# Oral presentations

**Yang Huilin**, chief doctor, professor and supervisor of PhD candidates. Currently, he serves as director of Orthopaedics Department and General Surgery Department in the First Affiliated Hospital of Soochow University, dean of Orthopedic Institute of Soochow University and president of Clinical and Medical Research College of Soochow University. He is board member of Chinese Medical Association, executive board member of Chinese Orthopedic Association (COA) and president of MISS (Minimally-invasive Spine Surgery) of COA, vice president of SCI of Chinese Association of Spine and Spinal Cord, vice president of SAS--Chinese Branch, vice president of SICOT Chinese Committee, secretary general of International Chinese Spine Society (ICSS) and etc... He also serves as deputy editor of IJSS, vice editor or member of editorial committee of journals. He has got more than 40 sci-tech awards, including one second prize of National Sci-Tech Advance Award. He now assumes 14 state or provincial projects. He has published more than 500 papers, among which more than 300 are included in SCI index, compiled 39 books and got 25 patents.

# Title: Never neglect subsequent refractures of augmented vertebrae following percutaneous kyphoplasty in osteoporotic vertebral fracture patients


## ABSTRACT

Osteoporotic vertebral fracture (OVF) which is one of the most common complications of osteoporosis has a significant negative impact on patients' quality of life. Percutaneous vertebroplasty (PVP) and percutaneous balloon kyphoplasty (PKP) with immediate pain relief and early mobilization are widely used as an effective therapeutic strategy for OVFs. The instantaneously recovered stability and strength of fractured vertebrae prevent continuous micro-motion and further collapse of the fractured vertebrae. However, complications also have been reported following PVP and PKP, of which the most common ones are cement leakage and refractures in adjacent vertebrae. Refractures in augmented vertebrae are rarely reported in the literatures whereas they are not uncommon in clinical work. In the literatures, the definition and diagnostic criteria of refractures of augmented vertebrae are not unified, thus the incidence of refractures varie from 0.56% to 63%. The aim of this study is to review the related articles reporting refracture of augmented vertebrae and discuss the characteristics, diagnosis, risk factors and prevention of refracture of augmented vertebrae.

Marc Bohner holds a MSc and PhD degree in Materials Science from the Swiss Federal Institute of Technology of Lausanne (1990 & 1993). His career included positions as postdoctoral fellow at the University of Utah (1994-1995), and the Swiss Federal Institute of Technology of Zurich (ETH Zurich; 1995-1998). Currently he leads the "skeletal substitute group" of the RMS Foundation in Bettlach, Switzerland. He is also a member of the management board.

Dr. Bohner's career focus has been biomaterials, in particular bone graft substitutes / calcium phosphates. He holds over 15 distinct patents, is the inventor of four commercial products, and has published and presented widely in his field (≈100 articles; h = 48 in Google Scholar). His teaching positions have included adjunct and affiliated appointments at the University of Sherbrooke, University of Bern, and ETH Zurich. His professional service has included: President, Swiss Society for Biomaterials, (2009-2012); President, GRIBOI (2009-present); Secretary, European Society for Biomaterials (2013-present); Co-chair of the annual congress of the European Society for Biomaterials (2009); and membership on a number of societies. He has been an Editor of Acta Biomaterialia (IF 6.0) since 2008. In 2014, he was awarded the “Racquel LeGeros Award” of the “International Society for Ceramics in Medicine” for his “contribution to Calcium Phosphate Research”

# Title: Doping calcium phosphates inorganic drugs to trigger a specific biological response: where do we stand?


## ABSTRACT

Doping calcium phosphates (CaPs) has been very popular since the publication of Gibson et al (1999) reporting the synthesis of Si-substituted hydroxyapatite [1]. Generally, the main aim of this approach is to modify CaPs biological response by adding a biological relevant anion or cation in the CaP crystal structure. In other words, CaPs are used as carrier for inorganic drugs, and the release of these inorganic drugs occur during CaP resorption. Unfortunately, the incorporation of foreign ions in the CaP crystal structure modifies the crystal shape and solubility, and as such, it is not possible to univocally attribute a biological effect to the release of the incorporated inorganic drug. Indeed, it could also be due to a change of surface topography or a change Ca or phosphate release [2]. Another difficulty related to CaP doping is associated with the determination of the location of the doping agents. Most studies conclude from a change of crystal lattice parameters that all inorganic drugs are incorporated in the crystal lattice even though part of the ions could be located at grain boundaries [2-3]. The aim of this communication is threefold: (i) briefly summarize this field of research, (ii) describe the difficulties to produce pure calcium phosphate powders, and (iii) show some dissolution results of undoped and Na-doped β-tricalcium phosphate (β-TCP).

**Name:** **Wouter Habraken**

**Date of Birth: 04.12.1979**

1998-2003: Master of Science at the Department of Chemical Engineering and Chemistry at the Eindhoven University of Technology (Eindhoven, The Netherlands).

2003-2008: Doctoral Thesis: "Development of Biodegrable Calcium Phosphate Cement for Bone Tissue Engineering" at the Radboud University Nijmegen Medical Center (Nijmegen, The Netherlands) Supervised by John .A. Jansen in cooperation with Antonios G. Mikos (Rice University, USA).

2008-2010: Postdoctoral Scientist at the Laboratory of Materials and Interface Chemistry and Soft Matter Cryo-TEM Research Unit (Nico A.J.M. Sommerdijk, Eindhoven University of Technology, Eindhoven, The Netherlands)

2010-2012 Postdoctoral Scientist at the Bio-Inspired Hybrid Materials and Synchrotron Research group (Barbara Aichmayer, Department of Biomaterials, Max Planck Institute of Colloids and Interfaces, Golm, Germany)

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# Title: Control over Biomineralization pathways: relevance for Bone regeneration and Calcium Phosphate Cements

**ABSTRACT**

Recent evidence indicates that the formation of complex hierarchical mineral structures in Nature proceeds via an amorphous intermediate phase, which upon arrival at the site of interest, fuses with the growing mineralized matrix and crystallizes. To understand what happens in this process, in our studies we investigate the stability and crystallization pathways of synthetic amorphous minerals by systematically changing the intrinsic properties of the material. Hereby we focus on particle size, chemistry and presence of trace amounts of organic and inorganic additives. Additionally, we investigate the influence of crystallization conditions by performing crystallization experiments in solution, under controlled humidity and upon heating. In such a manner we try to determine the underlying mechanisms of biomineralization processes that lead to complex hierarchical structures like bone. Additionally, in the current talk a comparison will be made with calcium phosphate cements where similar processes might play an important role regarding setting time and mechanical properties.

**Professor Jiang Chang** is Director of the Biomaterials and Tissue Engineering Research Center at the Shanghai Institute of Ceramics, Chinese Academy of Sciences, Fellow of International Union of Societies for Biomaterials Science and Engineering (FBSE), Fellow of Royal Society of Chemistry, Fellow of American Institute of Medical and Biological Engineering, Ph.D in 1991 in Chemistry from the Technical University of Darmstadt in Germany. Post Doctoral Research Fellow at the Medical University of Luebeck in Germany (1991-1993); Research fellow at the School of Medicine, University of Auckland in New Zealand (1993-1997); Research Assistant Professor at New York University (1997-1999); Research scientist at Johnson&Johnson (1999-2000); and Adjunct Professor at Shanghai Jiaotong University. He is the vice president of the Interdisciplinary Research Society for Bone and Joint Injectable Biomaterials. He is also an associate editor of the Journal of Materials Chemistry B. His research focuses on bioactive materials for tissue regeneration and tissue engineering. He has published over 300 scientific papers, and received 52 patents in the field of biomedical materials.

# Study on injectable bioactive hydrogels for tissue regeneration


## ABSTRACT

Alginate hydrogels have been widely studied due to good biocompatibility and ability to encapsulate living cells. However, the in situ injectability of alginate hydrogels is poor due to the fast crosslinking reaction with Ca ions, which is the traditional method to obtain alginate hydrogels. Here, a novel injectable alginate (SA)-based hydrogel is developed by combining actions of Ca2+ and Mg2+ released from akermanite (Aker) to crosslink SA instead of single type of divalent ions. The gelling process and injectability of the hydrogel could be well controlled by adjusting the amount of Aker in the composite hydrogels. In vitro results indicate that the SA/Aker composite hydrogel with optimized physicochemical properties is not only able to maintain the viability and proliferation of human bone marrow mesenchymal stem cells (hBMSCs) and human umbilical vein endothelial cells (HUVECs) but also shows stimulatory effects on osteogenic differentiation of hBMSCs and vascularization of HUVECs. In addition, in vivo results indicate that the SA/Aker composite hydrogel possesses osteoinductivity when it is injected subcutaneously in nude mice, which is the first time demonstration of osteoinductivity of silicate based biomaterials. Furthermore, when hBMSCs are encapsulated within the hydrogels, the composite hydrogel further stimulates the osteogenic differentiation and mineralization of hBMSCs and the ingrowth of blood vessels into hydrogels, indicating the stimulatory effects of the material on the interactions of seeded cells with recruited host cells.

# Professor Hala Zreiqat

Professor Zreiqat is recognised internationally as a leading authority in orthopaedic biomaterials research and is regularly invited to speak at national and international conferences. She has published 94 research papers, 8 book chapters and 7 review articles and is primary editor of A Tissue Regeneration Approach to Bone & Cartilage Repair (Springer, 2015). Professor Zreiqat has obtained >$8.2M in competitive funding. Her pioneering development of innovative biomaterials for tissue regeneration has led to one awarded (US) and 6 provisional patents, 5 as a lead inventor, and several collaborations with inter/national industry partners.

She received 3 honorary professorial appointments at universities in the USA, China, and Lebanon. She is the immediate Past President, ANZORS; Founder & Chair, Alliance for Design & Application in Tissue Engineering (partners include Harvard, Columbia, Stanford, Tufts, Hong Kong); Co-founder: Sydney Bone Group (2002 onwards). Awards: The Rebecca Cooper Research Foundation PhD Scholarship (2016-18), John & Eileen Haddon Award (2015), Leopold Dintenfass Memorial Award (2012) for Excellence in Research, and Australia-Harvard Fellowship (2013).

# Title: Development of unique 3D printed ceramic scaffolds and nanoparticles with versatile modular platform for regenerations of large bone defects


## ABSTRACT

The repair and regeneration of large bone defects under load has remained a major clinical challenge for orthopaedic surgeons. This presentation will provide our strategies in using three dimensional (3D) printing biomaterials platform and/or biologics to develop a cell-free therapeutics to promoting bone healing in these challenging situations.

The presentation will discuss our optimised 3D printing technology and mathematical modelling to fabricate highly porous (70%) and interconnective (100%) three dimensional novel glass-ceramic scaffold (Sr-HT Gahnite) with distinct pore geometries (hexagonal), with compressive strength approaching that of cortical bone (110MPa). Using computational modelling we demonstrated that hexagonal pore geometry profoundly increased the mechanical strength of the scaffolds to reach that of cortical bone. The Hexagonal scaffolds with design demonstrated a high fatigue resistance (1,000,000 cycles at 1-10MPa compressive cyclic load), failure reliability and flexural strength (30MPa) compared with those for conventional architecture.

The presentation will discuss our novel injectable ceramic-phosphate cement, with favourable physical and chemical properties compared to currently available injectable bone void fillers. Key advantages of the developed cement filler include cohesiveness, macroporosity, low exo-thermicity, appropriate setting time and bioactivity. In addition, the developed cement has a compressive strength and modulus matching that of cancellous bone.

We have developed a multi-porous inorganic and biocompatible nanoparticle (NPs) with chemical composition similar the inorganic component of natural bone. The presentation will highlight the novelty of these nano/meso particles.

Our developed scaffold and 3D printing technology, injectable and NPs platforms opens avenues for bone regeneration in load bearing bone defects in various clinical applications including orthopaedics, spine, dental and maxillofacial.

# Prof. Jean-Michel BOULER, Ph.D

Deputy-Director of CEISAM CNRS Research Center 6230

'Interdisciplinary Chemistry: Synthesis, Analysis, Modeling' – University of Nantes - France

Keywords : calcium phosphate biomaterials, bone tissue engineering, orthopaedics, osteoporosis.

Jean-Michel Bouler was appointed as full-Professor in the Department of Materials Science (Dental College – University of Nantes) in September 2005 after serving as Assistant-Professor since 1999. Prof. Bouler completed a BSc and a MSc in chemistry respectively at University of Nantes, France and University of Wales, U.K. before joining a Biomaterials Research Center at INSERM to study for a PhD in bioceramics.

Then, Prof. Bouler has been running the "Biomat Research Group" for 15 years within two INSERM labs involved in Bone & Dental Tissue Engineering (EMI 99-03 and UMR 791 respectively). He joined recently a new CNRS research center to focus on the synthesis and characterization of drug-combination systems based on calcium phosphate biomaterials. Research in this field was funded through numerous ANR Programs involving academic laboratories, clinical centers and private companies. These collaborations have led to 8 patent families and patent applications and some specific systems are now being taken forward to clinical applications and CE/FDA markings.

Jean-Michel Bouler has been consulting for orthopaedics companies for more than 20 years and has co-funded two of them (Biomatlante in 1995 and Graftys in 2005).

Jean-Michel Bouler has been involved in several scientific panels evaluating biomaterials programs for 'Agence Nationale pour la Recherche' (ANR, France), 'Fundação para a Ciência e a Tecnologia' (FCT, Portugal) and for 6th / 7th European Frame Programs. He co-chaired several major conferences in the field in Europe including the 24th GRIBOI Conference in 2014

Scientific record

140 articles in peer-reviewed journals

Sum of the times cited : >3500 (Webscience – jan 2016)

h Index = 32

8 patent clusters

# Title: Organic-inorganic injectable hybrid materials: possible strategies to improve calcium phosphate cements properties.


## ABSTRACT

Since first reported in the 1980s, calcium phosphate cements (CPCs) have attracted great interest as bone substitutes for the reconstruction of hard tissues owing to their excellent biocompatibility, bioactivity and osteoconductivity. Compared with calcium phosphate ceramics, CPCs have the advantage that they can be easily manipulated and shaped, and, in some cases, can be injected into a defect area, thereby not only avoiding invasive surgical procedures but also providing intimate adaptation to the bone cavity even for irregular shapes. The injected paste sets in situ to form nano or micro-crystallized calcium phosphates materials exhibiting biological reactivity and providing possible applications in tissue engineering or drug delivery.

However, despite the above advantages, pure inorganic CPCs have some critical drawbacks which limit their potential clinical applications. They generally present: (i) poor injectability, (ii) weak cohesion, (iii) fragile mechanical behavior and (iv) limited cell-mediated resorption ability due to, at least, a non-optimal porosity structure.

Since few years, one of the chosen way to improve those features consists in adding various organic compounds (e.g. block copolymers, hydrogels, fibers, emulsifiers etc..). We will present some of the strategies that have been conducted in that domain by a consortium of European laboratories.

**Short Academic Biography**

# Liming Bian, Ph.D.

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**Research interests:**Biomaterials;Stem cell tissue engineering ;Effect of microenvironment cues on stem cells;Nanomaterials for drug/gene delivery and molecular imaging

Dr. Bian received his B.Eng and M.Sc degree from the National University of Singapore in 2002 and 2004, respectively. Dr. Bian completed his Ph.D. study in Biomedical Engineering under the advisory of Dr. Clark T. Hung and Dr. Gerard A. Ateshian at Columbia University in 2009. Dr. Liming Bian completed his postdoctoral training under the advisory of Dr. Jason A. Burdick at the Polymeric Biomaterial Laboratory in the Department of Bioengineering, the University of Pennsylvania since 2009. Dr. Bian's research focuses on the development of novel biomaterials for the regeneration of musculoskeletal tissues. Dr. Bian is also interested in developing biomaterial platforms to investigate the role of cell microenvironment factors including mechanical, chemical and cell-matrix interactions in stem cell differentiation.

Dr. Bian is an affiliated member of the Orthopedic Research Society, American Chemical Society and Society for Biomaterials. Dr. Bian also served as an active reviewer for Tissue Engineering, the Journal of Biomedical Materials Research and the Journal of Biomechanical Engineering.

## Title: Robust injectable biopolymeric hydrogels for regenerative medicine

### ABSTRACT

In the most recent decade, increasing research emphasis has been placed on the "bio" part of biomaterials. Rather than being biologically inert, novel biomimetic biomaterials with intricate biological functions which can proactively interact with living cells and tissues by design have been presented by a number of recent studies. Insights gained from these studies also feedback to the optimization of biomaterial design leading to the development of novel biomimetic biomaterials with enhanced functions and efficacy. In our lab, we have shown that functionalization of the hydrogels with biomimetic peptides promotes the differentiation of the hMSCs [1, 2]. In addition to the biofunctionalization, the physical functions of the biomaterials are also critical to the successful translation of biomaterials to clinical treatment of cartilage diseases. We have developed a series of injectable hydrogels with unique properties such as resilient mechanical property, bioadhesiveness, injectability, and promoting recruitment of endogenous cells that are desirable for potential clinical applications in cartilage resurfacing[3].

**Dr. Changsheng Liu** is currently vice president of East China University of Science and Technology and a director of Engineering Research Center of Biomedical Materials under Ministry of Education. Dr Liu has devoted for decades into the fundamental and application research of biological materials and has obtained a series of outstanding achievements in the field of bone regeneration. He won an international Fellow of Biomaterials Science and Engineering (2012).
Dr Liu has a Ph.D. in Chemical Engineering in East China University of Science and Technology in 1996. He has visited University of Pennsylvania, Philadelphia, USA as a visiting professor from March to Sept, 2005. He has successfully applied 50 invention patents (including 2 international PCT Patent and 3 USA Patent) and published more than 170 SCI articles. He won the Second-prize of National Award for Natural Science (China) in 2014 (the first rank) and the Second-prize of National Award for Science and Technology Progress (China) in 2003 (the first rank), the First-prize of Award for Natural Science (Shanghai) in 2013 (the first rank).

# TITLE: Injectable calcium phosphate-based cement and its clinic applications


## ABSTRACT

SINCE their initial formulation in the 1980s, calcium phosphate cements (CPCs) have been increasingly used in minimally invasive interventions to treat vertebra and fragility fractures. It consists of aqueous slurries of calcium phosphate compounds which set to form a nanocrystalline hydroxyapatite (HA) or brushite matrix by a continuous dissolution / precipitation reaction. A promising advantage of CPCs is their self-setting nature, which makes them injectable and allows the use of a minimally invasive surgical procedure during clinical use. Moreover, CPCs have better biocompatibility than PMMA cement, which has drawbacks such as tissue heat necrosis and release of toxic monomers into the surrounding tissue. However, the clinical use of CPC is limited to non-load bearing defects since they are comparatively mechanically weak and susceptible to brittle fracture, unsatisfied setting speed, and a relative slow degradation rate. Herein, we give a systematic review on the recent progress of injectable calcium phosphate-based cements, including the forming of hybrid networks to obtain improved mechanical strength and modulated setting time; modification of both anti-washout performance and degradation rate via addition of polymeric compositions; and its clinic applications in minimally invasive vertebroplasty in surgical treatment of osteoporosis fracture.


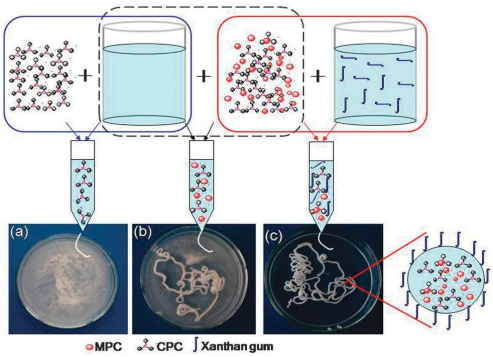


Fig. 1: Improved anti-washout of injectable calcium phosphate-based cement by addition of polymeric composition


REFERENCES: 1 M. Bohner (2010) European Cells and Materials 20:1-12 2 J. Wang, CS. Liu, et al (2010) Advanced Functional Material 20: 3997-4011 3 FP. Chen, CS. Liu, et al (2015) Journal of Materials Chemistry B 3: 9173-9181

**Dr. Wenguang Liu** is a full Professor of School of Materials Science and Engineering at Tianjin University. He received his bachelor degree in polymer chemical engineering in 1991, master degree in polymer materials in 1996 and PhD in Biomedical Engineering in 1999 from Tianjin University. Dr. Liu was a visiting scholar at The University of Hong Kong from July 2003 to January 2004. He did postdoctoral research at the Department of Cellular and Molecular Medicine, University of Ottawa (Canada) from March 2005 to November 2006. Dr Liu's current research work is at the interface of biomedical engineering and materials science ( soft-wet biomaterials, regenerative medicine and non-viral vector for gene delivery He has published more than 100 peer-reviewed papers. Dr Liu is the recipient of 2013 National Natural Science Funds for Distinguished Young Scholar.

