17th Interdisciplinary Research Conference on Biomaterials

Injectable and Implantable Biomaterials and Biologics for Tissue Regeneration

GROUPE DE RECHERCHE INTERDISCIPLINAIRE SUR LES BIOMATÉRIAUX OSTEO-ARTICULAIRES INJECTABLES

GRI BOI

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1st April – 3rd April 2007
Oxford, United Kingdom

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Contents

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Introduction

GRIBOI is an acronym for the "Groupe de Recherche Interdisciplinaire sur les Biomatériaux Ostéoarticulaires Injectables" (Interdisciplinary Research Society for Bone and Joint Injectable Biomaterials). It was originally set up in 1989 as an informal group of clinicians from Northern France who were concerned with minimally-invasive bone therapies based on percutaneous injection of bone substitutes. This group of clinicians included Hervé DERAMOND (Amiens) and Hervé LECLET (Berck) radiologists, Bernard DUQUESNOY (Lille) a rheumatologist and Pierre HARDOUIN (Berck) a rheumatologist and researcher in the biomaterials field. At this time the concept of "injectable biomaterials" in orthopaedics was new. The best known of such therapies is Acrylic Vertebroplasty (AVP), which was being initiated at this time by Prof Deramond, who was the first President of Griboi, and his group at Amiens. Although the use of PMMA as a bone substitute is still highly relevant for the palliative treatment of collapsed osteoporotic or metastatic vertebrae, new more sophisticated, resorbable and osteoconductive materials have now come into existence.

The initiators of GRIBOI realized very soon the great promise of injectable bone grafting materials for future clinical applications, for they open new avenues to preventive bone therapies. However, their success relies on a subtle alchemy of optimal material performance, adequate surgical technique and long-term favorable biological and biomechanical responses. Hence, the development of efficient therapies based on injectable bone materials is obviously a demanding interdisciplinary work well beyond the exclusive expertise of clinicians. Therefore, members of GRIBOI decided many years ago to broaden its membership to non-medical people, including biologists, materials scientists, engineers and others. This 17th GRIBOI meeting is the sixth one taking place out of France and meetings were previously held in Lausanne, Shanghai, Baltimore, Shanghai again and Bern. This is a consequence of the increasing popularity of the concept of "Injectable Bone Materials", which has been gaining more and more credibility and international recognition over the past fifteen years. This also manifests the will of GRIBOI to open its membership to the international community of physicians, biologists, engineers and industrial companies active in the field of minimally-invasive skeletal therapies. GRIBOI is particularly proud to be hosted by the UK in 2007. We are delighted to welcome you to the splendid City of Oxford for this two-day conference on biomaterials and related research. I hope you will enjoy not only the scientific program, but also the numerous cultural attractions offered by Oxford City and its surroundings and the University of Oxford itself.

The Executive Committee of GRIBOI wish here to express our deepest thanks for the efforts developed by the Local Organizing Committee and by the Royal Microscopical Society in preparing this exceptional meeting. Each Chairman gives a special colour to the annual meeting and the inclusion of implantable materials and biologics as well as injectable biomaterials in the present programme emphasizes the expanding knowledge in all aspects of biomaterials and vital biological agents that is required to promote tissue regeneration whilst developing minimally invasive methods. We hope that you will enjoy a very successful GRIBOI - OXFORD - 2007 Conference!

Jacques Lemaître
President of GRIBOI

Pierre Hardouin
Secretary of GRIBOI

International Scientific Committee

2006-07

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GRIBOI meetings

From 1990 to 1997 the meetings were organized in Berck (France), twice a year, and subsequently every two years. Attendees came from several countries, and in 1997 it was decided to meet annually, each time in a different location. Meetings were endorsed by several scientific societies and by Biomat.net. Griboi is a component of Biomat, the separate French society affiliated to the European Biomaterials Society.

NUMBER	DATE	LOCATION	Chair	Publication
1	1990 September 28th	Berck sur Mer France	P Hardouin, H Leclet, H Deramond	
2	1991 March 1st	Berck sur Mer France	P Hardouin, H Leclet	
3	1991 October 4th	Berck sur Mer France	P Hardouin, H Leclet	
4	1992 October 9th	Berck sur Mer France	P Hardouin, H Leclet	Proceedings
5	1993 November 19th	Berck sur Mer France	P Hardouin, H Leclet	Proceedings
6	1995 March 30-31st	Berck sur Mer France	P Hardouin, H Leclet	ITBM 1995, 16, S1
7	1997 March 20-21st	Berck sur Mer France	P Hardouin	ITBM 1997, 18, S1.
8	1998 March19-20	Lille France	B Duquesnoy	Proceedings
9	1999 March 1-2nd	Lausanne, Switzerland	J Lemaitre	Bone 1999, 25,2
10	2000 March 13-14th	Toulouse France	P Sharrock	Proceedings
11	2001 March 8-9th	Calais France	P Hardouin, J Jeanfils	Applied biomaterials 2002, 63,4
12	2002 March 14-17th	Shanghai, China	CS Liu	Proceedings
13	2003 March 14-15th	Baltimore, USA	S M Belkoff	Proceedings
14	2004 March 25-26th	Limoges France	D Bernache-Assolant, E Champion	Sauramps Medical ed 2004
15	2005 March 18-20th	Shanghai	K Dai, C Ding	Journal of Medical Biomechanics 2005, 20
16	2006 March 16-18th	Bern, Switzerland	M Bohner, S Ferguson, P Heini	Proceedings
17	2007 April 2-3nd	Oxford, UK	J Triffitt	Tissue Engineering
18	2008 June	Montreal, Canada	G Baroud	

The next meeting

18th Multidisciplinary Griboi Conference on Injectable Biomaterials and Biomechanics

June 2008

Quebec, Canada

Organizers: G. Baroud (chair) and F. Gitzhofer (vice chair)
BioMechanics Laboratory, Universit´e de Sherbrooke

Griboi 2008 brings together medical, biomaterials, and biomechanics researchers with a common interest in understanding the clinical, physical, biological, and chemical processes underlying the minimally invasive applications of Injectable biomaterials, with a focus on hard tissue implantation.

The conference also draws engineers from both industry and academia. A unique feature of the conference is that participants from varied backgrounds, but with similar interests in mechanisms related to Injectable biomaterials, interact on an equal footing. The format is designed to encourage in-depth discussion in a stimulating and relaxed forum for an interdisciplinary exchange of ideas.

Junior researchers and particularly graduate students are encouraged to attend the meeting. A few awards for young researchers are provided to encourage strong attendance of the Jung researcher.

Scientific Topics

- The main scientific topics of the meeting will include the following:
- Clinical applications
- Dental applications
- Science and engineering of injectable biomaterials
- Biomechanics of injectable biomaterials
- In vivo, in vitro, and in silico models of injectable biomaterials
- Instrumentation of injectable biomaterials
- Processes in biomaterials
- Bone cements
- Injectable biomaterial in soft tissue
- Nano-synthesis and characterization of Injectable biomaterials
- Micro-imaging, flow, and transport analysis of hard tissue
- Plasma technology and clinical applications

Exhibitors

The following companies are exhibiting at this meeting. Please take time to visit their stands during lunchtimes and during the tea and coffee breaks. Their contact addresses can be found overleaf.

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Synthes Ltd	20 Tewin Road, Welwyn Garden City, Hertfordshire AL7 1 LG Contact: Dawn Shore Tel. +44 1707 33 22 12 Email: shore.dawn@synthes.com www.synthes.com

Scientific Programme

	SUNDAY APRIL 1ST 2007
5.00 PM - 7:00 PM	Conference Registration at St Catherine's College Welcome and wine with the hosts sponsored by the Journal of Microscopy

	MONDAY APRIL 2nd 2007
8:00 am - onwards	Registration
8.55 am - 9:00 am	**OPENING OF CONFERENCE** ***JIM TRIFFITT***
SESSION 1	***ASPECTS OF STEM CELL THERAPY*** ***CHAIRS: PIERRE HARDOUIN*** (Boulogne sur Mer, France) **&** ***HARI REDDI*** (California, USA)
9.00am - 9.30 am	**INVITED LECTURE No. 1** **PAMELA ROBEY** National Institutes of Health/NIDCR, Bethesda Maryland, USA ***SKELETAL (AKA "MESENCHYMAL") STEM CELLS: HOW CAN WE MAKE THEM WORK FOR US?***
9.30 am - 9.45 am	**Jon Dawson** Bone Marrow Stromal Cells and Biomimetic Collagen-Hydroxyapatite Scaffolds for Skeletal Tissue Engineering (Abstract 48); *Bone and Joint Research Group, University of Southampton, United Kingdom, Department of Materials, Oxford University, UK.*
9.45 am - 10.00 am	**Kerong Dai** Proliferation and osteoblastic differentiation of human mesenchymal stem cells under different perfusion flow rates (Abstract 25): *Department of Orthopaedics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, P.R.China*
10.00 am - 10.15 am	**James Gallagher** Differential Growth of Human Osteoblastic Cells on HA and TCP with Different Surface Charge (Abstract 37): *Department of Human Anatomy & Cell Biology, University of Liverpool, UK*
10.15 am - 10.30 am	**Gang Li** Repair critical-sized bone defect in rabbit using different resorbable silicon stabilized HA/TCP bioceramics combined with fresh bone marrow cells (Abstract 18): *Department of Orthopaedic Surgery, Queen's University Belfast, Belfast, UK*
10.30am - 11.00 am	Coffee, Posters & Exhibits

	MONDAY APRIL 2nd 2007
SESSION 2	***TISSUE REGENERATION*** ***CHAIRS:- JAMES TRIFFITT*** (Oxford, UK) **&** ***PAMELA ROBEY*** (Maryland, USA)
11.00am - 11.30 am	**INVITED LECTURE No. 2** **A HARI REDDI** Orthopaedic Surgery, UC Davis School of Medicine, University of California at Davis, Davis, California, USA ***ARTICULAR CARTILAGE REGENERATION : SIGNALS, STEM CELLS and SCAFFOLDS***
11.30am - 11.45pm	**Aurelie Quintin** Fetal spine cells for intervertebral disc regeneration: Preliminary characterization (Abstract 17); *Laboratory of Biomechanical Orthopedics EPFL-HOSR, Swiss Federal Institute of Technology Lausanne, Switzerland*
11.45am - 12.00pm	**Amir Haze** In vivo bone, Periodontal Ligament and Cementum Regeneration, Using a Recombinant Human Amelogenin Protein- Possible Biological Mechanisms (Abstract 2); *Hebrew University – Hadassah, Jerusalem, Israel*
12.00PM - 2.00PM	LUNCH, POSTERS AND EXHIBITS
SESSION 3	***BIOMATERIALS*** ***CHAIRS: JACQUES LEMAITRE*** (Lausanne, Switzerland **&** ***JAN CZERNUSZKA*** (Oxford, UK)
2.00 pm - 2.30 pm	**INVITED LECTURE No. 3** **JAN CZERNUSZKA** Department of Materials, University of Oxford, Parks Road, Oxford, UK ***BIOMATERIALS:SCAFFOLDS FOR TISSUE REGENERATION***
2.30PM - 2.45PM	**Xin Zhao** Injectable Biodegradable Poly (ester-co-ether) Methacrylate Monomers for Bone Tissue Engineering and Drug Delivery Applications (Abstract 3); *Biomaterials and Tissue Engineering Division, UCL Eastman Dental Institute*
2.45PM - 3.00PM	**Christele Combes** Calcium carbonate cements in vitro and in vivo: influence of the liquid phase composition (Abstract 21); *CIRIMAT UMR UPS-INPT-CNRS 5085, ENSIACET, 118 route de Narbonne, France*
3.00PM - 3.15PM	**Tingting Tang** Bioactive glass /nano-HA gradient coating on metal substrate (Abstract 24); *Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai 200011,China*
3.15PM - 3.30PM	**Tobias Brunner** Comparison of amorphous TCP nanoparticles to micron-sized α-TCP as starting materials for calcium phosphate cements (Abstract 15); *Institute for Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, CH-8093 Zurich, Switzerland*
3.30PM - 3.37PM	**Nihan Tuncer** Production of titanium foams for implant applications (Abstract 32); *Department of Materials Science and Engineering, Anadolu University, Turkey*
3.37PM - 4.00PM	Coffee, Posters & Exhibits

MONDAY APRIL 2nd 2007

SESSION 4	***TISSUE GROWTH FACTORS*** ***CHAIRS: GRAHAM RUSSELL*** (Oxford, UK) **&** ***ALAN BOYDE*** (London, UK)
4.00 pm - 4.30 pm	**INVITED LECTURE No. 4** **KUBER T. SAMPATH** Discovery Research Initiative, Genzyme Corporation, One Mountain Road, Framingham, MA, USA ***GROWTH FACTOR STIMULATION OF REGENERATION IN THE MUSCULOSKELETAL SYSTEM***
4.30 PM - 4.45 PM	**Gary Balian** Bone targeting peptides potentiate osteogenesis (Abstract 9); *University of Virginia, Department of Orthopaedic Surgery, PO Box 801016, Charlottesville, VA 22908*
4.45 PM - 5.00 PM	**Monika Rumpler** Impact of a three-dimensional hydroxylapatite scaffold on differentiation of osteoblasts and tissue formation in vitro (Abstract 4); *Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, Potsdam, Germany*
5.00 PM - 5.30 PM	**GENERAL ASSEMBLY OF GRIBOI AGM - FOR ALL REGISTRANTS TO THIS MEETING**
5.30 PM	CLOSE OF FIRST DAY
6.45PM FOR 7.30PM	RECEPTION IN THE JCR LOUNGE WITH EARLY ENGLISH MUSIC PROVIDED BY *DOWNWIND* ***DOWNWIND*** **Early Music Consort** Downwind is a small group of amateur musicians from Oxfordshire and Berkshire who play early music on recorders and wooden wind-cap instruments The consort has performed at mediaeval banquets, mystery plays, for early dance groups and in various local concerts . This evening the consort, dressed in 17th century costume, will be playing early English music on recorders, crumhorns, cornamusa, Glastonbury pipe and three hole pipe and tabor.
7.30 PM	**CONFERENCE DINNER SPONSORED BY SYNTHES**

Thank you to Synthes for its kind and generous sponsorship of the Conference Dinner. Please take time to visit the Synthes stand during the meeting.

