are present and are taking part in the remodelling process, which was supported by areas of bone growth within the CPC.

CONCLUSIONS:

MF provided a means of improving both the mechanical and setting properties of CPC for load bearing applications. Incorporating MF into the CPC did not compromise the biological properties. Thereby highlighting the potential for a marine fibre reinforced CPC as a minimally invasive solution for burst fracture treatment.

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Vertebral Microstructure, Failure and Augmentation

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Although vertebral fractures are the hallmark of osteoporosis, their pathogenesis remains poorly understood. In contrast to osteoporotic fractures in the hip and wrist, those in the spine often have a slow onset and evade clinical detection for long periods of time. Clinical and biomechanics studies have identified many factors contributing to vertebral strength and fracture risk. These factors include the macro- and microstructure of the vertebra, distribution of mineral density within the vertebral body, properties of the mineralized tissue, and nature of the in vivo loading. With recent advances in the ability to quantify, or at least estimate, all of these properties, there is now tremendous opportunity to integrate this information towards a better understanding of vertebral failure. This talk will review some of the key findings from the body of literature on the contribution of vertebral microstructure to bone failure. Subsequently, recent work on direct, 3-D visualization of vertebral failure will be presented. Applications of this work and implications of the findings for vertebral augmentation will be discussed.

Effect of cement injection on mechanical properties in fractured and prophylactically augmented multiple myeloma vertebrae

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INTRODUCTION: Over 85% of patients with multiple myeloma (MM) have bone disease, mostly affecting thoraco-lumbar vertebrae [1]. Vertebral fractures can lead to pain and spinal deformities requiring application of vertebroplasty (PVP). Possibly prophylactic augmentation can be performed to minimize fracture of weak vertebrae occurring. Additionally application of Coblation to dissolve lesions, using radiofrequency energized plasma, from compromised MM vertebrae prior to cement injection (C-PVP) could help with cancer management.

The aim of this study was to evaluate biomechanical effects of augmentation in fractured and intact MM vertebrae, using PVP and C-PVP augmentation techniques.

METHODS: 57 single vertebrae with lesions, were dissected from 9 MM cadaveric spines. 29 MM vertebrae were initially fractured up to 75% of its original height on a testing machine using modified test set-up [2], with rate of 1mm/min. Load was applied at 25% of AP-diameter, from anterior. The remaining 28 intact MM vertebrae were used for prophylactic augmentation. Two augmentation procedures were investigated in both groups: PVP and C-PVP. All vertebrae were augmented with 15% of the vertebral volume using PMMA cement. Augmented MM vertebrae were fractured, following the same protocol as for initial fracture. Failure load (FL) was defined as 0.1% offset evaluated from slope fitted to the linear region (Stiffness) of load displacement curves. The load at the End of the tests were compared.

RESULTS: There were no statistically significant differences between the PVP and C-PVP augmentation techniques, hence the data was pooled for further analysis (Fig 1). The FL of augmented-initially-fractured vertebrae was 32% higher than for initial fracture ($p=0.028$, repeated ANOVA). There was no significant difference in FL of prophylactically augmented vertebrae. The End of test load for initial fracture was 55% and 49% lower than for augmented-initially-fractured and prophylactically augmented vertebrae, respectively ($p<0.001$). Stiffness of augmented-initially-fractured vertebrae was 50% and 59% lower than for initial fracture and prophylactically augmented vertebrae, respectively ($p<0.001$).

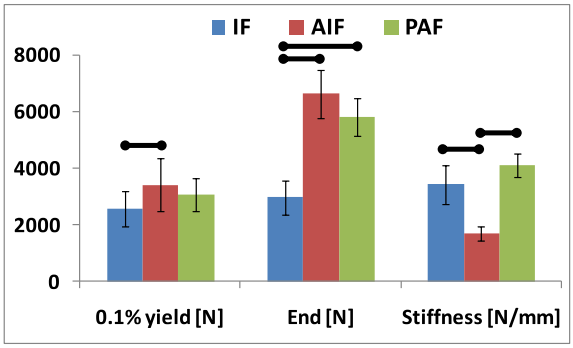

Fig. 1: Mean data ±95% confidence interval, with marked significant differences (IF – initial fracture; AIF – augmented-initially-fractured; PAF - prophylactic augmentation fracture).

DISCUSSION & CONCLUSIONS: Increase in FL and reduced stiffness of augmented-initially-fractured vertebrae is related to relatively small percentage fill (15%). In earlier study it was shown that smaller PMMA cement volumes are required to restore strength but larger volumes are needed to restore stiffness [3]. Prophylactic augmentation changed pattern of failure: initially it was similar to initially fractured vertebrae but at the End of test similar to augmented-initially-fractured vertebrae. Coblation treatment did not compromise stiffness or strength of augmented MM vertebrae and can be an important tool for surgeons to treat MM infiltrated vertebrae.

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Is morphology alone an effective indicator when planning prophylactic vertebroplasty in multiple myeloma patients?

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INTRODUCTION: Multiple myeloma (MM) is a neoplastic pathology resulting in structural weakening of a bone due to lytic infiltration to the bone. Minimally invasive operation techniques such as vertebroplasty(PVP) show great potential to prevent debilitating fractures in weakened bone. Previous studies indicated possible efficacy of prophylactic PVP[1,2]. However, non-destructive assessment of the vertebral body remains a key factor. The unique morphological character of bone affected by MM cancer hampers use of approaches defined for osteoporotic pathology. This study was performed to identify suitable methods for assessment of cancer samples prior to prophylactic augmentation.

METHODS: Forty-three samples from three MM spines were disarticulated, cleaned of soft tissue and underwent CT scanning ([microCT80, Scanco medical AG], voxel size 70.8µm3), followed by uni-axial eccentric compression test to induce the anterior wedge fracture. The failure load (*Fu*) was defined as the first zero slope on the load displacement curve. Bone density images were binarized with the threshold value based on an iterative selection method[3], The microstructural morphology was quantified using Image Processing Language (IPL, [Scanco Medical AG]). Trabecular Spacing (*Tb.Sp* and *Tb.Sp_VB*), thickness (*Tb.Th*), number of trabeculae (Tb.N), bone mineral density (*BMD*), bone fraction (*BVTV* and *BVTV_VB)*, degree of anisotropy (*DA*), structural model index (*SMI*) and connectivity density (*Conn.D*) were assessed in representative cylinder inside the vertebral body and for whole vertebral body (*_VB*). Subsequently, the weakest slice and the minimal fracture load (*Fz*) was estimated using an analytical beam theory applied on CT images[4,5]. Trabecular microarchitecture indices and the predicted *Fz* were compared to experimental data.

RESULTS: Morphometry indices (*BMD, Tb.Th, DA, BV/TV, Tb.Sp, Tb.N, SMI* and *Conn.D*) showed low or no correlation with *Fu*; varying from r=0.06(*BMD*) to r=0.44(*Conn.D*). Only *min.CSA*(r=0.69) and *Fz*(r=0.91) was found as good standalone predictors in all 43 samples. Furthermore, traditionally good predictors of strength (*BMD* and *BV/TV*) appeared to be successful in two out of the three spines (r=0.12, r=0.70 and r=0.73 for *BMD* and r=0.30, r=0.63 and r=0.72 for *BV/TV*). Where *Fz* remained consistently high regardless the difference between spines (r=0.86, r=0.85 and r=0.95). The product of microstructure indices and *min.CSA* was found to increase the correlation, varying from r=0.39 (*min.CSA*Tb.Sp*) up to r=0.76 (*min.CSA*Conn.D*).

Table 1 Coefficient of determination (R^2) of Failure load (Fu) versus Predicted failure load (Fz) and selected morphometric indices.

	Spine1	Spine2	Spine3	**All spines**
Beam theory(Fz)	0.74	0.73	0.9	**0.82**
BMD	0.01	0.49	0.53	**0.003**
min.CSA*BVTV	0.40	0.66	0.22	**0.36**
min.CSA*BVTV_VB	0.58	0.70	0.55	**0.56**
min.CSA*BMD	0.34	0.67	0.27	**0.25**
min.CSA*DA	0.35	0.29	0.72	**0.48**
min.CSA*Conn.D	0.17	0.51	0.20	**0.58**

DISCUSSION & CONCLUSIONS: Prior to conducting a study on the effectiveness of prophylactic augmentation in a MM cancer patient a detailed morphometrical assessment of this pathology had to be conducted. Results suggest that the lytic nature of the disease and interspinal variability (including due to treatment history) hampers using the conventional approach in benchmarking the cancer specimens. Next to a detailed morphological assessment of MM samples the results show that combination of both the geometric distribution and measure of bone deterioration is needed for reliable assessment of strength.

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Investigation of facet joint model properties for the assessment of interventions in the functional spinal unit.

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INTRODUCTION: The complex motion and geometry of the spine in the cervical region make it difficult to determine how loads are distributed through adjacent vertebrae or between the zygapophysial (facet) joints and disc. Spinal interventions such as vertebroplasty and disc/nucleus replacement have been reported to affect this load distribution [1] and have highlighted a requirement for finite element modeling to more fully investigate how the biomechanics in the functional spinal unit (FSU) is affected by such interventions. The aim of this study was to develop a method to validate specimen specific FE models of FSUs prior to spinal intervention, using local measurements as well as the overall stiffness**.**

METHODS: Three FSUs were excised from three-year old ovine spines from within the cervical region. Small markers were then affixed to the facet joints to track motion. The vertebrae were mounted in PMMA cement. Another marker disc was fixed to the upper PMMA surface to indicate the position of load application for the experiments and the corresponding FE models. The specimens were then μCT imaged (μCT100, Scanco, Switzerland).

Prior to testing, the facet joint capsules were cut, allowing a thin film pressure sensor (6900, Tekscan, USA) to be positioned between the cartilage surfaces. The specimens were then tested under axial compression, using a materials testing machine (Instron 3365, USA). A loading rate of 1mm/min was applied, and halted after 2mm of deflection had been reached. Photographs were taken at every 0.5mm of deflection using a tripod mounted camera (Canon 550d, Japan).

To determine the material properties of the disc, the specimen was then retested following removal of the posterior elements and facet joints. The microCT image data was used to create specimen specific FE models of the FSUs. The models were generated using image processing and meshing software (ScanIP, Simpleware, UK). Bone material properties were determined by the image grayscale obtained from the μCT [2] Cartilage material properties such as friction and Young's modulus were varied to investigate the models sensitivity to these parameters. The intervertebral disc was assigned material properties in the form of nine engineering constants [3] following a process of tuning specimen specific FE models (without posterior elements) to the corresponding experimental data.

RESULTS: Load displacement data from a single specimen is presented in Figure 2. The optimum value for friction coefficient for the cartilage interaction (μ_c) was 0.05, this corresponds well to values found experimentally for cartilage on UHMWPE [4].

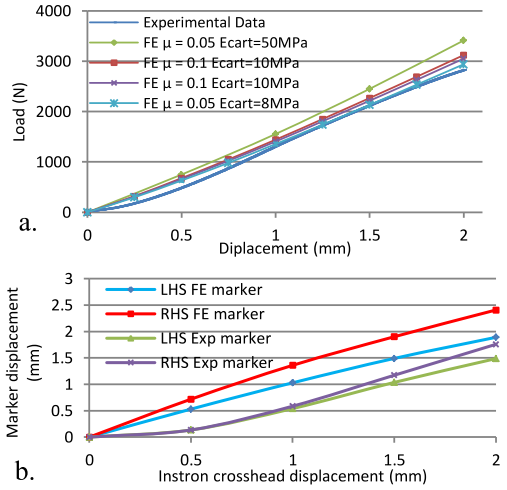

Fig. 2a) FE vs. Experimental data showing model sensitivity of E_{cart} &μ_c. b) FE predicted vs. experimental marker position (E_{cart}=50MPa, μ_c=0.05).

DISCUSSION & CONCLUSIONS: The models appeared to be more sensitive to the Young's modulus of the cartilage surface compared to the friction coefficient. Models predict the overall stiffness of the FSU and marker location well. This technique will now be used to investigate how the influence of spinal interventions affects natural FSU biomechanics.