# Title: High strength injectable supramolecular polymer hydrogels


## ABSTRACT

Hydrogels consisting of water swollen chemically or physically crosslinked polymer networks are being exploited as a wide range of biomedical applications such as cell scaffolds, soft tissue substitutes and bioactuators. However, their load-bearing applications are often limited by the poor mechanical performances. Chemical crosslinkers are generally essential for the construction of high-strength hydrogels; however, the resultant gels tend not to be injectable or reprocessable. Herein, we reported high strength injectable supramolecular polymer hydrogels synthesized from amino acid derivative-based monomer containing multiple hydrogen bonding interactions on side chains. The SP hydrogels demonstrated thermoplastic processability, injectability and self-healability. Also an autolytic thermoresponsive high strength hydrogel capable of responding to calcium ions and pH can be readily fabricated by tuning the end-groups on side chains. The SPs were implanted into the articular cartilage defects of rabbits. Initial in vivo results suggest that the SPs hydrogels are promising to be novel biomedical scaffolds for potential biomedical applications.

**Professor Bin Li** received the bachelor degree in Polymeric Materials Science and Chemical Engineering from the Department of Chemical Engineering of Tsinghua University in 1996. He received the PhD degree in Materials Science from Tsinghua University in 2001. He then worked as a Research Associate at the Institute of Materials Research and Engineering, Singapore from 2001 to 2004. After that he pursued postdoctoral training at the Department of Orthopaedics, University of Pittsburgh School of Medicine in USA from 2005 to 2009. He also completed two short-term trainings as a visiting research scientist at Carnegie Mellon University in 2004 and Harvard University in 2009, respectively. He joined Soochow University in 2009 as a Specially Appointed Professor and director of the Biomaterials and Cell Mechanics Laboratory (BCML) of Orthopedic Institute. He is the recipient of a number of awards such as the Orthopaedics Research Award (1st class) from Chinese Orthopaedic Association, Xu Guangqi Program from the French Embassy in China, and France Talent Innovation from the Consulate General of France in Shanghai. He currently serves as the chair of China Development Committee of the International Chinese Musculoskeletal Research Society (ICMRS). He is a fellow of Chinese Orthopaedic Research Society (CORS), Chinese Association of Orthopaedic Surgeons (CAOS), Chinese Association of Rehabilitation Medicine (CARM), and International Society of Orthopaedic Surgery and Traumatology (SICOT). He has delivered more than 40 invited talks and is the author of over 80 journal articles and book chapters. His research interests include biomaterials for orthopaedic applications, degenerative disc disease, stem cells and tissue engineering, smart molecular recognition and controlled release, surface modification and functionalization, cellular biomechanics and mechanobiology.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Calcium phosphate-silk fibroin composites: bone cement and beyond

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Calcium phosphates are promising self-curable, biocompatible, and osteoconductive materials which hold promising potential in orthopaedic surgeries. Especially in the area of bone cements for vertebral augmentation, calcium phosphate cements may be used as biodegradable fillers to achieve orchestrated cement resorption and new bone formation without the potential risks of traditional non-biodegradable poly(methyl methacrylate)-based bone cements. However, the applications of calcium phosphates are relatively limited due to the inferior mechanical strength of them. In order to improve their mechanical property, we have prepared composites of calcium phosphates and silk fibroin, a silk-derived protein which has been widely used in a lot of biomedical applications taking advantage of its remarkable biocompatibility, biodegradability, and mechanical properties. We found that supplementing silk fibroin could improve the mechanical strength of calcium phosphates, but not to a level that is sufficient for vertebral augmentation purposes. Therefore, we synthesized hydroxyapatite-silk fibroin complex. When supplemented to calcium phosphate/silk fibroin composites at a small amount, the complex helped markedly improve the mechanical strength, likely as a result of the enhanced interfacial integrity and oriented growth of hydroxyapatite crystals in the composite. Further, we developed stronger calcium phosphate cements using calcium hydroxide-treated silk fibroin as curing solution, the mechanical strength of which was dramatically improved with the increase of pH.

In addition to being used as bone cement, calcium phosphate/silk fibroin composites in the form of microspheres have also been used as an effective carrier system for delivering cells and drugs to facilitate bone regeneration. For instance, we have prepared hydroxyapatite/silk fibroin microspheres loaded with growth factors and anti-cancer drugs for repairing tumor-associated bone defects. The microspheres had good drug package efficiency, low burst release, and long-term release capability. Being composed of osteogenic materials and chemotherapy drugs, such a microsphere system is expected to prevent rejuvenation of tumor and in the mean time, to promote bone defect repair. We also prepared antibody-functionalized microspheres for recruiting bone marrow mesenchymal stem cells, which were used as the building blocks to promote new bone formation and bone defect repair.

In summary, calcium phosphate/silk fibroin composites, with the decent handling properties and biocompatibility, hold great promise for a variety of orthopaedic applications, including filler materials for minimally invasive surgeries to treating vertebral fractures and other bone defects.

**ACKNOWLEDGEMENTS:** These studies were supported by the National Natural Science Foundation of China (81471790, 31530024), Jiangsu Provincial Special Program of Medical Science (BL2012004), and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

**Dr. Weiping Ren** received his medical degree from Shanghai Second Medical University in China, and PhD in Biochemistry from Yamagata University, Japan. Dr. Ren is currently Associate Professor of Orthopedic Surgery and Biomedical Engineering, Wayne State University, Director of Orthopedic Research, Providence Hospital. The focus of his research is to develop implant surface fabrication technology, bioceramics for drug delivery and bone graft substitutes.

# Title: Development of self-setting polyphosphate cements as new bone graft substitutes


## ABSTRACT

Segmental bone defects remain a major clinical and socioeconomic problem. Calcium phosphate cements (CPCs) have been widely used as a bone-filler for the treatment of bone defects. CPCs, however, are brittle, weak and release drugs too fast. There are still no good solutions for these limitations though many efforts have been made in this field including using nanomaterials. We have developed injectable self-setting polyphosphate cements as a new bone graft substitutes. Calcium polyphosphate (CPP) possesses a polyphosphate chain-like structure and represents promising bone filler because of its biocompatibility and stimulation of bone healing. The advantages of CPP cements include controllable setting, excellent anti-washout, strong mechanical strength and sustained drug release. This talk will focus on the physiochemical and biological performances of CPP cement both in vitro and in vivo. The setting mechanism and the interaction between drugs and CPP cements during drug elution will be also discussed. We believe that this new CPP cement represents a unique injectable bone graft substitute with a plethora of applications to musculoskeletal injury.

**Lei Yang** is currently a Professor at the Orthopaedic Institute of Soochow University and anAssociate Director of the International Research Center for Translational Orthopaedics (IRCTO) at Soochow University. He received Ph.D. (2011) in Engineering and M.S. (2011) in Innovation Management and Entrepreneurship from Brown University (Providence, USA), and both M.E. (2006) and B.E. (2004) in Materials Science and Engineering from Tsinghua University (Beijing, China). Before joining in Soochow University, Dr. Yang worked as a Lecturer and Postdoc in the School of Engineering at Brown University. His research interests focus on novel biomaterials for orthopedic applications and mechanistic study on cell-material interactions. He has authored 1 book, 3 book chapters, over 30 journal papers, and 10 patents. He is anAssociated Editor of the *International Journal of Nanomedicine* (SCI IF=4.383) and an Editor of the Book Series of Biomaterials published by China Higher Education Press. He is also the recipient of many awards, including the 2nd place in the China Innovation and Entrepreneurship Competition (2015), the National 1000 Young Talents of China (2013), the US Fresh Face Young Scientist of Sigma Xi (2011), the US Society For Biomaterials Student Award for Outstanding Research (2011), and the US Materials Research Society Graduate Student SilverAward(2010).

# Mechano-activeBiomaterials forTissueRepairandRegeneration


**ABSTRACT:**

On-demand delivery of molecules and cells with high precision is a key topic in controlled release, yet is difficult to achieve in ceramic-based macroporous systems that receive great interests in the fields of drug delivery, tissue repair and regeneration. Recent advances in active biomaterials automatically in response to external stimuli lead to an integrative solution of bio-inspired mechano-active system that can deliver molecules and cells with ultra-high precision. In this talk, I will discuss the design and fabrication of such mechano-active biomaterials for the applications in regenerative medicine.

**Dr. Yufeng Zheng**, received his Ph.D in materials science from Harbin Institute of Technology, China in 1998. From 1998 to 2004 he was Assistant Professor (1998-2000), Associate Professor (2000-2003), Full Professor (2003-2004) at Harbin Institute of Technology, China and since 2004 he has been a Full Professor at the Peking University in Beijing, China. Dr. Zheng has authored or co-authored over 300 scientific peer-reviewed articles, with the citation of over 6900 times, and a H-index of 41 (http://www.researcherid.com/rid/A-4146-2010). He served as the Editor-in-Chief of Bioactive Materials (http://www.keaipublishing.com/en/journals/bioactive-materials/ ), Editor of "Materials Letters" (Elsevier), Member of the editorial board of the Journal of Biomedical Materials Research-Part B: Applied Biomaterials (Wiley), "Journal of Biomaterials and Tissue Engineering" (American Scientific Publishers), "Intermetallics" (Elsevier), "Journal of Materials Science & Technology" (Elsevier), "Acta Metallurgica Sinica (English Letters)" (Springer) and Journal of Orthopaedic Translation (Elsevier). His areas of special interest include the development of various new biomedical metallic materials (biodegradable Mg alloys and Fe alloys, beta-Ti alloys with low elastic modulus, bulk metallic glass, bulk nanocrystalline materials, etc). Dr. Zheng has received several awards including New Century Excellent Talents in University awarded by MOE of China (2007) and Distinguished Young Scholars awarded by NSFC (2012).

# Title：Is it feasible for introducing biodegradable metals into injectable biomaterials or combination devices?


**ABSTRACT:**

Biodegradable metals (BMs) are metals expected to corrode gradually in vivo, with an appropriate host response elicited by released corrosion products, then dissolve completely upon fulfilling the mission to assist with tissue healing with no implant residues. Mg-based BMs demonstrated potential feasibility in the application of bone repair, with the WE43 and Mg-Zn-Ca alloy staples being clinically used for foot/hand surgery in Europe and South Korea, in the form of bulk metallic materials. Since the biodegradable materials had been considered as new kind of bioactive materials, and it may bring some potential chances to be used in the bone augmentation procedures in orthopedics and dentistry. There is the following feasibilities for introducing biodegradable metals into injectable biomaterials and combination devices. The first one is to incorporate biodegradable metal powders into the fabrication of injectable biomaterials, and the second one is to combine the porous biodegradable metal implant with the injectable inorganic bio-ceramic or bioglass. In this talk, we will demonstrate the preliminary studies in literatures towards this target, and expect that it may bring some new opportunities for the design of injectable biomaterials or combination devices.

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FACULTY/HOSPITAL APPOINTMENTS

2005-Present: Professor & Head, Dept. of Spine Surgery, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou,China

2001-2005: Associate professor, Dept. of Orthopedics, The Second Affiliated Hospital, Sun Yat-sen University, Guangzhou,China

1998-2000: Associate professor, Dept. of Surgery, the Second Affiliated Hospital, Henan Medical University, Henan, China

1997-1998: Attending, Dept. of Surgery, the Second Affiliated Hospital, Henan Medical University, Henan, China

1989-1991: Resident, Dept. of Surgery, the Second Affiliated Hospital, Henan Medical University, Henan, China

TRAINING

Minimally invasive spine surgery, 6 months in USA, 2000
Clinical fellowship and research on spine surgery in Orthopedic Department of Hong Kong University, 2004.10-12
AO Spine fellowship., University of California, Los Angeles, USA, 2008.9-11
SRS global outreach traveling fellowship, Hospital for Special Surgery, New York, USA, 2010.Jan-Feb.
Visiting Fellow with Dr Lenke, Washinton University in S. Louise, USA 2015 Jan

AWARDS & HONORS

Maurice E. Müller/SICOT Award, by the International Society of Orthopaedic Surgery and Traumatology(SICOT), 2008.8
SRS Global Outreach Traveling Fellowship, 2009
ISSLS Fellowship, 2012

MEMBERSHIP IN PROFESSIONAL SOCIETIES

Board member of the International Society for Minimal Intervention in Spinal Surgery (ISMISS)
Board member of the Pacific & Asian Society of Minimally Invasive Spine Surgery (PASMISS)
Faculty of the China Chapter, AO Spine International

# Title: Biomaterials in percutaneous vertebraplasty and kyphoplasty:the current usesand the future innovation


## ABSTRACT

Vertebroplasty (PVP) and Kyphoplasty (PKP) are both minimally invasive surgical interventions in treatment of vertebral compression fracturessecondary to osteoporosis, metastatic tumor and multiple myeloma. The key procedure is to inject filling biomaterials into the target vertebra to restore the stability of the vertebra in a certain degree.Polymethylmethacrylate(PMMA) remains one of the most commonly used materials inPKP and PVP due to the acceptable injectability and mechanical properties. Also, for metastatic tumor, PMMA is reported to inactivate tumor cells, reduce local inflammation and innervate local nerve endings by its thermogenic effect during polymerization.However, the local bone regeneration is inhibited as PMMA is neither bioactive nor biodegradable. Also, the potential long-term effect of PMMA in the body is still unknown. Biodegradablecalcium phosphate cement (CPC) and calcium sulfate cement (CSC) are also reported use in PKP and PVP. The clinical outcome in terms of pain release and functional recovery are comparable to those use PMMA cement. Furthermore, the degradability of the cement makes them an alternative use in young patient. However, the injectability and mechanical properties of CPC and CSC are suboptimal. Re-collapse of the vertebra after the initial surgery is reported. Modification of cement by incorporating bioactive irons (e.g. strontium, magnesium), proteins (e.g. BMPs,) and drugs (e.g. bisphosphonate)areinvented and used in PVP or PKP, with encouraging preliminary results. However, long-term effects of these novel cements are unknown. In conclusion, in these recent years, thought efforts have been made to improve the physical and biological properties of biomaterials for the use in PVP and PKP, further studies are still needed to verify the clinical safety and efficacy. The research should direct at making a biomaterial withgood viscosity and injectability, together with optimal biodegradability that match the bone regeneration, and stiffness that balanced the stress between adjacent levels.

# Brief Curriculum Vitae


Family Name: **Kelekis**

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Place of Birth: Psychiko, Attiki Nationality:Greek


High School: Athens College "Apolytirion" Athens, 1989

University Degrees:

Medical Degree: Medical School of Athens

Ph D: University of Athens on the subject: "Application for the transfer of data and images at the 2nd Radiology Dpt of the University of Athens "

12/2014- Associate Professor of Diagnostic and Interventional Musculoskeletal Radiology at the 2nd Radiology Dpt of the University of Athens

6/2009-12/2014 Assistant Professor of Diagnostic and Interventional Musculoskeletal Radiology at the 2nd Radiology Dpt of the University of Athens

7/2004-6/2009 Lecturer on Diagnostic and Interventional Musculoskeletal Radiology at the 2nd Radiology Dpt of the University of Athens

5/2003-7/2004 Consultant on Radiology and Interventional Radiology at the 2nd Radiology Dpt of the University of Athens.

10/2000-10/2002 Intern at the Radiology Department of the Geneva University Hospital of Geneva, Switzerland (Département de
Radiodiagnostique et Radiologie Interventionelle)
Hôpital Cantonal Universitaire de Genève
Professor: F. TERRIER

10/1999-10/2000 Intern at the Radiology Dpt. of the "Free University" of
Brussels, Belgium (Service d'Imagerie Médicale, Hôpital Erasme) Université Libre de Bruxelles, Belgique
Professor: J.STRUYVEN

10/1998-10/1999 Intern at the University Hospital, Liege, Belgium
(Service d'Imagerie Médicale, Hôpital Universitaire,
Université de Liège)
Professor: R.F.DONDELINGER

09/1997-08/1998 Intern at the Radiology Dpt. of the Naval Hospital of Athens (During Military Service)
Director: N.Goudanis, Captain

01/1997-06/1997 Intern at the Internal Medicine Dpt (during Military Service)
Salamina Navy Hospital

Foreign Languages:English (Proficiency) French (Sorbonne I)

# Title : Novel bone augmentation technique on peripheral skeleton


## ABSTRACT

As opposed to spine where cranio-caudal and compression forces are applied, in the peripheral skeleton tensile, shear, torsion and bending forces limit the use of acrylic material. In peripheral skeleton existing polymers do not suffice for structural support and pathologic fractures constitute a significant and common delayed complication (especially in case of cortical bone involvement) of standard osteoplasty. Although recent studies report plain cement injection in the femoral neck as a safe and efficacious technique for pain reduction, large meta-analyses conclude that fracture is the most common delayed complication of such cases. Nowadays various hybrid techniques have been evolved including combinations of PMMA to metallic screws or to a metallic mesh of micro-needles (rebar concept) in order to achieve both pain reduction and structural support in peripheral locations.

If a fracture has occurred, screws can be percutaneously inserted in order to bridge the fracture line and bring the bony elements together. In order to avoid screw loosening and migration insertion should be performed in resistant bone. Alternatively cement injection should be additionally performed around the end of the screw for further stability. In oncologic patients with lytic lesions where therapeutic goals include pain reduction, bone consolidation and impeding fracture prevention, combining PMMA with a metallic mesh of micro-needles (rebar concept) constitutes an attractive alternative. The extent of cortical destruction limits the long-term stability of the rebar since optimal cement dispersion will be rendered by an early leakage.

Both techniques can be combined to ablation for local tumor control, pain reduction and structural support. They can be of extreme use in patients with painful oligometastatic disease improving survival and life quality.

Long term structural support along with pain reduction is the goal in both techniques. In vitro biomechanical evaluation will provide the necessary evidence for the maximum bending power and bending strength of such combinations in order to reach a safe conclusion concerning their efficacy in structural support. Both combinations have unique characteristics which render them ideal for specific peripheral locations and lesions.

**Professor Frankie Leung** is the Chief of Service of Orthopaedics and Traumatology at the Hong Kong University Shenzhen Hospital (HKUSZH) and Chief of Division of Orthopaedic Trauma at Queen Mary Hospital in Hong Kong. His professional appointments include Chairperson of AOTrauma Global Research Commission, member of Trauma sub-committee of SICOT and National Delegates of SICOT and Asia Pacific Orthopaedic Association. He is specialized in complicated fracture management and fracture fixation surgery.Professor Leung has published in various international orthopaedic journals and has authored book chapters in 'AO Manual of Fracture Management- Internal Fixator', 'Rockwood and Green's Fractures in Adults', etc. He has been invited speaker for international conferences and faculty for AOTrauma courses for education of surgeons in the field of orthopaedic trauma.Professor Leung's research interests are bone regeneration and anti-infective therapy for bone infection. Currently he and his team at HKUSZH is establishing a municipal Key Laboratory of Innovative Technology in Orthopaedic Trauma, with the support of the Shenzhen Science and Technology Innovation Commission.

## Title: Clinical application of bone cement in orthopaedics


### ABSTRACT

Polymethylmethacrylate (PMMA) remains one of the most enduring materials in orthopaedic surgery. It was first employed by orthopaedic surgeons over 60 years ago and remains a key component of modern practice. The material is not strictly a cement, but a grout. has a central role in the success of total joint replacement and is also used in newer techniques such as percutaneous vertebroplasty and kyphoplasty. The use of acrylic by orthopaedic surgeons is likely to continue, and knowledge of the properties and applications of this material remains essential. Besides using it as a structural support, it is also being used as a drug carrier.

In traumatic wounds, antibiotic impregnated cement beads or spacers are often used for local antibiotic administration to the soft tissue bed. In addition, the advantages of inserting such a spacer include maintaining a well-defined void to allow for later placement of graft, providing structural support, offloading the implant, and inducing the formation of a biomembrane.Masquelet and Begue proposed that this membrane prevents graft resorption and improves vascularity and corticalization. It has been described that, after the initial placement of the antibiotic impregnated spacer, an interval of 4 to 5 weeks is needed for development and maturation of a biologically active membrane that is suitable for grafting. The spacer also maintains the defect and inhibits fibrous ingrowth. For future development, the release properties and presence of bio-absorbability of the cement spacer would be of great interest. Another big issue is how to avoid multiple surgeries by altering the properties of the spacer.

Another potential use of bone cement is in fracture surgery. Fracture augmentation for osteopenic bone is a widely accepted concept but not commonly used due to the difficulty with application of the materials in conjunction with surgical implants in fracture repair. Augmentation of the surgical construct with a flowable hardsetting material to replace the structural voids and deficiencies seems to be an optimal solution. There are newer implants with perforations which allow cement to flow through and into neighboring bone. However, the efficacy and side effects still need to be demonstrated.