	TUESDAY 3RD APRIL 2007
SESSION 5	***CLINICAL AND BIOMECHANICAL FEATURES*** ***CHAIRS: K.DAI*** (Shanghai, China) ***& MARC BOHNER*** (Bettlach, Switzerland)
8.50 am - 9:10 am	**INVITED LECTURE No. 5** **JEREMY FAIRBANK** Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, UK ***CLINICAL REQUIREMENTS FOR SPINAL BIOMATERIALS RESEARCH***
9.10 am - 9.30 am	**INVITED LECTURE No. 6** **GAMAL BAROUD** Universite de Sherbrooke, Faculté de génie, Département de génie mécanique, 2500 boul. Universite, Sherbrooke, QC, Canada ***BIOMECHANICAL RESEARCH AT THE INTERFACE OF ENGINEERING AND MEDICINE-CEMENT AUGMENTATION OF OSTEOPOROTIC BONE***
9.30 am - 9.45 am	**Vassilis Likomitros** Biomaterial injection under local anesthesia in a case of spinal compression fracture (Abstract 23); *3rd Department of Orthopaedics, Aristotle University, Thessaloniki, Greece*
9.45 am - 10.00 am	**Arthour Gevargez** An injectable high strength bioceramic for treatment of vertebral compression fractures (Abstract 16); *Doxa AB, Uppsala, Sweden and Materials Science Department, The Angstrom Laboratory, Uppsala University, Sweden*
SESSION 6	***BIO-IMAGING*** ***CHAIRMEN - ZHANFENG CUI*** (Oxford, UK) ***& RICHIE GILL*** (Oxford, UK)
10.00am - 10.30 am	**INVITED LECTURE No. 7** **RALPH MULLER** Ralph Mueller, Institute for Biomechanics, ETH Zurich, Moussonstrasse 18, 8092 Zurich, Switzerland ***HIERARCHICAL MICRO-IMAGING IN BIOMATERIALS RESEARCH AND TISSUE REGENERATION***
10.30 am - 10.45 am	**INVITED LECTURE No. 8** **ALAN BOYDE** Biophysics OGD, Queen Mary, University of London, Dental Institute, New Rd., London, UK ***HIGHER ARCHICAL IMAGING OF BIOMATERIAL - TISSUE INTERACTIONS***
10.45 am - 11.15 am	Coffee, Posters & Exhibits
11.15 AM - 11.30 AM	**Jianping Wu** 3D Study of Microstructure of Articular Cartilage for Development of a Real Time Histology for Articular Cartilage (Abstract 29); *3D Image Laboratory, School of Mechanical Engineering, the University of Western Australia, Australia*
11.30 AM - 11.45 AM	**Uday Tirlapur** High Resolution 3D Resolved Non Invasive In situ Nonlinear Optical Imaging (NLOI) of Ex Vivo Tissues and Various Scaffolds Adopted for Tissue Engineering Applications with Human Mesenchymal Stem Cells (Abstract 36); *Oxford Centre for Tissue Engineering and Bioprocessing, University of Oxford, UK*

	TUESDAY 3RD APRIL 2007
SESSION 7	***PHARMACEUTICAL AGENTS AFFECTING BONE METABOLISM*** ***CHAIRS:KUBER SAMPATH*** (Massachusetts, USA) **&** ***JEREMY FAIRBANK*** (Oxford, UK)
11.45am - 12.15pm	**INVITED LECTURE No. 9** **GRAHAM RUSSELL** The Botnar Research Centre and Oxford University Institute of Musculoskeletal Sciences, Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, UK ***BISPHOSPHONATES AND TISSUE RESTORATION*** ***CURRENT & FUTURE OPPORTUNITIES***
12:15 pm - 12:30 pm	**Pascal Janvier** Characterization of new injectable biomaterials containing bisphosphonates (Abstract 20); *Université de Nantes, UMR 6513, 2 rue de la Houssinière - BP 92208 - 44322 Nantes Cedex 3 - France*
12.30PM - 2.25PM	LUNCH, POSTERS AND EXHIBITS
2.25 pm-2.30 pm	***GRIBOI-MONTREAL-2008***
SESSION 8	***ENGINEERING SCIENCE AND ORGAN RECONSTRUCTION*** ***CHAIRS: G BAROUD*** (Sherbrooke, Canada) **&** ***RALPH MUELLER*** (Zurich, Switzerland)
2.30 pm - 3.00 pm	**INVITED LECTURE No. 10** **RICHIE GILL** Oxford Orthopaedic Engineering Centre, Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, UK ***ENGINEERING REQUIREMENTS FOR TISSUE REPLACEMENT***
3.00PM - 3.15PM	**Michael Liebschner** Biomechanical efficacy of intervertebral disc augmetation (Abstract 10); *Department of Bioengineering, Rice University, Houston, TX, USA*
3.15PM - 3.30PM	~~**Gamal Baroud**~~ Bashoor Zakeh Geometric and Fluid Transport Analysis of Calcium-Phosphate Scaffolds (Abstract 47); *Biomechanics Laboratory, Sherbrooke University, Sherbrooke, Quebec, Canada*
3.30PM - 3.45PM	**Jaroslaw Wasikiewicz** The new packaging systems for implantable electronic devices (Abstract 30); *IRC in Biomedical Materials, University of London,UK*
3.45PM - 4.00PM	**Mark Thompson** Mechanical characterisation of the Bioflex® system (Abstract 64); *Department of Engineering Science, University of Oxford, Oxford, UK*
4.00PM - 4.05 PM	POSTER AND ORAL AWARDS KINDLY DONATED BY the Dr Robert Mathys Foundation Bischmattstrasse 12, CH-2544 Bettlach, Switzerland
4.05 PM	CLOSE OF MEETING

Thank you to the Robert Mathys Foundation for its kind and generous donation of prizes for the best poster and oral presentations.

Session 1 ASPECTS OF STEM CELL THERAPY

CHAIRS: PIERRE HARDOUIN & HARI REDDI

Invited lecture No. 1

PAMELA ROBEY

National Institutes of Health/NIDCR, Bethesda Maryland, USA

SKELETAL (AKA "MESENCHYMAL") STEM CELLS: HOW CAN WE MAKE THEM WORK FOR US?

It has long been known that bone marrow stroma contains a subset of multipotent skeletal stem cells that have the ability to reform a bone/marrow organ (bone/cartilage, hematopoietic stroma and marrow adipocytes). Similar but not identical cells have also been isolated from other related mineralized tissues. Major advances have been made in the last decade to develop methods to harness their unique properties for tissue regeneration. Using appropriate scaffolds, different populations of ex vivo-expanded cells can be used for bone regeneration in a variety of skeletal defects, and for the reconstruction of periodontal tissue in the oral cavity. While systemic injection has been envisioned for the treatment of generalized skeletal disorders, whether or not cells are able to escape from the circulation into the extravascular spaces is debatable at this time. Further work is needed to determine how to motivate these cells to not only escape, but to incorporate into a pre-existing structure at a high enough level to have a biological effect. Due to their ability to self renew and persist upon in vivo transplantation, skeletal stem cells may also be vehicle for gene transfer and therapy. Remarkably, it has been extremely difficult to apply molecular engineering techniques to human stem/progenitor cell populations. But with the advent of new viral and non-viral vectors, it is now possible to conceive of their use in serving as a stable source of a missing or deficient protein, or even to correct gene defects. Much has been learned, more still to come.

Bone Marrow Stromal Cells and Biomimetic Collagen-Hydroxyapatite Scaffolds for Skeletal Tissue Engineering

J.I. Dawson[1], D.A. Wahl[2], J.T.Czernuszka[2] R.O.C. Oreffo[1]
[1] *Bone and Joint Research Group, University of Southampton, United Kingdom* [2] *Department of Materials, Oxford University, UK.*

INTRODUCTION: The extracellular matrix (ECM) is of defining importance for skeletal tissue engineering. As well as bestowing upon the tissue structure and function, the ECM regulates cell proliferation and differentiation through direct cell receptor interaction, controlled diffusion of soluble factors and attenuated transmission of mechanical signals. The importance of collagen in the extracellular matrix and the role it plays in the temporal cascade of events leading to new bone from progenitors suggests it as a strong candidate material for tissue engineering scaffolds.
In a bio-mimetic approach, type I collagen matrices of defined mean porosity and incorporating precipitated nano-sized, carbonate substituted hydroxyapatite (HA) crystals [1, 2], were assessed for osteo- and chondrogenic conduction of unselected and STRO-1 immunoselected human bone marrow stromal cells (HBMSCs).

METHODS: HBMSCs were selected for expression of the STRO-1 antibody using magnetic activated cell sorting (MACS) and culture expanded over 2 passage prior to seeding onto a collagen-HA composite scaffold ($\bar{X}$ pore size = 135μm) or a pure collagen scaffold ($\bar{X}$ pore size = 64μm) to assess osteo- or chondrogenesis respectively. Cells were cultured *in vitro* in basal, osteogenic or chondrogenic conditions over 21 – 28 days with positive controls. Cell viability, scaffold penetration, tissue formation and gene expression was assessed using immunohistochemical, biochemical and μCT techniques and PCR.

RESULTS: After 21 days in chondrogenic conditions STRO-1-seeded collagen only scaffolds displayed extensive proteoglycan-rich ECM deposition around the scaffold periphery (Fig. 1A). In addition immuno-

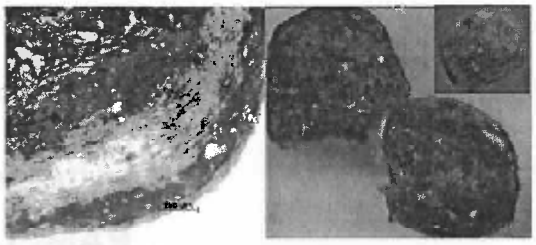

Fig. 1: (A) Chondrogenesis: Alcian blue stain for Proteoglycan rich matrix (B)Osteogenesis: Red stain for alkaline phosphatase activity in basal (inset) and osteogenic conditions.

histochemistry displayed ubiquitous Type II collagen synthesis and Sox-9 expression, the latter being expressed in cells toward the centre of the scaffold. Pronounced chondrogenic gene expression equivalent to the pellet culture positive control was confirmed by PCR. HBMSC seeded collagen-HA scaffolds cultured for 28 days in osteogenic conditions yielded high expression of alkaline phosphatase throughout (fig 1B). Extensive cell penetration into the scaffold was established by histology and μCT and an osteogenic gene expression profile confirmed with PCR.

CONCLUSIONS: Both collagen matrices proved highly conductive to growth and differentiation of HBMSCs. Substantial proteoglycan and Collagen II rich matrix synthesis and high alkaline phosphatase expression under chondro- and osteogenic conditions respectively indicate the potential of ECM cues for osteo and chondrogenesis.

REFERENCES: [1] D.A.Wahl, J.T.Czernuszka, *Eur. Cell Mater.* **2006**, *11*, 43. [2] E.Sachlos, *et al.*, *Tissue Eng* **2006**, *12*, 2479.

ACKNOWLEDGEMENTS: This work was supported by a grant from the BBSRC.

Proliferation and osteoblastic differentiation of human mesenchymal stem cells under different perfusion flow rates

Ke-rong DAI, Xiao-jiang SUN, Ting-ting TANG
Department of Orthopaedics, Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, P.R.China
krdai@163.com

INTRODUCTION: Our previous work has suggested that the flow perfusion culture is superior to the static culture for MSCs proliferation in critical-size β-TCP scaffold. It is well documented that the flow rate plays a key role in flow perfusion culture for its effects on the transfer of nutrients and waste products to and from cells, the retention of newly synthesized extracellular matrix components within the construct, and the fluid induced shear stresses within the scaffold pores. In the present study, we cultured hMSCs with a perfusion bioreactor and aimed at assessing the proliferation and osteoblastic differentiation of hMSCs in large scale scaffold under different perfusion flow rates.

METHODS: hMSCs isolated from iliac bone marrow aspiration were loaded with crtical-size porous β-TCP scaffold (H=30mm, φ=14mm) and cultured in osteogenic medium with a perfusion bioreactor under perfusion flow rate of 3ml/min, 6ml/min or 9ml/min for 15 days. The culture media were collected for D-glucose consumption assay every 3 days. After 15 days perfusion culture, the cell-scaffold composites were harvested for assessment of cell viability by MTT colorimetric method, SEM observation and osteogenic gene expression by real-time PCR.

RESULTS: The cell viability indicated that the cell-scaffold composites under 6ml/min exhibited the most viable cells ($P<0.01$). The proliferation of hMSCs assayed by daily glucose consumption showed that at early stage of culture cells proliferated faster under 9ml/min than 3ml/min and 6ml/min ($P<0.01$), while at late stage of culture cells proliferated faster under 6ml/min ($P<0.05$) (Fig.1). SEM indicated that all the macropores of the scaffold under different flow rates were filled with cellular layer. All cellular layers under 3ml/min were incompact, however, almost all cellular layers under 9ml/min were compact; under flow rate of 6ml/min, the cellular layers were either compact or incompact. Real-time PCR revealed that after 15 days perfusion culture, the mRNA expression of osteobalstic genes including ALP, OP and OC, enhanced with the increase of flow rate.

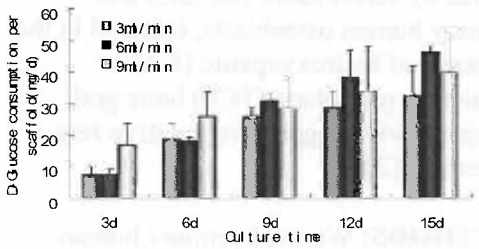

Fig.1: Daily D-glucose consumption

CONCLUSIONS: At early stage of perfusion culture, the proliferation rate of hMSCs was faster under 9ml/min, while at late stage, it was faster under 6ml/min. The osteoblastic differentiation of hMSCs was facilitated with the increase of perfusion flow rate.

ACKNOWLEDGEMENTS: This work was funded by the National Basic Science Research Program of China (973 Program) (2005CB522700).

Differential Growth of Human Osteoblastic Cells on HA and TCP with Different Surface Charge

J.A. Gallagher1, J.P. Dillon1, P.J.M. Wilson1, W.D. Fraser2, B.K. Mwaura3, M.J. Hayton3, J.J Cooper4,

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INTRODUCTION: The physicochemical properties of implant materials influence their interactions with cells, either directly or via adsorption of proteins. Surface charge, or zeta potential, is one of the critical characteristics which have the potential to influence the biological responses to materials. Although there are some preliminary reports on the effects of surface charge, for example on osteoinductivity (1), the effects of magnitude and polarity of charge on osteoblastic cells remain largely unexplored.

The objective of the present study was to investigate the growth and synthesis of matrix by osteoblastic cell lines and primary human osteoblasts, cultured in the presence of hydroxyapatite (HA) or tricalcium phosphate (TCP) bone graft materials with negative or positive zeta potentials (ZP).

METHODS: We used primary human osteoblasts isolated from explants of human bone and three different human osteosarcoma cell lines, MG-63, TE85 and SaOS-2, which represent different stages of osteoblastic maturation. Cells were seeded in to tissue culture wells containing HA or TCP with negative or positive zeta potentials, denoted HA-, HA+, TCP- and TCP+ respectively. Proliferation of cells on the materials and adjacent plastic was determined, and the synthesis of two osteoblastic markers, collagen I (P1NP) and osteoprotegerin (OPG) was measured.

RESULTS: Osteoblastic cells grew better in wells containing HA than TCP. There was also a marked effect of surface charge; HA- supported cell growth better than HA+ and TCP- better than TCP+. Although this pattern of results was obtained with all of the cell types used, the effects were most apparent with TE85 cells (see Fig 1). The effects on synthesis of collagen, but not osteoprotegerin, reflected the effects on cell growth.