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Session 2.5

Biomechanics 2

Chairmen: N. Dunne, M. Liebschner

Simulation of patient variance in computational models of the spine

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INTRODUCTION: Back pain remains a major clinical and societal problem. One barrier to the successful development of spinal interventions has been the lack of robust methods of testing new treatments before they are introduced clinically. Testing tends to be undertaken on standardised laboratory or computational models, or on small numbers of *in vitro* specimens. Our previous work on vertebroplasty has highlighted the difference in mechanical behaviour between patients after cement augmentation because of their different anatomies [1]. In order to improve preclinical testing tools, there is therefore a need to be able to represent these variances across the patient population. The aim of this work was to characterise the differences in vertebral anatomy, architecture and properties and to develop computational models that represent these variances.

METHODS: Fifty human cadaveric vertebrae (T12-L1) were imaged using microCT (uCT80, Scanco Medical, CH; Sykscan 1172, Skyscan, BE). The images were segmented using a consistent threshold and smoothing procedure. A new algorithm was developed to extract the cortical shell region. Both the shell and the trabecular bone regions were then divided into subregions and characterised using standard morphometrical measurement algorithms. Finite element (FE) models were generated from the segmented images (ScanIP, Simpleware, UK). The models had a maximum element size of 1mm and the material property of each element was based on the bone volume fraction (BV/TV) of the underlying image. Standardised loads and boundary conditions were applied and the models were solved to predict the vertebral stiffness (Abaqus, Simulia Corp, USA). Validation of the method along with sensitivity and convergence studies has been reported previously [1, 2].

RESULTS: Large differences in both the vertebral morphology and the internal bone structure were found, even in the same age group and spinal level. An example for the BV/TV is shown in Figure 1. Some regional trends were observed in terms of BV/TV distribution and cortical shell thickness, but not across all the spinal levels. The FE results demonstrated a large range in specimen stiffness, with a twofold difference even at the same level and in the same age group (Fig 2).

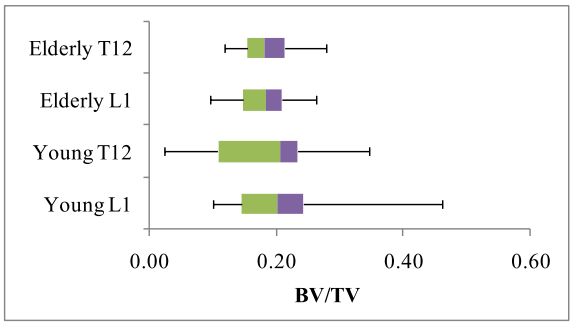
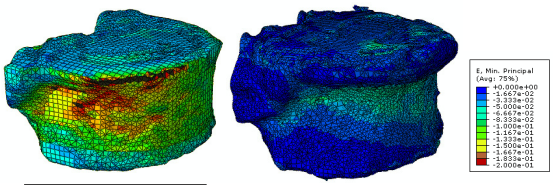


Fig. 1: Box plots of BV/TV across whole trabecular region for four subsets of the vertebrae.



0.87 kN/mm | 1.67 kN/mm

Fig. 2: Maximum compressive strain distribution on two example T12 models from the elderly population group. The stiffness values are shown.

DISCUSSION & CONCLUSIONS: Large variations in the geometry and bone properties from one vertebra to another were found. The FE results illustrate how these variances lead to big changes in the structural behaviour. The results highlight the need for treatments to be assessed across a clinically relevant range of specimens, rather than in a standardised model. This model dataset provides a potential tool, and further work is now underway using principal component analysis techniques to generate models representing specific variances in the patient population.

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Novel 2D bone surrogate models for assessing cement injection behaviour in vertebroplasty

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INTRODUCTION: Concerns related to cement extravasation, embolism, irregular or insufficient filling in vertebroplasty provide the motivation for the development of methodologies for assessing new cements and delivery systems. Reproducible and pathologically representative two-dimensional flow models are used to understand the influence of cement properties on injection behavior.

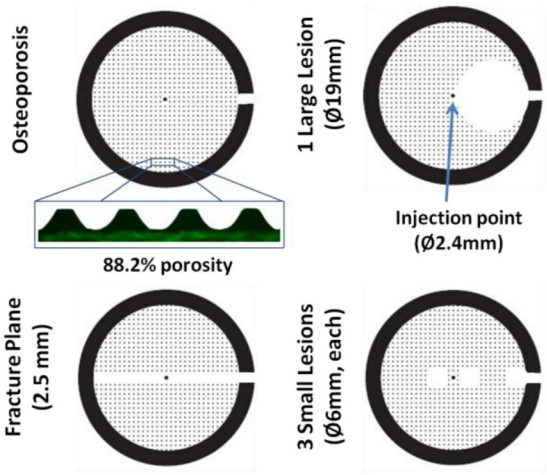

Fig. 1: Manufactured using photolithography, the 2D models have 1mm channel spacing, 0.25mm column thickness, 40mm outer diameter, and a solid boundary representing the cortical wall with 2.5 mm opening representing the flow exit point.

METHODS: A profile projector was used to test model variability. The 2D models, sandwiched between an upper glass window and a lower aluminium plate, were filled with bone marrow substitute (Carboxymethyl cellulose 1.25 % in water) then 1mL of PMMA bone cement (cold cure / rapid repair, WHW Plastics, UK) 1-to-1 liquid-to-powder ratio was injected (3, 5, 7, 9, 11 and 14 min) after cement mixing at a constant flow rate (3mL/min) using a syringe pump. Experiments were repeated three times. Labview was used to control the syringe pump and acquire LVDT, load cell and video data. The LVDT was used to measure the displacement of the syringe plunger. The load cell was used to measure the force applied on the plunger. The video data were analyzed in Matlab to quantitatively describe the resulting flow patterns and calculate parameters including the time to reach the hole in the boundary, the % filled area and the roundness.

RESULTS: The inter- and intra-model variability was very low. The pressure in the system was below 2 MPa for all injections. All parameters used to quantify the resulting flow patterns increased with the time of injection and reached an asymptotic behavior at 11 min after cement mixing.

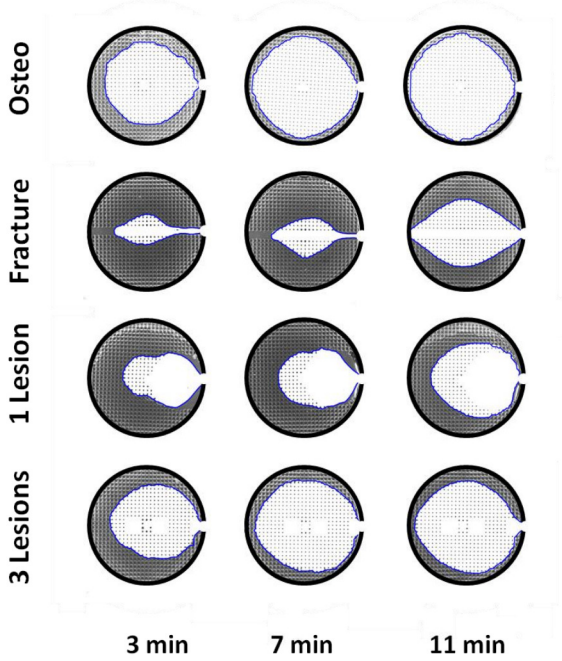

Fig. 2: Flow contours when the cement reached the hole in the boundary at various injection times after cement mixing.

DISCUSSION & CONCLUSIONS: There is a critical upper viscosity at which the injection pressure remains clinically relevant while the cement spreading is uniform and not affected by variations in the geometrical structure. The models provide a tool for quick, robust differentiation between various cement formulations, quantitative analysis of cement spreading at each time point until the cement reaches the boundary and prediction of the uniformity of cement distribution.

ACKNOWLEDGEMENTS: Funding is provided by the European Union under the Marie Curie Action - Grant number - 238690.

Mechanical and *in vitro* evaluation of low-modulus bone cement - Osteopal®V modified with linoleic acid

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INTRODUCTION: Adjacent vertebral fractures are a common postoperative complication upon vertebroplasty [1]. Some clinical and biomechanical investigations associate the risk for adjacent vertebral fractures with the usage of high stiffness bone cements [2]. The development of viable low-modulus cements has been a major challenge, since modification of commercial formulations typically results in poor cell viability or particle release [3]. Literature concerning *in vitro* evaluation of acrylic cements is scarce. In this work, we evaluate the mechanical behaviour of a commercial bone cement that, with minimal modification, can reach more bone-compatible mechanical properties. In addition, we report indirect cytotoxicity *in vitro* using a human osteoblast-like Saos-2 cell line.

METHODS: Osteopal®V (OP, Heraeus Medical, Hanau, Germany) cement was used as a base and modified with 9-*cis*,12-*cis*-linoleic acid (LA) dissolved in the liquid component. The following concentrations, with respect to the total weight of material, were analysed: 0 (OP), 0.75 (OP-0.75), and 1.50 (OP-1.50) wt% LA. Six groups of 14 specimens (∅=10 mm, h= 20 mm) were prepared. Three groups consisted of cement only, whereas the other three consisted of cement-augmented bovine bone cores. The stress-strain behaviour was determined by uniaxial compression testing using an Instron machine at a crosshead speed of 6 mm/min. For the cytotoxicity assay, three groups of 15 specimens (∅=12 mm, h=2 mm) were prepared. Extracts were prepared by placing specimens in cell medium at 3 cm^2/ml [4]. Every 1, 6, 12 and 24 h, extracts were collected and medium was refreshed. Cells were seeded at 20000 cells/cm^2 and after 24 h, extracts were added undiluted or 4-fold diluted [4]. The cells were incubated with extracts or complete media for 24 h or 72 h. Cell viability was quantified using the AlamarBlue assay.

RESULTS: Figure 1 illustrates the general stress-strain behaviour of all cement groups. The Young's modulus (*E*-modulus) and yield strength (σ_y) decreased as the LA concentration increased up to 1.50 wt%. For the cement-only group, the *E*-modulus and σ_y decreased by 74% and 83%, respectively.

For the bone-cement group, the *E*-modulus and σ_y decreased by 33% and 47%, respectively.

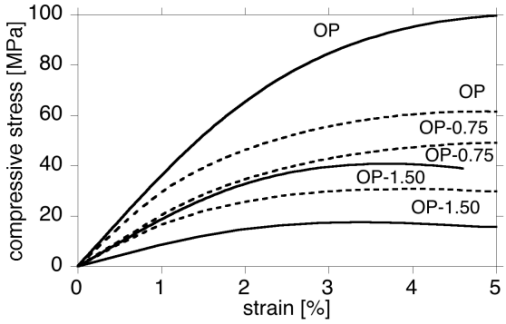

Fig. 1: Stress-strain curves for the cement-only (full) and bone-cement (dashed) groups.

Regarding cytotoxicity, for undiluted extracts, cell viability decreased for LA-modified cements in a dose-dependent manner. However, no significant differences were observed for LA-modified cements in 4-fold diluted extracts. In diluted extracts, the cells proliferated over time.

DISCUSSION & CONCLUSIONS: The addition of very small concentrations of LA can significantly reduce the *E*-modulus of commercial bone cement making it more bone-compatible. This is accompanied by a reduction of the *E*-modulus of the treated bony structure. The change in properties is thought to occur due to incorporation of LA into the polymer network by methacrylation via chain transfer mechanism. Although LA reduced the cytocompatibility of Osteopal®V, it is expected that fluid exchange would compensate this effect as observed for diluted extracts.

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Compressive fatigue properties of acrylic bone cement for vertebroplasty

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INTRODUCTION: Acrylic bone cements based on poly(methyl methacrylate) (PMMA) are commonly used for the fixation of joint prostheses as well as for vertebroplasty. The fatigue properties of the cement are important to the long-term success of the implant, and the ASTM F2118-03 standard for fatigue testing of acrylic bone cements – originally developed for cements used in joint fixations – specifies a fully reversed tension-compression test. However, the same standard is commonly used for testing of cements intended for vertebroplasty, despite the fact that vertebrae mainly experience compressive loads. Until now, purely compressive fatigue studies of PMMA are scarce [1, 2] and none has studied vertebroplastic cement, which e.g. contains a higher amount of radiopacifier. The aim of this study was to evaluate the compressive fatigue properties of vertebroplastic cements, as well as the effect of frequency on these properties. Due to the time consuming character of fatigue tests – the standard cites a frequency of 2 Hz – there is a desire for increasing the frequency to higher values.