**Dr. Songlin (James) Peng** is an assistant professor in the Departmental of Spine Surgery,Shenzhen People's Hospital, Jinan University School of Medicine, which is the top medical center in Shenzhen city. Dr. Peng obtains his M.D degree from the medical school from mainland of China and his Ph.D degree from the University of Hong Kong. As a spine surgeon, he treats a diverse array of degenerative spinal conditions. He has a specific research interest in cellular and molecular mechanisms for pathogenesis of Osteoporosis and the treatment of osteoporotic vertebral fracture with minimal invasive PVP/PKP. He has published more than 20 peer-reviewed articles in the area of bone and mineral disorders. Dr. Peng is the only spine surgeon representing China to attend one-year research training with the support from AO-Spine in 2015. He is also the only spine surgeon from mainland of China to obtain the AO Start-up grant till now.

## Title: Management of Osteoporotic Vertebral Fracture Recurrence after Percutaneous Kyphoplasty


### ABSTRACT

Osteoporosis is a growing health concern as the number of aged population is growing worldwide. Vertebral compression fractures due to osteoporosis is quite common and cause great morbidity to the aged population in China. Various percutaneous methods have been developed to aid in treatment, including percutaneous vertebroplasty or kyphoplasty (PVP or PKP). In the clinical setting, one of the challenges for the spine surgeons is the recurrent vertebral fracture after PVP/PKP. A number of risk factors contribute to this phenomenon, among which the deficiency/insufficiently of vitamin D is the most common problems for those postmenopausal women in southern of China with recurrent vertebral fractures after the procedures. After the sufficient vitamin D supplement and the anabolic treatment with teriparatide. Those cases with recurrent vertebral fractures have a great improvement in terms of pain relief, falls and fractures.

**Xu Bao-Shan**, Medical Doctor, MD, PhD

1998.9-2001.6: Postgraduate Department of Suzhou University. Doctor

**PROFESSIONAL EXPERIENCE**

**Internship and Residencies**

1994.6-1995.6: Affiliated Hospital of Shandong Medical University. Interne

1996.3-1998.6: Departments of surgery, Orthopaedics, Shandong Provincial Hospital. Resident

1999.3-2001.6: Department of Orthopaedics, First Affiliated Hospital of Suzhou University. Resident

**Fellowship**

2006.3-2007.4: Spine Unit Pr Le Huec, CHU Pellegrin Tripode, Univ Bordeaux 2, France. Visiting scholar

2006.11-2007.4: Service de Neurochirurgie, Dr. Jean Destandau, Hopital Bagatelle, Talence, France.

2011.7-2011.8: Dep. Spine surgery, Prof. Wong chung-chek, Sarawak general hospital, Kuching, Malaysia.

**Licenses**

2001.7-2003.12: Department of Orthopaedic oncology, Tianjin Hospital. Attending doctor

2004.1-2008.18: Department of Spinal Surgery, Tianjin Hospital. Associate professor

2009.1-Present: Department of Spinal Surgery, Tianjin Hospital. Professor

2015.4-present: Dept. minimally invasive spinal surgery, Tianjin Hospital, Director

**Academic Appointment**

2005-Present: Tutor of master postgraduate of Orthopaedics, Tianjin Medical University

2014-Present: Tutor of doctor postgraduate of Orthopaedics, Tianjin Medical University

**Professional Appointment**

Committeeman of Minimally Invasive Spinal Surgery Group of Chinese Rehabilitation Medical Association

Committeeman of Minimally Invasive Surgery Group of Chinese Orthopaedic Association

Committeeman of Chinese Geriatric Spine and Joint Disease Society

Committeeman of Minimally Invasive Surgery Group of Chinese Association of Orthopaedic Surgeon

Committeeman of Spinal Endoscopic Expert Group of Chinese Association of Orthopaedic Surgeon

Editorial Board member of Chinese Journal of Orthopaedics

Chairman of Minimally Invasive Surgery Group of Tianjin Orthopaedic Association

**Academic Interests**

Minimally Invasive Spinal Surgery, Intervertebral Disc Bioengineering, Degenerative Spinal Diseases, Spinal Deformity&Scoliosis

**Awards**

The second grade of “Science and Technology Advancement Prize” in Tianjin.

The third grade of “Science and Technology Advancement Prize” in Jiangsu Province.

## Abstract:

## The application of injecatble biomaterials for verterbroplasty and its related research.

**Dr. Nikos Mattheos** graduated from the Dental Faculty University of Athens. He completed his PhD degree in the University of Malmö, in Sweden where he also received specialist training in Periodontology. He has completed a 3-year residency with focus on Implant Dentistry and Fixed Prosthodontics in the department of Periodontology and Fixed Prosthodontics in the University of Bern, Switzerland under Professor N.P Lang. He received the position of Associate Professor (docent) in the University of Malmo, Sweden in 2008. His research is disseminated through more than 70 publications in international peer reviewed journals and he has received the IADR researcher's award in 2003 and 2013. He is currently Associate Professor in the Faculty of Dentistry, the University of Hong Kong, where directs two postgraduate programmes in Implant Dentistry and is active with teaching, research and patient care.

# Title: Guided Bone Regeneration of the dentoalveolar ridge: Potential, limitations and future directions


## ABSTRACT

Resorbtion of the dentoalveolar ridge is one direct consequence of edentulism, which can have significant implications for tooth replacement and patient's quality of life. The principles of Guided Bone Regeneration (GBR) include the separation of tissues with osseogenic potential through a barrier resorbable of non-resorbable membrane and application of a space maintaining bone substitute. Current reconstruction techniques of the edentulous ridge involve typically the application of xenografts, synthetic grafts or autologous bone block grafts, all however with significant limitations. This presentation intends to review in brief the current techniques and devices for dentoalveolar reconstruction, as well as point to the most promising directions for future developments in this field.

**Prof. Chengtie Wu** is now working in Shanghai Institute of Ceramics, Chinese Academy of Sciences (SIC, CAS). He completed his Ph.D in 2006, and then he worked in the University of Sydney, Dresden University of Technology, Germany and Queensland University of Technology where he was awarded Vice-Chancellor Research Fellow, APDI Fellow and Alexander von Humboldt Fellow. In 2012, Dr Wu has been recruited to work in SIC, CAS, as One-Hundred Talent Program of Chinese Academy of Sciences. Then he was awarded Recruitment Program of Global Young Experts of China (One-Thousand Young Talent Program), Shanghai Pujiang Talent Program and Shanghai Outstanding Academic Leaders. Prof Wu's research focuses on bioactive inorganic materials for bone tissue engineering. Up to now, Prof Wu has published more than 140 SCI peer-review journal papers, including Mater Today, Adv Funct Mater, Biomaterials, Chem Sci, Adv Mater Interface, Small, J Control Release, Acta Biomater, Carbon, J Mater Chem, ACS Appl Mater& Interface, Bone, Tissue Eng. etc. The papers have been cited more than 3400 times, H Index 34 via SCI, Web of Science. Prof Wu has been awarded 13 patents, in which 3 of them have been transferred to companies.

# 3D-Printing of bioscaffolds for bone therapy and regeneration

Chengtie Wu
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**ABSTRACT**

For therapy and regeneration of bone defects resulting from malignant bone disease, it is of great importance to develop multifunctional biomaterials for bone therapy and regeneration. Conventional biomaterials always lack multifunctional properties, limiting their application for treating and repairing bone disease (e.g. bone tumors)-initiated defects. How to design and prepare bioscaffolds with favorable microenvironments for disease therapy and tissue regeneration is one of interesting topics in the fields of biomaterials and tissue engineering. We developed several strategies, including harnessing nutrient elements, biomimetic structure and functional interface as well as thermo-therapy to construct multifunctional scaffolds by 3D-Pringting method for therapy and regeneration of bone tissues. It is interesting to find that both nutrient elements and biomimetic structure of the printed bioscaffolds have important effect on the stimulation of osteogenesis and angiogenesis of stem cells, and thermotherapy plays an important role to treating bone tumors. Therefore, we put forward new concept that 3D-Printed bioscaffolds combined bone therapy and regeneration could be a new direction of bone tissue engineering.

**Dr. Zhidao Xia** is a Senior Lecturer in Regenerative Medicine in Swansea University Medical School, Swansea, United Kingdom. He was awarded PhD in Oxford University in 2005 and worked as a Senior Research Fellow in Oxford University before he moved to Swansea University in 2010. His main research interests are skeletal tissue regeneration, including the application of human mesenchymal stem cells, biomaterials and tissue engineering techniques to improve tissue regeneration of bone, cartilage and tendon. He also has a particular interest and expertise on cell-biomaterial interface, such as macrophagic response to biomaterials, and the fate of stem cell/biomaterial construct in vivo. His current research focus on the development of biodegradable bone implants using 3D printing technology.

# Title: Development of Biodegradable Bone Graft Substitutes Using 3D Printing Technology


## ABSTRACT

Bone defects caused by tumor, bone diseases and trauma require bone graft substitutes as scaffolds to fill the voids, initiate osteogenesis and restore the structure and function of bone tissue. Autografts are the gold standard for repairing bone defects, but their usage is limited by donor-site morbidity and availability. Allografts and xenografts may have potential immunogenic responses by the host and the potential for disease transmission. Synthetic biomaterials such as ceramics, bone cements and bioglass have been widely used but their composition, porosity, biodegradation and mechanic property are different from bone tissue. Chemically converted natural coral has unique bone-like microstructure and porosity, and also been used successfully as bone graft substitute but their application is restricted due to limited natural resources. 3D printing is a new technology that provides rapid prototyping and fine control of complex structure at micrometer scale. We have developed a new type of biodegradable bone graft substitutes using selective laser sintering (SLS) 3D printing technology. The composition, porosity and microstructure of this biomaterial are similar to coralline hydroxyapatite, but it can be produced at low costs with unlimited supplying. In vitro assessments of material characterization, cytotoxicity using L929 cells and osteogenic potential using human osteoblasts and mesenchymal stem cells were performed which demonstrated comparable results with coralline hydroxyapatite. Further research is warranted to improve the mechanic property and in vivo osteogenic capacity before potential clinical application.

**Dr. Haobo Pan**, was born in Shanghai, China in 1978. He obtained his BS degree from the Department of Materials Science and Engineering at Shanghai University. Then, he continued his MS and PhD degree respectively at the University of Missouri (USA) and theUniversity of Hong Kong, focused on the study of bioceramics and dental biomaterials. In 2007, he started his research career as a postdoctoral fellow at the Li Ka Shine Faculty of Medicine in the University of Hong Kong, and promoted to Research Assistant Professor at the Department of Orthopaedics and Traumatology in the University of Hong Kong in 2010. In 2012, he was appointed as the Director of the Research Center in Human Tissues and organs Degeneration, Shenzhen Institute of Advanced Technology, Chinese Academy of Science. Later, he was promoted as the Vice Director of the Institute of Biotechnology and Biomedicine and appointed as the Director of Shenzhen Key laboratory in Marine Biomaterials. Now, he is appointed as Vice Director of the National Unit of Orthopaedic Biomaterials in China. In the past ten years, Dr. Pan focused in the study of Marine Biomaterials for Biomedical Application. He has published more than 60 papers in qualified international journals and served as board member of ISRN Biomaterials and Journal of Osteoporosis and Physical Activity. In 2013, Dr. Pan created Shenzhen Healthemes Co. Ltd. for biomaterials commercialization.

## Title：Alkaline biodegradable implants for osteoporotic bone defects—importance of microenvironment pH


### ABSTRACT

The biocompatibility and bioactivity of orthopaedic implant materials are directly influenced by the local microenvironment generated after implantation. The microenvironment pH (μe-pH) is critical to the effectiveness of the implants in repairing osteoporotic bone defects, but has seldom been discussed. The purpose of this study was to determine the μe-pH for some orthopaedic implant materials in vivo, and to investigate its effect on the bone defect healing process. Ovariectomized rat bone defects were filled with one of three materials (β-tricalcium phosphate, calcium silicate, 10% strontium-substituted calcium silicate), and μe-pH was measured by pH microelectrode at various intervals post-surgery. The specific tissue responses, new osteoid and Tartrate-Resistant Acid Phosphatase (TRAP)-positive osteoclast-like cells were visualized by histological staining. Results from parallel in vitro experiments were consistent with the in vivo outcome. The intermediate layer between implants and new bone area was studied using energy-dispersive X-ray spectroscopy (EDX). Regardless of material, higher initial μe-pH was associated with more new bone formation, late response of TRAP-positive osteoclast-like cells, and the development of an intermediate ‘apatitic’ layer in vivo. EDX suggested that residual material may influence μe-pH even 9 weeks post-surgery. In vitro, weak alkaline conditions stimulated osteoporotic rat bone marrow stromal cell (oBMSC) differentiation, while inhibiting the formation of osteoclasts. Thus, the μe-pH of implanted materials directly affects their effectiveness in healing bone defects. We therefore propose for the first time to determine specifically the μe-pH for implants as part of the design of orthopaedic implant materials to improve their bioactivity and efficacy.

# PROFESSOR DAVID R HAYNES, Ph.D

Deputy Head of the School of Medicine
University of Adelaide

**PROF. HAYNES** has published well over 100 publications in respected peer reviewed journals. Over the past two decades he has developed a respected international reputation in the fields of bone pathologies, biomaterials and inflammation. Over the past decade he has been President and Secretary of the Australian and New Zealand Society of Orthopaedic Research (www.ANZORS.org.au). In these roles he has help organise more than 5 national and 3 international meetings. Associate Professor Haynes has been chief investigator on more than 17 successful major national and international grants (NH&MRC, European Union Framework 7 and ARC) grants since the early 1990's as well as several commercially funded studies on pharmacological regulation of inflammatory cytokines, pathogenic bone loss and implant loosening. He also makes a significant contribution to the training of medical students (MBBS), Health Science students and Nursing students in the Faculty of Health Sciences.

# Bioengineering of hydroxyapatite coatings for optimal performance

Professor David R Haynes, ANZORS
School of Medicine, University of Adelaide, Australia

For many elderly people breaking a hip is not an inconvenience, it is a death sentence. Lack of physical mobility results in decreased fitness and a rapid decline in heath. In Australia approximately ½ of women and 1/3 of men over 60 will have a fracture at some stage with recurrent fracturing being very common (www.osteoporosis.org.au). Repair often requires the use of bone implants, such as, various fixation devices and artificial joints. In order to be effective these structures need to be well integrated into the bone tissues and in the elderly, who often have poor bone health, this is not always the case. While the use of HA coatings has been relatively positive an approach to improve these coatings that promotes development of healthy bone cell metabolism is now required.

Two important functions are needed for the formation and maintenance of healthy bone. Firstly, the proliferation and differentiation of mesenchymal stem cells (MSC) into bone forming cells and secondly, the differentiation of cells of the monocyte/macrophage lineage into bone resorbing osteoclasts. It is important to realize that we need both the anabolic and catabolic activities as both osteoclast resorption and osteoblast bone formation are intimately related [2]. However, MSC and monocyte/macrophage cell populations can have considerable variability of morphology, proliferation and differentiation. Bone marrow derived MSC have the potential to mature into several cell types [1] and osteoclasts formation is complex.

This talk describes tests we currently use to identify potential hydroxyapatite coatings. We can now manipulate hydroxyapatite to vary in many ways, such as, surface texture, chemical composition or the attachment of various molecules/factors. Our recent findings confirm that such variations can influence osteoclast maturation and functions. In addition, this can also influence MSC transcriptional activity of markers associated with proliferation, differentiation and maintenance of ‘stemness’ (undifferentiated state) and ability to differentiate into bone forming cells.

[1]. Arthur A, Zannettino A, Gronthos S. The therapeutic applications of multipotential mesenchymal/stromal stem cells in skeletal tissue repair. J Cell Physiol 2008.

[2]. Osteoclast-derived activity in the coupling of bone formation to resorption. T. John Martin, Natalie A. Sims Trends in Mol Med. 11 76-81 2005.

**Dr. Qin** is Professor and Director of Musculoskeletal Research Laboratory in the Department of Orthopaedics & Traumatology, the Chinese University of Hong Kong (www.ort.cuhk.edu.hk). Dr. Qin also holds joint professorship in Shenzhen Institutes of Advance Technology (SIAT) of Chinese Academy of Sciences (CAS) and serves Director of the Translational Medicine Research & Development Center of Institute of Biomedical & Health Engineering of SIAT (www.siat.cas.cn). He received his B.Ed and M.Ed. in sports medical sciences at the Beijing University of Physical Education in China, and his Ph.D. at the Institute of Experimental Morphology at the German Sports University, Cologne, Germany and postdoc in AO-Research Institute, Davos, Switzerland. Dr. Qin was research scientist in the Department of Trauma & Reconstructive Surgery, University Clinic Rudolf Virchow, Free University Berlin (now known as Charite Medical University), Germany, before joining CUHK in late 1994.

Dr. Qin has been working on advanced diagnosis, prevention and treatment of bone metabolic disorders, especially osteoporosis and osteonecrosis, in collaboration with research and clinical scientists in medicine, geriatrics, rheumatologists, traditional medicine, and biomaterials. Dr. Qin is the past President of the International Chinese Musculoskeletal Research Society (ICMRS) (www.icmrs.net) and member of a number of journal editorial boards, including Editor-in-chief of Journal of Orthopaedic Translation (http://ees.elsevier.com/jot); Associate Editor of Clinical Biomechanics and Chinese Journal of Orthopaedic Surgery; editorial member of a number of international journals, including Journal of Bone and Mineral Research (www.jbmr.org) and International Journal of Sports Medicine (http://www.thieme.de/sportsmed). He holds memberships in several international and national orthopaedic and related research organizations, including collage fellow of American Institute of Medical and Biological Engineering (http://www.aimbe.org). As Principle Investigator, Dr. Qin has received over 30 competitive research grants (including CRF, GRF, ITF, HMRF, NSFC-RGC, and EU-NSFC) and over 30 research awards. Dr. Qin also holds 8 new invention or new utility patents.

Dr. Qin published 9 monographs as editor or associate editor, 5 conference proceedings, 80 book chapters, over 400 journal papers in English, German, and Chinese, including 300 SCI articles published in Nat Med, JBMR, Osteoporosis Int, Bone, A&R, Biomaterials, Acta Biomaterialia, Am J Sports Med, Int J Sports Med, etc. with citation >5000 and a H-index of 42.

# Title: R&D of Mg-based Biometal for its translation for orthopaedic applications

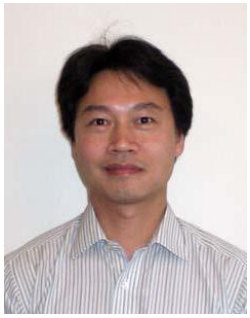

## ABSTRACT

It is known that magnesium (Mg) is the eighth most common element in the crust of the earth and now attracts great attention to become biodegradable or biocorrosive medical implants for both cardiovascular and orthopaedic applications.

In orthopaedics, Mg and its alloys are mainly considered suitable for degradable bone implants with good initial stability that avoid a second surgical procedure to remove the temporary metallic parts for fixation after the tissue has sufficiently healed, apart from lowering overall associated health care costs. Safety concerns are also raised although Mg dissolution is unlikely to have adverse or side effects since Mg is the fourth most plentiful cation in the human body, including involvement in the formation of biological crystal apatite; it is also a co-factor for many enzymes and stabilizes the structures of DNA and RNA; beneficial from a physiological standpoint, since Mg deficiencies in human body will result in disorders of metabolic organs and cardiovascular system as well.

The author's group is conducting R&D of orthopaedic implants, in collaboration with biomaterial scientists for developing Mg and its alloys as biocorrosive orthopaedic implants in terms of fixation implants, scaffolds or injectable materials. We are investigating their bone stimulation effects physiologically and biologically using both in vitro and in vivo preclinical experimental models. Human pilot or Phase I studies are also conducted to investigate its biosafety as well as its efficacy for adequate orthopaedic indications, including its potential for its application or contra indication in bone tumor.

The presentation generated from this R&D program on Mg or its alloys is based on a translational R&D model and roadmap, i.e. from Observations to Mechanisms and then to Proof-of-Concept before clinical tests, followed by clinical trials. Testing standards and guidelines are also essential for this translational roadmap and the author's group has also been working on necessary modification for current ISO standard for testing cytotoxicity relevant for biodegradable implants.