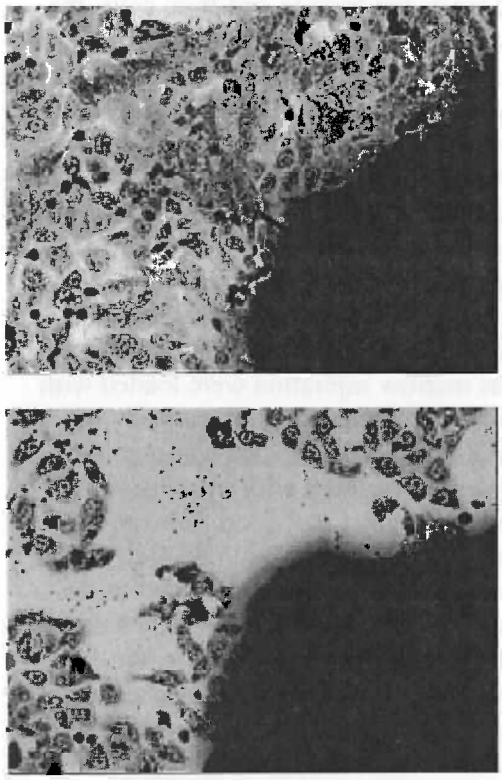

Fig. 1: TE85 cells growing in tissue culture wells containing HA– (upper panel) or HA+ (lower panel).

CONCLUSIONS: Surface charge has profound effects on the growth of osteoblastic cells and the synthesis of bone matrix constituents. Modifying the zeta potential is a potential route to design of better bone replacement materials.

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Repair critical-sized bone defect in rabbit using different resorbable silicon stabilized HA/TCP bioceramics combined with fresh bone marrow cells

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Introduction: Synthetic ceramic grafts possess required characteristics of an ideal bone substitute, i.e., osteointegration and osteoconduction. Osteointegration depends upon the intrinsic properties of the ceramic, such as size, morphology, crystallinity of the granules, porosity, composition, and the calcium-phosphate ratio. Osteoconduction mostly depends upon the porosity and the microstructure of the scaffold. In this study, we evaluated the performance of a silicon-containing mineral biomaterial (Skelite™, Millenium Biologix, Canada) with different crystallinity and microstructure in repair of critical-sized bone defects in rabbit.

Materials and Methods: Bilateral 15mm critical-sized bone defects were created in the ulnae of fourteen NZW rabbits. The defects were filled with Skelite™-based biomaterials loaded with freshly isolated allogenic bone marrow mononuclear cells (5 million per implant site). The ulnar defects were divided into 6 groups with at least 4 defects in each group. Cylinders groups, the Group A cylinders were made with the standard manufacturing process. The Group B cylinders were made by sintering Group A cylinders at 1050°C for 2 hours. The Group C cylinders were made by re-sintering the Group B cylinders at 1250°C for 2 hours. Group D was filled with Skelite mini-granules, which were produced in the same manner as the Group A cylinders, and were re-sintered for 1 hour at 1200°C. Group E was crushed Skelite scaffolds, where mini-granules were crushed and then sieved to particle size between 1.4 and 3.35 mm. Group F was filled with autologous cancellous bone granules. Serial radiographs were taken and all rabbits were sacrificed at 9 weeks following implantation. Upon sacrifice, the defects were evaluated by peripheral quantitative computed tomography, and histological examinations.

Results: The defects in all groups had united by 9 weeks. Serial radiographs showed that the cylinder groups (Groups A-C) had better osteointegration and greater amount of bone formation than those of Group D and E. There was no significant difference in the speed or amount of bone formation in Groups A-C. For the total bone mineral density (BMD) in the defect regions, there was no difference among the groups, except group F was significantly lower ($p<0.04$). Group A and E had significantly higher trabecular BMD than those in groups B, C, F suggesting more new bone formation and/or less bone remodeling in Groups A and E. Histology data (Figure 1) revealed that the quality and quantities of newly formed bone in groups A-C was superior than those in Groups D-F. Among the cylinder groups, the amount of bone formation was similar; however, the implants were resorbed faster in Group C.

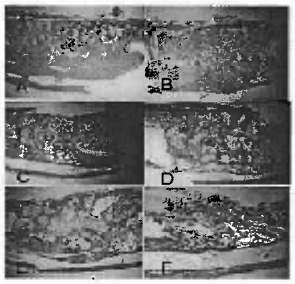

Figure 1. Representative histology of Groups A-F at 9 weeks following implantation.

Discussion: Unlike most bone substitutes, Skelite™ contains silicon. Present in the form of silicon-stabilized tricalcium phosphate, the silicon substitutes for phosphorus is distributed throughout the material's crystal structure and provides Skelite with a multiphase composition, consisting of approximately 67% Silicon-TCP and 33% HA/TCP, with more than 70% porosity. The different sintering profiles for manufacturing did not significantly affect the *in vivo* osteointegration and osteoconduction capabilities of the tested biomaterials, but they varied the material strength and resorption rate. The Skelite™ in cylinder shape which filled exactly into the defect gap was much superior in facilitating bony in-growth comparing to the granule formats.

Session 2 TISSUE REGENERATION

CHAIRS: JAMES TRIFFITT & PAMELA ROBEY

INVITED LECTURE No. 2

A HARI REDDI

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ARTICULAR CARTILAGE REGENERATION : SIGNALS, STEM CELLS and SCAFFOLDS

Regeneration and Tissue Engineering of tissues is dependent on Signals, stem cells and scaffolds. Bone and cartilage are adjacent tissues. The regenerative potential of bone is legendary. However, articular cartilage is feeble in this regard. Bone morphogenetic proteins (BMPs) are the molecular basis of bone regeneration. All BMPs induce chondrogenesis and therefore are also cartilage morphogenetic proteins. In addition cartilage derived morphogenetic proteins (CDMPs) were isolated from articular cartilage. These are also known as GDFs 5-7. BMPs and CDMPs initiate, promote and maintain chondrogenesis. The responding stem/progenitor cells for cartilage are found in bone marrow, synovium, perichondrium, periosteum adipocytes and in the articular cartilage itself. Recent work has identified a side population (SP) cells with stem/progenitor cells with lineage potential for surface and middle/deep chondrocytes. The scaffolds for cartilage include but not limited to collagens, proteoglycans and a variety of natural and synthetic hydrogels. Current work on cartilage regeneration is proceeding at an exponential phase and is exciting.

FETAL SPINE CELLS FOR INTERVERTEBRAL DISC REGENERATION: PRELIMINARY CHARACTERIZATION

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[2]Orthopedic Cell Unit Therapy, UNIL, Lausanne, Switzerland
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INTRODUCTION: Degeneration of the intervertebral disc (IVDD) is though to be one main causes of low back pain. IVDD begins early in the nucleus pulposus (NP) with decreased cellular content and a loss of proteoglycan and water. Our hypothesis is that matrix synthesis could be stimulated by proteoglycan producing cells. Fetal disc cells could be a promising cell source for regeneration of the degenerated disc. The aim of this study was to study the feasibility of using fetal disc cells for disc tissue engineering.

METHOD: Fetal spinal column tissues (1 cm) were obtained from fetuses after voluntary interruption of pregnancy at 14-16 weeks of gestation (n=3). The spinal tissue was cleaned of adherent tissue, dissected and put into culture in tissue culture dishes. Cells were routinely cultured in DMEM with 10% FCS and 200 mM L-Glutamine. Cell proliferation in monolayer was measured with the CellTiter colorimetric method and compared to adult NP cells (individuals aged 30 to 40 years). HLA I and II expression monolayer culture was measured by flow cytometry. Sulphated glycosaminoglycan (sGAG) production by fetal spine entrapped in alginate beads was measured by DMMB assay and normalized to DNA content.

RESULTS: Isolated cells proliferated faster than adult NP cells. They uniformly express HLA-I but not HLA-II proteins. HLA-I and -II expression were constant through *in vitro* passages up to 11. Compared to monolayer culture, fetal spine cells in alginate beads showed a rounded morphology. They synthesized matrix as shown by the increase in GAG/DNA ratio during culture in alginate beads up to 26 days.

DISCUSSION: Fetal spine cells can be cultured in vitro with minimal requirements. They proliferate fast and do not express HLA-II proteins. When cultured in a 3-D environment, they synthesize sGAG, which are responsible for the high water content in NP matrix. Optimization of the cell type and their associated isolation and proliferation will be essential for assuring large numbers of patients benefiting for tissue engineering in the future.

Acknowledgements: Supported by AO Research Grant 04-S33.

In-Vivo Bone, Periodontal Ligament and Cementum Regeneration, Using a Recombinant Human Amelogenin Protein- Possible Biological Mechanisms.

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Introduction: We have shown, for the first time, that a single recombinant enamel matrix protein – the Recombinant Human Amelogenin Protein (rHAM$^+$) (Taylor et al. 2006) alone, can bring about significant progressive regeneration of all tooth supporting tissues: bone, periodontal ligament and cementum, after induction of chronic periodontal disease in-vivo, in a dog model (Deutsch et al. 2003, 2006 Haze et al. 2003, 2005, 2006 submitted in revision, Haegewald et al. 2003). The objectives of the current study were to explore possible molecular mechanisms associated with the regeneration process.

Methods: Micro-CT, Immunohistochenistry, in-situ hybridization, confocal microscopy, Western blot analysis, RT-PCR and DNA sequencing, were used to study the regeneration process and the expression of amelogenin in normal and regenerating dog periodontal tissues and normal rat periodontal tissues.

Results: In the normal and regenerating periodontal tissues amelogenin was found to be expressed in osteoblasts and osteoclasts lining the alveolar bone, in part of the osteocyts, periodontal ligament (PDL) cells and in clusters of cementoblasts along the root. Amelogenin expression was found in specific bone marrow cells, and in cells surrounding the blood vessels. Stronger amelogenin expression was observed adjacent to the regenerating area and to a lesser extent in distal alveolar bone. We examined the recruitment of mesenchymal stem cells (MSCs) two weeks after treatment with rHAM+ as compared to control, using two MSCs markers: CD105 and STRO-1 antibodies. The results showed significantly many more CD105 and STRO-1- positive stem cells in the granulation tissue, in the PDL as well as the bone and bone marrow of the experimental tooth treated with rHAM+, compared to control. Our recent results, examining normal dog and rat long bones, have shown that amelogenin is expressed by the endosteum cells, periosteum cells, and in specific cells of articular cartilage and epiphyseal growth plate. In long bone marrow amelogenin expression was detected in a number of morphologically different cells, some of which were expressing mesenchymal stem cell markers. We have established, for the first time, the mRNA sequence encoding for dog amelogenin protein in enamel organ and in bone marrow. The amelogenin alternatively spliced transcript encoding for LRAP, which has been suggested to have osteoinductive activity (Veis 2003), was also identified in rat bone marrow

Conclusions: We suggest that amelogenin might serve as a key protein in mesenchymal progenitor cell recruitment from the bone marrow and periodontal tissues; and/or in their differentiation, which brings about regeneration of all three periodontal tissues. These results also imply a role for amelogenin in long bone formation and remodeling.

Supported by DFG grant no. Be 1142/4-1 and ISF grant no. 597/02.

Session 3 BIOMATERIALS

CHAIRS: JACQUES LEMAITRE & JAN CZERNUSZKA

INVITED LECTURE No. 3

JAN CZERNUSZKA

Department of Materials, University of Oxford, Parks Road, Oxford, UK

BIOMATERIALS: SCAFFOLDS FOR TISSUE REGENERATION

Biomaterials are having a tremendous impact in healthcare applications and are used as total hip replacements, arterial stents and heart valves, for example. The next challenge in the development of medical devices is in the regeneration of tissue – tissue engineering.

We manufacture a range of scaffolds for use in tissue engineering. One major area is in the development of bone tissue. Bone analogues that comprise collagen and hydroxyapatite – the two major components of bone - organised on several length scales to mimic natural bone, act as the matrix of our scaffolds. This matrix is then manipulated into the desired shape using solid freeform fabrication technology

The scaffolds can be independently controlled over several length scales:

i) at the molecular level we control the stoichiometry and substitution levels of the hydroxyapatite.

ii) at the nanoscale we control the mineral size, shape and crystallographic orientation.

iii) at the microstructural level we control the volume fraction and distribution of the hydroxyapatite within the collagen scaffold.

iv) at the mesoscale we control the porosity of the collagen and the extent and dimensions of microchannels to aid vascularisation.

v) at the macroscopic level we control the external shape to fit the proposed defect site.

We collaborate extensively with several groups. Examples of current work will be presented to display our capabilities. These studies will highlight the importance of collaboration between materials scientists, biological scientists and surgeons, in helping the health care sector and patients.

Injectable Biodegradable Poly (ester-co-ether) Methacrylate Monomers for Bone Tissue Engineering and Drug Delivery Applications

Xin Zhao, Sze Man Ho, Anne Young (Biomaterials and Tissue Engineering Division, UCL Eastman Dental Institute)

Introduction: The project aim was to develop a new range of, systematically varying fast photocuring, strong polymer adhesives for bone repair that can degrade to cell compatible components and release drugs at a controllable rate after set.

Methods: Adhesives consisting of poly (propylene glycol) of molecular weight of 425, 1000 and 2000g/mol to which 2, 4 or 8 degradable lactide units were attached each end were synthesised. These were then terminated with polymerisable methacrylate groups. Polymerisation setting rates of the adhesive monomers were quantified using FTIR and Raman. Viscoelastic properties, degradation products, water sorption characteristics and release of chlorhexidine diacetate, ketoprofen and prednisolone from the solid set discs as a function of time of immersion in deionised water at 37 °C were characterized using dynamic mechanical analysis (DMA), FTIR, pH determination, gravimetric investigations and UV spectroscopy. Use of the monomers with steriolithography was also demonstrated. Finally mechanical, degradation and cell compatibility studies of the set adhesives filled with various calcium phosphates were investigated using DMA, ion chromatography, gravimetric analysis, Raman mapping and proliferation of MG63 cells.

Results: For all adhesives, 240s of light exposure was sufficient for over 90% conversion of the methacrylate groups. Shorter exposure times were required for full reaction with the smaller molecules. The modulus of the set materials also increased significantly as the adhesive molecular weight was reduced. The set material degradation rate was either linear with time or proportional to the square root of time. The drug release rates varied with drug type and initial level as well as adhesive chemical structure and its water sorption characteristics. The addition of inorganic fillers could both increase or decrease material modulus as well as affect cell compatibility.

Conclusion: The novel formulations synthesised in this study provide a promising new range of injectable, fast-curing, biodegradable, cell compatible, slow drug-releasing adhesive materials for various applications in bone tissue engineering and drug delivery but may additionally be used with steriolithography to generate 3D implants from computer NMR or ultrasonic tomography images of a patient bone deformity.