METHODS: A commercially available acrylic cement developed for use in vertebroplasty, Osteopal V® (Heraeus Kulzer GmbH), was used for all tests. The powder phase contains 45 wt% of ZrO_2 as a radiopacifier. The powder and liquid phases were manually mixed, and moulded in cylindrical moulds (6 mm diameter, 12 mm height). The specimens were left to cure at ambient temperature. Prior to fatigue testing, specimens were visually and radiographically examined and samples containing defects greater than 1 mm were discarded. Fatigue testing was performed under ambient conditions with a constant-amplitude sinusoidal wave profile, at a frequency of 2 or 10 Hz, using a dynamic material testing system (MTS Axial 858 Mini Bionix II). Each specimen was subjected to a small pre-load of 20 N, and run-out was set at 5 million cycles. Failure was defined to occur at a cumulative strain of 15%, as vertebral compression fractures are detected at a vertebral height reduction of 15-25% [3].

RESULTS: The S-N curve for the experimental data together with fits to an Olgive-type equation are shown in figure 1. The statistical analysis of the fatigue results showed significant differences between the tested frequencies at stress levels of 55 and 60 MPa. However, at higher stress levels the diffferences between 2 and 10 Hz were no longer significant. The estimated fatigue limits from the Olgive equations were 54.5 and 41.3 MPa, for 2 and 10 Hz, respectively.

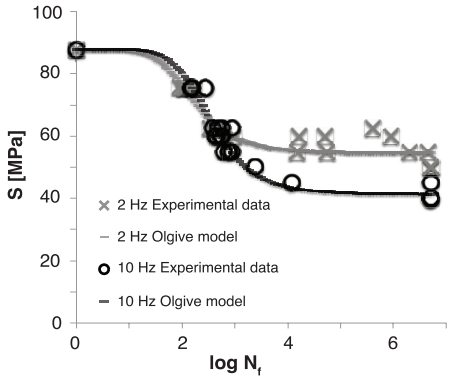

Fig. 1: Applied stress (S) as a function of the number of cycles to failure (N_f).

DISCUSSION & CONCLUSIONS: The fatigue strengths presented here are lower than previous findings for acrylic cement in compression (CMW1®) [2]. However, the cement composition differs and this will influence the mechanical properties. The results presented here also show significant differences in fatigue properties at different frequencies. It has previously been shown that localized thermal heating of PMMA may shorten the fatigue life of the specimen [1], and increasing the frequency increases the thermal softening of the material. Hence an increase from 2 to 10 Hz may lead to an apparent decrease in fatigue life of acrylic bone cements under compressive loading. The estimated fatigue limit is approximately five times as high as that for the same cement (to the authors' best knowledge) in full tension-compression at 2 Hz [4]. This indicates that tension-compression fatigue testing may substantially underestimate the performance of cements intended for vertebroplasty.

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ACKNOWLEDGEMENTS: Funding from VR – Swedish research council, Vinnova and EU is gratefully acknowledged.

Augmentation of cadaveric vertebrae with an α−TCP/ α−CSH ceramic cement: a biomechanical study on the effect of injected volume

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INTRODUCTION: More than two million vertebral compression fractures (VCF) occur annually in the EU [1], with increasing health-care costs currently exceeding 30 billion €/year[2]. Percutaneous vertebroplasty (PVP) as a cheaper and minimally invasive treatment tool shows promising results in improving patients' quality of life. Predominantly there are two types of materials used in PVP, biocompatible calcium-based materials (CaP) and acrylic PMMA materials that lack osseointegration. The injectable biphasic α−TCP (Tri-Calcium Phosphate) / α−CSH (Calcium Sulfate Hemihydrate) material used in this study is osteoconductive and resorbable, thus possessing a bone remodelling capacity. This biomechanical study provides evaluation for the initial stages after implantation with focus on optimization of cement volume.

METHODS: Thirty samples from three osteoporotic spines were disarticulated, cleaned of soft tissue and underwent CT scanning ([microCT80, Scanco medical AG], voxel size 70.8µm^3). Samples were allocated to three equal groups (A, B, C) using a beam-theory based fracture prediction method[3].
Samples underwent an eccentric axial compression at 25% of vertebral body depth, at 1mm/min rate to induce a wedge fracture. Stiffness was defined as the maximum slope on the load-displacement curve. Strength was estimated based on the proof load at 0.5% apparent strain offset. Groups A, B and C were injected bi-pedicularly with the ceramic cement with 10, 20 and 30% of vertebral body volume, respectively. The augmented samples were soaked in 0.04% sodium azide solution and left to cure for 72h, at 37°C, with sequential re-fracturing. Pre/post augmentation strength and stiffness were compared.

RESULTS: Groups A, B and C were injected with 2.1±0.7, 4.3±1.2 and 6.4±2.0ml of cement, respectively. No significant difference was found in either strength or stiffness between the groups (p>>0.05). Initial stiffness was higher for all groups (box plot in fig.1). Combining all data, the initial strength (1.05±0.61kN) increased to 2.02±1.0kN, whilst the stiffness was found to decrease from 1.98±1.66kN/mm to 1.18±0.55kN/mm (fig.2).

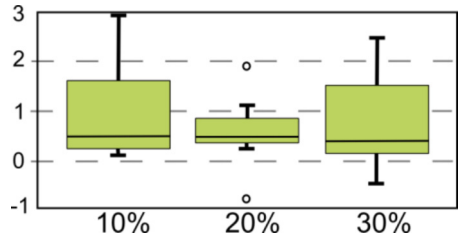

Fig.1 Difference between pre and post augmentation stiffness [kN/mm] grouped by filling volume.

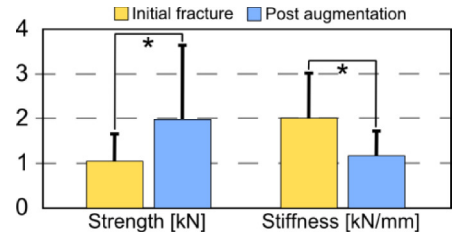

Fig.2 Comparison between pre/post augmentation.

DISCUSSION & CONCLUSIONS: The results of this study show that this ceramic cement almost doubled the strength of the cadaveric vertebrae, while stiffness was almost halved when compared to pre-augmentation values, which is in agreement with previous studies[4,5,6]. Regarding the effect of different cement volume, the reported results indicate that even the smaller volumes (~2ml, 10% of vertebral body volume), may be sufficient to stabilize the fracture suggesting that the volume of introduced cement is not crucial in terms of biomechanical improvement. The findings imply that volume is not a crucial factor in enhancing vertebral strength and stiffness. Hence, the volume can be adjusted rather according to the bioactive character of the cement to achieve the optimal bone-healing conditions.

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ACKNOWLEDGEMENTS: EU funded project Grant Agreement n° PITN-GA-2009-238690-SPINEFX.

Session 3.1

Beyond PMMA and CPC

Chairmen: S. Guelcher, A. J. Wagoner-Johnson

Novel Resorbable Calcium Phosphate Putty for Bone Tissue Engineering

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[1]Center for Craniofacial Regeneration, School of Dental Medicine, [2]Department of Bioengineering, [3]McGowan Institute for Regenerative Medicine, [4]Department of Oral and Maxillofacial Surgery, University of Pittsburgh, Pittsburgh, PA

INTRODUCTION: There is a need to develop safe and effective craniofacial/orthopedic bone regeneration material. The objective of this study is to determine the efficacy of a novel biodegradable nano-structured Calcium Phosphate (CaP) based putty for bone regeneration. This putty will contain nano-sized CaP nanoparticles (NanoCaPs), as carriers, with or without BMP-2 to enhance bone regeneration in a critical sized bone defect model.

METHODS: Nano-structured porous calcium phosphate based putty carrying nanosized CaPs nanoparticles (NanoCaPs) were prepared and characterized prior to their in vivo use. Critical size defects (CSD) in rabbit ulnae (1.5-cm segmental defect) and a 15mm diameter rabbit craniofacial defect were created to test the regeneration potential of the putty alone or with BMP-2. X-rays were taken immediately following the surgery as well as 2, 8 and 26 weeks post-op. Rabbit specimens were harvested at designated time points and Micro-CT as well as histological, and histomorphometry analysis were performed to quantify bone regeneration.

RESULTS: The putty shows excellent cell attachment and cellular migration. The nanostructured nature and the high specific surface area of the HA formed as a result of the setting reaction are added factors contributing to the likely observed faster resorption kinetics of the implanted putty. Our results of the radiographical, micro-CT and histological/histomorphometrical assessment of the new regenerative bone showed that with or without BMP-2 addition to the CaP-putty yielded higher bone regeneration compared to the control groups.

DISCUSSION & CONCLUSIONS*:* Our results to date thus indicate that the CaP-putty with or without the incorporation of BMP-2 does yield bone healing enhancement compared to controls. Our current on-going research is focused on exploring further the development of resorbable putties that are amenable to the addition of growth factors.

Keynote No 31

Market needs and regulatory challenges associated with bone void fillers

Kerem KALPAKCI

Medtronic Spine & Biologics, USA

Injectable, settable lysine-derived polyurethane grafts for bone regeneration

SA Guelcher

Vanderbilt University, Nashville, TN

INTRODUCTION: Within the US more than 650,000 bone grafting procedures are performed each year. Although autologous bone grafts have the best capacity to stimulate healing, explantation risks donor-site morbidity. Thus, the development of synthetic therapeutics exploiting minimally invasive surgical techniques has substantial benefits for treatment of orthopaedic injuries. We are developing biodegradable bone grafts and drug delivery systems comprising two reactive liquid components that harden in situ as an injectable alternative to autograft.[1,2] These materials cure in <10 min and support infiltration of cells and ingrowth of new tissue *in vivo*. The injectable grafts are also an efficient delivery system for biologics, including antibiotics and growth factors such as recombinant human bone morphogenetic protein (rhBMP-2). We aim to create a family of injectable grafts for treatment of orthopaedic conditions that are difficult to heal, such as tibial plateau fractures in which articular congruence must be maintained as well as open extremity fractures contaminated with bacteria.

METHODS: Polyurethane (PUR) biocomposites were prepared from a lysine triisocyanate–poly(ethylene glycol) prepolymer, a polyester triol (70% caprolactone, 20% glycolide, 10% lactide polyol, M_n 300 - 900 g mol^{-1}), triethylene diamine catalyst in dipropyl glycol, and a particulated matrix, such as allograft bone, calcium phosphates, bioactive glass, or sucrose. BG particles were functionalized by surface grafting of polycaprolactone (PCL) to enhance interfacial bonding.[3,4] Compression and torsion testing were performed on cylindrical specimens. For the *in vivo* study, a 3mm x 5mm unicortical defect was created in the diaphysis of the rat femur. Biocomposites were injected into the defect and allowed to cure *in situ* for ~5 min followed by wound closure. Rats were sacrificed 4 and 8 weeks after implantation (n=5). After harvesting the defects, µCT images were taken and radial analysis was conducted for µCT evaluation. Specimens were embedded in PMMA, ground-sectioned (~50 µm), and stained with Sanderson's Rabid Bone Stain in conjunction with Von Gieson Solution.

RESULTS: Under static compression, surface-modified BG/PUR biocomposites have static compressive and torsional strengths of 68 and 28 MPa, respectively (Fig. 1A-B), which are twice those of unmodified BG composites (C-BG) and substantially greater than those of host bone. After 8-weeks implantation time, cellular infiltration and new bone formation were observed throughout the entire BG/PUR biocomposite (Figure 1C-D). No signs of prolonged negative inflammatory response that hindered bone growth were observed. Both radial histomorphometric and µCT quantification of bone within the biocomposite showed a comparable increase in amount of new bone between the 4- and 8-week time points.