# PROFESSOR JIAKE XU, PH.D

Winthrop Professor and Head of Laboratory in the School of Pathology and Laboratory Medicine at the University of Western Australia

Founding Fellow, Faculty of Science, the Royal College of Pathologists of Australia

**PROF. XU** completed his PhD studies at UWA in 1994, and carried out his postdoctoral research at Stanford University from 1994 to 1998. He returned to UWA in 1998, and has since undertaken research and teaching in the Schools of Pathology and Laboratory Medicine. He has served as the President of the Australian and New Zealand Orthopaedic Research Society (ANZORS, 2012-2015). His current research activities are focused on gene discovery, molecular mechanisms of osteoclast functions and the intercellular communication between osteoclasts, osteoblasts and endothelial cells in bone microenvironment, which have significant implication in bone diseases; including osteoporosis, Paget's disease of bone and malignancy-related osteolysis. He has published over 130 SCI papers, including Nature Medicine, Endocrine Reviews, PNAS, Annals of the Rheumatic Diseases, J Bone Miner Res., Mol. Cell. Biol., J Biol. Chem., Arthritis Rheum., Stem Cells, and Biomaterials.

# Angiogenic factors in bone microenvironment: potential therapeutic targets for bone repair

Jiake Xu

School of Pathology and Laboratory Medicine, the University of Western Australia, Perth, Australia

Angiogenesis plays an important role in physiological bone growth and remodeling, as well as in pathological bone disorders such as delayed fracture repair, osteonecrosis, and tumor metastasis to bone. Angiogenic factors, produced by cells from a basic multicellular unit (BMU) within the bone remodeling compartment (BRC) regulate local endothelial cells and pericytes. The expression of angiogenic factors by osteoclasts, osteoblasts and osteocytes in the BMU and in the cartilage-bone interface is evident. These include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), BMP7, and epidermal growth factor (EGF)-like family members. In addition, the expression of EGFL2, EGFL3, EGFL5, EGFL6, EGFL7, EGFL8 and EGFL9 has been recently identified in the bone local environment, giving important clues to their roles in angiogenesis and bone homeostasis. Understanding the role of angiogenic factors in the bone microenvironment may help to develop novel therapeutic targets and diagnostic biomarkers for bone and joint diseases, such as osteoporosis, osteonecrosis, osteoarthritis, and delayed fracture healing.

## ASSOCIATE PROF. WANG GUOCHENG，PH.D

Center for Human Tissues and Organs Degeneration
Institute of Biomedicine and Biotechnology
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# Refining nanotopographical features on bone implant surfaces

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Plasma sprayed is a well-known surface modification technique, it has been widely used in many industry fields. In biomedical field, it has been commercialized to produce hydroxyapatite and titanium coated orthopaedic implants. In the last decades, a large amount of studies have been carried out to develop new coating products with plasma spraying technique, aiming to overcome the drawbacks of the currently used hydroxyapatite coated implants. Most of the studies have been conducted using coating materials with different chemical compositions, however, seldom of them focused on the exploration of how to make innovative application of this traditional surface technique for better serving the development of new biomedical coatings. The effects of nanotopography on responses of bone-related cells have been well-documented, making "how to generate and refine nanotopography of plasma sprayed biomedical coatings as well as how they influence their biological performance" especially important in the modern biomedical coating design. In our previous work, attempts were made to address these questions by several independent studies. In this proceeding, we are going to summarize our previous studies with a purpose to address the following three questions: 1. the effects of nanotopography on bone cell adhesion, 2. the effects of nanotopography on bone-like apatite formation in simulated body fluid. 3. How to refine the nanotopography of plasma sprayed coatings.

# Posters

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Mapping the evolution of brushite cement degradation

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**INTRODUCTION:** Brushite calcium phosphate (CaP) cements have attracted interest as injectable bone graft biomaterials due to their oestoconductive and resorbable properties[1]. However the degradation and bioactivity of the implanted cement is highly dependent on the aqueous environment, and is poorly understood having been shown to vary significantly in the literature[2]. Analysis of samples aged *in-vitro* and *in-situ* has largely depended on methods that give high resolution morphological, and overall bulk chemical information on the material at a given time point, but with low overlap of information types limiting the understanding[1, 2].

In this study we aimed to fill the information gap of the current analytical methods, and provide further means to monitor cement degradation and help elucidate the bioactivity. Using confocal Raman microscopy (CRM) to map and show the evolution of phase changes in brushite cement cylinders that were dynamically aged under physiological conditions in a range of media.

**METHODS:** Cylinder ageing: brushite cement cylinders (12x6mm) were produced from β-tricalcium phosphate (TCP) and 3.5M orthophosphoric acid mixed at a ratio of 1.75g/ml and left to set overnight. Cylinders were sterilised under UV light then aged in 20ml of either phosphate buffered saline (PBS), Dulbecco's modified eagle medium, or fetal bovine serum, maintained at 37°C and changed every 24 hrs. Image Mapping: cylinders were removed from media at staggered time points, dried, cut to expose the cross section and planed to a smooth surface. Image mapping was performed using confocal Raman microscopy with a 514nm 60mW laser. Computational analysis: Data was exported to Matlab and baseline corrected. Clustering was performed to assign each pixel to the dominant phase of its' associated spectrum. Observations were compared with XRD, micro-CT and SEM.

**RESULTS:** Mapping of $PO_4$ peaks associated with brushite, β-TCP and octacalcium phosphate (OCP) allowed the evolution of each to be seen. The time series using PBS media that showed a clear development from an initially almost homogeneous distribution of brushite, to a distinct three phase structure, as the outer layer of brushite underwent dissolution via hydrolysis, leaving a TCP scaffold that was subsequently seeded by OCP (Fig 1).

Comparison to micro-CT, XRD and SEM data validated these results. In media containing serum the degradation of brushite and subsequent seeding of OCP was not observed.

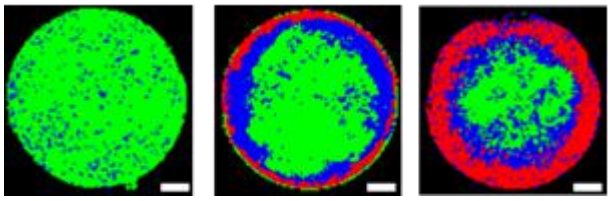

*Fig. 1: CRM mappings of the cylinder cross-section showing the dominant CaP phase at each spatial location for (A) 0, (B) 10 and (C) 50 days. Colour key: Green - brushite, blue - TCP, red -OCP. Scale bar 1mm.*

**DISCUSSION & CONCLUSIONS:** This study has shown important steps in improving cement degradation understanding. In particular the results showing a gap between the receding brushite and precipitating OCP where the scaffold is formed only of TPC could have important consequences on the mechanical properties and biological activity across the cement. This provides a complementary addition to the currently used imaging and analysis modalities, by providing spatial chemical information that is not dependent on complex processing or the quantity of crystalline material in the sample.

We have shown that high resolution chemical mapping of calcium phosphate cements can be used to visualise and quantify chemical changes resulting from ageing under physiological conditions. This level of information may allow a significant insight into the degradation of implants, enabling improved material optimisation.

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**ACKNOWLEDGEMENTS:** The authors thank the EPSRC for providing financial support via the PSIBS DTC for this project.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Study on akermanite/PMMA composite bone cement

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**INTRODUCTION:** Polymethyl methacrylate (PMMA) bone cement has been widely used for prosthetic fixation or as a bone substitute in orthopedics owing to its desirable mechanical strength, relatively low toxicity and good handlibility in the surgical room [1]. However, the major shortcomings of PMMA bone cement are its low osseointegration rate and harmful exothermic reactions [2]. An effective approach to solve the clinical problems of PMMA cement is the incorporation bioactive inorganic fillers [3]. Akermanite ($Ca_2MgSi_2O_7$, AKT) possesses excellent osteostimulation ability and controlled biodegradability [4], making it an ideal bioactive filler for PMMA cement. The purpose of this study is, therefore, to harness the advantages of both PMMA and AKT in order to prepare a new kind of composite bone cements with comparable mechanical strength, weakened exothermic effect and improved osteogenic activity as compared with PMMA cement.

**METHODS:** Akermanite powders were synthesized by the sol–gel process. A commercial PMMA cement (Tianjin Synthetic Material Research Institute®) was used for the study. Composite bone cements with different akermanite weight ratio, namely 0, 10%, 30% and 50%, were prepared by mixing the powder with the liquid at a PMMA powder-to-liquid ratio of 2:1(g/ml) under ambient conditions at room temperature. The setting properties and mechanical strength of the bone cements were investigated according to the standard ISO 5833/1. The *in vitro* bioactivity of the composite cements was assessed by soaking in simulated body fluid. Furthermore, the effect of the composite cement on the proliferation and differentiation of osteoblast cells was investigated by culturing the cement with cells.

**RESULTS:** AKT powders were uniformly dispersed and interacted with the PMMA matrix, and there was no discernible defect within the composite bone cements. The peak temperature of polymerization of bone cements, which was lower than that of pure PMMA bone cement, decreased with the increasing AKT content. The AKT/PMMA composite bone cements still maintained desirable setting time (12~14 min) and compressive strength (~ 100MPa). GIXRD and SEM witnessed that the AKT/PMMA composite bone cement induced the formation of hydroxyapatite on its surface in simulated body fluid. Both PMMA and AKT/PMMA bone cements were able to support cells adhesion, while the cells on the surface of AKT/PMMA bone cements showed a better spreading behavior. Furthermore, significantly higher increase in cell number and ALP

activity was observed for MC3T3 cells cultured on AKT/PMMA than on PMMA.

**DISCUSSION & CONCLUSIONS:** New bioactive AKT/PMMA composite bone cements were successfully prepared. The incorporation of AKT into PMMA significantly lowered the polymerization temperature of the PMMA matrix, whilst the as-formed composite cement possessed proper setting time and high mechanical strength. Furthermore, AKT/PMMA bone cements showed better osteogenic activity. The improvement of cell proliferation and bone-related differentiation on the surface of the AKT/PMMA composite cement can be attributed to its advantages over PMMA cement, including the ability to induce the formation of apatite layer, releasing Ca, Mg and Si ions from the surface, and beneficial pH microenvironment. Our results indicated that the new AKT/PMMA composite bone cement may be a promising candidate material for orthopedic applications.

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**ACKNOWLEDGEMENTS**: This work was supported by grants from the Natural Science Foundation of China (grants no. 81190132), the Funds of the Clinical Research Center for Biomaterials, Shanghai Institute of Ceramics, CAS (grants no. BMCRC2010001), and One Hundred Talent Project, SIC-CAS (Y36ZB1110G).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Discovery of a novel injectable starch gel for use in heart and joint repair

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**INTRODUCTION:** The field of gels has been avidly studied due to its potential to revolutionize healthcare, electronics and materials science.[1] A novel gel was synthesized by combining $Ca^{2+}$ ionic crosslinkers and starch in aqueous solution. This innovative biomaterial is not only cheap and easy to synthesize, but its conductivity, injectability, and high stretchability have the capacity to play an important role in treating myocardial infarction and alleviating cartilage damage in joints via minimally invasive injection methods.

**METHODS:** The samples were elongated to investigate the stretchability. Self-healing tests were performed by elongating the sample until fracture, restoring the fractured halves to their original state, and then rerunning the test to examine the overlap of the resultant graphs.

Stickiness data was obtained by covering two stainless steel probes with paper, aluminium, or pig's heart tissue. A sample was placed in the middle, compressed to a specific force, and then elongated until fracture. Maximum stress was measured to evaluate the stickiness.

Injectability was measured by inserting the gel into a syringe and pressing with a maximum force of 50 N to simulate the average human grip strength. The final weight of the injected gel was measured against the initial weight to quantify the ease of injection.

Conductivity measurements were performed using an AUTOLAB 86631 electrochemical workstation (Metrohm AG) with Pt electrodes and rheology measurements were performed using an AR2000 rheometer. All aforementioned experiments were performed on 33wt% (full), 20wt% (half), and 14wt% (third) amounts of $Ca^{2+}$ ionic crosslinker.

**RESULTS:** The gel demonstrated a combination of self-healing properties and high stretchability with stretch ratios of the full sample consistently reaching $\lambda = 22$ or higher before fracture.

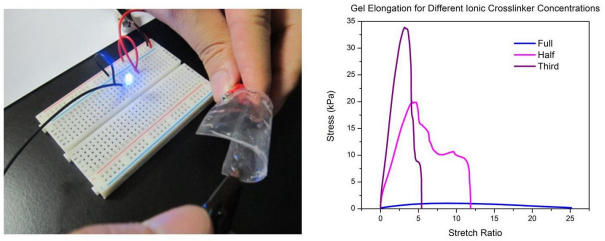


*Fig. 1: **(a)** Full gel sample conducting electricity to illuminate an LED. **(b)** Stress vs. stretch ratio graph showing elongation of all three samples until fracture.*

Stickiness and stiffness were found to be closely correlated: the maximum stress values from the stickiness test increased from 2.38 kPa to 6.86 kPa (on an aluminium surface) for the full and half samples, respectively. In parallel, the maximum stress values in Fig 1b also increased with decreasing ionic crosslinker content, belying higher stiffness.

Conductivity of the gel sample was measured to be between $10^{-2}$ and $10^{-4}$ $S \cdot cm^{-1}$ for the full and half samples, respectively. The injectability test showed 98% extrusion for the full sample, and decreased with decreasing ionic crosslinker.

**DISCUSSION & CONCLUSIONS:** This novel gel exhibits high stickiness, stretchability, and conductivity, and is stable at gel point for all ionic crosslinker concentrations. The high correlation between the ionic crosslinker content and mechanical and electrical properties is most likely due to the competing mechanisms of $Ca^{2+}$ and starch. This can provide opportunities to tailor the mechanical and electrical properties of the gel to better treat myocardial infarction and aid in joint repair using approaches involving injection.

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European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Characterisation and properties of silver-loaded injectable bone cement

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**INTRODUCTION:** With the development of minimally invasive surgical techniques, there is a growing interest in the development of injectable bone cements especially for orthopedic applications [1-2]. Some important issues such as improvement of resorption capability and better protection against implant-associated infections are major challenges in this field [3-4]. The objective of this study is to evaluate the impact of introducing silver and carboxymethylcellulose (CMC) into a calcium phosphate – calcium carbonate mixed cement on its physico-chemical and biological properties.

**METHODS:** Reference cement was prepared by mixing the liquid phase (deionised water) with the solid phase composed of equal mass of vaterite-$CaCO_3$ and brushite $CaHPO_4$, $2H_2O$. Silver-loaded composite cements were made by mixing an appropriate amount of silver phosphate salt ($Ag_3PO_4$) and carboxymethylcellulose (CMC) powder with the solid phase of the mineral cement. The composite cements were characterised *in vitro* in terms of physico-chemistry and handling (composition, structure, microstructure, setting reaction kinetics, setting time, injectability), biological activity (antibacterialness on *S. aureus* and *S. epidermidis;* activity of human bone marrow stromal cells (HBMSC)), and after implantation *in vivo* in rabbit femoral condyle.

**RESULTS:** The results showed that the introduction of a silver phosphate and CMC into the cement composition did not change the setting reaction mechanism: brushite dissolved and reacted with some vaterite to form an apatite phase. However, depending of its concentration the presence of CMC can delay or accelerate the dissolution-reprecipitation reactions while the setting time was delayed in all cases. The introduction of CMC (2 and 10% w/w) in the mineral matrix greatly improved the injectability of the cement paste: the phase separation (filter-pressing) phenomenon no longer occurs during paste extrusion and the force needed to extrude the paste remained constant and around 50 N.

*In vitro* biological studies showed that silver-loaded $CaCO_3$-CaP-CMC cements had antibacterial properties (anti-adhesion and anti-biofilm formation) without a toxic effect on HBMSC cells and allowed determining an optimum range of silver concentration (minimum of Ag content: 0.0375 % w/w in the solid phase). The *in vivo* implantation of selected compositions presented very promising results in terms of resorbability: indeed, after six weeks of implantation, the residual composite cement accounted for 35% of the surface of the explant histological cross-sections, and new bone was present in about 20% of the surface, not only in the periphery of the implant but also in the bulk of the material.

**DISCUSSION & CONCLUSIONS:** This study contributes to understanding the complex physical-chemical reactions and/or interactions involved in silver-loaded $CaCO_3$-CaP-CMC composite cement paste.The possibility to control cement handling, physico-chemical and biological properties by introducing silver and CMC additives makes this biomimetic composite cement a promising candidate especially for the prevention of bone implant-associated infections. In addition, we will discussed the interesting properties of these composite cements in terms of cohesion, ductility, and toughness, thanks to the entangled polymeric CMC network, which could prevent the release of mineral particles in the body fluids after implantation.

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**ACKNOWLEDGEMENTS:** The authors thank the French Agence Nationale de la Recherche for supporting this research (grant n°BIOSINJECT-ANR-09-TECS-004).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Preparation, handling properties and bioactivity of an injectable borate bioactive glass cement

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**INTRODUCTION:** Large bone defects resulting from trauma, malignancy, infection and congenital diseases are a common occurrence in orthopedic and craniofacial surgery. There have increased the need for synthetic bone graft substitutes [1]. Injectable cements composed of bioactive glasses (BGs) represent a new class of bone substitutes [2]. The objective of this study was to develop an injectable bone cement composed of borate BG particles dispersed in a chitosan solution that served as the hardening liquid, and the properties of the cement relevant to potential clinical application such as handling properties and bioactivity were studied.

**METHODS:** The cement was prepared from a mixture of bioactive borate glass particles and an aqueous solution of chitosan used as the setting phase, details as described previously [2]. The cement was formed by mixing the glass particles (solid) and chitosan solution (liquid) in varying solid to liquid (SL) ratio (weight to volume) of 1.0, 1.5, 2.0 and 2.5 g $ml^{-1}$. The injectability, cohesiveness and compressive strength of the cement was tested in vitro using a procedure described previously [3]. The degradation of the cements with different SL ratios was studied as a function of immersion time in PBS at 37 °C. The presence of crystalline phases in the cements after immersed in PBS was determined by X-ray diffraction (XRD), and composition analysis of the cements was performed using FTIR. The morphological features of the cements before and after immersion in PBS were examined in a field emission scanning electron microscope.

**RESULTS:** The average value of the injectability decreased slowly as the SL ratio increased from 1.0 to 2.0 g/ml but then decreased more steeply to as the SL ratio increased to 2.5 g/ml. The initial setting time of the cement decreased almost linearly with increasing SL ratios. The compressive strength of the cement increased almost linearly with the SL ratios. There was no noticeable change in the external dimensions of the cement with an SL ratio of 2.0 g/ml after immersion in PBS for up to 60 min, indicated the excellent cohesiveness. After immersion in PBS, a layer of spherical particles enriched in Ca and P (determined by

EDS analysis) formed on the surface of the samples and covered the whole surface, showed bioactivity and biodegradation. This surface layer of precipitated material became denser with the increase of SL.

**DISCUSSION & CONCLUSIONS:** The injectable bioactive borate glass cement created in this study is a promising biomaterial for healing bone defects with a regular or irregular shape by minimal invasive surgery.

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**ACKNOWLEDGEMENTS:** This work was supported by the National Natural Science Foundation of China through projects 51072133, 51372170, 81201377, 81371938, 81201384 and 51272274.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Injectable calcium silicate-based bone cements

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With the increasing popularity of minimally invasive techniques, the development of injectable systems that can mould to the shape of the bone cavity and polymerize (or harden as solid implanted materials) when injected in situ has attracted a great deal of attention. Such devices should shorten the surgical operation time, minimize the damaging effects of large muscle retraction, reduce the size of scars, and lessen post-operative pain, allowing the patients to achieve rapid recovery. Thus, a great number of injectable bioceramic-based bone substitute grafts such as calcium phosphate, calcium silicate, calcium carbonate, and calcium sulphate, have been developed [1-3]. Among injectable cements, calcium silicate has attracted significant interest, due to its excellent osteogenesis, sealing ability and regenerative capability. Exposure of calcium silicate-based material surfaces to a physiological solutions leads to the precipitation of a bone-like apatite layer, which may assist the material to integrate into living tissue. Calcium silicate bone cements may not only induce the differentiation of human mesenchymal stem cells, bit also have considerable bacteriostatic activity, as revealed by inhibition zones and the bacteriostasis ratio against Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacterial strains [3]. The translation of the cements from the bench to bedside has been quite successful, but there are several documented shortcomings of the cement systems, including anti-washout ability, radiopacity, osteoinduction, and angiogenesis.

In general, the unmodified ceramic cements have some difficulty maintaining the original grafted shape at defect sizes when implanted because they do not have enough washout resistance (or viscosity) to the body fluid within their hardening periods. To overcome the disadvantage of anti-washout, polymers, such as cellulose, alginate, gelatin, chitosan, and polylactic acid, can be added to ceramic cements. The use of vertebroplasty and endodontics require good visualization during injection and post-treatment, such as in situ monitoring of cement microleakage. Bismuth oxide, zirconium oxide, barium sulphate, and strontium carbonate can be introduced to improve radiopacity due to their high molecular weight. However, the addition of radiopacifiers may be detrimental to some of the physical, mechanical, and biological properties.