Calcium carbonate cements in vitro and in vivo: influence of the liquid phase composition

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Introduction: Filling bone defects with materials that generate resorption activity and lead to neoformed bone tissue represent an important concern for orthopaedic and dental surgeons. As biodegradation and bioactivity properties of cements have often been related to the solubility of their constitutive phases, calcium carbonates are interesting candidates for the design of cements with improved biodegradation rates due to their higher solubility compared to apatite. Recently, calcium carbonate-based cements have been presented as promising biomaterials for bone reconstruction. Improved bioactivity can be achieved by associating cements with biologically active macromolecules (growth factors, specific protein). With this aim in view we investigated the possibility of using blood serum, which naturally contains such bioactive components, to prepare calcium carbonate cement paste. This physical-chemical preliminary investigation is based on the use of different liquid phase compositions (from water to serum) to evaluate the possibility of preparing calcium carbonate cements with patient blood. A comparison with the composition and structure of a cement tested in vivo (in contact with biological fluids) will be presented.

Materials and Methods: The cement paste was prepared by mixing the appropriate amount of liquid phase (either deionised water, solution containing one or several blood serum components with a concentration close to that in vivo: 0.9 % NaCl, albumin (50 $g.L^{-1}$), glucose (1 $g.L^{-1}$), or newborn calf serum) with a powder mixture of metastable calcium carbonate phases (amorphous $CaCO_3$ and vaterite). After setting and hardening at 37°C, cements were characterised using FTIR spectroscopy, X-ray diffraction and scanning electron microscopy (SEM) techniques. An in vivo test of this cement, prepared with water, was also performed filling two midparietally transosseous calvarial defects in a rat. After one week, the explanted cement was analysed using diamond ATR FTIR spectroscopy and SEM.

Results: Cements prepared with a solution containing one component (either water, isotonic NaCl, albumin or glucose) mainly led to a hardened body made of aragonite whereas cement prepared using serum led mainly to calcite. The use of a liquid phase combining several blood serum components ((NaCl+albumin), (NaCl+albumin+glucose) for example) led to a cement final composition consisting of a mixture of vaterite and aragonite, indicating that the setting reaction was not complete. This result can be compared to the analyses of a cement after one week of implantation in vivo. FTIR analysis and observation by SEM showed that the setting reaction did not occur in vivo, as most of the vaterite crystals remained untransformed and the formation of needle-like aragonite crystals can not be seen. Among several hypotheses that could explain such behaviour when cement is in contact with biological fluids, the effect of phosphate ions known as inhibitors of aragonite crystallisation can be considered.

Conclusions: The final composition of the $CaCO_3$ cement is highly sensitive to the composition of the aqueous environment and can be adapted depending on the composition of the liquid phase used. It seems likely that there can be a synergic effect of blood serum components on the setting reaction and further investigations need to be performed to determine which component(s) mainly controlled the crystallisation of aragonite particularly to explain cement behaviour observed in vivo. In vitro tests showed that it is possible to prepare $CaCO_3$ cements with the patient's blood which could have interesting advantages in adding potentially active components such as platelets to the cement.

Bioactive glass /nano-HA gradient coating on metal substrate

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INTRODUCTION: Hydroxyapatite(HA) has been widely used as coating material on metallic implants to promote the osteointegration, but the delamination or crack coating in vivo have been reported which may caused by the difference of the thermal expansion coefficients of the HA with metal. Compositional changes and structure transformation of HA caused by the high flame temperatures in the process of plasma spray will also resulted in poor bonding strength between coating and metal substrate. In this paper, bioactive glass(BG) /nano-hydroxyapatite(nano-HA) composite gradient coating with thermal expansion coefficients that match those of Ti6Al4V were prepared and used to coat Ti6Al4V by sintering method with comparable low temperature. The bonding strength and osteointegration of the BG/nano-HA gradient coatings were evaluated both in vitro and in vivo.

METHODS: The gradient coating was prepared on the surface of Ti-6Al-4V cylinder substrate by the sintering technology at about 800□. The coating surface and the interface between coating and titanium alloy substrate were observed by Scaning Electron Microscope(SEM). The bonding strength between the gradient coating and the substrates was evaluated by standard bonding test (ISO 13779-4:2002). The titanium alloy implants with gradient coating and the non coating implants were randomly implanted in each side of the tibial of the New Zealand rabbits. At 2, 4 and 12 weeks after implantation, the rabbits were sacrificed after X-ray examination and the mechanical push-out tests were carried out to measure the bonding strength of interface between implants and host bone tissue. Undecalcified sections were prepared to evaluate the osteointegration of the implant.

RESULTS: The SEM showed that the coating surface was composed with needle-like nano-hydroxyapatite crystal and well connection existed in the interface between the coating and titanium alloy. The bonding strength between coating and Ti-6Al-4V was 39.6±3.5Mpa. The push out test indicated that the bonding strength between bone and gradient coating implants were higher than that between bone and non coating impalnts at each time interval□$p<0.05$□. No delamination of coating were observed after the push out test by SEM. The histological study indicated that there were well integration between the the coating and surrounding bone(Fig1).

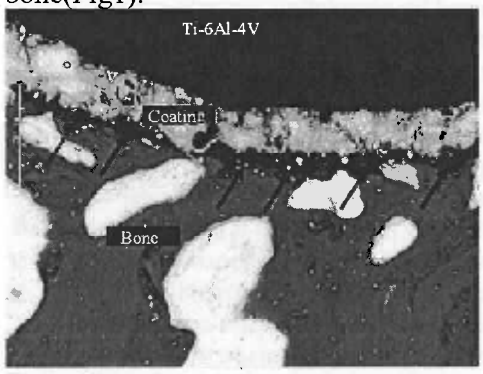

Fig1. Close integration between the coating and the surrounding bone (arrows) could be found on the section at 12 weeks after implantation. Van Gieson's picro-fuchsine.

CONCLUSIONS: Though some histomorphometry data is still processed, these results had indicated that the bioactive BG /nano-HA gradient coating could improve the bonding strength between the implants and surrouding bone tissues. The BG/nano-HA gradient coating may have good potential to be used in practical applications.

ACKNOWLEDGEMENTS: This work was supported by the Fund from the Science and Technology Commission of Shanghai(0552nm024).

Comparison of amorphous TCP nanoparticles to micron-sized α-TCP as starting materials for calcium phosphate cements

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INTRODUCTION: Bone repair and regeneration of defects arising from trauma, tumor or bone diseases display a serious clinical problem in orthopedic surgery. Injectable and resorbable calcium phosphate compounds (CaP) have gained great importance due to their biocompatibility, bioactivity and osteoconductivity and the possibility of minimal invasive surgery. Apatitic calcium phosphate cements (CPC) cure by the reaction of a metastable CaP (e.g. α-TCP). This work describes the use of amorphous TCP, a high-temperature, metastable TCP in the form of nanoparticles for application in apatitic CPCs.

METHODS: The XRD-amorphous TCP (ATCP) nanoparticles were synthesized by flame spray synthesis [1] whereas α-TCP was prepared by solid state chemistry [2]. Reactivity of pure and mixtures of ATCP and α-TCP were tested using isothermal calorimetry. Further analyses included XRD, specific surface area and electron microscopy before and after setting as well as compressive strength and setting time.

RESULTS: Isothermal calorimetry results showed that the ATCP material reacted considerably faster when hydrated with Na_2HPO_4 than the micron-sized α-TCP. The energy release for the ATCP cement was short and intensive (finished after 40 min) whereas the α-TCP reacted for several hours to days. The specific surface area of the set cements, which is very important for the interaction with the implantation site, followed the addition of amorphous material and reached values of up to 160 m^2/g for pure ATCP [3]. The high surface areas are in agreement with the nanostructure of the newly formed apatite crystals shown in Fig. 1.

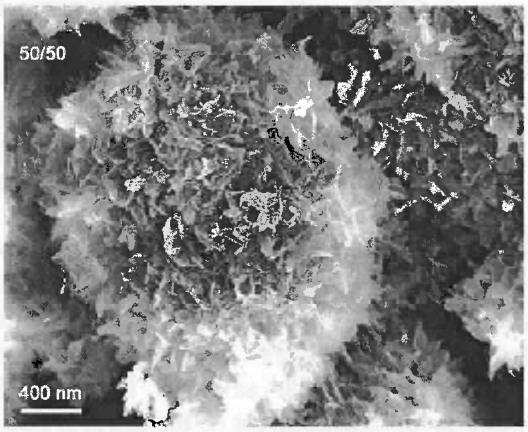

Fig. 1: SEM image of a 50/50 (w/w) mixture of ATCP/α-TCP after setting showing the formation of nanocrystalline apatite.

CONCLUSIONS:
The results clarified the importance of both particle size and phase of TCP on the reaction kinetics of apatitic CPCs. The described aerosol-derived ATCP nanoparticles are an interesting and promising starting material for the use in apatitic cements.

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ACKNOWLEDGEMENTS: Financial support by the Gebert Rüf Foundation is kindly acknowledged.

Production of titanium foams for implant applications

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Department of Materials Science and Engineering, Anadolu University, Turkey.

INTRODUCTION: Despite its excellent biocompatibility, commercially pure titanium (CP-Ti) is considered as a disadvantageous material due to its low strength and hardness. To achieve better properties alloying and coating are usually applied. However, potential toxic effects of alloying elements limit their use in implant applications [1]. This fact necessitates the use of CP-Ti with improved mechanical properties. In literature it is reported that the difficulties in processing contamination free Ti foam with desired properties can be overcome by the use of creep expansion processes. However, these processes are costly since they include HIPing. Furthermore, they are limited to low porosity levels when compared to simple sintering [2]. Simple sintering with fugitive space holder offers a wide range of properties and cost savings. The crucial point is the arrangement of the process such that the contamination level is kept at minimum.

METHODS: After Ti and different sized carbamide powders were dry mixed in ball mill for 30 min they were first pressed uniaxially and then CIPed to form a powder compact. Removal of the space holder was conducted either by heat treating at a temperature determined by the TG-DTA analysis, or by dissolution in hot deionized water. After the removal, the compacts were sintered at different temperatures between 900-1400 °C for 2 h in Ar atmosphere. Contamination was minimized using Mg and Ti sacrifices, shielding and the addition of TiH_2. The foams were characterized by using SEM, EDX and XRD. Compression tests were carried out at a strain rate of 10^{-3} s^{-1}.

RESULTS: Ti foams with suitable pore structure for both bone ingrowth and strength of the total implant were produced by simple sintering with space holder powders (Fig. 1).

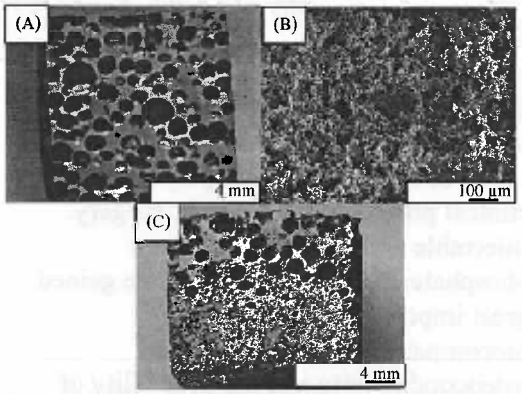

Fig. 1: Ti foams with (A) coarse, (B) fine pores. (C) FGM structure.

CONCLUSIONS: Carbamide can be removed either thermally or by dissolution in water, which renders it possible to minimize contamination of Ti. When carbamide is removed thermally, foaming temperature should be chosen carefully to avoid reaction with Ti. By changing processing parameters foams with a wide range of properties could be obtained. Foams with coarser pores were found to be more strain-rate sensitive. Additionally, FGMs with graded porosity and pore size were easily produced and characterised. Coating of resulting foams with antibacterial HA is being carried out.

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Session 4 TISSUE GROWTH FACTORS

CHAIRS: GRAHAM RUSSELL & ALAN BOYDE

INVITED LECTURE No. 4

KUBER T. SAMPATH

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GROWTH FACTOR STIMULATION OF REGENERATION IN THE MUSCULOSKELETAL SYSTEM

The bone morphogenetic protein (BMP) containing osteogenic devices BMP-2 (InFuse™) and BMP-7 (OP-1™) have been used as bone graft substitutes for the treatment of long bone fractures and inter-body fusion of vertebrae in humans. While BMPs offer tremendous promises for orthopedic tissue engineering, there are still numerous challenges. There is a need for optimal delivery systems for surgical approximation and BMP retention in order to promote localized osteogenesis. Furthermore, adequate vascular supply is a prerequisite for the stimulation of tissue regeneration. As BMP devices are expensive and faced with reimbursement issues for their wider usage, autologus bone marrow derived mesenchymal stem cells (MSCs) could be engineered in the surgical suite to deliver the BMP via *ex vivo* gene therapy with an appropriate carrier matrix. Alternately, BMP genes could be delivered locally via viral and/or non-viral vectors with biomaterials. The 'cell-gene-matrix' and 'gene-activated-matrix' devices could exploit the donor callous site to make the protein for a period of time sufficient enough to induce the differentiation of adjacent mesenchymal stem cells from bone marrow, periosteum and muscle into bone and restore the function thus providing a cost effective and readily customizable therapy for regeneration of the musculoskeletal system in the future. BMP signaling as *ex vivo* gene therapy will also have an advantage for repair and regeneration of articular cartilage and intervertebral disc in humans.

BONE TARGETING PEPTIDES POTENTIATE OSTEOGENESIS

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Orthopaedic Research Laboratories Department of Orthopaedic Surgery and Department of Biochemistry & Molecular Genetics, University of Virginia

INTRODUCTION: The objective of this study is to determine if bone tropic peptides isolated from a phage display peptide library can promote osteogenesis in bone defects and to understand the molecular mechanism of peptide-mediated bone regeneration.

METHODS: Peptide Synthesis: Peptides were selected from a Ph.D. 12-mar phage display library kit by *in vivo* biopanning. The unique peptide sequences eluted from bone and marrow were then identified by sequencing phage DNA. Based on initial studies, two of the peptides, designated R1 and L7, were selected for further investigation. Cell Staining: The biotinylated form of peptides R1 and L7 were synthesized and fluorosceinisocyanate-labeled avidin was used to localize peptide binding to mesenchymal cells in culture. Microarray: Using the Affymetrix GeneChip system, a cDNA microarray analysis was performed to examine gene expression profiles in mesenchymal cells treated with R1. Affinity Chromatography: To identify the molecular targets of osteotropic peptides, lysates prepared from mesenchymal cells were applied to biotinylated peptide immobilized on avidin agarose. The bound proteins were eluted and analyzed by SDS-PAGE.
Surgical procedure: Peptides were initially tested in 3mm unicortical defects. To determine the osteogenic effect of peptides in a critical size defect, a gelfoam cylinder (8x3x3 mm3) was soaked in either L7 or R1 (20 mM) solution or in PBS only (control) and placed in the unicortical defect (8x3x3 mm3) created bilaterally on the antero-medial aspect of the tibia of 9-month Fischer 344 rats. The tibias were harvested at 3, 5 and 12 weeks after surgery, fixed in 10% formalin, decalcified in rapid decalcifier for 2 weeks and embedded in paraffin. Serial 10 µm sections were stained with H&E, examined microscopically and analyzed histomorphometrically.