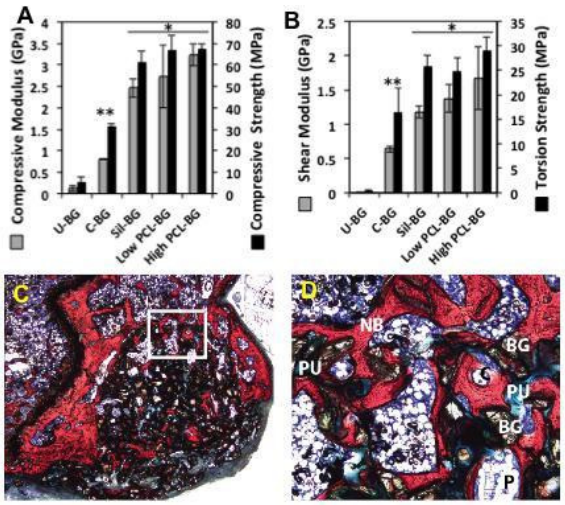

Fig. 1: (A) Compressive and (B) torsional properties of BG/PUR composites. (C) 1.25X and (D) 10X images of histological sections showing new bone (red, NB) and cellular infiltration (C), as well as resorption of residual polymer (PU) and bioglass (BG) at 8 weeks implantation time in rats.

DISCUSSION & CONCLUSIONS: Composites fabricated from surface-modified BG particles exhibited compressive and torsional strengths exceeding those of unmodified BG and host trabecular bone. After 8 weeks implantation time, the biocomposites supported balanced remodeling, as evidenced by histomorphometric and µCT analysis of new bone growth.

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ACKNOWLEDGMENT: NSF DMR0847771.

A Clinical Case Study: Using a Strontium Substituted Bioactive Glass - Stronbone® - to Fill Alveolar Sockets

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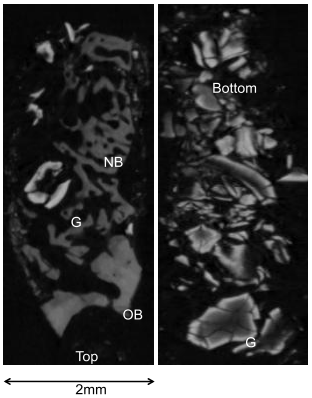
Dental Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London

INTRODUCTION: Strontium is known to stimulate osteoblast and inhibit osteoclast activity. Strontium Ranelate is used as a drug for treating osteoporosis and the active component is the Sr^{2+} cation. Stronbone® is based on the 45S5 bioactive glass composition, but where 10 mole percent of Calcium is replaced by Strontium. The substitution of Sr for Ca in bioactive glasses expands the glass network and results in faster glass dissolution and hydroxycarbonated apatite formation, improved bioactivity as well as the release of strontium [1,2]. Stronbone® has irregular shaped glass particles of 100-800 micron diameter.

METHODS: A patient with carious UR4, UL3, and UL4 was identified and informed consent was obtained. The teeth were extracted using an atraumatic technique and the sockets curetted. Stronbone® was mixed with the patient's blood and packed into the alveolar sockets Following implantation, a barrier membrane was placed over the grafted socket prior to suturing without achieving primary closure.
After two months, a bone sample was obtained from UR4 using a 3mm internal diameter trephine. The same process was repeated for the two other sockets at three months. The alveolar socket model is one of the few ways material from bone substitutes can be obtained clinically.
The trephined cores were fixed with formalin and then examined by X-ray Microtomography (XMT) using our in house built muCAT Scanner. Following XMT, the samples were decalcified, wax embedded and sectioned for histology.

RESULTS: The two month implanted core showed little evidence of new bone formation, though there was extensive evidence of glass dissolution and apatite formation on the surface of the glass particles. The histology showed a fibrous matrix surrounding the particles. The UL4 area (Figures 1 and 2) at three months showed extensive new bone formation and almost complete dissolution of the bioactive glass particles in the apical half, but there was limited new bone and many residual glass particles in the coronal half of the core.

DISCUSSION AND CONCLUSION: Strontium containing Bioactive glass does not inhibit bone formation and has the capability to stimulate new bone but the results obtained depend on the size, geometry of the socket, and the healing time.



- On Left.Original bone OB can be seen with new trabecular bone (NB) forming. Most of the glass particles have disappeared.
- On the Right the bottom halve contains lots of glass particles (G) and relative little new bone. The strontium substituted apatite can be seen forming on the glass particles

Figure 1 XMT slices of Stronbone® implanted in UL4. at 3 months. The top is the basal part and the bottom the alveolar

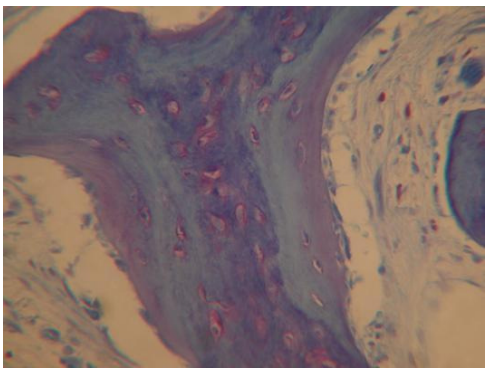


Figure 2 New bone formation. Light Microscopy of aToluidine Blue Stained Section from an UL4 .

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In vivo rhBMP-2 Release from Degradable Polyurethane Biocomposites

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INTRODUCTION: Growth factors incorporated into scaffolds for tissue engineering promote the infiltration of cells and tissue. Recombinant human bone morphogenetic protein-2 (rhBMP-2) stimulates osteoblast differentiation and new bone formation. Biodegradable polyurethane (PUR) biocomposites incorporating allograft bone particles have been reported to be effective carriers for rhBMP-2 and support new bone growth[1]. However, delivery of even a low dose of rhBMP-2 combined with allograft can result in transient resorption.[2] Mastergraft (15% hydroxyapatite, 85% tricalcium phosphate) and sucrose beads are biocompatible substitutes for allograft. In the present study, we investigated the *in vivo* release of rhBMP-2 from PUR biocomposites containing Mastergraft or sucrose and compared this to new bone formation at 8 weeks.

METHODS: The rhBMP-2 was radiolabeled with 2 mCi $Na^{125}I$ prior to use and mixed with non-labeled protein at a hot:cold ratio of 1:8. The biodegradable polyurethane (PUR) was synthesized from a lysine triisocyanate (LTI) prepolymer and polyethylene glycol (PEG), a polyester triol (900 g/mol), and triethylene diamine catalyst. The lyophilized rhBMP-2 was hand mixed into the PUR and injected into bilateral, non-critical defects of approximately 5mm diameter by 11mm depth made in the metaphysis of the distal femurs of New Zealand White rabbits. Treatment groups included a collagen sponge (positive control) and the PUR with 45% Mastergraft or 40% sucrose all with a dose of 35µg rhBMP-2 per defect (n=4 defects per group). Porosity of the PUR samples was 40-50%. Release profiles of rhBMP-2 were measured with a scintillation probe collimated by a 3cm hollow tube wrapped in leaded tape and were normalized to initial data and decay. The animals were sacrificed at 8 weeks and new bone formation and polymer degradation evaluated by µCT, histology, and histomorphometry.

RESULTS: The rhBMP-2 release profiles for both PUR groups were significantly different than the collagen sponge group (Fig 1). Within the first two days, almost 90% of the rhBMP-2 released from the sponge whereas about only 20% is released from the PUR groups. The release profile from the PUR scaffolds does not change based on the type of filler even though the sucrose dissolves within the first few days based on *in vitro* data.

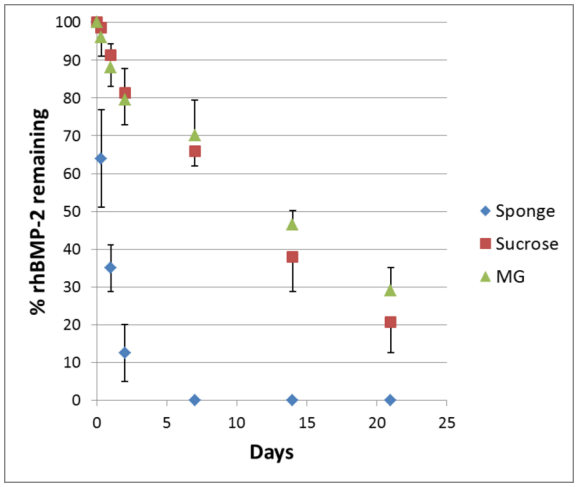

Fig. 1: rhBMP-2 release profile for the collagen sponge, PUR with sucrose, and PUR with Mastergraft (MG) groups

DISCUSSION & CONCLUSIONS: The PUR groups extend the release of rhBMP-2 when compared to the collagen sponge. In addition, the filler type does not change the release profile, which alludes to the fact that the rhBMP-2 release is controlled by diffusion from the PUR. In ongoing experiments, we are testing the effect of different molecular weight polyester triols on PUR degradation and new bone formation. We aim to tailor the degradation rate of the PUR by changing the molecular weight and composition of the polyesterol triol to match the rate of cellular infiltration.

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Effects of rhBMP-2 and mineralized matrix composition on osteoclastic differentiation and resorptive activity

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INTRODUCTION: Settable biocomposite (BC) bone grafts (polyurethane/mineralized matrices) with the potential of maintaining initial bone-like strength during healing undergo balanced remodeling when injected into femoral plug defects in rabbits. The grafts were augmented with recombinant human Bone Morphogenetic Protein-2 (rhBMP-2) to reduce gaps between newly formed bone and the resorbing matrix. Delivery of rhBMP-2 at low (L) and high (H) doses increased new bone formation at 6 and 12 weeks. However, it also increased polymer degradation and matrix resorption at 6 weeks, which suggests osteoclast activation. Thus, the aim of the present study was to investigate the effects of rhBMP-2 delivery and matrix composition on the differentiation and function of osteoclast-like cells.

METHODS: Mouse bone marrow cells positive for CD11b were seeded on several mineralized materials: bioactive glass, tricalcium phosphate (TCP), and dentin. The cells were then treated with nuclear factor kB ligand (RANKL, 50 ng/ml), macrophage colony stimulating factor (M-CSF, 25 ng/ml), and rhBMP-2 (50 ng/ml) for up to 28 days. Cellular attachment and differentiation into osteoclast precursors was verified by intracellular TRAP staining. The effect of rhBMP-2 and matrix composition on osteoclast differentiation was monitored through the gene expression of osteoclast specific markers (TRAP, Cathepsin K, Atp6v0d2 proton pump). Resorptive activity of the cells was quantified by measuring secreted TRAP into the culture medium, as well as number and area of resorption pits generated on the materials imaged using SEM.

RESULTS: Cells seeded on mineralized matrices differentiated into osteoclast precursors positive for intracellular TRAP. After 7 days of culture without rhBMP-2, dentin promoted significantly higher gene expression of osteoclastic markers than all the other groups. When rhBMP-2 was added to the culture, the expression of osteoclastic gene markers changed most significantly in dentin (Fig. 1). Resorptive activity, which correlates to TRAP secreted into the extracellular media [1], was initially lower than dentin for both TCP and bioglass (day 7). However, after 15 days of culture, osteoclasts were activated on the synthetic matrices and had higher activity than the dentin control (Fig. 2).

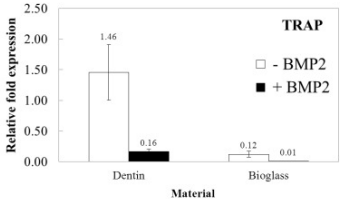

Fig. 1. TRAP gene expression normalized to GAPDH after 7 days of culture. Cathepsin K and ATP6v0d2 followed the same trend.

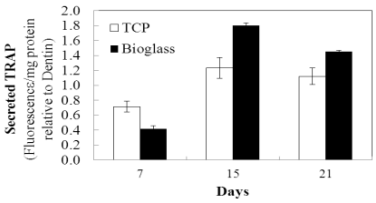

Fig. 2. TRAP produced by osteoclasts and secreted into the media as a measure of resorptive activity. Bars represent values relative to dentin and error bars represent standard error of the mean.

DISCUSSION & CONCLUSIONS: The results above suggest a combined effect between substrate composition and rhBMP-2 delivery on the differentiation and activity of osteoclasts, and support the observations of other groups related to osteoclasts expressing rhBMP-2 receptors [2]. Additional longitudinal studies of the gene expression of cells seeded on the different matrices is required to verify the suggested lag in differentiation/activation generated by synthetic matrices (ongoing studies in our lab). This work underscores the importance of taking into account changes in the differentiation process of cells due to their interaction with synthetic vs. natural matrices during the design of functional scaffolds for bone tissue engineering.