A bone graft that induces vascularization by supporting endothelial cell migration, adhesion, and proliferation is more conducive to bone defect healing. Therefore, a promising strategy to promote angiogenesis within the cement is the local and sustained delivery of angiogenic factors by the material itself. The use of stable and low-cost inorganic ions (e.g. Cu) or polymer (e.g. gelatin) to induce a regenerative response within the materials offers a complementary approach to the use of recombinant or concentrated protein or peptide growth factors. The kinetics of ion release from any scaffold must be tailored because high doses of metallic ions could be toxic. On the other hand, the osteoinductivity is also desired because the cement can recruit the primitive, undifferentiated and pluripotent cells from the surrounding tissues and stimulate their differentiation into osteoprogenitor cells, which develop into differentiated bone cells over time. The incorporation of an osteoinductive factor, such as bone morphogenetic proteins, could be one of the most effective ways to improve the efficacy of cement materials, but this is difficult to apply in a clinical setting. Therefore, a material that can provide a potentially osteoinductive micro-environment without the addition of exogenous growth factor would be challenges facing clinical applications.

In conclusion, although the significant advances made in the injectable calcium silicate-based bone cements for tooth and bone regeneration and repair, a great deal of work remains to be conducted. The synergistic combination of biofactors and ceramics has led to a promising class of bone grafts as the next-generation materials with unique properties for specific clinical applications.

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European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# The alginate/silicate bioceramics composite: preparation and evaluation of its behavior as bioactive injectable hydrogels

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**INTRODUCTION:** The injectable hydrogels have the abilities to effectively and homogeneously encapsulate drugs or cells to fit any defect size or shape and to create an integrative implant-tissue interface to maintain the continuity of the tissue. The sodium alginate (SA) 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. Silicate based bioactive ceramics 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 . Since calcium-containing silicate ceramics (SC) 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 SC into alginate may lead to an in-situ forming and injectable SA/SC composite hydrogel, which may combine the advantages of SA and SC, especially maintaining the bioactivity of SC and the injectability and porous structure of SA hydrogel.

**METHODS:** The gelling time and mechanical properties of the hydrogels with different amount of SC and GDL were systematically analyzed. Thereafter, the swelling behaviors of 5% SA/SC hydrogels with different contents of GDL were investigated. FTIR spectroscopy and SEM was used to analyze the formation of bone-like apatite that formed on the surface of each specimen after soaking in SBF. Furthermore, the effect of the composite hydrogel on cell proliferation and differentiation was evaluated by culturing the hydrogel with cells. Human bone mesenchymal stem cells (hBMSCs) and human umbilical vein endothelial cells (HUVECs) were chosen to investigate the effect of the SA/SC composite hydrogel on cell proliferation, cell osteogenic and angiogenic differentiation.

**RESULTS:** The gelling time could be controlled by the addition amount of the silicates, which could be adjusted between about 30 seconds and 20 min by varying the amount of silicates and GDL. 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, hBMSCs and HUVECs cultured with the composite hydrogels were able to maintain the viability and proliferation. Furthermore, the composite hydrogels not only stimulated hBMSCs to produce alkaline phosphatase, but also promote angiogenesis of HUVECs in the both cell coculture system.

**DISCUSSION & CONCLUSIONS:** Injectable bioactive composite hydrogels were prepared by in situ releasing Ca ions from silicate ceramics to crosslinking SA in the presence of pH regulator GDL. The gelling time and swelling behavior of the composite hydrogel system could be controlled and regulated by varying the contents of silicate components and GDL. The composite hydrogel possessed good bioactivity, which was revealed by the ability to induce formation of bone-like apatite on its surface in SBF. Furthermore, the composite hydrogels could stimulate the osteogenic differentiation of hBMSCs and angiogenic differentiation of HUVECs in the coculture system. The combination of good injectability, bioactivity and angiogenic ability suggests that SC/SA composite hydrogels have great potential as injectable system for applications in bone regeneration and bone tissue engineering.

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**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).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Design of injectable CPCs loaded with local analgesic: preliminary "catwalk" gait analysis results

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**INTRODUCTION:** Postoperative pain following bone reconstruction is considered as one of the major undesirable complications largely described in literature especially in bone iliac crest procedure. This pain (which can become chronic) substantially disrupts patients recovery by reducing their mobility, delaying their functional recovery, increasing their hospital stay, which impairs their quality of life and autonomy. The administration of local anaesthetics has proven to be an effective analgesic technique for the treatment of postoperative pain with a significantly reduced drug use. In this clinical context we have focused our studies in developing new injectable combined calcium phosphate cements (CPCs) that deliver "in situ" local anaesthetics such as bupivacaine and ropivacaine. This study follows previous promising experiments related to the association of bupivacaine with calcium deficient apatite (CDA)[1].

**METHODS:** Different formulations of commercial apatitic cements, mainly constituted by α-TCP (78%), were loaded with bupivacaine and ropivacaine. The cement paste was prepared by mixing the obtained powder with an aqueous solution of $Na_2HPO_4$. The liquid / solid ratio was adapted to obtain a cement with the desired properties after hardening. The final product after the setting process was in all cases CDA loaded with a local anaesthetic. Eighteen Wister female rats were unilaterally implanted for eight weeks with 0%, 8% of bupivacaine and 8% ropivacaine, in critical cylindric defect in distal femur (right hind limb). The implantation impact on functional recovery and locomotion of the animals was studied using a "Catwalk" gait analysis system (Noldus).

**RESULTS:** Incorporation of bupivacaine and ropivacaine into the solid phase of a cement affects its setting reaction and therefore its intrinsic properties in a dose dependant manner. However differences appeared in the behavior of ropivacaine-loaded cement vs bupivacaine-loaded cement. The release of the drugs (60%) is effective in the critical first four days period after the operation lowering the risk of developing chronic pain. The use of a "Catwalk" gait analysis system has been proposed

as a method to study the consequences induced by pain during normal behavior such as walking. In the rat, the implantation of cement loaded with local anesthetics in a calibrated bone defect and its impact on the animals have been studied with this unique tool. A clear analgesic effect in favor of the loaded cements versus cement alone was observed.

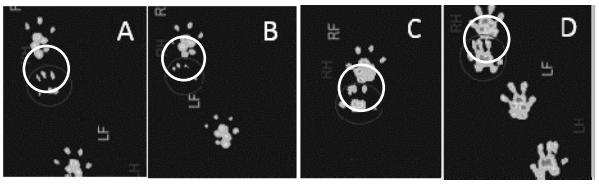


*Fig. 1: Paw prints from "Catwalk". All circles relate to right hind paw. Cement alone (A,B), Bupivacaine-loaded cement (C,D) A and C, 6H post-op ; B and D, 24H post-op.*

For example, figure 1 presents the print of the right hind paw after the implantation of the bupivacaine-loaded cement. The print is clearly visible twenty-four hours after surgery (D) whereas the paw print remains barely visible after implantation of the control cement (B) that does not allow the complete placement of the paw. Such analgesic effect seems to be in accordance with the release profile of bupivacaine.

**CONCLUSIONS:** These injectable combined CPCs are well tolerated and provide a controlled release of local anaesthetics that could be a part of the global management of pain following bone reconstructive surgery.

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European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Percutaneous vertebral augmentation for extreme vertebral fractures and malignant lesions in the thoracic spine: an 18 months follow-up upon pain reduction and mobility improvement

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**INTRODUCTION:** Preliminary results of vertebroplasty upon cement stability, widening of the fracture line and subsequent fracture in extreme vertebral fractures and extensive malignant lesions, seem to be moderate [1]. Purpose of our study is to assess clinical outcome (on terms of pain reduction and mobility improvement) and safety (potential complications and implant migration) of patients with symptomatic extreme vertebral fractures and malignant lesions in the thoracic spine treated by percutaneous vertebral augmentation with biocompatible polymer (KIVA implant).

**METHODS:** During the last 2 years, 10 patients with symptomatic extreme vertebral fractures and malignant lesions in the thoracic spine underwent percutaneous augmented vertebroplasty [a biocompatible polymer was introduced within the vertebral body and filled with PMMA]. Under fluoroscopic guidance and unilateral, transpedicular approach, a working cannula and coaxially the KIVA system were introduced in the vertebral body of interest. Initially a coil was advanced to create a path through the cancellous bone guiding the KIVA Implant which deploys as a stacked, cylindrical column centered at vertebral body's midline. Cone beam CT was performed immediately after percutaneous vertebroplasty augmented with the KIVA implant. Standard x rays and Computed Tomography scans were performed during follow-up. Pain prior, the morning after and at the last follow-up (average follow-up 12 months) were compared by means of a numeric visual scale (NVS) questionnaire. Cement extravasation and implant migration were also recorded..

**RESULTS:** Comparing the pain scores of questionnaires prior (mean value 8.9 NVS units) and post (mean value 1.5 NVS units) treatment, there was a mean decrease of 7.4 NVS units on terms of pain reduction and life quality. Overall mobility improved in 10/10 (100%) patients. No complication was observed. No symptomatic or clinically significant extravasations occurred. No implant change or migration was observed during the 18 months follow-up period.

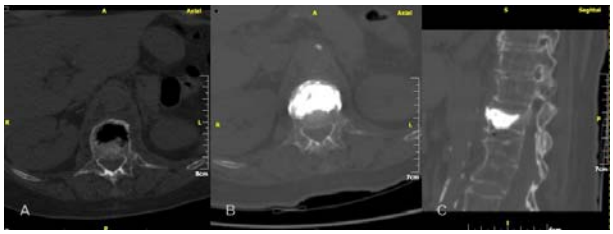

*Fig. 1: A – Computed Tomography axial scan illustrating a large sized osteonecrotic cavity in T12 vertebral body. B, C - Computed Tomography axial and sagittal reconstructions post vertebral augmentation with KIVA implantillustrating complete filling of the cavity and anchoring to surrounding normal bone*

**DISCUSSION & CONCLUSIONS:** Preliminary results in treatment of symptomatic extreme vertebral fractures and malignant lesions in the thoracic spine report significant pain reduction and mobility improvement with no proof of implant migration [2]. The implant seems to function as an internal cast which anchors the fractured segments and in addition provides structural support and the potential of height restoration. Further studies however, are required.

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European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Influence of carboxylmethyl cellulose on the handling and biodegradation properties of calcium phosphate cements

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**INTRODUCTION:** Calcium phosphate cements (CPCs) are self-setting, injectable cements considered to be a favourable bone substitute primarily due to its excellent biocompatibility, osteoconductivity, and injectability [1]. However, their clinical applications are limited due to their relatively poor cohesion upon injection into the body. To this end, carboxymethyl cellulose (CMC), a nontoxic and biocompatible derivative of cellulose, was incorporated into CPCs in order to improve their cohesion and injectability [2].
The aim of this study was to evaluate the effect that different concentrations of CMC would have on the injectability, cohesion, and setting time of the CPC. Animal studies were later performed to determine the influence of CMC on the biodegradability of CPCs in vivo.

**METHODS:** CPCs were prepared by mixing a solid phase (α-tricalcium phosphate and CMC) with a liquid phase (4 wt% aqueous solutions of $NaH_2PO_4 \cdot 2H_20$). The amount of CMC varied from 0 to 1.5 wt%, with increments of 0.25wt%. The injectability was determined by measuring the mass percentage of CPC paste that could be extruded by hand from a 2.5 ml Terumo syringe with a nozzle orifice diameter of 2 mm. The initial and final setting time was measured using a Gillmore needle apparatus, while the cohesion was evaluated qualitatively using an in-house grading scheme and quantitatively by measuring the mass loss of CPC particles upon a washout procedure. Finally, the *in vivo* biodegradation and new bone formation was evaluated histologically and histomorphometrically upon implantation of CPC containing 1.5 wt% of CMC with or without 40 wt% PLGA (as a porogen) into adult female New Zealand rabbits after 4, 12, and 26 weeks.

**RESULTS:** Incorporation of CMC, irrespective of the amount, showed an immediate improvement of the injectability to upwards of 90%, versus just 68% for the control (Fig. 1). The addition of CMC also showed a positive improvement on the cohesion, particularly once a wt% of greater than 1% was achieved (Fig 2). CPCs without or with PLGA were replaced by 1 and 6 vol% of new bone, respectively, after 26 weeks of implantation.

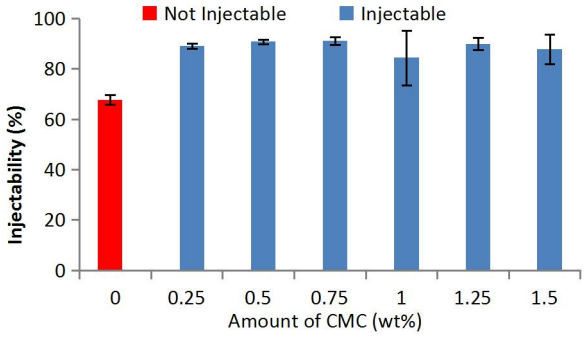


*Fig. 1: Injectability of CPC containing CMC.*

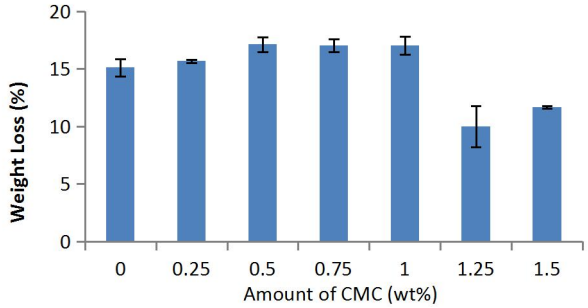


*Fig 2: Weight loss percentage attributed to the washout effect of a CPC containing CMC.*

**DISCUSSION & CONCLUSIONS:** Overall, the incorporation of CMC, in particular >1 wt%, clearly shows a strong improvement in the injectability and cohesion properties of CPCs, thus providing favorable handling properties for its use as a bone substitute in applications involving minimally invasive surgical techniques. This improvement is attributed to the fact that CMC acts as a viscosifier, or suspending agent, that is capable of holding the calcium phosphate particles together as one cohesive unit, thus minimizing washout and filter-pressing upon injection from occurring [3]. This observation might also explain the reduced in vivo degradation rate as compared to previous in vivo studies of comparable experimental design.

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**ACKNOWLEDGEMENTS:** This study was funded through a grant provided by Cam Bioceramics BV, Leiden, The Netherlands.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# A Novel Fracture Mechanics Model for Osteoporotic Bone: Enabling the Design of Safer and more Effective Orthopaedic Implants for Elderly Patients

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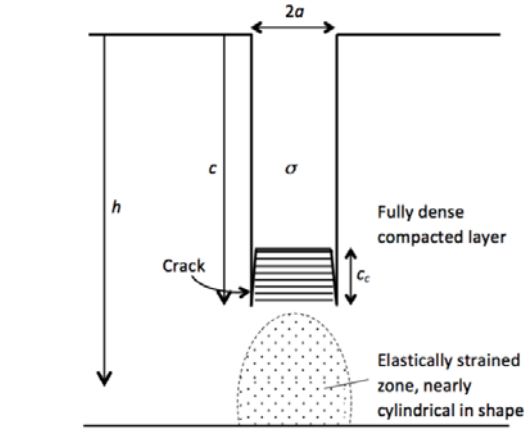
**INTRODUCTION:** Osteoporotic fractures are experienced by half of women and one-quarter of men over 50 years of age, requiring millions of fracture fixation devices each year.[1] Such implants are designed, however, using incomplete and inaccurate models of osteoporotic bone fracture mechanics.[2-5] The fracture behavior of porous and highly compressible material in general remains an open area of research in the field of solid mechanics.[2] As a result, typical implants fail at high rates in elderly patients, leading to complications such as "cut out," in which implants penetrate through bone into surrounding tissues of the shoulder and hip.

**METHODS:** We present a novel fracture mechanics model for osteoporotic bone verified by empirical evidence. A biomechanical study was carried out to observe the penetration performance of various fracture fixation screws in natural and synthetic bone. Femoral heads from human cadavers (15 matched pairs, female, median age 75 years), and synthetic bone tissue (Sawbones, 0.16g/cc), were scanned using micro-CT and analyzed for density and defects. Typical bone screws for femoral and humeral neck fixation (3.5 and 5.0mm, 316LVM steel) were fabricated with several tip designs: conical "sharp" tip, flat "blunt" tip, hemispherical tip, and designs ranging between these extremes. Each screw was pushed into the substrate axially at a constant penetration rate of 5mm/min. Micro-CT scans were repeated for each sample to observe fracture and compaction patterns. All screws were then sawn in half axially. Fresh substrates were also sawn axially and clamped between transparent slides, creating a "window" view. Using a high-resolution camera, each half-screw was recorded during penetration. The video was then analyzed using digital image correlation (DIC) software to create a strain field.

**RESULTS:** Load was recorded over 2 minutes, generating the following mean values at 10mm depth for 5mm diameter screws: 161N sharp tip; 112N blunt tip; 130N hemispherical tip ($p$<0.01). This empirical data was used to validate a new mathematical osteoporotic bone fracture model based on the energy absorption mechanisms of crack creation and material compaction.




Simplifying, we have

$$\sigma = \sqrt{2E\left(\frac{4\gamma}{a} + \sigma_0 \epsilon_c\right)} \qquad (1)$$

The prediction is therefore that, in the "deep crack" limit, the applied stress $\sigma$ to the screw tends to be a constant given by eqn. (1) above. This is a constant with respect to crack length $c$, and specimen thickness $h$, but varies inversely with screw radius $a$.

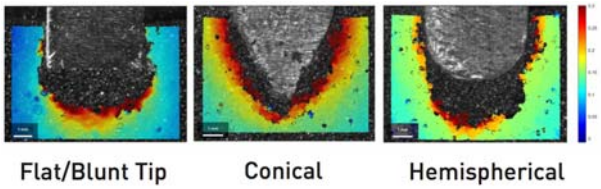



*Fig 1. (Top) Extract of our mathematical model for the cracking and compaction of osteoporotic bone*
*Fig 2. (Bottom) Strain fields of various implant tips in osteoporotic bone specimens.*

**DISCUSSION & CONCLUSIONS:** Our novel osteoporotic bone fracture model may be used to optimize the design of future implants to better serve patients with osteoporosis. An initial counterintuitive insight gained is that implants with so-called "anti-cutout" blunt tips may in fact lead to increased cutout rates for osteoporotic patients in comparison to other tip designs. We offer alternative design concepts that may lead to superior safety and efficacy for implants intended for such patients.

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European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Long-circulating polymers as nucleic acid and drug delivery system for cancer therapy

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**INTRODUCTION:** When it comes to the *in vivo* application of drug and gene carriers, the long circulating ability is the most important property which could enhance the accumulation of drug and gene in the target site. However, nonspecific protein adsorption is the first step and the proteins in the blood are intended to interact with carriers especially the cationic drug and gene carriers following intravenous injection. The presence of nonspecific adsorption accelerates the blood clearance of carriers due to the recognition by the reticuloendothelial system, resulting in negatively effect of therapeutic efficiency[1]. Therefore, it is an important requirement of the drug and gene carriers to resist the nonspecific protein adsorption to extend the circulation time for systemic intravenous and leave enough time for the carriers to interact with the target tissue and cells. The most commonly used protein-repelling material is water soluble polymer, such as poly(ethylene glycol) (PEG). Although PEGylation is believed to be beneficial for systemic circulation, it also suppresses the electrostatic cellular interaction and uptake of the drug carriers, thereby dramatically reducing their biological activity[2]. In this study, we developed two kinds of long-circulating carriers without PEG modification, which showed excellent protein resistance ability and enhanced cell uptake efficiency. Addition, the tumor suppression effect of these two systems was evaluated *in vivo*.

**METHODS:** Chitosan-agmatine bioconjugates, termed as CS-DM-Agm, were prepared by dimethylmaleic anhydride modification and thenucleophilic reaction between tosyl of tosylated chitosan and primary amine of agmatine. Another star-shaped polymer consisting of a cationic poly[2-(dimethylamino) ethyl methacrylate] (PDMAEMA) shell and a zwitterionic poly[N-(3-(methacryloylamino) propyl)-N,N-dimethyl-N-(3-sulfopropyl) ammonium hydroxide] (PMPD) corona was grafted from a polyhedral oligomeric silsesquioxanes (POSS)-based initiator via atomic transfer radical polymerization (ATRP), termed as POSS@PDMA-b-PMPD.

**RESULTS:** Zeta potential analysis confirmed that CS-DM-Agm/VEGF siRNA complexes could be transformed from a negatively charged form into a positively charged form in the slightly acidic tumor extracellular environment. This charge conversion enhanced the cellular uptake of the VEGF siRNA complexes, which further led to remarkable gene silencing efficiency and a high apoptotic rate of tumor cells both *in vitro* and *in vivo*. The micelles formed by POSS@PDMA-b-PMPD could co-deliver tumor-suppressor p53 gene and anticancer drug DOX into MCF-7 cells, resulting in high tumor cell apoptosis. Furthermore, this co-delivery system not only enhanced the antitumor efficacy but also reduced the side effects.