RESULTS: Biotin-labeled R1 was shown to bind to marrow mesenchymal cells in culture. L7 promotes the formation of clusters similar to cells that undergo matrix mineralization *in vitro*. Results from microarray showed changes in the expression of several bone related genes. Visualization of affinity chromatography fractions on SDS-PAGE showed that peptide R1 binds to two components with a molecular mass ~200 kDa and ~ 45 kDa, and that L7 binds three proteins with a molecular mass in the 40-50 kDa range. Histological examination of tibial defects at all time points demonstrated that empty defects without gelfoam or without peptide showed no bone repair. In the smaller (3mm) defects, histomorphometry showed that defects treated with peptides contained 2-3 times more bone than gelfoam without peptide. In the larger (8mm) defects, the gelfoam control without peptide showed repair at the edges of the defect but none at the cortex and no bone marrow within the defect site. At 3 weeks, defects with gelfoam+R1 showed extensive cortical bone repair. By 5 weeks, the cortex had thickened and the marrow has begun to reform. By 12 weeks the defect was completely healed. The thickness and continuity of bone repair in the groups that were treated with R1 peptide was greater than in the control group without peptide. With L7 by 3 weeks, a single layer of bone formed at the middle of the defect and a minor amount of gelfoam remained. By contrast to R1, defects treated with L7 showed an abundance of bone with a callus and no bone marrow at 12 weeks.

CONCLUSIONS: Peptides R1 and L7 stimulate bone regeneration *in vivo*. R1 and L7 show distinct properties. R1 binds to marrow mesenchymal cells *in vitro* and promotes cortical bone and marrow *in vivo*. By contrast, L7 potentiates bone repair but without marrow regeneration at 12 weeks. Further analysis and characterization of the molecular targets will demonstrate the mode of action of the peptides on cells *in vitro* and tissue regeneration *in vivo*. Synthetic peptides may have advantages over larger molecules such as speed of preparation, molecular stability, long shelf lives and potentially easier bioengineering applications. These peptides, therefore, may offer attractive alternatives to existing growth factors that stimulate bone regeneration.

Supported by NIH grant AR053579.

Impact of a three-dimensional hydroxylapatite scaffold on differentiation of osteoblasts and tissue formation in vitro

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We used hydroxylapatite (HA) scaffolds for in vitro studies with cell biology, mimicking the three-dimensional natural environment. The aim of this study was firstly, to illuminate the impact of the three-dimensional environment on the gene expression pattern of osteoblastic marker genes under normal and hormonal treatment. Secondly, we studied tissue formation kinetics within a pore system of varying geometry.

The HA scaffolds for gene expression experiments were designed with fully interconnected porosity and were realized by rapid prototyping combined with ceramic gel casting. The scaffolds for tissue formation studies were realized by a combination of wax printing and ceramic gel casting. Murine osteoblast-like cells (MC3T3-E1) were seeded onto these scaffolds and treated optionally with triiodo-L-thyronine (T3) and 1,25-vitamin D3 (D3). At defined time points, RNA was isolated, reverse transcribed and the gene expression profile was analyzed by PCR. Beyond that, we studied the kinetics of tissue formation within the pore system and quantified it (without hormonal treatment) over a time period of 6 weeks by image analysis of phase contrast microscopic pictures.
Osteoblast-like cells cultured on HA scaffolds started to express early marker genes of the osteoblastic differentiation process, alkaline phosphatase (ALP) and collagen, and also a marker for the mature phenotype, osteocalcin (OCN). Furthermore, PCR analysis revealed a high expression of osteoprotegerin (OPG), the osteoblast-specific transcription factor Runx2 and RANKL, whereas their expression is near the detection limit in two-dimensional standard culture systems. However, hormonal treatment of cells with T3 and D3 yielded a gene expression pattern similar to that known from two-dimensional culture systems.
Beyond the level of gene expression, we also studied tissue formation kinetics within the pore system of a three-dimensional scaffold. Based on these results, we postulate a biphasic tissue growth behaviour starting with a slow growth modus and switching to an accelerated amplification rate based on the circumference of the pore system.
Gene expression analysis demonstrates that the three-dimensional environment, which is similar to the natural situation, influences gene expression. Additionally, tissue formation dynamics is also strongly affected by the spatial environment, more precisely by the perimeter of the pore system. This insight could be applied for future scaffold design.

Session 5 CLINICAL AND BIOMECHANICAL FEATURES

CHAIRS: K.DAI & MARC BOHNER

INVITED LECTURE No. 5

JEREMY FAIRBANK

Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, UK

CLINICAL REQUIREMENTS FOR SPINAL BIOMATERIALS RESEARCH

Introduction: This presentation reviews areas of spinal disorders where interventions are used and biomaterials might be applied.
Spinal deformity: Scoliosis and kyphosis are corrected using sophistically engineered implant systems. Mostly these are titanium based which seems to produce less tissue reaction and possibly lower infection rates than stainless steel. Long-term stability is achieved by obtaining a solid bone graft. Currently we expect very high fusion rates (95-99%?) although a solid fusion is difficult to measure. Many surgeons harvest bone during the procedure from the spine itself and from the ribs if combine with a costoplasty (throacoplasty). If bone is taken from the pelvis than bone donor pain can be a problem and is used to justify supplementary biomaterials. It is easier to justify in patients with neuromuscular deformities where large quatities of bone a re required. It is very difficult to prove that bone graft supplements work *in vivo*.
Back pain: Spinal fusion for back pain remains controversial. Fusion works better than conventional physiotherapy but is nearly equivalent to intensive rehabilitation. Fusion rates are falling in the UK but rising in the US. The intellectual basis for immobilising segments of the lumbar spine is flawed, and the success rate of fusion is around 50% with out anyone having found a way to predict success. In this background it is difficult to introduce a new technique.
Insufficiency Fractures: Vertebroplasty and kyphoplasty have become widely used for managing osteoporotic fractures. Methyl methacrylate has been widely injected with considerable success. Other cements and bone substitutes have been tried, but MMA's are a hard act to beat. I shall discuss the difficulties in designing trials to show that new materials are better and what to do with young people with burst fractures.
Tumours and metastases: Many of the same issues that have been raised for fractures are relevant to cancer. The short life expectancy probably means that it will be near impossible to show the superiority of new materials.
Infections: The management of serious infection with vertebroplasty is controversial. Antibiotic carriers may have an application.
Conclusion: Biomaterials are widely used in the spine but it is difficult to establish a scientific proof for new materials.

INVITED LECTURE No. 6

GAMAL BAROUD

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BIOMECHANICAL RESEARCH AT THE INTERFACE OF ENGINEERING AND MEDICINE-CEMENT AUGMENTATION OF OSTEOPOROTIC BONE

The purpose this work is to conduct biomechanical research at the interface of engineering and medicine. The current focus is on the cement augmentation of osteoporotic bone. This is an emerging procedure for the treatment of fragility fractures related to osteoporosis. In this procedure, bone cements are used to strengthen and augment the bone that has been affected by osteoporosis.

Working with mechanical engineers, chemical engineers, and orthopaedic surgeons, the aim is to make the cement augmentation process safer and more predictable. To do this, I and my team break the process down into its components, which are then analyzed, using a wide array of methods ranging from mathematical models and computer simulations to laboratory testing.

The research is relevant in that it is developing guidelines, techniques, biomaterials, and/or devices for a safe cement injection procedure.

Biomaterial injection under local anesthesia in a case of spinal compression fracture.

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INTRODUCTION: Injection of PMMA cement for the treatment of osteoporotic fractures with kyphoplasty is widely accepted. However there is a lot of scepticism to expand this treatment for acute traumatic fractures for young patients due to the fear of adverse effects in the long term because of the use of PMMA cement.
METHODS: This is a case report of a 32 year old male working in a heavy duty construction job. He had a traumatic compression fracture of T11 and T12 vertebra, type A1 according to A.O. Grading system, with no neurology (Frankel E). The patient was evaluated with plain x-rays, CT and MRI of the thoracolumbar junction for proper fracture grading and exclusion of canal compromise or soft tissue damage. The patient reported a back pain V.A.S. score of 85. Under local anaesthesia, and image intensifier control, a trocar was inserted through the skin and a full kyphoplasty procedure performed using a novel synthetic injectable bone graft substitute composed of Calcium sulphate and β-tricalcium phosphate (Genex, Biocomposites Ltd. UK). Two small balloons were inserted through the pedicles from both sides. 1cc of Genex was inserted each side, a total of 2cc per vertebra.
RESULTS: Next day the patient had significant pain (VAS 35) improvement and was fully mobilised. He was discharged after two days and given the order to stay at home with rest. After 4 weeks he returned to work reporting no pain. At the three months evaluation he had no deterioration of pain or the deformity (local angulation).
CONCLUSIONS: Kyphoplasty for the treatment of vertebral osteoporotic fractures has good results in 95% of fractures treated. Improvement of pain, correction of deformity and patient mobilisation are the main advantages.

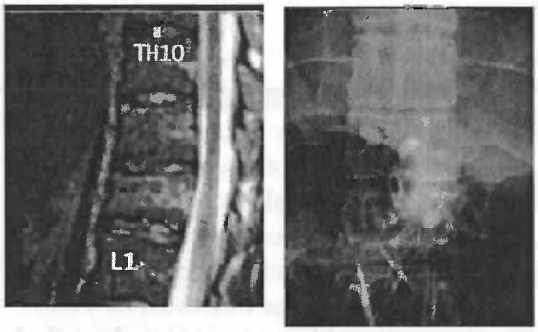

Fig. 1: (A) Fracture and bone oedema T11-T12 (B) Genex inserted bipedicular.

The use of PMMA cement is the major disadvantage because of (1) a great difference in strength compared to neighbouring osteoporotic bone causing further damage to the broken and adjacent vertebra, (2) exothermic reaction (85oC) causing damage to neurovascular tissue, (3) and cement debris in the long term causing an inflammatory reaction. We decided to use Genex, a synthetic, radio-opaque bone graft, presented as an injectable setting paste which is resorbed in approximately 8 to 12 months. It use prevents long term problems of PMMA cement debris and it exhibits a low exothermic reaction with a maximum temperature of 37oC. When prepared, Genex is a cohesive paste, which requires careful preparation as it starts to set in 2 to 3 minutes. Therefore speed and experience is required to use it through the long kyphoplasty cannula. For this young patient, it was strong enough to support and prevent the deformity from further deterioration. We believe Genex warrants further study as a bone substitute for kyphoplasty for the treatment of spinal compression fractures.

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AN INJECTABLE HIGH STRENGTH BIOCERAMIC FOR TREATMENT OF VERTEBRAL COMPRESSION FRACTURES

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INTRODUCTION

Vertebral compression fractures (VCF) caused by low-energy trauma are very common in osteoporotic patients. Over the last decade, vertebroplasty, a treatment that includes injection of cement into the affected vertebrae, has developed into an established method for treatment of VCF. The conventional bone cement, polymethyl-methacrylate (PMMA), is commonly used for this procedure, despite several potential drawbacks such as inadequate viscosity, extensive exothermic reaction during curing and foreign body response. The aim of this study was to evaluate the clinical use of a novel injectable high strength calcium aluminate based bioceramic, for treatment of osteoporotic vertebral compression fractures using percutaneous vertebroplasty.

MATERIALS AND METHODS

This open, prospective trial investigated the use of an injectable calcium aluminate bioceramic material (Xeraspine™, Doxa AB, Uppsala, Sweden) for treatment of osteoporotic vertebral compression fractures. The material is specially designed for vertebroplasty with an optimal viscosity and cohesiveness, high mechanical strength and high radio-opacity. Eight patients (aged 75, range 37-85 years) with a total of 10 VCFs due to low-energy trauma at T10 to L5 were included. The fractures had occurred within 8 weeks before enrolment. All fractures were treated by one of the authors (AG) using a unilateral percutaneous transpedicular injection of Xeraspine™ into the vertebral body under guidance by fluoroscopy and computer tomography (CT). The average volume injected was 3.5 ml ± 0.6 ml (SD). The patients were followed for twelve months with clinical assessments following a specific protocol and CT assessments for evaluation of material performance.

RESULTS

Prior to treatment, pain at rest was on average 76 (range 60-85) on a 100 mm VAS. At discharge about 4 hours after the procedure, the average pain at rest was 11 (range 0-40) with 5/8 patients reporting no relevant persisting pain. At one week the average pain at rest was 11 (range 0-60) with 5 patients still having no pain. At three months 6 out of 7 patients reported no pain and one patient reported 10 for pain. Due to concomitant disease, not related to this study, one patient could not be seen at the three-month follow-up. At twelve months the average pain at rest was 6 (0-30) with 6/8 patients reporting no persisting pain.

There was no major leakage of material during injection. Limited leakage without clinical relevance into the disc space was observed during the injection in one patient and CT also later verified this. No relevant fragmentation of the material or any reaction within the bone surrounding the injected material was observed during the follow-up period.

CONCLUSIONS

The results from this clinical pilot study with Xeraspine™ verified a solid safety profile as well as very encouraging and homogenous therapeutic effect in terms of immediate as well as lasting pain reduction following treatment of vertebral compression fractures.

Session 6 BIO-IMAGING

CHAIRS: ZHANFENG CUI & RICHIE GILL

INVITED LECTURE No. 7

RALPH MULLER

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HIERARCHICAL MICRO-IMAGING IN BIOMATERIALS RESEARCH AND TISSUE REGENERATION

INTRODUCTION: With recent advances in tissue engineering and regenerative medicine there is a need for quantitative image processing of three-dimensional natural and engineered biomaterials. A number of new microstructural imaging modalities have been put forward recently allowing quantification with high precision and accuracy. Although biomedical imaging technology is now readily available, few attempts have been made to expand the capabilities of these systems by adding quantitative analysis tools as an integrative part of biomedical information technology. Nevertheless, engineering endpoints have become an important factor for success in basic research and the development of novel therapeutic strategies in biology and biomedicine.
METHODS: Computed tomography is an approach to image bone in a hierarchical fashion providing multi-scale imaging capabilities with isotropic resolutions ranging from a few millimeters (clinical CT), to a few micrometers (μCT) down to one hundred nanometers (nanoCT). Typically, we are not only interested in static imaging of bone structure but also dynamic imaging of material function. To that end we have developed an image-guided technique that utilizes micro-compression in combination with tomography and allows, for the first time, the direct three-dimensional visualization and quantification of failure initiation and progression on the microscopic level. Developments in structure characterization of biomaterials using non-destructive imaging and analysis tools allow monitoring and eventually control of biomaterial processing such as to facilitate better cell in-growth and survival in applications of trauma and orthopaedic surgery (Fig. 1 and 2).

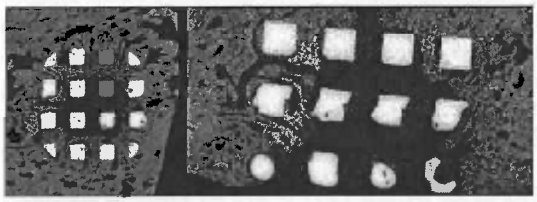

Fig. 1: Bone ingrowth of scaffold in minipig mandibula as measured by μCT providing a resolution of 30 μm.