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In vivo ovine animal study of bioglass-based calcium phosphate cement

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INTRODUCTION: The first successful calcium phosphate cement (CPC) was developed by Chow and Brown[1] and was based on a composition comprising of ground $Ca_4(PO_4)_2O$ and $CaHPO_4$ salts that formed apatite. This study investigates CPCs produced using melt derived bioactive glasses as one of the initial reagents. These cements are produced through reactions between bioactive glass (table 1) and $Ca(H_2PO_4)_2$. Three bioglass cement compositions were implanted with a commercial composition HydroSet®. Three bioglass cement compositions of two forming octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_4.5H_2O$) cements (PaG04 & PaG15) and the third containing fluoride (PaG08). Fluoride was added in order to produce a fluorapatite cement which would potentially be osteoinductive but non-resorbable.

METHODS: Glasses were produced via the melt-quench route[2]. The glass powder and $Ca(H_2PO_4)_2$ powders were mixed together to give an overall calcium to phosphate ratio of 1.67. The cement powders were then mixed with a 2.5% Na_2HPO_4 solution to give a liquid to powder ratio of 0.6. The cement paste was then mixed for 30 s. A six week (n=1) and twelve week (n=6) ovine animal study was conducted. Four cement compositions (PaG04, PaG08, PaG15, and HydroSet) were implanted into proximal and distal femur sites. Cement compositions were chosen based upon working and setting times, compressive strength, and cements phase formed. Distal and proximal sites were drilled into the right a left femur, the size of the defect was 8 mm diameter and a depth of 15 mm. After implantation tetracycline labelling was injected into each animal at eight weeks. X-ray microtomography (XMT) using an in-house built MuCAT scanner, with time-delay integration and a voxal size of 30 µm, was conducted on the harvested samples.

Table 1: Glass compositions of produced glasses (mole %).

Name	SiO_2	P_2O_5	CaO	Na_2O	CaF_2
PaG04	38.00	6.00	50.40	5.60	0.00
PaG08	36.80	6.00	49.23	5.47	2.50
PaG15	42.00	4.00	39.00	15.00	0.00

RESULTS: Cements were all implanted without complication and no infection was witnessed in any of the animals. All animals had mobility within two hours of the operation. XMT showed new bone formation surrounding the implant site was at six weeks on all compositions, there was also new bone beginning to form over the top implant. Clear interdigitation with trabecular bone was identified with a clear bond between the cement and host bone.

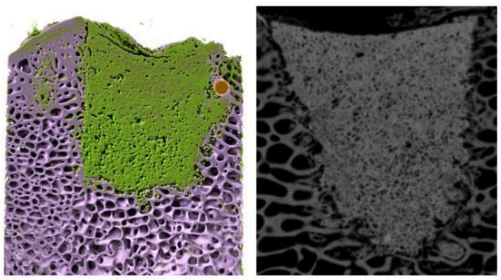

Fig. 1: 3DReconstructed XMT images of implant at six weeks showing the cement PaG04 (left) and HydroSet (right). Green shows cement implant and new bone formation pink shows host bone.

DISCUSSION & CONCLUSIONS: There appeared to be more interdigitation associated with the bioglass based compositions compared to HydroSet®. The bioglass compositions showed a higher degree of apparenrt osseointegration compared to the HydroSet®, where there was a distinguishable gap between the trabecular bone and the cement. It appeared the PaG08 cement showed increased resorption and increased bone formation compared to the other compositions. The results indicated that all the cement samples studied are osteoconductive and are able to osseointegrate with host bone. The developed bioglass cements showed potentially improved osseointegration with host bone compared to HydroSet® cement.

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Effects of an osteoporotic condition on the biological performance of injectable calcium phosphate/PLGA cements

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INTRODUCTION: Calcium phosphate cements (CPC) are promising candidates for bone regenerative applications, especially due to the control over degradation by implementing a degradation platform based on poly(lactic-co-glycolic acid) (PLGA) microspheres[1]. The PLGA microspheres are prone to rapid hydrolytic degradation, which creates porosity within the CPC matrix and increases the surface area in contact with the biological surroundings to allow faster degradation of the CPC. With an increasing elderly world population, the number of osteoporotic patients is rapidly rising[2]. For the treatment of bone defects in osteoporotic patients, no data are available regarding the biological performance of synthetic bone substitute materials. In view of this, the aim of the present study was to comparatively evaluate the biological performance of CPC/PLGA in healthy and osteoporotic rats using a femoral condyle bone defect model.

METHODS: PLGA (Purasorb®; Purac BV, Gorinchem, NL) was used for the preparation of PLGA-microspheres as described previously[3], which were characterized, combined with CPC at 30 wt.% in a 2 ml syringe (containing 1 g CPC/PLGA) and sterilized using gamma irradiation (>25 kGy; Synergy Health BV, Venlo, NL). Female Wistar rats were used for pre-clinical experiments after ethical approval and according to national guidelines for the care and use of laboratory animals. Half of the animals underwent bilateral ovariectomy (OVX) and the other half received sham surgery (SHAM). Six weeks later, all animals were used for the creation of unilateral femoral condyle defects (Ø 2,5 mm, depth 3 mm). CPC/PLGA was injected into the bone defect and left to heal for 4 and 12 weeks. Retrieved specimens were histologically processed, embedded in MMA (n=7) or paraffin (n=1) and stained with methylene blue/basic fuchsin or HE/TRAP. The sections were histologically and -morphometrically analysed.

RESULTS: CPC/PLGA was created with PLGA microspheres of 36±20 µm and a porosity of ~75% (microporosity: ~49%). Histological analysis showed no inflammatory responses and a faster degradation of CPC/PLGA in OVX condition. For both SHAM and OVX, new bone was trabecular-like and associated with bone marrow formation. TRAP staining revealed similar osteoclastic activity for SHAM and OVX. Quantitative measurements showed significantly faster CPC/PLGA degradation and delayed new bone formation (Fig. 1) for OVX compared to SHAM.

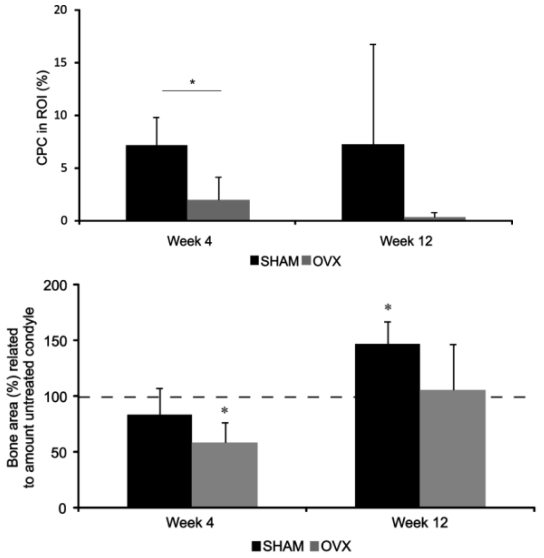

*Fig. 1: Histomorphometric analysis of CPC (top) and new bone (bottom) in the defect area. Dashed line indicates amount in untreated contralateral femoral condyle.(*p<0,05)*

DISCUSSION & CONCLUSIONS: This study clearly shows that an osteoporotic condition significantly accelerates CPC/PLGA degradation and delays new bone formation compared to healthy controls. The rat femoral condyle bone defect model allows screening for empowerment strategies for bone substitute materials to be used in osteoporotic conditions.

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Session 3.2

Hydrogels and Biologics

Chairmen: W. Wagner, P. Weiss

Thermoresponsive hydrogel development for the interruption of adverse remodelling processes in ischemic cardiomyopathy

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INTRODUCTION: Ischemic cardiomyopathy is characterized by an adverse remodelling process wherein the infarcted ventricular wall thins and stiffens, while the ventricular cavity dilates. Mechanically, the thinning wall is exposed to increasing stress, which may be involved in driving further wall thinning and scar formation. It is hypothesized that reducing this wall stress during the remodelling period may serve to alter the outcome towards a more functional structure, characterized by less dilation and wall stiffening. One way this might be accomplished is through the injection of material into and around the infarct region to reduce the wall stress.

METHODS: Thermoresponsive hydrogels are a class of materials that have attractive features for ventricular wall injection. With a lower critical solution temperature below body temperature, these materials could be injected in soluble form through a small bore needle, yet gel in situ to avoid regurgitation and to provide mechanical support. A variety of synthetic thermoresponsive hydrogels have been synthesized that have tunable phase change behaviour, hydrolytic lability, as well as the capacity for serving as reservoirs for controlled release of bioactive agents [1-4]. These materials have been characterized in vitro and evaluated in a rat model of ischemic cardiomyopathy.

RESULTS: Echocardiographic assessment of rat ventricular function has demonstrated the benefit of thermoresponsive hydrogel injection versus saline control material in terms of end diastolic area and fractional area change. Histological assessment shows maintenance of a thickened wall with the injected material being infiltrated by macrophages as the degradation process proceeds.

DISCUSSION & CONCLUSIONS: The injection of synthetic thermoresponsive hydrogels into the ventricular wall has shown benefit in altering the remodelling course of ischemic cardiomyopathy, at least over the first several months following myocardial infarction in the rat model. Fundamental questions remain as to the optimal properties of such hydrogels, for instance in terms of degradation rate, stiffness, and the need for concurrent pharmaceutical agent delivery. If this approach can be effective in the clinic, it would be attractive in that it might be accomplished in a minimally invasive manner and serve to delay or halt the progression towards end-stage cardiomyopathy, where current options remain limited to hospice care, cardiac transplantation or possibly the implantation of a ventricular assist device for chronic circulatory support.

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Study on injectable silicates/alginate composite hydrogels

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INTRODUCTION: Silicate based bioactive ceramics and glasses have received much attention as potential biomaterials for bone regeneration and bone tissue engineering. The recent studies showed that the calcium silicate bioceramics possessed excellent bone regeneration ability, biodegradability and the ability to induce angiogenesis. However, the common drawback of the bioactive ceramics and glasses is the brittleness of the materials and the difficulty in shaping. The sodium alginate (SA), an injectable hydrogel, has been widely investigated for tissue engineering applications. SA has a distinctive ability to form hydrogels via ionotropic crosslinking in presence of divalent cations such as calcium. However, alginate inherently lacks mammalian cell-adhesivity and bioactivity to stimulate cell differentiation, which hampers the applications of alginate in bone tissue engineering applications. Since calcium containing silicate ceramics and glasses have been reported to be biodegradable and are able to release calcium ions under physiological environment, it is reasonable to assume that the addition of CS into alginate may lead to an in-situ forming and injectable CS/SA composite hydrogel, which may combine the advantages of SA and CS, especially maintaining the bioactivity of CS and the injectability and porous structure of SA hydrogel.

METHODS: Silicate bioglasses and calcium silicate bioceramic were incorporated into algeinate solution to for composite hydrogels. The gelling time, the hydrogel stability and the structure of the composite hydrogels were evaluated. The in vitro bioactivity of the composite hydrogels was examined by soaking in simulated body fluid. Furthermore, the effect of the composite hydrogel on cell proliferation and differentiation was evaluated by culturing the hydrogel with cells.

RESULTS: The gelling time could be controlled by the addition amount of the silicates, which could be adjusted between about 30 seconds and 10 min by varying the amount of silicates. SEM observation of the composite hydrogels showed an optimal interconnected porous structure with pore size ranging between 50 and 200μm. FTIR and SEM witnessed that the composite hydrogels induced the formation of hydroxyapatite on the surface of the materials in simulated body fluid. In addition, rat bone mesenchymal stem cells (rtBMSCs) cultured in the presence of ion extracts of hydrogels were able to maintain the viability and proliferation. Furthermore, the ion extracts of the composite hydrogels stimulated rtBMSCs to produce alkaline phosphatase and to promote angiogenesis of human umbilical vein endothelial cells.

DISCUSSION & CONCLUSIONS: Injectable bioactive composite hydrogels were prepared. The gelling time and swelling behavior of the composite hydrogel system could be controlled and regulated by varying the contents of silicate components. The composite hydrogels possessed good bioactivity, which demonstrated by the ability to induce formation of bone-like apatite on its surface in SBF and to stimulate the osteogenic differentiation of rtBMSCs and angiogenic differentiation of HUVECs. The combination of good injectability, bioactivity and angiogenic ability suggests that silicate/SA composite hydrogels have great potential as injectable system for applications in bone regeneration and bone tissue engineering.