**DISCUSSION & CONCLUSIONS:** The high antitumor efficiency of CS-DM-Agm/VEGF siRNA complexes was attributed to the negative-to-positive charge conversion. In the blood surroundings, the circulation time of gene complexes was prolonged due to less interaction with the negatively charged serum components. When circulating around the slightly acid tumor extracellular environment, the charge reversal behavior would facilitate the cellular uptake by tumor cells. This charge conversional siRNA delivery system could cross the blood barrier and cell uptake barrier effectively, resulting in the remarkable antitumor efficiency. The introduction of antifouling zwitterionic PMPDSAH segments not only could improve the serum stability of the complexes, but also facilitate the cellular uptake, together resulting in enhanced the antitumor efficacy.

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**ACKNOWLEDGEMENTS:** This project is financially supported by National Natural Science Funds for Distinguished Young Scholar (No.51325305).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Evaluation of an in-syringe mixer design capable of mixing and delivery of both PMMA and CaP cements via a long cannula.

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**Introduction:** Whilst there has been substantial progress in the development of metallic orthopaedic implants, their use in combination with biomaterials has been slow to mature. The clinical use of biomaterials to augment fixation of implants in compromised bone is an obvious application and of particular interest in fragility fracture indications such as hip fracture or proximal humeral fracture. However whilst there are many preclinical and clinical reports of the use of injectable materials to augment implant fixation (PMMA and CaP) there is still insufficient evidence that the improvement in clinical outcomes justifies their use. As an example[1] in 7 augmentation studies that qualified for inclusion in the meta-analysis, there were different implant types (nail or plate), different injectable materials (PMMA or CaP), and different injection sites (either in the trochanteric region or around the lag screw). Biomaterials manufactures often work independently of implant makers so the mixing and delivery to operative sites can prove challenging.
In review with clinicians, a major factor that limits their use of biomaterials is the lack of a standardised mixing and delivery system, and in particular, for the hip fracture application, a cannula system of sufficient length to deliver the cement. The authors consider that these unmet needs can be addressed. Accordingly the author's goal was to demonstrate effective mixing and delivery of both PMMA and CaP cements through a standardised mixing system and additionally prove the feasibility of injection through a long cannula.

**Method:** An off the shelf in-syringe mixing system, developed by Medmix AG Switzerland. In this design (P System) an internal paddle is used to agitate and blend cement (or other biomaterials) components that are placed in the syringe body.The paddle is then transformed into a syringe plunger for delivery of the cement. By fitting a spindle drive to the plunger it is then possible to pressurise the cement and assist delivery via a range of specialty cannulae. To prove the feasibility of using the same device for mixing and delivery of PMMA and CaP, cements from Wego Company (Weihai China) were evaluated under laboratory test conditions. In a hip screw augmentation indication model, both types of cement were injected through a 50 cm long cannula into a sawbones femoral head model via the lag screw.

**Results** The laboratory test series showed that both cement types could be successfully mixed and effectively delivered via the in-syringe mixing system. The addition of a spindle drive enabled delivery of the injectable cements under a wide range of test conditions, for example in the hip screw augmentation test. In this the lag screw was advanced short of its final position to leave a 2 cm long cavity that was successfully filled with each type of cement via a side port at the cannula tip. The screw was then advanced to its final position simultaneously pressurising the cement into the cancellous bone around the screw.

**Discussion** It was demonstrated that a single standardised mixing system could be used, in principle, for the mixing of both PMMA and CaP cement types. Further it was demonstrated in an invitro lab model that it was feasible to undertake lag screw augmentation, with both cement types, via a long (hip fracture specific) cannula. The cannula featured a side port at the tip.

**Commentary** This sets the scene for a more robust standardised mixing and delivery method to enable the clinical effectiveness of screw augmentation to be more thoroughly assessed. Unstable hip fractures (AO classification 31-A.3) are an ideal target and using the Swedish RSA (Radiosteroanalysis) method as few as 60 patients (30 control) would be sufficient to confirm that there was a beneficial effect or not.

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# Injectable monodisperse PLGA/MgO microspheres for in-situ bone regeneration

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**INTRODUCTION:** Osteoporosis is disease that mainly affects elder people over age of 50 around the world. The use of bisphosphonates (BPs) is the common drug treatment for Osteoporosis. However, long-term medication of BPs reported to be likely associated with atypical bone fractures recently. Other biological agents eg. bone morphogenetic proteins (BMP-2) and insulin-like growth factors, are costly, easily deactivated by enzymes and rapidly degraded. Our previous studies proposed that magnesium ions in specific concentration can stimulate bone regeneration in vivo[1]. Therefore, this study aims at designing a drug delivery system by using a FDA approved polymer namely poly(lactic-co-glycolic acid) (PLGA) and magnesium oxide in order to constantly deliver magnesium ions for *in situ* bone regeneration.

**METHODS:** The PLGA/MgO (w:w=1:0.25) microspheres were fabricated by the technique of microfluidics. PLGA was dissolved in dichloromethane (DCM). Afterwards, MgO nanoparticles were suspended to DCM solution that defined as inner phase(oil phase). The mix solution flowed through the capillary. 3% polyvinyl alcohol(PVA) solution was defined as outer phase(water phase) to shear the inner phase to form PLGA/MgO droplets, followed by drying overnight. The size and morphology of PLGA/MgO microspheres were characterized by Scanning Electron Microscope (SEM). The biocompatibility of PLGA/MgO microspheres was evaluated by MTT and ALP assays.

**RESULTS& DISCUSSION:** The SEM images showed that the fabricated PLGA/MgO microspheres were monodisperse with a size of 150μm and the inner structure of these microspheres was porous resulting from MgO naoparticles set as porogen. On the other hand, Mg ions were evenly distributed in the inner phase of the microsphere, indicating that these PLGA/MgO microspheres can consistently release Mg ions in vitro. Consequently, the MC3T3-El pre-osteoblasts cultured with the extracts of these PLGA/MgO microspheres achieved higher cell viability and ALP activity compared with the extracts of PLGA microspheres alone.

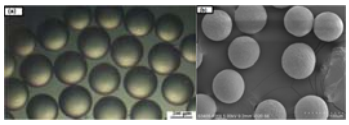

*Fig.1 The morphology of the PLGA/MgO (a) droplets and(b)microspheres fabricated by microfluidic technique*

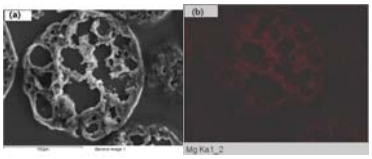

*Fig2 (a)Inner structure and (b)Mg ions distribution within PLGA/MgO microspheres*

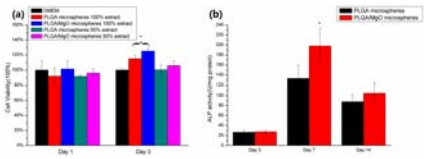

*Fig. 3(a) The cell viability and (b) ALP assay of MC3T3-El pre-osteoblasts cultured with the extracts of PLGA/MgO microspheres that immersed in DMEM for 24h) DMEM . *denotes the significant difference between PLGA microspheres group and PLGA/MgO microspheres group($p<0.05$) ;*denotes ($p<0.01$)*

**CONCLUSIONS:** The injectable PLGA/MgO microspheres fabricated by microfluidic technique exhibit excellent biocompatibility high ALP activity in vitro. With the use of constant release of Mg ions, it is anticipated that these magnesium based microspheres may stimulate local bone formation vivo, thereby potentially reducing the bone fracture healing time.

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**ACKNOWLEDGEMENTS:** This work was financially supported by National Natural Science Foundation of China No. 31370957 and Hong Kong Research Grant Council General Research Fund (#718913).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Effect of the acid type on the cohesion and the cytotoxicity of chitosan/β-cyclodextrin/hydroxyapatite hydrogels

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**INTRODUCTION:** Hydrogels are promising materials for osteoarticular applications [1]. Their capacity to be functionalized and their suitability for minimal invasive surgeries are among their main advantages [2]. This work presents the effect of different acidic solutions on the cohesion and on the cytotoxicity of physical chitosan hydrogels loaded with hydroxyapatite (HA).

**METHODS:** Hydrogel powders consisted of chitosan (CS; Sigma Aldrich, France), HA and water soluble β-cyclodextrin polymer (PCD). HA with a particle size of 2 µm was synthesized and provided by the LMCPA. PCD was synthesized from the polymerization between cyclodextrin and citric acid with $NaH_2PO_2$ as a catalyst according to Martel *et al.* [3]. CS was milled in a rotor mill (PULVERISETTE 14, Fritsch, Germany) and PCD was hand-milled with a mortar, both powders were sieved to 125 µm (Fisher Scientific, France). Next, CS and PCD were co-milled by hand in a mortar at a 1:1 ratio and sieved to 125 µm. Hydrogels were prepared by adding the CS:PCD mixture (6 % w/v) and HA (3% w/v) into a syringe. Demineralized water ($H_2O_d$) was added and the whole was mixed. Then, 1 % v/v acetic acid (AcOH; Merck, France), 1 % v/v lactic acid (LA; Sigma Aldrich, France) or a 0.2 M HCl (Panreac, France) solution was added and the whole was mixed again. The zeta potential (ZP) was measured for the components in solution. The pH of the hydrogels was followed for 2.5 h and the cohesion was monitored for 1 h in $H_2O_d$, phosphate buffer solution (PBS; Sigma Aldrich) or DMEM medium (Gibco, France) at 37 °C. The cytocompatibility was assessed according to the ISO 10993-5 standard and with osteoblasts cells (MC3T3-E1).

**RESULTS:** The ZP and the pH values were affected by the type of acid used for obtaining the gel. No significant differences in the consistency of the hydrogels after injection were observed with the different acids used (Fig. 1). Cohesion behavior was affected by the solution used for evaluation. Cohesion was poor in $H_20_d$ and better in PBS and DMEM. Cohesion was better for hydrogels obtained with AcOH, followed by LA and HCl, respectively. The cytocompatibility of hydrogels produced with HCl had the less cytotoxic effect, followed by LA and AcOH. Compared to the control the percentages of the cell viability were 73.6, 56.8 and 46.4, respectively. Nevertheless, after 1 week of storage, hydrogels produced with LA and HCl became liquid.

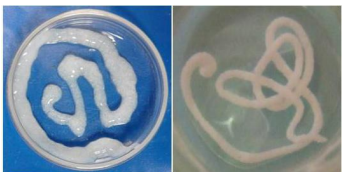

*Fig. 1: Images, after injection, of a hydrogel made with AcOH (left) consistency and (right) cohesion in PBS at 37 °C.*

**DISCUSSION & CONCLUSIONS:** Physical hydrogels were used and formed by the electrostatic interaction between $NH_3^+$ (CS) and $COO^-$ (PCD). These interactions depend on the acidity of the solution used as this is related to the availability of the $NH_3^+$ and $COO^-$ groups. All three acids used have been reported as good candidates for dissolving the CS [4]. HCl is a strong acid while AcOH and LA are weak acids which main difference is the presence and orientation of the OH group pointing out of the plane at their skeletal formula. Even if an initial gel was formed the stability of the hydrogel for longer term was only obtained by using AcOH. Nevertheless, this acid presented the lowest pH value after mixing and the highest cell cytotoxicity. Hence, alternative solutions are needed in order to obtain a stable hydrogel with better properties for their use with cells.

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**ACKNOWLEDGEMENTS:** Authors thank Prof. Betbeder for ZP measurements. Authors thank the financial support of the Nord-Pas-de-Calais region via the ARCir program for the *BioCerMed* project.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Injectable, settable, and resorbable nanocrystalline hydroxyapatite/ polyurethane hybrid polymers with strength comparable to PMMA

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**INTRODUCTION:** Bone cements utilized for bone fractures at weight-bearing sites, such as intra-articular joints, are subjected to repetitive, dynamic physiological loading from daily activities and must have adequate strength [1]. The performance requirements for non-resorbable poly(methyl methacrylate) (PMMA) are compressive strength >70 MPa, bending modulus >1800 MPa, and bending strength >50 MPa [2]. However, resorbable weight-bearing cements are not currently available. In this study, we designed resorbable nanocrystalline hydroxyapatite (nHA) polyurethane (PUR) inorganic-organic hybrid polymer networks with mechanical properties exceeding that of PMMA. Remodeling of nHA/PUR hybrid polymers was assessed in a rabbit femoral plug defect model.

**METHODS:** Hydroxyapatite nano-particles (nHA) (<200 nm, Sigma) were reacted with lysine triisocyanate (LTI) to yield a viscous nHA-LTI prepolymer (0-65 wt% nHA). nHA/PUR hybrid polymer networks were synthesized by crosslinking the nHA-LTI prepolymer with either poly(ε-caprolactone) triol or poly(thioketal) diol and an iron acetylamide catalyst. nHA/ Poly(thioketal-urethane) (nHA/PTKUR) hybrid polymer was injected into rabbit femoral plug defects. At 6 and 12 weeks following the surgery, the animals were sacrificed and and the defects analyzed by microCT and histology for new bone formation and cellular infiltration.

**RESULTS:** The yield strength of the hybrid polymer networks increased from 80 MPa to 113 MPa as the nHA loading increased from 0 wt% to 52 wt% total (65 wt% nHA loading in prepolymer). However, when 52 wt% nHA was blended with the polyurethane as a powder with no prepolymer step, the compressive strength was 91 MPa, which is lower than that of nHA-PUR hybrid polymers with >16 wt% nHA loading. In the rabbit femoral plug defect model, grafts maintained mechanical stability at 12 weeks. Images of histological sections showed evidence of graft resorption, new bone formation near the host bone interface, and slow integration of the graft with host bone (**Fig 1**).

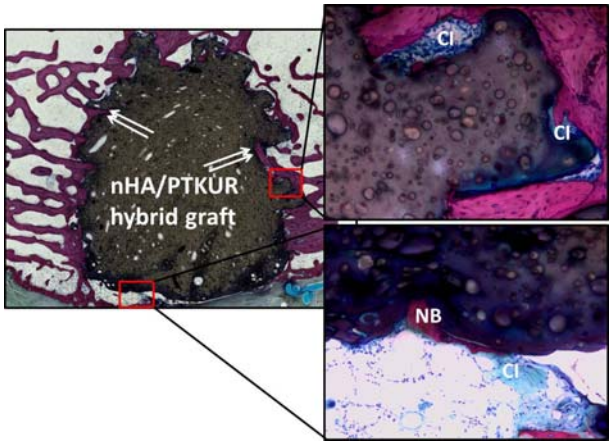


*Fig. 1: Cellular infiltration and new bone formation at the interface between host bone and the hybrid bone graft at 12 weeks. Arrows indicate new bone integrating with the graft. Abbreviations. CI: cell infiltration, NB: new bone*

**DISCUSSION & CONCLUSIONS:** Covalent bonding of LTI to nHA significantly enhanced the mechanical properties of nHA/PUR hybrid polymers compared to physically blended composites. Furthermore, the compressive strength of the hybrid polymers exceeded that of PMMA bone cement. When implanted in femoral condyle plug defects in rabbits, nHA/PUR hybrid bone grafts resorbed slowly, integrated with host bone, and supported new bone formation. These findings highlight the potential of nHA/PUR bone cements for healing of weight-bearing bone defects.

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**ACKNOWLEDGEMENTS:** This work was supported by the National Institutes of Health (R01 AR064304) and Medtronic Spine and Biologics.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Local Delivery of rhBMP-2 from a Compression-Resistant Graft in a Canine Lateral Ridge Augmentation Model

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**INTRODUCTION:** Large mandibular defect reconstruction presents a continual challenge in oral and maxillofacial surgery. Growth factors such as recombinant human bone morphogenetic protein-2 (rhBMP-2) incorporated in scaffolds for tissue engineering promote cellular infiltration, induce osteoblast differentiation, and enhance new bone formation. RhBMP-2 is often delivered via an absorbable collagen sponge, which requires a secondary space maintenance system such as titanium mesh[1,2]. Biodegradable polyurethane (PUR) biocomposites are effective carriers for rhBMP-2 and support new bone growth[3]. Mastergraft ceramic (CM) is an osteoconductive ceramic with mineral content similar to bone. In the present study, we assessed space maintenance and new bone formation with an injectable PUR/CM composite with and without rhBMP-2 in a canine lateral ridge augmentation model.

**METHODS:** The PUR was synthesized from a lysine triisocyanate (LTI) and polyethylene glycol (PEG) prepolymer, a polyester triol (450 g/mol), and triethylene diamine catalyst. Treatment groups included the composite containing 45 wt% CM with no rhBMP-2, a low dose of 200 µg/mL rhBMP-2, or a high dose of 400 µg/mL rhBMP-2 (n=4/group). An absorbable collagen sponge with a titanium mesh incorporating 400 µg/mL rhBMP-2 was used as a clinical control. Lyophilized rhBMP-2 was mixed with the PUR and injected into a lateral ridge defect measuring approximately 13-14 mm mesiodistally by 8-9 mm apico-coronally by 3-4 mm bucco-lingually. For the clinical control samples, the rhBMP-2 was absorbed on the collagen sponge and placed in the defect with a piece of titanium mesh fastened over the sponge. Animals were sacrificed at 16 weeks and new bone formation evaluated by radiographs, µCT, histology, and histomorphometry.

**RESULTS:** The canine lateral ridge augmentation study was designed to answer the following questions: (1) Will the PUR/CM grafts maintain space and prevent prolapse in mandibular lateral ridge defects with protective membranes? (2) What is the optimal dose or rhBMP-2 from the PUR/CM composites? The high dose of rhBMP-2 led to more new bone formation and better

maintenance of the host ridge width than the low dose (Fig 1). Space maintenance of the ridge was comparable

for the compression-resistant PUR/CM high-dose group without the titanium mesh and the absorbable collagen sponge with the titanium mesh (clinical control). At the zero or low dose of rhBMP-2 in the PUR/CM graft, new bone formation and space maintenance were reduced.

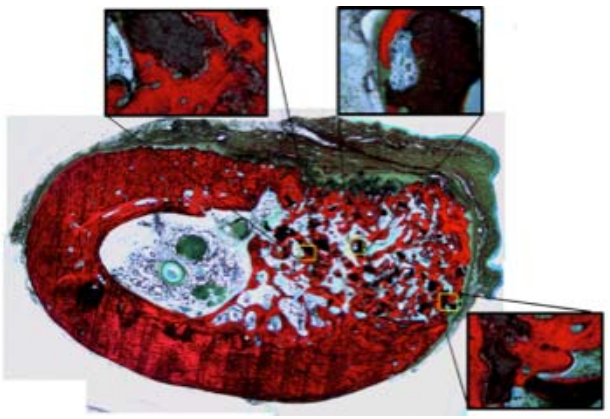

*Fig. 1: PUR/CM grafts augmented with 400 µg/mL rhBMP-2 support new bone formation (red), ceramic (black) resorption, and maintain the contour of the ridge at 12 weeks.*

***DISCUSSION & CONCLUSIONS:*** *PUR/CM grafts with 400 µg/mL rhBMP-2 supported new bone formation and space mainetnance in a canine lateral ridge augmentation model. Space maintenance was comparable to the absorbable collagen sponge with titanium mesh. Elimination of metal hardware is anticipated to achieve more predictable outcomes with fewer complications.[4]*

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**ACKNOWLEDGEMENTS:** This work was supported by the Department of Defense and Medtronic Spine and Biologics.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# An injectable miRNA-activated matrix for effective bone regeneration *in vivo*

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**INTRODUCTION:** An ideal miRNA delivery system for bone regeneration should protect miRNA from inactivation throughout the process of matrix formation, storage and release *in vivo*. Matrix mediated local gene delivery has interesting properties for addressing these requirements[1]. However, the matrix, especially that with high negative charge such as hyaluronic acid, can compete with genes from positively charged vectors, causing polyplex decomplexation and finally low transduction efficiency[2]. Here, we designed an injectable miRNA in situ delivery system. miRNAs were encapsulated within nanocapsules which were further entrapped into an O-carboxymethyl chitosan (CMCS) network via electrostatic interactions. microRNA-21 (miR-21) is an osteogenic-related miRNA which was chosen as the gene model.

**METHODS:** After internalization in the nanocapsules and mixture with biocompatible CMCS, the miR-21 nanocap-sules (n(miR-21)) coating was used for the intracellular delivery of miR-21. Cell viability analysis and the effect of this coating on osteogenic differentiation were evaluated *in vitro* using human umbilical cord mesenchymal stem cells (hUMSCs). The expression of osteogenesis-related genes, including runt-related transcription factor 2 (RUNX-2), alkaline phosphatase (ALP) were evaluated by qRT-PCR. For the *in vivo* study, 60 male Sprague-Dawley (SD) rats were used. A 3 mm tibial plateau bone defect was made. Samples were collected and prepared for hematoxylin and eosin (H&E) and immunohistochemical staining.