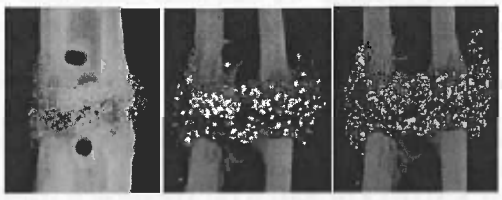

Fig. 2: Radiography, high-res-CT (90 μm) and μCT (30 μm) of tibial defect in sheep.

CONCLUSIONS: Microarchitectural bone imaging is a nondestructive, noninvasive, and precise procedure that allows the measurement of natural and engineered biomaterials as well as the repetitive 3D assessment and computation of microstructural and micromechanical properties in animal bone and patients.
Hierarchical bioimaging in combination with biocomputational approaches are well suited to investigate structure-function relationships as well as failure mechanisms in a variety of applications of tissue engineering and regenerative medicine. We expect these findings to improve our understanding of structure function relationships in natural and engineered biological tissues and with that to also allow improved quality control and more successful outcomes in tissue engineering and regenerative medicine.
ACKNOWLEDGEMENTS: Partially funded by the EU/NoE EXPERTISSUES (NMP3-CT-2004-500283).

INVITED LECTURE No. 8

ALAN BOYDE

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HIGHER ARCHICAL IMAGING OF BIOMATERIAL - TISSUE INTERACTIONS

INTRODUCTION: Our aim is to investigate hard tissue matrices and the cells which produce them using novel microscopic techniques. We may study tissues in the live animal or, usually, in tissue retrieved at operation or post mortem. The best approaches for good resolution imaging of the tissues and cells in bone organs leave the sample more or less intact, which is impossible for physical sections of mineralised tissues. The best approaches for good resolution imaging of the tissues and cells in bone organs leave the sample more or less intact, which is impossible with physical sections of mineralised tissues. Thus when we section a tissue it is to obtain an optical section in the plane of, or deep to, a cut and polished or micro-milled surface. Otherwise, we make 3D samples which extract surfaces and interfaces and image them with one or another deep field imaging system.

METHODS: Microscopies we consider include both light microscopy (LM) and scanning electron microscopy (SEM) and their cross correlation when imaging block surfaces. We also seek suitable methods either for 3D visualisation, where continuous motion parallax is particularly valuable, or for the extraction of information from interfaces which are anything but planar. *In vivo*, video rate confocal LM is especially important. *In vitro*, confocal 3D LM is rivalled by 3D imaging using conventional (non-confocal) deep field imaging means, including rotation of the sample or changing the direction of observation. Binocular stereoscopic visualisation is excellent for the single observer. The rotating condensor aperture method provides a solution for transporting all histological imagery to the lecture theatre in 3D. It also demonstrates that polarised light images need to be interpreted with great caution.

Real time stereo-imaging in SEM and stereophotogrammetric analysis both use secondary electron imaging. In present SEM, we exploit mostly backscattered electron (BSE) imaging, both for quantitative mineral density measurement at high resolution (eg Boyde & Firth 2005) and with multiple detectors, tilts, focal planes or accelerating voltages (to alter information depth) to extract 3D (eg Boyde 2003). However, cathodoluminescence should be important in studying materials.

RESULTS: In mature bone, most of the cells in contact with mineralised bone tissue - whether a resting mineralised surface left as formed by osteoblasts or a resorbed surface previously sculpted by osteoclasts – are adipocytes, which therefore themselves merit the handle 'bone lining cells': evidence for this comes from many different lines of 3D microscopy. The ex- and future osteoblasts are squeezed in between the adipocytes, but do not pave the bone

CONCLUSIONS: We shall make comparisons with x-ray microtomography, where laboratory based systems have the great advantage that most of the tissue 'sectioning' is optical and a complete 3D reconstruction is obtained, but the disadvantages of insufficient resolving power, such that even the osteocytic lacuna is unresolved, and of any means of discriminating soft tissue elements and cells.

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ACKNOWLEDGEMENTS: The author is grateful to the Horserace Betting Levy Board for financial support.

3D Study of Microstructure of Articular Cartilage for Development of a Real Time Histology for Articular Cartilage.

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INTRODUCTION: Osteoarthritis (OA) is characterised by degeneration of articular cartilage (AC) as a result of deterioration of the internal microstructure of AC [1]. Therefore, the microstructure of AC is critical to the tissue's physiological status and development of a method for monitoring the pathological changes of the tissue.

AC contains mainly water, collagen, proteoglycans and chondrocytes [1]. A small amount of lipids has been reported to be distributed over the cartilage surface and to increase lubrication efficiency [2]. Traditional stereological study of the microstructure of AC requires physical sectioning and dehydrating the tissue, which can generate unknown artefacts. By contrast, laser confocal microscopy offers to study the 3D microstructure of a biological tissue while the tissue is not compromised of dehydration and sectioning. Using a fibre optic laser scanning confocal microscope (FOCM), the 3D microstructure of AC (chondrocytes, lipids and the collagen network) was studied.

METHODS: cylindrical AC specimens of approximately 3mm height and 3mm diameter were cut from the central bearing region of bovine femoral heads and condyles. They were stained respectively by Acridine Orange, Acriflavine, Nile Red and Picric Sirius Red. After they were washed by 9g/L saline water, the specimens were examined by a FOCM equipped with a 60x oil/1.4 lens.

RESULTS: the collagen fibres in the superficial zone of AC have a 3D structure, which is more sophisticated than previously observed in 2D optical microscopy. The chondrocytes are organised in a 3D heterogeneous manner from the surface to the deeper region. Lipids were confirmed to be distributed over the surface of AC.

CONCLUSIONS: the 3D collagen structure in the superficial zone of AC appears to be far more complex than previously thought. The surface structure is immediately relevant to our understanding of the degeneration of AC and the potential wear resistance and wear processes of AC during initiation of early OA. FOCM used in this study possesses a unique optical arrangement that has allowed development of confocal arthroscopy to study the chondrocyte of sheep articular cartilage *in vivo* [3]. Thus, successful imaging the 3D collagen structure, the chondrocytes and lipids in this study promises development of a 3D histology for *in vivo* monitoring the pathological changes in AC using confocal arthroscopy.

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High Resolution 3D Resolved Non Invasive *In situ* Nonlinear Optical Imaging (NLOI) of Ex Vivo Tissues and Various Scaffolds Adopted for Tissue Engineering Applications with Human Mesenchymal Stem Cells

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1Oxford Centre for Tissue Engineering and Bioprocessing, University of Oxford, UK. 2 Nuffield Department of Orthopaedic Surgery, Oxford, UK.

INTRODUCTION: Future advances in cell and tissue engineering require intricate knowledge of vital cellular, sub-cellular, extracellular (structural) and physiological (functional) changes in two-, three or even four-dimensions (in space and time). Precise high resolution monitoring of engineered cells and 3D tissue constructs can be accomplished by way of vital non-invasive near infrared (NIR) laser based optical techniques. Femtosecond NIR non-linear optical imaging (NIR-NLOI) is now emerging as a safe modality for imaging various living cells and tissue *in vivo* and *ex vivo* with minimal phototoxicity and photodamage. *In situ* NLOI enables simultaneous imaging of various endogenous and exogenous fluorophores by two-or multiphoton excitation using a single NIR (700 – 1100 nm) wavelength. Importantly NLOI facilitates concomitant imaging of extracellular structural components such as collagen/elastin by utilizing their intrinsic optical signals such as second harmonic generation (SHG) as well.

METHODS: We have used the recently established (Mulholland, et al)3 state-of-art femtosecond multiphoton laser scanning optical system modified and configured for the non-invasive 3D imaging of thick biological specimens with microspectral imaging capability. Sub-femtolitre excitation was realised using a high-numerical-aperture (N. A. 1.3) 60x water or 100 x Oil immersion objectives, with a working distance of c.a 1 mm and 200 micrometers respectively. In the Bio-Rad multiphoton dedicated system, a single 670 nm ultraviolet optimised long-pass dichroic mirror, placed in the excitation path within the infinity focus of the microscope head directed fluorescence emission signal in the UV to visible wavelength range towards non-descanned bi-alkaline and multi-alkaline Photomultiplier Tubes (PMTs). Advanced image analysis (Imaris) modalities was used to evaluate the ex vivo tissue samples and 3D cultures of stem cells as well.

RESULTS:

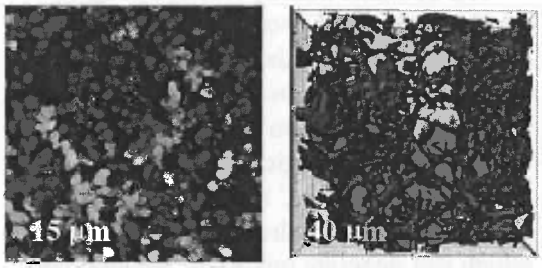

Fig. 1: Cell morphology and organisation of collagen in ex-vivo mouse lung (left) and hMSC in chitosan (right).

CONCLUSIONS: During the meeting we will briefly describe the NIR-NLOI system-setup and present a few of the recent applications of this state-of-art imaging technology for non-invasive monitoring of (1) 3D orientation of cells and collagen/elastin in various living *ex vivo* tissues by way of two-photon excitation and SHG imaging, as well as (2) *in situ* characterization of changes in cell morphology and viability of human bone marrow stem cells (hMSC) grown in various other 3D scaffolds including collagen and chitosan.

REFERENCES: Tirlapur et al., Microscopy Research and Technique (2006) 69: 767-775.

ACKNOWLEDGEMENTS: This work is funded by BBSRC/EPSRC: grant number Gr BB/D014751/1)

Session 7 PHARMACEUTICAL AGENTS AFFECTING BONE METABOLISM

CHAIRS: MEN - KUBER SAMPATH & JEREMY FAIRBANK

INVITED LECTURE No. 9

GRAHAM RUSSELL

The Botnar Research Centre and Oxford University Institute of Musculoskeletal Sciences, Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, UK

BISPHOSPHONATES AND TISSUE RESTORATIONCURRENT & FUTURE OPPORTUNITIES

Bisphosphonates are stable chemical analogues of inorganic pyrophosphate, which is a byproduct of many biosynthetic reactions, and also acts extracellularly as the body's natural water softener.
The ability of bisphosphonates (BPs) to inhibit bone resorption was discovered nearly 40 years ago. The recognition of the future clinical potential of these drugs emerged from studies of their effects on the physicochemical behavior of calcium phosphate crystals. The ability of BPs to bind strongly to bone mineral gives them the unique property of selective uptake by their intended target organ.
During the intervening period the BPs have become progressively well established as the major drugs used for the treatment of bone diseases associated with excessive resorption, including Pagets disease, multiple myeloma, and bone metastases Bisphosphonates are currently the leading drugs used for the treatment of osteoporosis; alendronate and risedronate can reduce vertebral fracture occurrence by 30-50%, and also significantly reduce nonvertebral fractures and hip fractures. Zoledronate is already widely used in oncology and is awaiting regulatory approval in osteoporosis after Phase 3 trials have shown that it reduces all fracture types when given just as a single iv dose of 5mg once a year.
The pharmacological effects of BPs in vivo are the result of two key properties, their affinity for bone mineral, and their inhibitory effects on osteoclasts. There is new information about both properties. Mineral binding affinities differ among the clinically used BPs, and may influence not only their potency but also their potentially prolonged duration of action. The cellular effects of the nitrogen-containing bisphosphonates (such as pamidronate, alendronate, risedronate, ibandronate, and zoledronate) on osteoclasts appear to result from inhibition of the enzyme farnesyl pyrophosphate synthase (FPPS), a key branch point enzyme in the mevalonate pathway, well known as the biosynthetic pathway leading to isoprenoids and sterols. BPs inhibit the biosynthesis of FPP and GGPP (farnesyl pyrophosphate and geranylgeraniol pyrophosphate), that are utilised for the post-translational modification of small GTP-binding proteins essential for osteoclast function. More background information about the discovery and development of BPs and their chemistry, pharmacology, and current clinical applications can be found in reviews [i, ii, iii, iv].
There have been many studies over the years to explore the potential use of bisphosphonates with implants and in preserving bone structure. There are now several reports suggesting that BPs may enhance fracture repair, probably by stabilizing the fracture callus [v]. Ibandronate can improve the osseointegration of metal implants in ovariectomised rats [vi]. Zoledronate can conserve bone architecture in experimental models of osteonecrosis [vii] and Perthes disease [viii]. There are other potential applications of BPs in orthopaedics, including protection against loosening of prostheses [ix], better integration of biomaterials and implants [x], and improved healing in distraction osteogenesis [xi]. In clinical trials alendronate has been shown to be effective in osteonecrosis [xii]. There is considerable potential for utilising these interesting drugs for these new applications.

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Characterization of new injectable biomaterials containing bisphosphonates

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

METHODS: 200mg of calcium phosphate support is placed in a BP solution (e.g. zoledronate) of desired concentration. After 48H of stirring, the solid is washed with water and the amount of incorporated BP is determined by measuring the residual amount of BP in the liquid phase. This solid can be incorporated per-operatively in an ether-cellulosic viscous solution allowing injection.

RESULTS: From SEM and ^{31}P MAS NMR experiments, the presence of various types of association modes was obvious, depending on the nature of the calcium phosphate support. In some cases (e.g. β-$Ca_3(PO_4)_2$, **I**), an acicular crystalline Zoledronate derivative forms onto the surface of the CaPs (fig1) providing a rapid BP release profile.

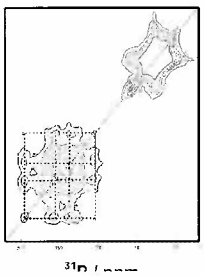

Fig 1 : ^{31}P MAS NMR and SEM of **I-Loaded**

On the contrary, in other cases (e.g. calcium deficient apatites noted CDA, **II**) a strong chemisorption of the BP takes place on the surface of the support driven by a PO_3/PO_4 exchange process (fig2).

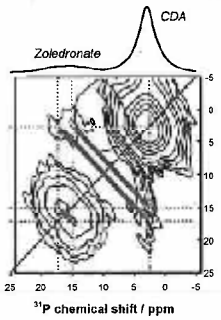

Fig 2 : ^{31}P MAS NMR of **II-Loaded**

In this latter case, Zoledronate is released at very low concentrations, making possible the evaluation of the biological activity of Zoledronate-loaded materials using *in vitro* bone resorption assays. Zoledronate-loaded CDA showed a dose-dependent inhibitory effect on osteoclastic activity similar to that observed with solutions of Zoledronate ranging from 10^{-6} to 10^{-9}M. A Langmuir-based model[3] was designed to describe the Zoledronate/ CDA interaction in water or phosphate buffers. We have demonstrated that the BP release profile (i) can be tuned by the BP loading ratio on CDA (ii) is dependent on the phosphate concentration in the desorption medium.