ACKNOWLEDGEMENTS: This work was supported by the National Natural Science Foundation of China (Grant Nos. 81190132, 31200714 and 30900299), the Natural Science Foundation of Shanghai Municipal (Grant No. 12ZR1413900) and the Opening Project of State Key Laboratory of High Performance Ceramics and Superfine Structure (Shanghai Institute of Ceramics, Chinese Academy of Science) (Grant No. SKL201203SIC).

Hydrogels and musculoskeletal applications

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Biomimetic extracellular matrices show bioactive properties, enable exchange of stimuli with its environment and induce specific cellular responses. We (INSERM U791) worked for 10 years on injectable self crosslinking hydrogel for cartilage and bone tissue engineering. We have developed a hydrogel with great potential in bone and articular tissue engineering. It is a pH-related auto- reticulating hydrogel that is made up of a hydroxypropylmethyl cellulose (HPMC) aqueous solution onto which silane groups are graft to enable the formation of covalent links between HPMC chains. This polymer is stable in aqueous solution at pH superior or equal to 12.5. Acidification of the solution results in progressive increase of the viscosity and the formation of a hydrogel. Hydrogels can be blended with calcium phosphate ceramics to do biomaterial composite for bone substitution or on the opposite can be used in calcium phosphate cements to make macropores pores inside the cement. Extracellular matrix is a non-uniform material combining proteins, glycosaminoglycans and fibers. Hydrogel alone is too soft if we want to use it for musculoskeletal tissue engineering with alive cells in it. We engineer matrices from hydrogels made of polysaccharides, blends with polylactic fibers, calcium carbonate and phosphates or silicates nano or micro particles in a non-homogenous manner. One pot assembly of hydrogels with nano and/or microparticles of organic or mineral composition is being studied and will provide modulation of mechanical strength and controlled release of bioactive molecules (for i.e. pO2 and cell adhesion) into niches within the matrices. Values of soft tissue elasticity (Young modulus E) could be in the range of 10 kPa for muscle, 20 kPa for cartilage and 30-40 kPa for precalcified bone.

Revisit immune responses to silkworm silk

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INTRODUCTION: Silkworm silk refers to silk produced by the domesticated silkworm, *Bombyx Mori (BM)*, or wild silkworms such as *Antheraea Perni (A P)* (Figure 1). Both types of silk (domesticated or wild) is made of two main components: fibroin, a fibrous protein that forms the core silk filament with strong mechanical strength, and serecin a glue-like coating which surrounds the silk fiber and joins the silk filaments together. It is reported that most immune responses are from reaction to sericin with the presence of fibroin. This study compared macrophage response to silks from *BM* and *AP*, and evaluated inflammatory responses elicited by raw silks and degummed silks of *BM* both *in vitro* and *in vivo*.

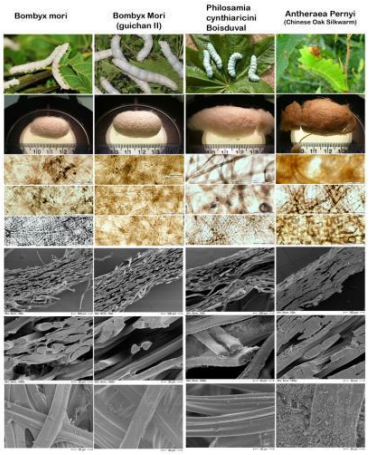

Figure 1 Silkworms, cocoons and the ultrastructure of raw silks

METHODS: For *in vitro* study, we cultured murine macrophages RAW264.7 in presence of these materials. Cellular responses to silks were observed by using bright field microscopy, scanning electron microscopy and transmission electron microscopy. TNF and IL 1β mRNA expression and TNF protein level were also tested in. For *in vivo* experiment, we implanted these materials into BALB/C mice subcutaneously for 10 weeks.

RESULTS: The results indicated that undegummed *AP* silk caused macrophage cell death *in vitro* (Figure 2); sericin-like structure appeared to exist within fibroin, which illustrated continuous macrophagic infiltration *in vivo*. For *BM* silk, macrophages do not respond to raw silks, degummed silks or white sutures tested in a short-term *in vitro* model. Throughout the *in vivo* study, the degummed silk fibres were tolerated well by the host animals. Interestingly, the degummed silks appeared to exhibit a higher degradation rate than raw silks.

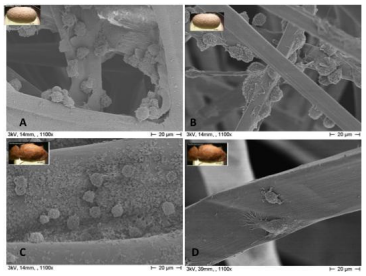

Figure 2 Macrophages on undegummed (A), degummed (B) B M silk; and undegummed (C) and degummed (D) A P silk.

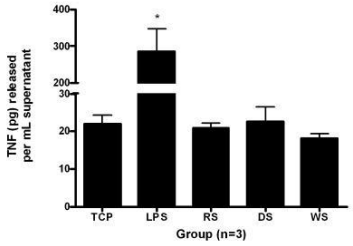

*Figure 3TNFα release from macrophages in response to TCP (control), LPS (positive control), raw BM silks (RS), degummed silks (DS) and white sutures (WS) after 4 days in vitro exposure. Data were shown as mean ± SE * compared with control p<0.01*

DISCUSSION & CONCLUSION: There are structural differences of silk from *Bombyx Mori* and *Antheraea Perni*. Silk from *Bombyx Mori* silkworms is easier to degum (remove of sericin) and less to provoke tissue response than that from *Atheraea Perni*. *Bombyx Mori* silk is biodegradable, or slowly biodegradable material. Macrophages and foreign body giant cells actively participate the biodegradation process, with very little evidence of consequent inflammation.

Selected Abstract No 29

Stem cells update

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Biomimetic collagen/apatite scaffolds for bone repair and regeneration

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INTRODUCTION: In recently years, bone tissue engineering has proved to be a promising approach for repair and regeneration of damaged bone. In this process, the architecture and geometry of scaffolds and progenitor cell sources play critical roles in directing new bone formation.

In the current study, a method combining the biomineralization gelation approach with controllable freeze casting was developed to prepare a novel biomimetic collagen/apatite (Col-Ap) scaffold. The effect of scaffold structure and cell type on bone forming capability was evaluated using a two-hole mouse calvarial defect model.

METHODS: Biomimetic Col-Ap hydrogel was synthesized using a collagen-containing modified simulated body fluid. The hydrogel was frozen using two freezing regimes: 1) cooled at a constant rate in a homogenous cold environment; 2) unidirectionally cooled from bottom to top at a fast cooling rate. It was then freeze-dried, cross-linked using EDC and rinsed.

The in vivo performance of the scaffold was evaluated using transgenic mice carrying a pOBCol3.6GFP transgene associated with osteoblast differentiation. The scaffolds were loaded with four different combination of osteoprogenitor cells, including mouse calvarial cells (mCalv), bone marrow cells (BMSC), mCalv+ BMSC, bone marrow stromal cells (BMSC), and tested in a two-hole calvarial defect model. Histological analysis was carried out to assess new bone formation.

RESULTS: Mineralized collagen hydrogel was prepared by a biomimetic gelation method. The rheological behaviour of Col-Ap hydrogel can be tailored by controlling collagen concentration in m-SBF and gelation temperature (see Table 1). Freeze-dried hydrogels with an isotropic equiaxed structure and a unidirectional lamellar structure were prepared. The pore size of these scaffolds could be easily adjusted by controllable gelation and freeze casting. In the equiaxed regime, the average pore size of the scaffolds was ranged from 69 to 120 μm. In the lamellar regime, the inter-lamellar spacing of the scaffolds ranging from 7 to 340 μm.

The scaffolds were evaluated *in vivo* using a two-hole calvarial defect model, which demonstrated that both of the scaffolds well supported osteoblast activities and new bone formation (see Fig. 1). Moreover, the structure of newly formed bone was closely related to the microstructure of scaffolds.

Table 1. The effect of collagen concentration and gelation temperature on the storage modulus (G').

Collagen concentration (g/L)	25ºC (Pa)	25ºC+40ºC (Pa)	40ºC (Pa)
2	280.9	547.2	324.7
4	773.6	1500.1	853.9

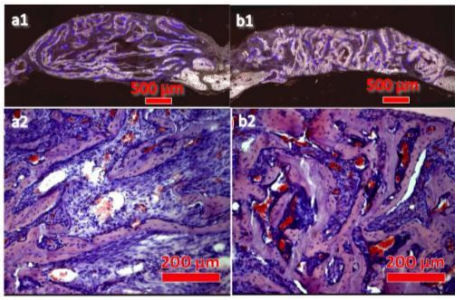

Fig. 1: Fluorescent imaging analysis of bone formation in the calvarial defects loaded with (a) lamellar scaffold+mCalv and (b) equiaxed scaffold+mCalv after 28 days of implantation.

DISCUSSION & CONCLUSIONS: We developed a novel biomimetic gelation method to prepare mineralized collagen hydrogel with tailored properties. Freeze-dried hydrogels with an isotropic equiaxed structure and a unidirectional lamellar structure were prepared and the pore size of which could be tailored by controlling hydrogel properties and freezing regimes.

In vivo evaluations have demonstrated that such prepared scaffolds have excellent bone forming capability. Also, the scaffold architecture plays an important role in guiding new bone formation. In addition, the study has demonstrated the importance of cell source in bone tissue engineering.

ACKNOWLEDGEMENTS: The authors would like to thank National Science Foundation (CBET 1133883) and the National Institute of Health (1R21AR059962-01A1) for their support.

Effects of surface modification of 45S5 bioactive glass on dynamic compressive fatigue mechanical properties of polymeric biocomposites

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INTRODUCTION: Injectable, settable bone grafts that possess dynamic mechanical strength exceeding that of host bone and maintain strength comparable to bone while remodeling could improve the clinical management of a number of orthopaedic conditions, such as repair of open tibial plateau fractures, screw augmentation, and vertebroplasty. Injectable polyurethane (PUR) biocomposites are an attractive alternative to calcium phosphate cements due to their tough mechanical properties and ability to facilitate active remodeling [1]. 45S5 bioactive glass (BG) has widely been used for bone regeneration purposes due to its osteoconductivity and bioactivity [2]. Although physiological loads are generally cyclic, fatigue properties of biomaterials utilized in load-bearing applications are rarely reported. In this study, we investigated the effects of BG surface-modification on the dynamic compressive mechanical properties of BG/PUR biocomposites. Prior to reaction with the PUR binder, BG particles were functionalized with the silane-coupling agent 3-aminopropyl-trietoxysilane (APTES), which has been shown to increase the mechanical compressive strength of BG, as well as surface grafting of polycaprolactone (PCL) to enhance interfacial bonding [3,4]. We hypothesized that a low-porosity BG/PUR biocomposite comprised of surface-modified BG would improve the fatigue properties compared to one made with cleaned BG, as well as native trabecular bone.

METHODS: Cylindrical biocomposites were prepared from a lysine triisocyanate–poly(ethylene glycol) prepolymer, polyester triol (70% caprolactone, 20% glycolide, 10% lactide polyol, Mn ~300 g mol^{-1}), triethylene diamine catalyst in dipropyl glycol, and BG (56.7 volume %). After pre-conditioning the specimen in phosphate buffer solution for 24 h, mechanical testing was completed in cyclic sinusoidal compression mode at a frequency of 5 Hz, reaching independently set physiologically relevant maximum stress levels (5, 10, and 15 MPa). Throughout the entire testing period, specimen were hydrated with water via a drip-system, additionally an extensometer tracked the percent strain. Complete mechanical failure was defined as strain >9%. Analysis focused on the mean fatigue life, change in modulus and residual strain, as well as mode of failure.