**RESULTS:** Nanocapsules had a moderate zeta potential level (~15mV). Due to biocompatibility of the CMCS matrix, both CMCS/lipo/miR-21 and CMCS/n(miR-21) had negligible cytotoxicity. The nanocapsules released from the CMCS matrix were capable of delivering miR-21 into the hUMSCs with a high efficiency of 61.6%. At day 3, the ALP mRNA expression level was higher in CMCS/n(miR-21) treated cells (approximately 4.9-fold compared to the CMCS/lipo/miR-21 group). RUNX-2 mRNA expression level was also higher in the CMCS/n(miR-21) group (1.4-fold) compared to the CMCS/lipo/miR-21 group and this was significantly increase at day 7 (1.6-fold) compared

to the CMCS/ lipo/miR-21 group. Quantitatively, the CMCS/n(miR-21) treated hUMSCs had higher calcium nodule formation compared to the control group and approximately 1.1-fold more nodule formation than the control group. Immunohistochemistry to measure expression of possible miR-21 targets revealed that in contrast to controls less PTEN-positive staining was seen in the CMCS/n(miR-21) group.

**DISCUSSION & CONCLUSIONS:** The current study constructed a newly formed microRNA-activated matrix using nanocapsule polyplexes that was optimized for stability and efficient release of specific pro-osteogenic miRNAs for bone regeneration *in vivo*. miR-21 molecules were encapsulated within thin network polymer shells to form the n(miR-21) via in situ polymerization rather than sample surface adsorption, which enables the synthesis of polymeric-wrapped miRNA nanocapsules with high delivery efficiency and improved stability. Bone regeneration using the microRNA-activated matrix was confirmed with better transfection efficiency of hUMSCs and subsequent high calcium production as well as better bone formation *in vivo*. Thus, there is promise for future applications of CMCS/n (miR-21) as it offers low toxicity, high stability and stimulates osteogenesis for bone repair *in vivo*.

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**ACKNOWLEDGEMENTS:** This project is financially supported by Grant No. 2012DFA51690, 31370970, 13JCZDJC33300 and 14JCZDJC38500

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Injectable radiopaque and bioactive polycaprolactone-ceramic composite for orthopaedic augmentation

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**INTRODUCTION:** Injectable bone cements (IBCs) such as polymethylmethacrylate (PMMA) and calcium phosphate cement can be used to provide mechanical stability in weakened osteoporotic bone for hardware augmentation during fracture healing. Currently available IBCs do not have the required simultaneous combination of high flexural strength, high radiopacity, and high bioactivity. To address the shortcomings of currently available IBCs, composites based on biodegradable polycaprolactone (PCL) and with incorporation of baghdadite[1] (Bag, $Ca_3ZrSi_2O_9$) microparticles have been developed and studied[2].

**METHODS:** PCL (Mw: ~80,000) was cryogenically grinded and mixed with baghdadite particles, then melt extruded to form homogeneous PCL-Bag composites with varied vol.% baghdadite (PCL, PCL-1%Bag, PCL-5%Bag, PCL-10%Bag). Force of injection at injection rate of 0.75cm$^3$/min, and temperature of material at the nozzle exit was measured after melting the composite at 75$^o$C. Flexural stress vs. strain graphs were obtained from 3-point bending tests. Radiopacity of the composites in Hounsfield units (HU) were obtained by using cone beam computed tomography (CBCT) at 70keV. Primary human osteoblast (HOB) proliferation on PCL-Bag composites was measured via colorimetric method. Relative gene expressions of Runx2, osteocalcin (OC) and osteopontin (OPN) were obtained by a sequence of RNA isolation, cDNA synthesis, and real-time polymerase chain reaction.

**RESULTS:** PCL, PCL-1%Bag, and PCL-5%Bag required 600-700N, and PCL-10%Bag required ~900N of compressive force for injection. All formulations exited the nozzle between 55-60$^o$C, and cooled to below 50$^o$C after 1min. PCL-10%Bag showed significant increase ($p<0.05$) in flexural strength (29.7±2.0 MPa) compared to PCL (23.8±0.7 MPa). All groups remained intact at 0.3 flexural strain. Increased loading of baghdadite particles increased the radiopacity of PCL. PCL, PCL-1%Bag, PCL-5%Bag and PCL-10%Bag showed -108±25 HU, 389±104 HU, 1669±48 HU and 2971±195 HU at 70keV respectively, compared to 646 ± 162 HU for porous hydroxyapatite. There was significant increase in HOB proliferation on PCL-5%Bag compared to PCL at day 3, and on PCL-10%Bag compared to PCL at day 7 (Fig. 1a). PCL-1%Bag, PCL-5%Bag and PCL-10%Bag showed significantly increased Runx2 expression in day 3 compared to PCL. PCL-10%Bag showed significantly increased OPN and OC gene expression in day 7 compared to all other formulations. (Fig. 1b-d)

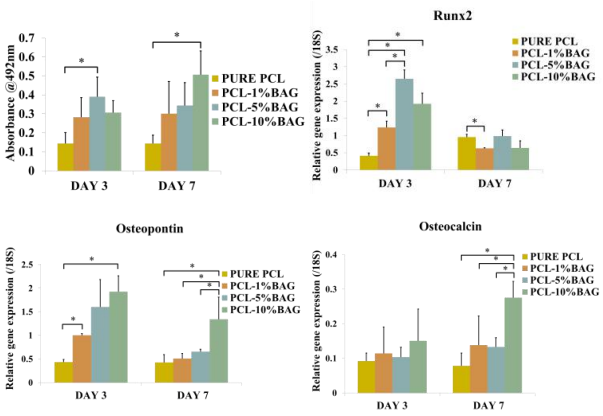


*Fig. 1. a) HOB proliferation; HOB gene expression of b) Runx2, c) OPN, d) OC. *: $p<0.05$*

**DISCUSSION & CONCLUSIONS:** PCL composites with variable baghdadite loading were characterized in vitro. In particular, PCL-10%Bag possesses high flexural strength, high radiopacity and good bioactivity for new bone formation, with lower thermal necrosis risk than PMMA. PCL-10%Bag formulation could be a putative candidate for a bone void filler or hardware augmentation where immediate mechanical loading could improve in patient outcome.

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**ACKNOWLEDGEMENTS:** The authors acknowledge the funding sources Australia National Health and Medical Research Council, Australian Research Council, Rebecca Cooper Foundation and Australian Postgraduate Award

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Vertebral body augmentation reliably reduces morbidity in osteoporotic spine fractures

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**INTRODUCTION:** The morbidity of osteoporotic vertebral fractures continues to plague us as the ageing of population sets in across many communities globally. Treatment options have ranged from simple immobilization to vertebroplasty with acrylate to instrumentation of the spine. We looked at the results of our procedure which stents the vertebral body from within before augmenting with acrylate.

**METHODS:** 60 patients with painful and symptomatic osteoporotic fractures were treated in 2012 and followed through for 36 months thereafter. All were female, post-menopausal and radiographically osteoporotic with at least moderate degrees of Genant-type fractures of the thoracic and lumbar spines. 42 received an image-guided percutaneous, transpedicular procedure that used a trocar to deliver a catheter-mounted, collapsed cobalt-chromium mesh into the corpus of the vertebra concerned, before expanding the mesh hydraulically within the body of the vertebra. 18 had the procedure as part of a more extensive open decompressive and instrumented approach. All patients were treated by the same surgeon. We studied the efficacy of pain relief and the chronology of activity resumption.

**RESULTS:** All patients experienced pain relief within 48 hours after the procedure. All percutaneous cases had speedy recovery within 24 hours. In the open cases, 4 patients had residual radiculopathy pain that lasted for 48 hours. 59 patients resumed pre-morbid physical activity while the sole exception suffered persistent post-traumatic, procedure-unrelated lower motor neuron damage that took 12 weeks to improve. Six cases in the open category showed cement leaks, compared to one in the percutaneous group. All leaks but one were asymptomatic and inconsequential right up till 36 months of follow-up. The sole exception suffered pulmonary embolism but recovered after 3 days of intensive care support. Five patients with percutaneous treatment suffered inconsequential adjacent segment fractures subsequently.

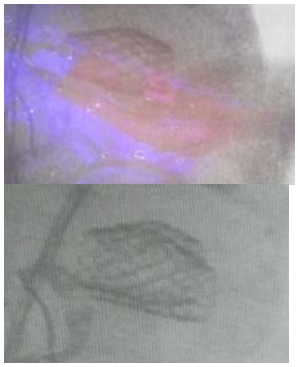

*Fig 1: Serial intra-procedural radiographs of mesh opening and restoring vertebral height before augmenting with polymethylmethacrylate*

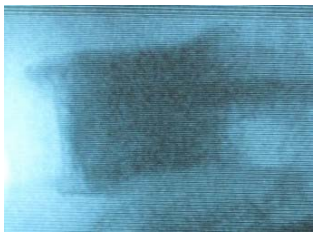

*Fig 2: Reconstituted corpus with polymethylmethacylate*

**DISCUSSION & CONCLUSIONS:** Vertebral body stenting has effectively relieved the symptoms of pain and radiographically restored stability in our patients. This procedure has been executed safely and without major complications, with the patients exhibiting continuity of symptom relief and spine stability at 36 months of follow-up. We are expanding this study into a wider pool of patients to achieve better statistical significance.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# MicroRNA in osteoporosis, osteoporotic fracture and its potential as early diagnostic biomarker

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**INTRODUCTION:** Osteoporosis (OP) is mainly caused by the unsuccessful maintenance of the balance between osteoblast-mediated bone formation and osteoclast-mediated bone resorption. Several miRNAs have been reported to regulate bone metabolism by modulating the differentiation and activity of osteoblasts and osteoclasts. Therefore, miRNAs are regarded as one of the important modulators in the bone remodeling.

**METHODS:** Eighteen subjects from south of China were recruited in this study. Six of them are osteoporosis patients (OP). Another six are patients with vertebral osteoporotic fracture (OPF). And six subjects are healthy people as healthy control (HC). The exclusion criteria for the patients are the following: tumor, known chronic, systemic, metabolic, endocrine diseases including polycystic ovarian syndrome, medication treatment effected bone metabolism and any medical history or signs of other inflammatory disease, acute trauma. This study was approved by the local ethical review committee and the subjects provided informed written consent. The whole blood were collected from each subjects and separated into plasma immediately. MicroRNA profiling was performed on the plasma samples which were divided into 3 groups: osteoporosis (OP) group (without frature, n=6), osteopororic fracture (OP F) group (n=6), healthy control (HC) group (n=6). MiRNAs from plasma were extracted using the miRNeasy Serum/Plasma Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions; then the miRNAs were transcribed to cDNA using the miScript II RT Kit (Qiagen, Hilden, Germany). RNA integrity were monitored by electrophoresis in 8% denaturing polyacrylamide gels. The total RNA concentrations were determined using Nanodrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE).The miRNAs expression was further validated by quantitative real-time RT-PCR (qRT-PCR).

**RESULTS:** Significant differentially expressed miRNAs between OP group and HC group or OP F group and HC group were identified through fold change and P-value which is fold change values ≥2 or ≤ 0.5 and P-values<0.05. 118 miRNAs were upregulated and 266 were downregulated in OP group compared to

HC group. In addition, there are 123 upregulated miRNAs and 218 downregulated in OP F group compared to HC group. Particularly, 41 upregulated and 164 downregulated miRNAs expressed significant differences both in the OP compared groups and the OPF compared groups. Among them, miR-4522 was upregulated 13.45-, 27.75-fold in the OP compared groups and the OP F compared groups, respectively ($p<0.01$ for both).

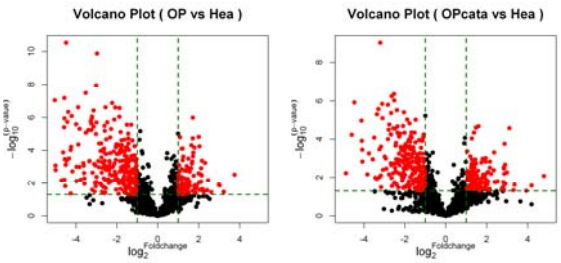


*Fig. 1: The volcano plot of miRNAs between each group. The red dots represent the miRNAs with P-values <0.05 and fold change values ≥2 or ≤0.5.*

**DISCUSSION & CONCLUSIONS:** MiR-4522 might play a crucial role in the molecular pathogenesis of OP and associated with the severity of OP. Further research will accelerate the understanding of the diagnostic and pathological mechanism of miRNA and miR-4522 might be an important biomarker in early diagnosis of OP.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Effects of tricalcium silicate composite material on the proliferation and mineralization behaviours of human dental pulp cells in vitro

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**INTRODUCTION:** Tricalcium silicate (C3S) has been proved to promote the proliferation and odontogenic differentiation of human dental pulp cells (hDPCs) via extracellular signal-regulated kinase 1/2 pathway[1-2]. However, C3S is not eligible as dentin alternative material due to its long setting time and poor mechanical strength. In our previous studies, we have demonstrated that the self-setting properties could be significantly improved by the addition of dicalcium silicate (C2S) [3]. However, the biological properties of as-formed C3S/C2S composites as dentin alternatives remain unknown. Therefore, the aim of this study was to investigate the effects of composite material on proliferation and mineralization of hDPCs, which were compared with those of calcium hydroxide and C3S, in order to evaluate the potential application of the material.

**METHODS:** The viability of hDPCs was measured by cell counting kit-8 (CCK-8) assay. According to different proportion of C3S and C2S, composite materials were divided into two groups: C3S/C2S (80/20) and C3S/C2S (60/40). Cells were cultured in material-containing medium (5mg/mL) and compared with untreated controls. Mineralization of hDPCs was evaluated by assessment of alkaline phosphatase (ALP) activity, real-time polymerase chain reaction of odontogenic marker genes such as dentin sialophosphoprotein (DSPP), osteocalcin (OCN), alkaline phosphatase (ALP), and collagen type 1 (COL-1), western blot detection of expression of DSPP and alizarin red S staining of calcium nodules formation. Statistical significance among groups was determined by using one-way analysis of variance. All data are presented as mean ± standard deviation (SD).

**RESULTS:** The results of CCK-8 assay showed that, for all the test groups, the viability of hDPCs decreased after incubation for 3 days, which was however followed by a signicant increase after 7 days. Cells cultured in the composite material groups possessed higher ALP activity than C3S and calcium hydroxide groups. Analysis of odontogenic marker genes indicated the composite materials enhanced the expression of these genes, indicating elevated mineralization of hDPCs in the presence of the materials. Such effect was most pronounced for the group C3S/C2S (80/20) and alizarin red S staining followed a similar trend as those in expression of odontogenic marker genes.

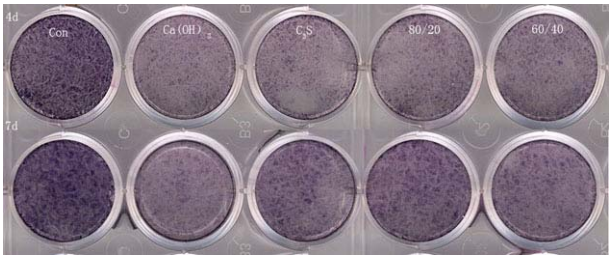


*Figure 1 ALP staining.*


*Figure 2 Alizarin red S.*

**DISCUSSION & CONCLUSIONS:** Our results reveal that composite material of C3S and C2S was advantageous over pure C3S by have an enhanced ability to stimulate the proliferation and odontogenic differentiation of hDPCs, which could be related to their difference in ion-releasing. Further investigations are ongoing to confirm our assumption. Nevertheless, our current in vitro study suggest that the C3S/C2S composite materials possess desirable biocompatibility and bioactivity, and might be a new type of pulp-capping agent and dentin alternative materials.

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**ACKNOWLEDGEMENTS:** The authors thank Hong-ya Pan for her excellent technical support.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# In vitro evaluation of a novel non-decay tricalcium silicate-based bone cement: self-setting properties and cytocompatibility

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**INTRODUCTION:** Previous studies have confirmed that tricalcium silicate ($Ca_3SiO5$, $C_3S$) cement possesses desirable biocompatibility and bioactivity, which make them prospective candidate for orthopedic applications.[1, 2] From the clinical point of view, however, more efforts are still needed to improve the washout resistance, handling properties and mechanical strength of the materials. The use of polymeric additives has been proven to be an effective approach to improve the self-setting properties of inorganic cements.[3] As a natural polymer, alginate with excellent biocompatibility has been extensively investigated for its gelling ability in the presence of calcium ions, which is known to be one of ionic products during the hydration of $C_3S$.[4] The purpose of this study is to develop a novel tricalcium silicate/alginate ($C_3S$/SA) composite cement, in which alginate was used as an additive to the cement. We assume that the in-situ formation of alginate hydrogel in the presence of tricalcium silicate would improve the washout resistance, handling properties and mechanical strength of the cement without causing adverse effect on its bioactivity and biocompatibility.

**METHODS**: $C_3S$ powder was synthesized by sol-gel method,[1] and SA solutions with the concentration of 1.0%, 1.5% and 2.0% in weight percentage were prepared. Cement pastes were prepared by mixing $C_3S$ powder with deionized water or SA solutions with a liquid to solid ratio of 0.6 mL/g. The washout resistance, handling properties, injectability, setting time, compressive strength of the composite cements were characterized and compared with those of pure $C_3S$ cement. In addition, the in vitro bioactivity, degradability and the cytocompatibility of the composite cements were also assessed.

**RESULTS**: Pure $C_3S$ cement disintegrated immediately when injected into water, and only 63% of the pastes could be injected after 5 minutes' standing. In contrast, the composite pastes with SA showed superior washout resistance, and more than 90% of the composite pastes could be injected out the syringes like a strip. Moreover, it was of special interest to note that the composite pastes also possessed good formability, which was not observed for pure $C_3S$ cement. The compressive strength of the $C_3S$/SA cements with optimal composition was up to 54 MPa, which was significantly higher than that of pure $C_3S$ cements (35.3 MPa). In addition, the $C_3S$/SA

composite cements showed good ability to induce apatite formation in SBF and to promote bone-related cell proliferation, which was comparable to that of pure $C_3S$ cement.

**DISCUSSION & CONCLUSIONS:** The improvement in washout resistance and handling properties of $C_3S$ cement in the presence of alginate was attributed to the fact that SA could chelate with $Ca^{2+}$ to form three-dimensional calcium alginate hydrogel. The hydrogel hindered the collapse of $C_3S$ pastes, which thus resulted in enhanced anti-washout ability of the pastes. In addition, the as-formed alginate hydrogel within the composite also reinforced the $C_3S$ cement by interpenetrating with calcium silicate hydrate (CSH) network, which was the hydration product of $C_3S$ and was known to play a critical role in the development of mechanical strength of the cement. The results of our study suggested that, with outstanding wash-out resistance, enhanced self-setting properties, good bioactivity and biocompatibility, the novel non-decay $C_3S$/SA composite bone cements might be considered as a prospective injectable/formable self-setting implant for orthopedic applications.

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**ACKNOWLEDGEMENTS**: The authors gratefully acknowledge the financial support from the Natural Science Foundation of China (Grant No.: 81190132 and Grant No.: 81401529), and Shanghai Pujiang Program (Grant No.: 14PJ1409400).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Preparation and properties of partly degradable Mg-PMMA complex bone cement

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**INTRODUCTION:** Poly(methyl methacrylate) (PMMA) bone cement has been primarily used in surgery for more than 50 years. However, there are some disadvantages which limited its application in clinical. Aseptic loosening of the prosthesis accounts for 52–55% of revision surgeries and occurs most frequently at the cement-bone interface. The objective of this study was to prepare partly degradable and bioactive PMMA bone cement with desired mechanical properties, which could degrade and allow for bone ingrowth.

**METHODS:** This bioactive bone cement was consisted of magnesium beads as degradable fillers, and PMMA as matrix. Solid component contains BPO and PMMA prepolymer, while liquid component contains N,N-dimethyl-p-toluidine (DMPT) and methyl meth-acrylate (MMA). Five bone cement specimens in each group were tested for compressive strength according to ISO 5833. MG-63 Cells were seeded onto the cements and allowed to adhere for 24 hours.

**RESULTS:** SEM images of the Mg-PMMA bone cement were shown in Fig. 1. The Mg beads were uniformly distributed in the polymeric matrix, then after degradation, the PMMA matrix became porous with the pore size at 300-500μm. The compressive strength of Mg-PMMA in different proportions didn't show significantly differences as shown in Fig. 2. Fluorescence microscopy analysis confirmed that MG-63 cells could be attached very well to the surfaces of all the Mg–PMMA cements in Fig. 3.