CONCLUSIONS: CDA is a suitable carrier for bisphosphonates, providing a bioactive drug delivery system whose release kinetics is compatible with the inhibition of bone resorption. On *In vivo* osteoporosis models, significant stimulation of new bone formation was observed for II-loaded versus control [4].

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Session 8 ENGINEERING SCIENCE AND ORGAN RECONSTRUCTION

CHAIRS: G BAROUD & RALPH MUELLER

INVITED LECTURE No. 10

RICHIE GILL

Oxford Orthopaedic Engineering Centre, Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford, UK

ENGINEERING REQUIREMENTS FOR TISSUE REPLACEMENT

The next generation of treatment modalities for joint disease will be a paradigm shift from the current use of metallic implants for joint replacement. Early joint disease manifests as lesions of the articular cartilage and the disease progresses to large scale erosion of the articular surface, leading to pain and impairment of function. The goal of tissue regeneration is to repair these early relatively small lesions before the large scale joint degeneration occurs. However, a functioning joint is a challenging environment for any implanted device. Along side the development of tissue repair technologies, understanding of the mechanical conditions within the joint is essential to practical solutions to be developed. Human joints, like other mammalian joints, are constrained by the biological mechanisms available to provide joint stability and motion. As a consequence human joints suffer from load amplification; the internal forces within a joint are generally many times larger than the load being carried by the limb moved by the same joint. For function many joints require a large range of motion, and thus have non-conforming surfaces. This gives rise to small contact areas, further increasing the load amplification. The natural tissues of the joint have evolved to have remarkable material properties. The joint environment needs to be characterised so that tissue repair devices can be designed to survive and carry out their function.

BIOMECHANICAL EFFICACY OF INTERVERTEBRAL DISC AUGMETATION

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INTRODUCTION: One of the major causes of back pain is degenerative disc disease [1,2]. One viable treatment modality for discogenic pain resulting from degenerative disc disease is nuceleous arthroplasty through nucleus augmentation. This procedure represents an alternative to spinal fusion and total disc arthroplasty, however, the biomechanical efficacy of disc augmentation has not been tested yet. The objective of this study was to evaluate changes in spine kinematics after nucleus augmentation.

METHODS: Four fresh frozen human cadaveric thoracolumbar spines (Males, age 72-82) were used. Two spinal segments from each spine were randomly distributed to the treatment (N=8) and to the control (N=8) group. Treatment included disc augmentation with a collagen based biomaterial. The maximum injection volume used was approximately 2 ml. The injection pressure was monitored during and after the injection, and throughout each experiment.

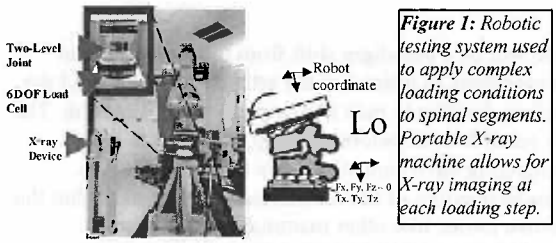

Figure 1: Robotic testing system used to apply complex loading conditions to spinal segments. Portable X-ray machine allows for X-ray imaging at each loading step.

All spinal segments underwent mechanical testing using a 6-DOF robotic testing system (KR150, KUKA Robotics Corp.). The peripheral path in response to three revolving bending moments of 7.5, 10, and 12.5 [N.m.] was determined. Using a hybrid control system, the compressive force, shear forces and other remaining moments were minimized. In addition to applying different bending moments, two axial compressive pre-loads, 0 [N] and 600 [N], were applied to all segments and the three bending moments [3]. Changes in motion area and motion patterns were calculated. Significant differences between test groups were determined and statistically tested.

RESULTS: Disc augmentation resulted in an increased motion field area compared to the same specimens before treatment (Figure 2). These differences were statistically significant ($p < 0.05$). Including the 600 N axial pre-load case, the average increased range of motion for the treatment group was 43 % compared to control. Furthermore, the irregular motion patterns in the spinal segment due to disc degeneration were smoothed after treatment.

Noteworthy was the finding that intradiscal pressure did not vary significantly with treatment, however, discal pressure was the lowest in bi-lateral bending.

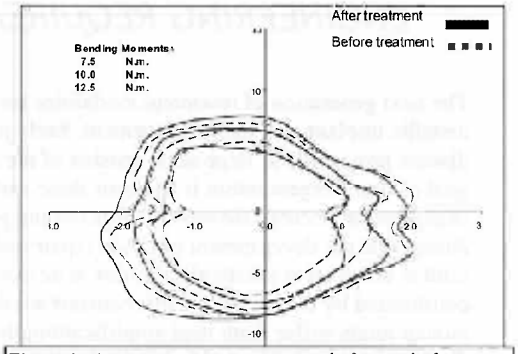

Figure 2: A comparison in motion area before and after treatment. In each case the motion field area was increased.

CONCLUSION: The results of our investigation indicated that intervertebral disc augmentation through a collagen based material is a promising alternative option to total arthoplasty. The increase in range of motion due to treatment did not depend on spinal levels and was mostly seen within the sagittal plane in flexion. The compressive axial preload reduced the flexibility of the spinal joint significantly, however, the benefit of the treatment still persisted.

ACKNOWLEDGMENT: Funding was provided by Medtronic Sofamor Danek

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Geometric and Fluid Transport Analysis of Calcium-Phosphate Scaffolds

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INTRODUCTION: A few years ago, a model was proposed to predict the time required for the cell-mediated resorption of mineral bone substitutes [1]. To assess the validity of this model, b-tricalcium phosphate (b-TCP) bone substitutes of four different (macro)pore sizes were synthesized, characterized [2], and implanted in sheep [3]. The application of the resorption model to the in vivo data requires, in particular, the characterization of the (macro)pore size, connectivity, porosity, surface area, and permeability of the four different b-TCP bone substitutes. The goal of this abstract is to describe how the samples were analyzed and what the results were.

MATERIALS AND METHODS: The calcium phosphate substitutes (8 mm in diameter, 13 mm in length) were produced by the so-called calcium phosphate emulsion method, in which oil is dispersed in a calcium phosphate cement paste [2]. Variations in the emulsifier concentration lead to four macropore sizes. The samples were scanned by μCT with a 30 μm resolution. The geometric analysis was performed as follows: (i) *fuzzification* was done using sigmoidal function, (ii) *3D fuzzy distance transform* (FDT) map of pore structure voxels was determined, (iii) *ridge points* were extracted based on the second derivative of the 3D FDT map, (iv) *pore size* and *connectivity* were calculated on the basis of FDT values of the local maxima and saddles of ridge points, (v) the *porous structure* was reconstructed for fluid flow analysis by growing spheres at each of the skeletal voxels with radii equal to their FDT value, and (vi) the *flow in a binary reconstructed scaffold* was simulated by a Lattice Boltzmann code (LB Development, Consortium, Erlangen). The fabrication and advanced images, in addition to the flow analysis tools, are novel.

RESULTS AND DISCUSSION: The variability in substitute fabrication was lower than 5%, except for the permeability. The new 3D image analysis tools delivered the distribution pore size and connectivity. Specific geometric and transport properties varied substantially among scaffolds, as depicted in Table 1. Bone surface density increased as the pore size decreased. Also, increasing the pore size and connectivity values increased permeability. Figure 1 shows the FDT map used for pore size and connectivity characterization, and velocity to determine the permeability representative for the results obtained in this study

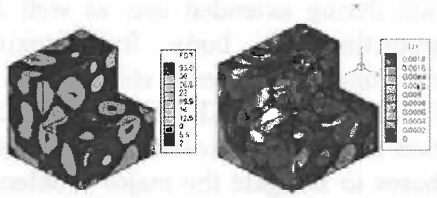

Figure 1: (left) FDT map and (right) velocity map with streamlines show flow in the scaffold pores.

Parameter	Sample A	Sample B	Sample C	Sample D
Pore size (mm)	109± 2.4	171.6± 5	319.6± 4	728± 10
Connectivity (mm)	46.2±0.7	48.5±2.7	52.8±2.8	98.9±5.4
Porosity (%)	51.5±1.8	54.9±1.5	55.5±0.7	54.4±0.6
Surface density (1/mm)	11.57±0.2	9.98±0.28	6.51±0.17	3.28±0.08
Permeability($*10^{-10}$ m^2)	1.28±0.02	2.71±0.39	3.6±0.4	4.0±0.7

Table 1

CONCLUSIONS: These image and analysis tools allow for both geometric and transport characterization of substitutes. This is turn may explain the in vivo behavior [2] and help design appropriate substitutes.

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The authors thank T. Zeiser for the support provided for the LB code.

The new packaging systems for implantable electronic devices

J.M. Wasikiewicz[1], D. Paul[1], N. Roohpour[1], P. Vadgama[1]
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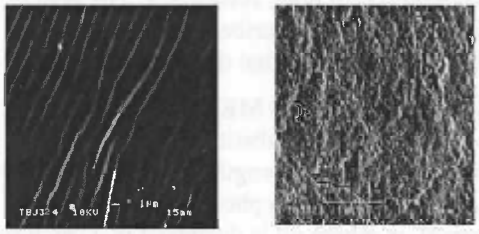
INTRODUCTION: 25 members of a major EU-consortium have been developing electronic devices for functional electrical stimulation, glaucoma and CNS pressure monitoring and also cochlear, retinal and urethral implants[1]. However, the packaging of such state of the art devices to enhance tissue biocompatibility, and to protect conducting elements from *in vivo* corrosion during extended use, as well as for protecting the body from toxins leaching from implant parts, still remains a concern. Potential candidate barriers as hydration resistant and solute impermeable interphases to mitigate the major problems of chronic implantation including lipid modified silicone, modified polyurethane and diamond like carbon were investigated.

METHODS: The membranes were examined by field emission scanning electron microscopy (Jeol JSM 6300F, Japan).
The water uptake by polymers was monitored by FTIR (Nicolet-800 FTIR) spectrometer.
Electrochemical surface characteristics were assessed by electrical impedance spectroscopy (EIS), (Gamry instruments PC4/300).
Contact angle measurements were performed on an in house setup combined with computer system.
Mechanical tests were carried out according to the following ASTM standards: D412-87, D1004-66, D2240-86.
.

RESULTS: A lipid was used to modify silicone rubbers in order to confer increased water resistance and ensure silicone materials to be useful for electronic device packaging. FTIR, EIS and contact angle measurements confirmed that optimised incorporation of lipid in silicone influences the water resistance of such materials.
Similar results were obtained for modified polyurethane. Analysis of mechanical properties of modified silicone and polyurethane shows that such modification also results in changes in tensile strength and elongation of tested materials.
DLC coatings led to ultrathin (<1μm), coherent, adherent films on both flexible and inflexible substrates.
Additionally, if we coated silicone containing lipid with DLC we observed significant increase in surface smoothing after coating (Fig 1).



a)
b)

Fig. 1: SEM images of layers a) silicon + IPM, b) silicon + lipid coated with DLC

DLC also has highly solute resistant properties and shows promise as a hydration barrier, depending upon the C-H ratio used for the source gas for deposition.

CONCLUSIONS: A range of new candidate test materials was established as possible packaging systems for passive micromolecular device surfaces. These presented variable H_2O permeability, and could be adapted alternatively for the active and passive components of microelectronic devices.

REFERENCES:
1. HealthyAims at www.healthyaims.org

ACKNOWLEDGEMENTS:
We acknowledge generous support from the EU (Grant no. IST-2002-1-001837)

Mechanical characterisation of the Bioflex® system

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[1] Department of Engineering Science, University of Oxford, Oxford, UK., [2] Center for Musculoskeletal Surgery, Charité - Universitätsmedizin Berlin, Germany, [3] Institute for Medical Genetics, Charité - Universitätsmedizin Berlin, Germany

INTRODUCTION: An essential prerequisite for the study of mechanotransduction is the ability to apply well-defined mechanical stimuli to cells. The Bioflex® system (Flexcell Intern. Corp., Hillsborough, NC) is claimed to apply equi-biaxial strain to cells cultured on a flexible silicone membrane. This is achieved by a pneumatic system that stretches the membrane on which the cells are cultured over a post. Recent work identified an optimal stimulation protocol for MC3T3-E1 cells and confirmed the homogeneity of the strain field across the membrane [1]. The aim of this study was to quantify the relationship between the nominal strains reported by the device and those measured on the surface of the membrane using digital image correlation (DIC). Additionally the influence of the number of cycles on this relationship and the biaxial quality of the strains were investigated.

METHODS: Optical texture on the membranes for the DIC analysis was obtained using an airbrush. High-resolution digital images of membranes in the reference state (nominal 0.2%) and at nominal strains of 2.5%, 5% 7.5% and 10% were made in all 6 wells of 3 Bioflex plates. The principal strains within the homogeneous strain region remaining at all times on the upper surface of the post were evaluated using DIC (Vic2D, Correlation Solutions, West Columbia, USA). These measured strains were compared with the strains reported by the device controller. Bioflex membranes were investigated in their unused state and after 10,000 and 20,000 load cycles at 2 Hz, 5% nominal strain.

RESULTS: The reported strains were highly correlated with the measured strains ($R^2 = 0.99$) on all membranes. On unused membranes, regression analysis showed measured strains 1.1 times higher than reported strains. This increased after 10,000 cycles loading, and after 20,000 the measured strains were up to 1.4 times the reported strains (Fig. 1). The DIC measured strain field was approximately biaxial, with an average difference between maximum and minimum principal strain of 9%.

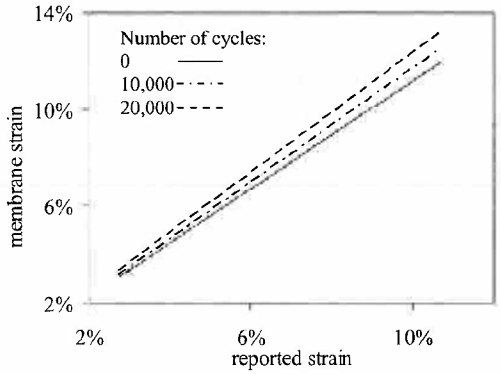

Fig. 1: Relationship between membrane strain determined using DIC and that reported by the Bioflex System after 0, 10,000 and 20,000 cycles.

CONCLUSIONS: The relationship between reported and DIC measured strain in the Bioflex system changes with increasing numbers of load cycles. On fresh membranes the reported strains are within 10% of the measured values, but after 20,000 cycles the measured strains are up to 40% larger than those reported, indicating that considerable change in the mechanical stimulation of cultured cells may occur over long stimulation periods. These changes may be due to plastic deformation of the membrane material. Small local variations in the lubrication under the membrane may contribute to the discrepancy between the principal strains.

REFERENCES: 1. Ott et al. 2006 *Calcif Tissue Int* 78, suppl.1, S62.

ACKNOWLEDGEMENTS: This study was supported by the Hansjörg Wyss AO Medical Foundation and by the German Research Foundation (grant DFG KFO 102/1).