RESULTS: Under static compression, cleaned and surface-modified BG/PUR biocomposites have static compressive strengths of 7.9 ± 3.2 and 53.8 ± 6.5 MPa, respectively. Consequently, the dynamic compression fatigue properties of the cleaned-BG/PUR biocomposite cannot feasibly be evaluated at maximum stress levels of 10 and 15 MPa. Under a maximum stress level of 5 MPa, the mean fatigue life of the surface-modified and cleaned-BG biocomposites was 899,953 ± 160,471 and 197 ± 257 cycles ($P<0.05$), respectively. The mean fatigue life of surface-modified BG/PUR biocomposite at 15 MPa was determined to be 85,666 ± 16,615 cycles. As shown in Fig.1A, at a max level of 15 MPa, the compressive modulus of the surface-modified BG/PUR biocomposite decreased as the number of cycles increased. Additionally, its residual strain increased throughout the testing due to plastic deformation (Fig. 1B).

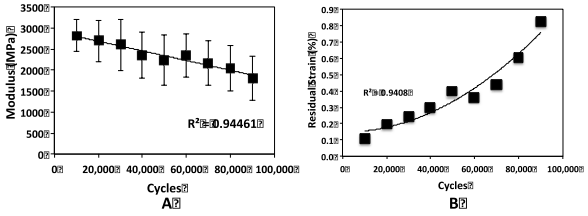

Fig. 1: Fatigue analysis of surface-modified BG/PUR biocomposite at a max stress level of 15 MPa. Second order polynomial fits correlate to respective R^2 values.

DISCUSSION & CONCLUSIONS: By comparing the fatigue life of PUR biocomposites made with cleaned versus surface-modified BG, we conclude that APTES-PCL surface modification significantly extends the BG/PUR biocomposite's ability to withstand physiologically relevant dynamic compressive stresses.

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Acceleration of gelation and increased mineralization of thermosensitive chitosan hydrogels by incorporation of alkaline phosphatase

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INTRODUCTION: Chitosan has been widely applied as a biomaterial for tissue engineering due to its biocompatibility, non-toxicity and biodegradability. Furthermore, injectable thermosensitive chitosan hydrogels are formed by neutralization of acidic chitosan solution using sodium beta-glycerophosphate (β-GP) at physiological pH and temperature. Bioactive substances such as Alkaline Phosphatase (ALP), a mineralization-promoting enzyme can be incorporated during gelation. To promote bone regeneration, the presence of calcium phosphate (CaP) mineral is desirable. The aim of this study was to investigate the effect of ALP on mineralization and gelation of chitosan-β-GP hydrogels.

METHODS: Chitosan/β-GP injectable gels were produced according to the protocol of Chenite et al.[1]. Briefly, 16ml of 2.5% (w/v) chitosan (Fluka) in 0.1 M HCl was mixed with 2ml of 1g/ml (β-GP) solution in water and 0.4 ml of an aqueous ALP solution of concentration 2.5, 1.25 or 0 mg/ml. Hydrogel mineralization was induced by incubation 0.1 M (aq) calcium glycerophosphate (Ca-GP) Hydrogel mineralization was characterized by monitoring mass changes, FTIR, XRD and SEM Gelation kinetics and viscoelastic properties were studied by rheometry at 37 ºC, oscillatory stress of 5 Pa and frequency of 1 Hz. The gelation point was defined as the point where storage modulus (G') exceeded the value of loss modulus (G'').

RESULTS: Addition of ALP shortened gelation time. An example is given in *Fig.1.* Increasing ALP concentration led to an increase in solid mass percentage. Mineralization with CaP was confirmed by FTIR, XRD and SEM.

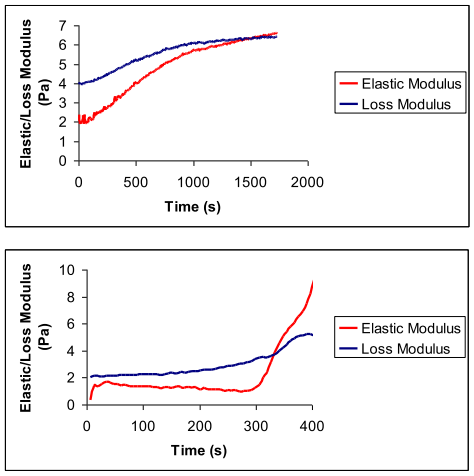

Fig.1: Determination of gelation time without ALP (top) and with ALP (0.23 mg/ml gel) (bottom) at 37ºC.

CONCLUSIONS AND OUTLOOK: Addition of ALP to thermosensitive chitosan/β-GP hydrogels accelerates gelation at 37 ºC and induces their mineralization. Acceleration of gelation is advantageous from a clinical point of view and mineralization is expected to promote bone regeneration. Hence addition of ALP makes chitosan hydrogels more attractive as injectable biomaterials for bone tissue engineering.

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Session 3.3

Calcium-phosphate platform

Chairmen: J. Chang, P. Sharrock

Hydroxyapatite made easy

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Hydroxyapatite, HA, the calcium salt closely resembling the minerals in bone, has been the subject of much research and controversy since its introduction in the medical field. Considered by most to be non-resorbable, difficult to manufacture and at best an inert biomaterial, others have found it to be degradable, having osteoinductive-like properties, and of easy synthesis. Some claim tricalcium phosphate is a better biomaterial with higher resoption rates, usually basing their arguments on in vitro small scale experiments. We will survey the clinical data available on these subjects, including plasma spraying of HA coatings on metal implants and review the numerous methods put forward for HA synthesis procedures including sol-gel, thermal combustion, hydrothermal, biomimetic, solid state and calcium phosphate cement proceses. Recent trends on HA modification by grafting organic molecules and extended substitutions will be exposed, as well as sintering investigations in the presence of bioglass additives. We will report on various methods used to manufacture porous ceramic or composite HA implants, and show the relation between porosity and drug release properties. We will end with the description of our latest results on carbonated HA synthesis at atmospheric pressure starting with calcium carbonate and phosphoric acid as sole reactants and describe the higher reactivity obtained with this procedure for making HA gel of nanometer scale and high specific surface area, using green chemistry.

Influence of iron oxidation state on magnetic properties of iron substituted hydroxyapatite

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INTRODUCTION: Materials with multi-functionality are auspicious for medical applications. Magnetic materials, in particular, are of high interest for the remote actuation. Particles injected intravenously are guided with a magnetic field to a targeted site for targeted drug delivery or hyperthermia treatments[1]. Many magnetic materials lack an intrinsic biocompatibility and vice versa for biocompatible materials. Current magnetic biomaterials rely on composites of a magnetic and a biocompatible material to achieve multi-functionality.

We have taken an alternative approach to this method. The properties of hydroxyapatite (HA), the mineral phase of bone, are readily modified through substitution of different atomic sites[2]. Exploiting these properties, magnetic elements can substitute the calcium site of HA and induce an intrinsic magnetism. We have achieved fabrication of biocompatible, magnetic HA through iron substitution.

METHODS: HA was synthesized via a wet chemical method; particle size was modified by controlling solution concentration, temperature and ageing time. Iron substituted HA was fabricated via ion exchange. Briefly, HA powder was immersed in iron salt solutions containing various amounts of Fe^{2+} and Fe^{3+}. Collected powders were then characterized using X-ray diffraction (XRD), Fourier-transform infra-red spectroscopy (FTIR), energy dispersive X-ray spectroscopy (EDXS) and X-ray photoelectron spectroscopy (XPS). Crystallite size of fabricated powders was calculated using Scherrer's formula, and the magnetic properties were measured by vibrating sample magnetometry (VSM).

RESULTS: The HA powders had a crystallite size of ~ 40 nm or ~ 300 nm. XRD and FTIR spectrums of iron-substituted HA are indicative of a phase of pure substitution. EDXS confirmed the presence of iron. High resolution XPS scan of the Fe 2p peak supported the observations from XRD and FTIR with observed peak positions of ~710 eV and ~712 eV, associated with iron phosphate bonding. Magnetic properties of iron substituted

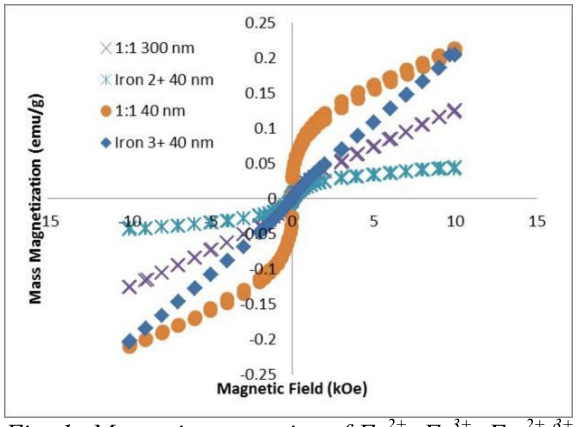

Fig. 1: Magnetic properties of Fe^{2+}, Fe^{3+}, $Fe^{2+}/^{3+}$ 40 nm, and $Fe^{2+/3+}$ 300 nm substituted hydroxyapatite.

HA varied based on crystal size and oxidation state. Figure 1 depicts the varying magnetic properties ranging from paramagnetic to super-paramagnetic to antiferromagnetic.

DISCUSSION & CONCLUSIONS: An intrinsic magnetic biomaterial based on iron substitution for calcium in HA has been successfully fabricated via ion exchange. Both Fe^{2+} and Fe^{3+} substituted calcium sites; the binding energy observed at ~710 and ~712 eV correspond to Fe^{2+} and Fe^{3+} bonded to phosphate. The magnetic properties can be tailored based on the oxidation state of iron. Paramagnetic properties arose from the spin-only electron contribution of Fe^{3+}. Anti-ferromagnetic and super-paramagnetic properties are attributed to the exchange interactions of iron through oxygen and or phosphate atoms. Biocompatible and magnetic iron-substituted HA has been successfully fabricated, which has the potential for various biomedical applications, such as intravenous injection for targeted cancer therapy.

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Development of ion substituted calcium phosphate cement for spinal repair

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INTRODUCTION:

Ion substituted calcium phosphate cement (CPC) offers the potential to improve the mechanical and bone remodelling properties of bone cement used in vertebroplasty[1]. Magnesium (Mg) has been shown to expedite the resorption process of bioceramics[2], Mg has also been shown to stabilise the crystal structure of tricalcium phosphate (TCP)[3]. Similarly silicon (Si) has been included in hydroxyapatite (HA) to increase the rate of bone repair. The aims of this study were two-fold; (i) Synthesise Mg and Si substituted TCP powders; (ii) Use these TCP powders to produce and characterise Mg-Si substituted CPC.

METHODS:

Solid-state hydrothermal reactions were used to synthesise Mg and Si substituted α-TCP. This was achieved via a stoichiometric reaction between either calcium hydrogen phosphate ($CaHPO_4$), calcium carbonate and magnesium oxide (MgO), or $CaHPO_4$ and calcium silicate. X-ray diffraction (XRD) was used to confirm the phase composition of the TCP powders produced. XRD also confirmed the extent of ion substitution into the crystal structure of TCP. These ion substituted α-TCP powders were mixed with di-sodium hydrogen phosphate producing injectable CPC, as per the methods described by O'Hara *et al*[1]. The weight loadings (wt.%) of Si used were 1.0, 2.5, 5.0, and 10.0. The wt.% of Mg used were 0.1, 0.25, 0.5, and 1.0. CPC containing Si and Mg (both individually and collectively) were produced. Setting, compressive and injectability properties were characterised with CPC (100% α-TCP) used as a control[5]. Statistical analysis using ANOVA with Gabriel's Pairwise Comparisons post hoc test was conducted (PASW Statistics 18, USA).

RESULTS:

Synthesis of ion substituted α-TCP powders was confirmed via XRD. Mg substituted CPC (Mg-CPC) provided non-significant reduction in setting properties; with significant improvements in compressive strength and injectability (Table 1). Si substituted CPC (Si-CPC) showed non-significant reductions for the properties measured (Table 2).

Table: *Percentage changes for Mg-CPC vs. Control (* denotes p<0.05, ** denotes p<0.01).*

	Percentage Improvement vs. Control			
wt.% Mg	**0.1**	**0.25**	**0.5**	**1.0**
Setting Time	-2.0	-4.0	-5.0	-5.0
Injectability	7.0	7.0	14.4	26.2*
Compressive Strength	47.5**	48.0*	46.0**	33.0*

Table: *Percentage changes for Si-CPC vs. Control.*

	Percentage Improvement vs. Control			
wt.% Si	**1.0**	**2.5**	**5.0**	**10.0**
Setting Time	-3.0	-4.0	-6.0	-8.0
Injectability	-1.0	2.0	3.0	7.0
Compressive Strength	-3.0	-5.0	-4.0	-7.0

The CPC with both Mg and Si substituted (Mg-Si-CPC) demonstrated no significant reductions in the setting times or extent of injectability, however significant improvements in compressive strength (43.4%) were recorded for Mg-Si-CPC containing 0.25wt.% Mg; and 10wt.% Si content ($p<0.001$ vs. Control).