**DISCUSSION & CONCLUSIONS:** Partly degradable and bioactive Mg-PMMA bone cement were obtained by incorporating Mg beads into PMMA. The porous structure would be seen after the degradation of the Magnesium beads which might promote bone ingrowth and improve the interfacial bonding strength. Mg-PMMA bone cement is expected as a new type of filling material for orthopedics surgery.

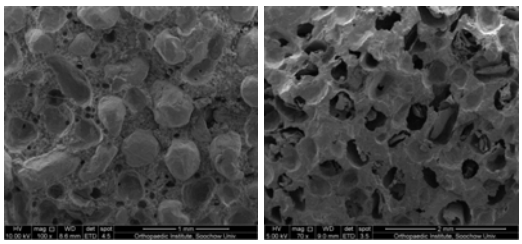

*Fig. 1: SEM images of the Mg-PMMA bone cement (left) before degrade and（right）after degrade.*

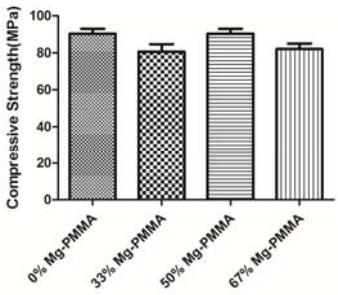


*Fig. 2: Compressive strength of Mg-PMMA bone cement*

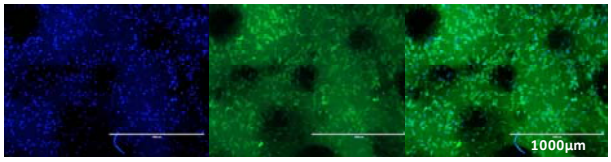


*Fig. 3: Images of MG-63 cells cultured on Mg–PMMA bone cements after 24 hours in vitro.*

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**ACKNOWLEDGEMENTS:** The authors greatly acknowledge the financial support from Startup Fund of Soochow University (Q4134924), NSFC (81471790, 31530024).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Calcium phosphate bone cement reinforced with modified silk fibroin (SF)

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**INTRODUCTION:** While calcium phosphate cement (CPC) as a biodegradable material may be used to achieve orchestrated cement resorption and new bone formation for vertebral augmentation without the potential risks of poly(methyl methacrylate) bone cement, it applications are limited due to the poor mechanical strength. In this study, we aim to develop a novel strong CPC by using treated silk fibroin (tSF).

**METHODS:** A $Ca(OH)_2$ solution (0.16wt%) was added into 20% aqueous silk fibroin solution with the volume ratio from 1:2 to 1:15 at different pH. and then after being rotated and placed in the 37℃ environment for 4 hours, treated silk fibroin (tSF) solutions were obtained. The tSF solution was used as a curing liquid to mix with α-TCP at the liquid-to-powder ratio of 0.4 ml/g. The 20% SF solution was also mixed with α-TCP as control group. After solidified for 3 days, the CPC samples were analysed by using mechanical testing machine and SEM, respectively. MC3T3 cells were seeded onto the tSF CPCs for 24 hours before SEM observation.

**RESULTS:** The strength of CPCs made from tSF solutions was dramatically reinforced with the increase of pH (Fig. 1). The compressive strength of CPC could reach 54.35±1.4 MPa as the top at pH8.5. We found that mass of cluster could be observed in both groups of CPCs (Fig. 2). At the same time, flower-like HA crystals section was distributed between the clusters in SF group, while smaller needle-like HA crystals section was distributed between the clusters in tSF groups, and further, the connection between the clusters in tSF group is closer than that in the SF group. SEM observation confirmed that MC3T3 cells could be attached very well to the surfaces of tSF CPCs (Fig. 3).

**DISCUSSION & CONCLUSIONS:** The mechanical properties of CPCs could remarkably been enhanced to 54 MPa by using tSF solutions as curing liquid, which was treated by $Ca(OH)_2$ to different pH (7.5-11). The reinforced CPCs made from tSF showed good biocompatibility.

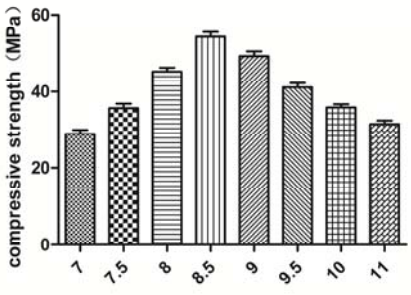


*Fig. 1: Compressive strength of CPCs at different pH of tSF solution (SF group: pH=7).*

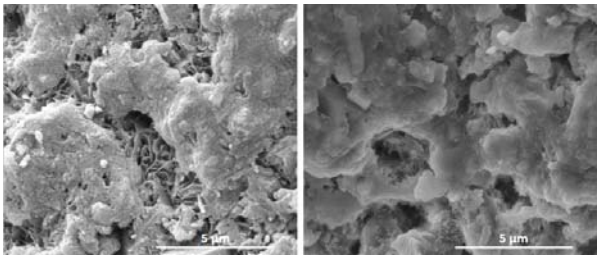


*Fig. 1: SEM images of the SF CPCs (left) and tSF CPCs (right).*

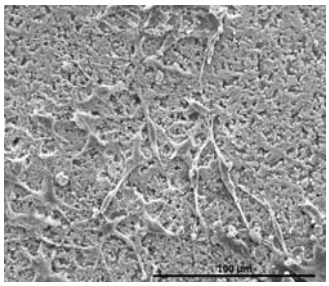

*Fig. 3: MC3T3 cells cultured on tSF CPCs after 24 hours in vitro by SEM observation.*

**ACKNOWLEDGEMENTS:** This study was supported by the NSFC (81471790, 31530024), Jiangsu Provincial Special Program of Medical Science (BL2012004), and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Synthesis of a novel injectable clay-based nanocomposite hydrogel with suitable mechanical properties

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**INTRODUCTION:** In our study a novel nanocomposite hydrogel (NC gel) was successfully prepared by in situ free-radical photo-polymerization of the acrylic acid derivatives and macromolecular crosslinker (PEGDA). The obtained hydrogel not only exhibits dramatic improvements in mechanical properties and can also be used as the injectable hydrogel which means the pre-hydrogel can in-situ forming hydrogel after injected.

**METHODS:** The acrylic acid derivative (monomer)/clay and acrylic acid derivative (monomer)/clay/PEGDA (macromolecular crosslinker) nanocomposite hydrogels were synthesized by in situ free-radical photo-polymerization of monomer and PEGDA in the presence of exfoliated clay. Firstly, clay was dispersed in water under ultrasonication at 37 °C for 1 h. Secondly, monomer, PEGDA and initiator were added to the clay suspension. After the solution was mixed well, it was transferred into plastic tubes with about 2 mm inner diameter. Photo-polymerization was carried out in a crosslink oven (XL-1000 UV Crosslinker, Spectronics Corporation, NY, USA) (Fig. 1). The clay content was varied from 1 to 10 wt % with respect to the monomer weight, and the solid content of the nanocomposite hydrogel varied from 20% to 30%. Tensile tests, compression tests, FTIR, XRD, SEM, TEM, XRD and Raman spectrum were used in our experiment.

**RESULTS:** Mechanical tests show that the obtained monomer clay-based hydrogel has the best tensile strength (about 400 kPa) and excellent stretch ability (higher than 5000%) when clay content and solid content are 5% and 20% respectively. The compression strength of the hydrogel is higher than 8 MPa and can recover to its original shape when compression ratio is less than 80% which will be very useful for tissue By adding macromolecular crosslinker (PEGDA) in to the system, the tensile strength of the hydrogel increases from 400 kPa to 800 kPa and the shape of the obtained hydrogel almost doesn't change after swelling in water which are very attractive for using as the tissue engineering candidate.

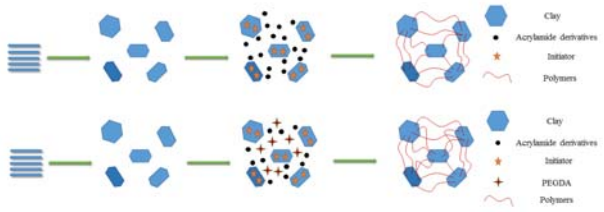


*Fig. 1: Preparation procedure of monomer/clay and monomer/clay/PEGDA nanocomposite hydrogels.*

**DISCUSSION & CONCLUSIONS:** Nanocomposite hydrogels composed of exfoliated clay showed attractive fracture strain up to 4000% and good compression strength. This kind of hydrogel was synthesized by in situ photo-polymerization of monomer in the presence of clay with or without macromolecular crosslinnker (PEGDA). More interestingly, the obtained hydrogel has good biocompatibility and suitalbe viscosity before photo-polymerization, which means this kind of hydrogel can be injected into the tissue defect site before polymerization and form hydrogel after photo-initiated by in-situ UV-light.

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**ACKNOWLEDGEMENTS:** The authors gratefully acknowledge the support for the work from the General Research Fund (GRF) (Grant No. 17205714).

European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# Injectable calcium phosphate bone cement reinforced by biocompatible diatomite

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**INTRODUCTION:** As vertebral compression fracture (VCF) becomes more prevalent, minimally invasive kyphoplasty that injects bone cement to repair fractured vertebrae has shown to be an effective treatment. However, bioactive calcium phosphate bone cement (CPC) is applicable to kyphoplasty due to its low compressive strength, poor injectability and inferior anti-collapsibility. It has been reported that adding biomineral diatomite (DT), whose composition is mainly silica to ordinary cement can improve its compressive strength [1]. Because silica with proper size has been reported to be biocompatible and already used in bio-related applications, we hypothesize that properly modified diatomite could be used to reinforce CPC (mixture of α-TCP and [$Na_2HPO_4 \cdot 2H_2O$] in this work).

**METHODS:** The raw DT was calcined at 600°C and cleaned by 5M HCl. Raw DT had different shapes and sizes and was separated using wet sieves with the different mesh sizes and then collected by sedimentation method. Cytotoxicity of DT and hydroxyapatite (HA, as a control) were tested using Cell Counting Kit-8 and Cytotoxicity LDH Assay Kit-WST with MC3T3 rat osteoblasts and NIH3T3 fibroblasts at different concentrations following the manufacturer's instructions.

The biocompatible DT was selected based on cytotoxicity results and then modified by two methods: Simulated Body Fluid (SBF) soaking and HA coating. DT (5%), CPC(90%) and starch (5%, added for anti-collapse purpose) was mixed with $NaH_2PO_4$ solution as liquid phases (Liquid solid ratio is 1:2). Samples were cast into columns (diameter: 6mm and length 12mm) for compression study on a mechanical tester at a cross speed of 0.5mm/min. The injectability was evaluated by injecting the cement paste through a syringe with maximum force of 100N and measuring the weight of the extruded paste against the initial weight of the samples.

**RESULTS:** Results showed that diatomite has a size-dependent cytotoxicity. Specifically, DT with average size of 30μm (DT30) had the least toxicity and DT with average sizes of 10μm (DT10) and 3μm (DT3) had the highest toxicity. DT30 revealed similar toxicity as HA and thus was used for the reinforcement of CPC. SBF- and HA-modified DT are shown in Figure 1, showing calcium phosphate deposited on the DT disk. Results of compressive strength, moduli and injectability of cements are shown in Table 1.

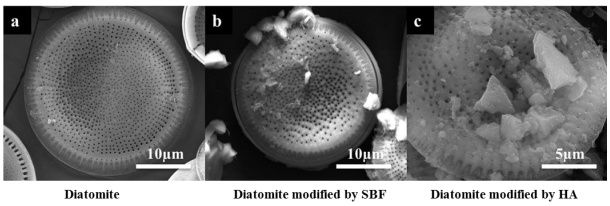


*Fig.1: SEM images of the diatomite.(a)Diatomite was calcined and exposed to HCl. (b)Diatomite modified by SBF for 48h. (c)Diatomite modified by HA with the molar radio 3:1.*

*Table 1,The mechanical properties of cements*

| | Strength（MPa） | Modulus（MPa） | Injectability（%） |
|---|---|---|---|
| CPC | 15.62±0.98 | 748.04±72.97 | 15±1 |
| Starch | 18.73±2.14 | 950.54±215.54 | \ |
| SBF soaking DT -CPC | 25.17±2.13 | 1404.96±188.22 | 21±0.5 |
| HA coating DT-CPC | 24.536±1.42 | 1496.9±231.92 | 28±2.2 |
| SBF soaking DT-starch -CPC | 29.47±2.45 | 1711.24±216.95 | 82±2.2 |
| HA coating DT-starch-CPC | 38.34±0.88 | 2131.3±49.24 | 90±2.2 |

**DISCUSSION & CONCLUSIONS:** Size-dependent cytotoxicity of DT at micrometer scale, was reported for the first time. DT with average size of 30μm was biocompatible and DT with sizes of 10μm and 3μm were highly cytotoxic. SBF- and HA-modified CPC, showed significant increases in compressive strength, injectability and anti-collapsibility of CPC. The maximum compressive strengths and moduli were increased from 15.6MPa to 38.3MPa and from 748MPa to 2131MPa respectively. The injectability of CPC was also satisfactory for kyphoplasty. This biomineral is therefore promising for the reinforcement of CPC bone cement.

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European Cells and Materials Vol. NN. Suppl. N, 20xx (page htu)
ISSN 1473-2262

# A simple and effective approach to prepare injectable macroporous calcium phosphate cement for bone repair: Syringe-foaming using a viscous hydrophilic polymeric solution

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**INTRODUCTION:** Calcium phosphate cements (CPCs) are attractive as bone substitutes due to their injectability and self-setting ability in physiological conditions, as well as to their higher similarity to biological apatites and higher reactivity than that of a ceramic hydroxyapatite. However, not only micropores but also macropores seem necessary to accelerate resorption of CPCs and bone ingrowth. CPCs without macropores, especially apatitic cements given their low physicochemical solubility, present a slow rate of resorption and can only be slowly replaced by new bone from periphery of the defect. In this study, a simple and effective approach is proposed to prepare α-tricalcium phosphate (α-TCP)-based macroporous apatitic CPCs through an original syringe-foaming process, using Si-HPMC as the foaming agent.

## METHODS:

α-TCP powder and Si-HPMC solution are the two main components used to prepare Si-HPMC foamed CPCs. The synthetic procedure of Si-HPMC powder was detailed by Fatimi et al. [1], according to a method described by Bourges et al [2]. The Si-HPMC solution and the $NaH_2PO_4$ solution were initially sealed in two commercial syringes, being ready for use. Subsequently, the desired volume of air was pumped into the syringe containing the $NaH_2PO_4$ solution. Both syringes were then joined by a connector, and the solutions and air were rapidly mixed by pushing the two plungers of the syringes alternately in opposite directions for twenty seconds until a homogeneous Si-HPMC foam was formed. The resultant Si-HPMC foam was then kept in a syringe for 15 minutes of gelling before the preparation of the foamed CPCs. To prepare the CPC paste, the solid phase (98 wt% α-TCP powder and 2 wt% CDHA) of CPC was manually mixed with 2.5 wt% $Na_2HPO_4$ solution in a mortar at an L/P ratio of 0.35 mL/g for 1 min, and the resultant paste was packed into a syringe of 5 mL, followed by removing the entrained air. Subsequently, the pre-prepared Si-HPMC hydrogel foam and CPC paste were rapidly mixed for 30 seconds until a homogeneous Si-HPMC foamed CPC paste was formed. The injectability, cohesion, microstructure and mechanical properties of this macroporous CPC are systematically investigated, and a preliminary *in vivo* study of this new biomaterial is carried out in distal femoral sites of rabbits.

## RESULTS:

The Si-HPMC foamed CPCs demonstrate excellent handling properties such as injectability and cohesion. After hardening the foamed CPCs possess hierarchical macropores and their mechanical properties (Young's modulus and compressive strength) are comparable to those of cancellous bone. Moreover, a preliminary in vivo study in the distal femoral sites of rabbits was conducted to evaluate the biofunctionality of this injectable macroporous CPC. The evidence of newly formed bone in the deep zone of implantation site indicates the feasibility and effectiveness of this foaming strategy that will have to be optimized by further extensive animal experiments.

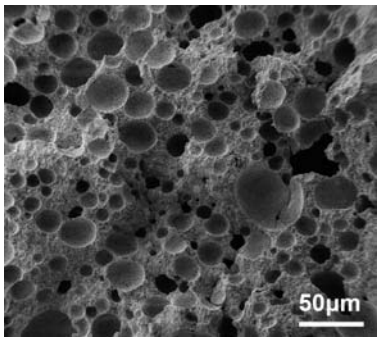


*Fig. 1:SEM image of the fracture surfaces of Si-HPMC foamed CPC injected into a saline solution immediately after preparation ($L/P_{total}$= 0.8, $V_{air}/V_{hydrogel}$ = 1.0).*

**DISCUSSION & CONCLUSIONS:** In the present study, a new and simple method to prepare macroporous CPCs is developed by foaming in connected syringes and using Si-HPMC as a foaming agent, taking advantage of its high viscosity and self-crosslinkable properties. Now we have to increase the polymer degradation properties before to go to a couplet preclinical study.

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**ACKNOWLEDGEMENTS:** We acknowledge financial support from the INSERM and the regional program BIOREGOS II (Pays de la Loire, France).

# Alendronate-modified liposome for bone targeting gene delivery

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**INTRODUCTION:** Aging is usually related to an increase in metabolic skeletal disorders such as osteoporosis and bone metastases, accompanied with the decrease in mesenchymal stem cells (MSCs) number.[1] For bone regeneration, directing MSCs toward the bone surface is a key step for further osteogenic differentiation.[2] Stromal cell-derived factor-1 (SDF-1) has been reported with the effect in inducing MSC migration.[3] Construction of the bone targeted gene delivery system would be a promising way to locally express SDF-1 on bone surface for attracting MSC migration and differentiation. This study aimed to develop bone targeting liposome by alendronate (Aln) modification for modulating MSC migration.

**METHODS:** Aln was conjugated to DSPE-PEG2000-COOH via carbodiimide chemistry through the reaction between carboxyl and amino. Aln-liposome was prepared using the thin-film hydration method with the component of DOTAP, DOPE, cholesterol, DSPE-PEG2000 and DSPE-PEG2000-Aln as shown in Fig 1a. The liposome diameter distribution and zeta potential were measured by a dynamic light scattering detector. The morphology of liposomes was observed by TEM. The transfection efficiency was examined by transfection of COS-1 cell with GFP reporter gene. Bone target ability was examined using fluorescence absorption test. Then Aln-liposome was applied to SDF-1 gene delivery to MC3T3 osteoblast cells for MSC migration.

**RESULTS:** Bone targeting Aln-liposome was prepared by conjugating liposome with alendronate. It showed the average diameter around 104 nm and zeta-potential at 41.5 mV. TEM image showed the sphere morphology of Aln-liposome (Fig 1b). The critical DNA complex ratio examined by gel retardation was 1:1 (N/P). The transfection efficiency of Aln-liposome was even higher than that of Lipofectamine™ 2000 showed by luciferase and GFP reporter gene expression in COS-1 cell (Fig 1c). Aln-liposome showed the bone targeting ability in vitro. The binded liposomes increased from 13.7% to 61.4% after modification with alendronate by examining the changes in fluorescence of NBD-labeled liposome after mixed with hydroxyapatite. Aln-liposome was then applied to deliver SDF-1 gene to MC3T3 osteoblast cells, and its expression was confirmed by PCR and

western blot. The expressed SDF-1 showed the effect in attracting MSC migration in transwell culture.

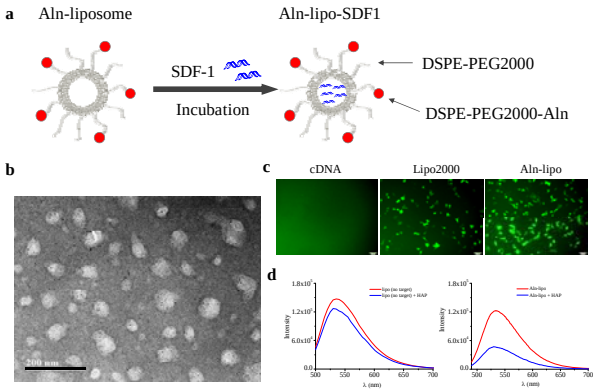


*Fig. 1: Schematic diagram for the preparation of Aln-liposome (a). TEM image of Aln-liposome (b). Transfection efficiency of Aln-liposome to COS-1 cells (c). Hydroxyapatite binding ability of liposomes by fluorescence absorption (d).*

**DISCUSSION & CONCLUSIONS:**

Alendronate modified Liposome showed the bone targeting ability and high gene transfection efficiency. By delivery SDF-1 gene, this system could attract MSC migration. This study provides a novel potential technique for bone regeneration.

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**ACKNOWLEDGEMENTS:** This work was supported by NSFC No.81301567 and Shenzhen Science and Technology Research Funding (CXZZ20140901004122089).