DISCUSSION & CONCLUSIONS:

Significant improvements in compressive strength were recorded for Mg-CPC and Mg-Si-CPC. It is postulated that Si ions substitute into the TCP crystal structure as $Ca_3(P_{0.9}Si_{0.1}O_{3.95})_2$,[5]. It is suggested that Mg is substituted into TCP in the formula $Mg_xCa_{3-x}(PO_4)_2$ (x=1 or 2). When Mg is substituted into TCP the Mg-O bond becomes stronger, whereas the Ca-O bonds are weakened by the increased bond length compared to the Mg-O interaction. It is hypothesised that this is the reason for the Mg stabilising the structure of TCP, and improving the compressive properties of CPC developed herein.

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Experimental and analytical investigations into the mechanism of filter pressing of calcium phosphate pastes during injection

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INTRODUCTION: The location or mechanism by which filter pressing occurs during the injection of calcium phosphate cement (CPC) remains unknown[1].The aim of this study was to establish if existing filtration theories correspond with experimental results obtained in order to identify the filter pressing mechanism.

METHODS: In this study beta-tricalcium phosphate (β-TCP) and deionised water were mixed to form a non-setting calcium phosphate paste (CPP), which was used to simulate the flow of CPC. The CPP was loaded into a syringe and extruded at a constant rate to a maximum force of 100 N. The variables investigated were liquid-to-powder ratio, LPR, (40, 42.5 and 45 %), extrusion rate (5, 10 and 20 mm/min), needle gauge (12, 13 and 14 G) and length (3, 7.5 and 11 mm). The level of injectability[2] and the LPR of sections of the extrudate and paste remaining in the barrel were determined[1].

In order to model the filter pressing of CPP, it was assumed that the paste in the barrel acted as a compressible cake. By exerting a mechanical force on the cake, the filtrate (i.e. extruded paste) is squeezed out, which is referred to as 'expression of the cake'[3]. A derivation of Darcy's law was used, see *Equation 1*:

$$\Delta P = \alpha_{av}\mu c_{av}\frac{Q^2}{A^2}t \qquad (1)$$

where ΔP is required pressure to compress the cake, α_{av} is the average specific cake resistance, c_{av} is the concentration, and A is area of the cake. Viscosity and flow rate of the filtrate are denoted by μ and Q respectively and t represents time.

RESULTS: Comparing the LPR of sections of the extrudate and paste remaining in the barrel, it was evident that phase separation occurred from the onset of injection and continued throughout the injection process until a non-injectable portion of CPP remained in the barrel. With regards to injectability, no significant difference (p-value <0.05) was observed between experiments conducted with or without cannulated needles. The model provided good agreement with regards to injectability (Fig.1 and Table 1) but under-estimated the extent of phase separation (Table 1).

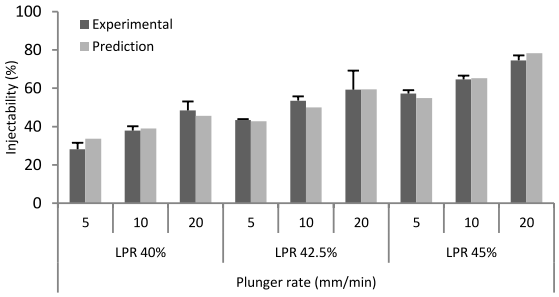

Fig. 1: Effect of plunger rate and initial LPR on injectability (Experimental and model results)

Table 1: Regression coefficients for predicted vs experimental injectability and LPR values (m and c are coefficients of the regression line and R^2 is the coefficient of determination)

	m	*c*	R^2
Injectability	0.99	0.36	0.96
$LPR_{Extrudate}$	1.37	-15.43	0.94
$LPR_{Paste\ in\ barrel}$	0.79	7.24	0.80

DISCUSSION & CONCLUSIONS: This study shows that the location of filter pressing is in the syringe barrel, as needle geometry does not have a significant effect on injectability. The correlation between experimental results and cake expression theory indicate that the mechanism of filter pressing is due to consolidation of particles forcing water to migrate to a region of less pressure, i.e. barrel exit. The migrating water has a lubricating effect enabling granular flow through the barrel exit. As the LPR of the paste is reduced the pressure required to induce granular flow is increased. The pressure required quickly exceeds pressure that can be produced by hand during injection.

By establishing that existing filtration theory corresponds with filtration of CPP during injection this study has given a potential new insight into the mechanism of filter pressing of CPC.

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Injectable lubricants for prosthetic articular joints

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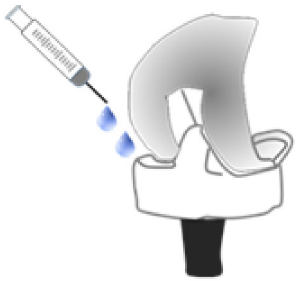
INTRODUCTION: Wear particles of orthopaedic implants have long been recognized as the principal cause of degradation and failure of the articular joint implants.[1] Thus, improvement of tribological properties of implant materials is a key requirement to improve longevity of prosthetic articular joints. The present study proposes to solve this problem by administering external lubricants to prosthetic joints.



This approach is primarily based upon recent development of lubricant additives that improve anti-wear properties of a variety of materials in aqueous solutions.[2-4]

Fig. 1: A schematic illustration of conceptual approach to improve the longevity of orthopaedic implants in this work.

A most outstanding novelty of this approach is that since the external lubricants are independently administered from the implant manufacturing and surgical processes, those who have already received prosthetic joint surgeries, not only the future patients, can benefit for longer lifetime of the prosthetic implants.

METHODS: External lubricants were formulated by dissolving commercial amphiphilic triblock copolymers, PEO-PPO-PEO, in aqueous buffer solution. Pin-on-disk tribometry was employed to assess the lubricating capabilities of external lubricants for the sliding contacts between UHMWPE and CoCrMo tribopair. Various biopolymers, including hyaluronic acid (HA), bovine submaxillary mucin (BSM), bovine serum albumin (BSA) etc., have also been tested. For in-vitro cytotoxicity tests, cell morphology and standard MTT tests have been performed.

RESULTS: Tribological studies have shown that all the PEO-PPO-PEO copolymers tested displayed immediate reduction effects in the coefficient friction upon injection for the sliding contacts between CoCrMo pin and UHMWPE disk in calf serum. A representative example, the case where 1 ml of F127 solution (20%) injected into 2 ml of serum is shown in Figure 2.

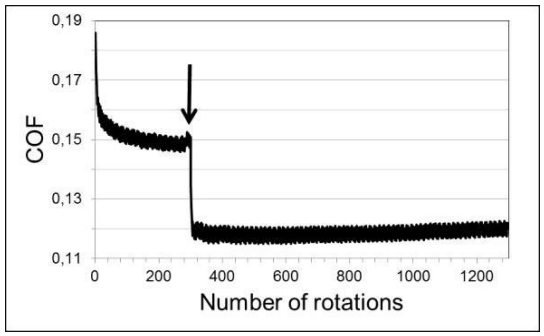


Fig. 2. 1 ml of F127 solution (20%) injected (arrow) into serum solution at 300th rotation immediately reduced the coefficient of friction, and persisted to the end of tests (1,300 rotations, ca. 38 m).

The efficacy of reducing the coefficient of friction was roughly proportional to the concentration of lubricant additives. But, none of the biopolymers tested show lubricating effects. In-vitro cytotoxicity tests have shown that more hydrophilic copolymers are more favourable in cell viability, as tested from both cell morphology and MTT tests.

DISCUSSION & CONCLUSIONS: The first test results in this work indicate that the new approach proposed i.e. injection of external lubricants to prosthetic joints, is very promising to improve the tribological properties of orthopaedic implants, thus potentially their longevity. In addition, in vitro biocompatibility tests also support that the selected lubricant additives reveal positive cell viability.

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Optimization of an acidic calcium phosphate cement with enhanced radiopacity

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INTRODUCTION: Calcium phosphate cements (CPCs) are commonly used in biomedical applications due to their resemblance to the mineral phase of bone. However, due to this resemblance the radiopacity of CPC is very similar to that of bone. The inorganic radiopacifiers (e.g. ZrO_2 and $BaSO_4$) commonly used in non degradable bone cements are not water-soluble and cements utilizing these radiopacifiers have shown an increased bone resorption [1]. To overcome this problem, other radio opaque agents need to be investigated. Studies have shown that strontium can enhance the osteoblasts activity, which could lead to increased bone formation [2]. Hence, soluble strontium salts could be an alternative for enhanced radiopacity. However, additives that do not (or only partially) take part in the setting reaction may have a negative effect on the cement's mechanical properties. In this study the influence of additions of strontium chloride ($SrCl_2$) on various properties of a CPC is evaluated. The objective is to find an optimal composition with regards to radiopacity, compressive strength (CS), and setting time.

METHODS: A screening study was first performed with three factors included; wt% sodium pyrophosphate (SPP), wt% $SrCl_2$ and liquid phase (phosphoric acid, citric acid, or water). The maximum amounts of SPP and $SrCl_2$ were fixed at 5 wt%, and 30 wt% respectively; and the concentration of the acids were kept at 2 M and 0.5 M for phosphoric acid and citric acid respectively. From the results of the screening study further testing was performed using 10wt% $SrCl_2$ and varying the amount of SPP from 0.25 to 1 wt%.

The CPC consisted of equimolar amounts of β-tricalcium phosphate and monocalcium phosphate monohydrate. The cement powders were mixed with the liquid in a powder to liquid ratio of 3.3 g/mL.

RESULTS: The CS measurements showed that the incorporation of 30 wt% $SrCl_2$ decreased the strength drastically (*Fig 1)*. However, as the amount of $SrCl_2$ decreased, the effect on CS was not as pronounced. It was also seen that the incorporation of all additives increased the setting time remarkably. Most distinct was the increase to approximately 400 minutes for cements containing 30 wt% $SrCl_2$ together with 5 wt% SPP. Furthermore, the setting of cements without any SPP or $SrCl_2$ is very short and molding was almost impossible. The radiopacity of the cements showed logarithmic relations, and 15 wt% and 30 wt% had a similar radiopacity of 1.4 and 1.5 mmAl/mm, respectively. To increase CS and decrease setting time it was desirable to have as low amounts of $SrCl_2$ as possible, which still showed radiopacity, and 10 wt% was hence chosen for further studies.

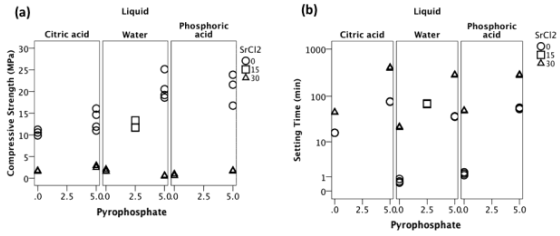

Fig. 1: (a) Compressive strength, and (b) setting time for cements investigated in the screening study. Three to four measurements were made for each group.

For cement compositions containing 10 wt% $SrCl_2$ in combination with varying amounts of SPP it was seen that the addition of SPP increased the CS exponentially from 4 MPa for 0.25 wt% to 20 MPa for 1 wt%. The setting time was also increased with increasing amount of SPP; however, between 0.5 wt% and 1 wt% the setting time was constant around 35 minutes.

DISCUSSION & CONCLUSIONS: This study has shown that compositions containing 1 wt% SPP and 10wt% $SrCl_2$ could be a feasible alternative for cements requiring enhanced radiopacity. These cements have a setting time of approximately 35 minutes and show CS of around 20 MPa, this can be compared to cement without any $SrCl_2$ or SPP, but with 0.5M citric acid to make moulding possible, which had CS of around 12 MPa and a setting time of approximately 10 minutes.

REFERENCES: [1] P Mitchell et. al. (2003) *Biomaterials* **5**:737–748 [2] PJ Marie, et. Al. (2001) *Calcif Tissue Int* **69**:121-9

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