Programme of Poster Presentations

Posters will be on display throughout the meeting in rooms on both floors of the Bernard Sunley Building. Please take time during lunchtimes and during morning and afternoon breaks to examine them and to speak with the presenters.

Posters are numbered and a key can be found on the following pages. Full abstracts are also included.

1 **Preparation of Hydroxyapatite-Gelatin Scaffolds Crosslinked by Glutaraldehyde for Bone Tissue Engineering**
aMehdi Kazemzadeh Narbat [1] , Mehran Solati Hashtjin [1] , Azadeh Goudarzi [2]
[1] Science & Research university, P.O. BOX 1476766981, Tehran, Iran, (a member of young researchers club) [2] Amirkabir university of Technology, P.O. BOX 15875-4413, Tehran, Iran

5 **What is the Role of MYSTIQUE Plating and Bioresorbable Technology in Anterior Cervical Fusion Procedures?**
Jason E. Garber [1], John S. Thalgott [2], Madilyne E. Fogarty [2], Stephani D. Christensen [2], Christopher D. Corona [2], Steven P. Caiazza [2]
[1] Nevada Neuroscience Institute, Las Vegas, NV, [2] International Spinal Development & Research Foundation, Las Vegas, NV;

6 **Evaluation of monomer polymerization by raman microscopy**
G. Mabilleau [1,2], C.Cincu [1,3], M.F. Baslé [1], D. Chappard [1]
[1] INSERM, EMI 0335 – LHEA, Faculté de Médecine, 49045 Angers Cedex, France, [2] Botnar Research Centre, University of Oxford, Nuffield Orthopaedic Centre, Oxford, UK, [3] Department of Macromolecular Chemistry, University Politehnica, 71101 Bucharest, Romania.

7 **Hydroxyapatite implantation affects bone growth and formation**
Luzin V., Ivchenko V., Ivchenko D., Skorobogatov A., Lubenets A.
State Medical University, Luhansk, Ukraine

8 **The Role of a Gore-tex (polytetrafluroethylene) in a CHARITÉ Artificial Disc Revision: A Case Report**
Jason E. Garber [1], John S. Thalgott [2], Stephani D. Christensen [2], Christopher D. Corona [2], Madilyne E. Fogarty [2], Earl D. Cotrell [3],
[1] Nevada Neuroscience Institute, Las Vegas, NV, [2] International Spinal Development and Research Foundation, Las Vegas, NV [3] General Vascular Specialists, Las Vegas, NV

11 **Biomechanics of vertebroplasty may be sensitive to intervertebral disc quality**
K. Sun [2], L. Rhines [3], A. Burton [3], E. Mendel [4], M. Liebschner [1]
[1] Department of Bioengineering, Rice University, Houston, TX; [2] Department of Veterans Affairs Medical Center, San Francisco, CA; [3] MD Anderson Cancer Center, Houston, TX; [4] Department of Neurological Surgery, Ohio State University, Columbus, OH

12 **Experimental study of bone integration between xeraspineä and sheep vertebrae**
Uhlin, T [1]; Engqvist, H [1,2]; Jarmar, T [1,2]; Bjorklund, K [1]; Höglund, A U [3]
[1] Doxa AB, Uppsala, Sweden, [2] Materials Science Department, The Angstrom Laboratory, Uppsala University, Sweden, [3] Visionar Biomedical AB, Uppsala, Sweden.

13 **A comparative evaluation of the in vitro hemocompatibility of orthopaedic cements**
A.U. Höglund [1], J. Sanchez [2], T. Persson [3], T. Uhlin [3], H. Engqvist [3,4], K. Bjorklund [4], R. Larsson [2]
[1] Visionar Biomedical AB, Uppsala, Sweden, [2] Uppsala University, The Rudbeck Laboratory, Sweden, [3] Doxa AB, Uppsala, Sweden, [4] Materials Science Department, The Angstrom Laboratory, Uppsala University, Sweden.

14 **Compressive strength development of xeraspineä due to aging in PBS**
Persson T. [1] and Engqvist H. [1,2]
[1] Doxa AB, Uppsala, Sweden, [2] Materials Science Department, The Angstrom Laboratory, Uppsala University, Sweden

19 **Dense HA bioceramics with a high compressive strength**
Sergey V. Dorozhkin
Kudrinskaja sq. 1-155, Moscow 123242, Russia

22 **Calcium phosphate biomaterials for a local-delivery of bisphosphonate: chemical association vs in vitro activity.**
E. Verron [1], H. Roussière [1,2], B. Bujoli [2], P. Janvier [2], JM. Bouler [1], J. Guicheux [1].
[1] INSERM, UMRS 791, Laboratoire d'Ingénieurie Ostéo-Articulaire et Dentaire, LIOAD, Nantes, France ; Univ Nantes, Faculté de chirurgie dentaire, F-44042 Nantes, France, [2] UMR CNRS 6513, Laboratoire de Synthèse Organique, LSO, Nantes, France ; Univ Nantes, Faculté des Sciences et des Techniques, F-44322 Nantes, France

26 **Cryopreservation of Human Mesenchymal Stem Cells – Effect of Different Cryoprotectants**
Xia Xu, Zhanfeng Cui
Oxford Centre for Tissue Engineering and Bioprocessing, Department of Engineering Science, Oxford University, England

27 **Process Capability of Scaffold Mould Using a 3D Printing Technique**
Chaozong Liu [1,2], David J Williams [1], Jan T Czernuszka [2]
[1] The Wolfson School of Mechanical & Manufacturing Engineering, Loughborough University, Loughborough LE11 3TU, UK [2] Department of Materials, University of Oxford, Oxford OX1 3PH, UK

28 **Engineered Collagen-Hydroxyapatite Scaffolds for Bone Tissue**
C. Z. Liu [1,3], Z. D. Xia [2], P. Hulley [2], J. Triffitt [2], J. T. Czernuszka [1]
[1] Department of Materials, University of Oxford, Parks Road, Oxford OX1 3PH, UK. [2] Botnar Research Centre, University of Oxford, Headington, Oxford OX3 7LD, UK. [3] The Wolfson School of Mechanical & Manufacturing Engineering, Loughborough University, UK.

31 **Hydroxyapatite-coated liposomes for potential use as local drug delivery devices.**
Qingguo Xu, Jan Czernuszka
Department of Materials, University of Oxford, United Kingdom

33 **Percutaneous Injectable Synthetic Calcium Sulfate for the Enhancement of Percutaneous Spinal Fusion**
D. P. Stathakopoulos, M.D.,[1] Ph.D, I. M. Kyratzoulis, M.D. [2] V.K.Bitounis FRCS [1]
[1] Spinal Surgery Department, Athens Medical Center, Athens, Greece, [2] Spinal Surgery Department, Mediterraneo Hospital, Athens, Greece

34 **Characterization of a biological bone substitute for vertebroplasty**
C. Ehrenborg, M. Nilsson
BoneSupport, Ideon Science Park, Lund, Sweden.

35 **Scaffold Characterisation for Human Mesenchymal Stem Cell Culturing**
N. Trainor [1], U. Tirlapur [1], J. Triffitt [2], Z. Cui [1]
[1] Department of Engineering Science, University of Oxford, UK. [2] Nuffield Department of Orthopaedic Surgery, Oxford, UK.

38 **Addition of polymeric resorbable phases to calcium phosphate bone cements**
V Jack [1], F Buchanan [1], N Dunne [1], D Farrar [2]
[1] School of Mechanical and Aerospace Engineering, Queen's University of Belfast, BT9 5AH, Northern Ireland, [2] Smith & Nephew plc Research Centre, York Science Park, York Y010 5DF, United Kingdom

39 **Oxygen monitoring in regenerating tissue**
T. Grünhagen [1], C.P. Winlove [2], J.P. Urban [1]
[1] Department of Physiology, Anatomy and Genetics, University of Oxford, Great Britain, [2] School of Physics, University of Exeter, Great Britain.

40 **The Mechanism of the adsorption of Fibrinogen on CP Titanium**
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41 **Modelling of oxygen, glucose, lactic acid and pH in engineered cartilage**
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42 **Spectrometric analysis of non-stoichiometric hydroxyapatite nanocrystals**
E. F. Brès [1], V. Serin [2], G. Carbajal de la Torre [1,3], C. Combes [4], G. Leroy [5], C. Rey [4]
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43 **Hydroxyapatite – catgut composite as a prospect bone implant**
A. Stepuk, V. Putliaev
Department of Material Sciences, Moscow State University, Moscow, Russia

44 **Spherical a-TCP Obtained by Suspension Induction-Plasma Spraying**
C. Damia [1],H. Park [2], M. Habib [1], M. Bohner [3], F. Gitzhofer [2], and G. Baroud [1]
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45 **Enhancing PMMA Bone Cement with Radiopacifier for Vertebroplasty**
A. Ahmari and G. Baroud
Biomechanics Laboratory, Mechanical Engineering Department, Université de Sherbrooke, Sherbrooke, Québec, Canada

46 **Enhancement of the Injectability of Calcium Phosphate Bone Substitute**
M. Habib [1], F. Gitzhofer [2], M. Bohner [3], G. Baroud [1]
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49 **Two methods to characterize calcium phosphate putties**
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50 **Lumbar Spondylolisthesis Treated by Bi-posterolateral Fusion Combined with Coralline Spinal Interbody Fusion**
Zhibin Meng, Kun Fu, Jun Li, Hongchao Li
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51 **Application of absorbable screw in treatment of the olecranon fractures**
Xing Danmou [1], FENG Eryou [2], Kan Wusheng [1], Peng Zhengren [1]
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52 **In Vitro Experimental Study on Chitosanas Carrier for Sustained Release of Recombinant Human Bone Morphogenetic Protein-2**
YU Aixi [1], Bao Tongzhu [2], ZHU Shaobo [1], WU Wuzhou [1], HE Xibin [1]
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53 **Biomechanical and clinical study of atlantoaxial instability in posterior atlantoaxial transarticular screw fixation**
Zhuang-hong Chen, Ji-feng Huang, Xian-hua Cai, Lunhong Yu, Ximing Liu, Feng Xu, Huasong Wang
Department of Orthopaedics, Wuhan General Hospital of PLA, Wuhan 430070, China

54 **Tissue Regeneration of Severely Infected Bone Exposure Wound by Combining Vacuum Sealing Drainage with Flap Transplantation**
Jiuhong Song [1], Aixi Yu [2], Guorong Yu[2], Xibin He[2], Kai Deng[2], Shengxiang Tao[2], Zhenyu Pan[2] and Jianhua Zhang[2]
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55 **Surgical Reconstruction with Internal Fixation for intra-articular calcaneal fractures with malunion**
Jiafu Qu, Lihai Cao, Yang Sun
Department of Foot Surgery ,the Second Hospital of Tangshan, Tangshan 063000, China

56 **The Preliminary Study of Navel Injectable Biomaterial to Repair Cartilage --High Viscous Chitosan/Glycerol Phosphate -Demineralized Bone Matrix**
Zhiqiang Wang, Bo Jing
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57 **Manufacture and application of implantation artificial skull**
Jinwu Chen, Yongsheng Yuan, Dan Zhou
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58 **Repair of Rabbit Growth Plate Defects with Tissue-engineering Cartilage: Compound of Autologous Bone Marrow Mesenchymal Stem Cells and Biological Scaffold**
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59 **Clinical Evaluation of Vacuum Sealing Drainage Effect in The Treatment of Severe Soft Tissue Defect of The Lower Limbs**
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60 **Effect on the osteoblastic differentiation from rabbit adipose-derived stem cells transfected by adenoviral vector mediated hBMP-2 gene in vitro**
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61 **Osteoclastic responses to phase pure hydroxyapatite (HA) and silicon (Si)-substituted HA**
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62 **Near surface damage characterisation of biomedical grade Y-TZP**
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63 **Comparative study between coralline hydroxyapatite and coverslips culture with mesenchymal stem cells**
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65 **Tissue Responses to Mesenchymal Stem Cells and Biomaterial Implants**
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67 **CAMC – a new imaging device for pedicle approach**
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68 **Injectable Biomaterials for the Regeneration of Intervertebral Disc**
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Poster abstracts

1. Preparation of Hydroxyapatite-Gelatin Scaffolds Crosslinked by Glutaraldehyde for Bone Tissue Engineering

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Introduction: Calcified tissue, such as long bone and jaw bone is considered a biologically and chemically bonded composite between HA (HA: $Ca_{10}(PO_4)_6(OH)_2$) and type-I collagen. Gelatins are compositmlamj
ionally virtually identical to the collagen from which they are derived. Therefore In this study, to mimic the mineral and organic component of natural bone, hydroxapatite[HA] and gelatin[GEL] composite scaffolds were prepared using solvent-casting method combined with freeze drying process. Glutaraldehyde [GA] was used as cross linking agent in the making of gelatin-tricalcium phosphate composites rendering them no longer water soluble but since GA is cytotoxic the bisulfite sodium was used as excess GA discharger. Prepared composite scaffold of HA and gelatin is expected to show increased biodegradation together with sufficient mechanical strength.

Methods: The slurry composites were prepared using solvent casting method. Definite GEL concentration was dissolved in deionized water [DI] at temperature $45\,^{\circ}C$. Desire volumetric content (30wt%, 40wt% and 50wt%) of fine HA particles relative to gelatin were added. The reinforced slurry composite was then heat treated on magnet stirrer under constant mixing for 1h at $45\,^{\circ}C$. The slurry was deagglomerated by magnet stirring meanwhile the temperature was monitored continuously. Then to avoid air bubbles the slurry immediately was injected by using a syringe into cylindrical Teflon molds. The molds were frozen at $-70\,^{\circ}C$ and were dried in a commercial freeze-dryer for 6h for solvent (DI) removal. After that, the white composites were removed and placed in room temperature for 24 h, they were immersed in a 8% solution of GA for 3 h then. To remove the residues of GA agent, the cylinders were washed with DI for 24 h, during which time the water was changed every 6 h. Besides, the sodium bisulfite was used to discharge the excess GA.

Results and Discussion: FT-IR spectroscopy was used to estimate the conformational change of the HA/GEL composite structure. FT-IR spectrum for the cross-linked HA-GEL composite indicates chemical bond formation between carboxyl ions in GEL and HA phases. The compressive strength, Young's modulus and elongation of composites were measured with an Instron materials testing machine. The compressive modulus of HA-GEL scaffolds increased with HA content. It was found that the mechanical properties of GEL/HA with ratio of 50wt% was similar to that of trabecular bone. Water absorption of HA-GEL composites with different HA content were studied to evaluate the effect of HA content on the size and stability of material. The water absorption of composites reduced with HA content. A liquid displacement method was used to measure the porosity and density of HA scaffolds. .It was found that the addition of HA results in more dense and thicker pore walls with lower porosity The morphology and microstructure of the scaffolds were examined using SEM and light microscopy. The scaffold prepared has an open, interconnected porous structure with a pore size of 80-400 μm. The biological responses of scaffolds carried out by L929 fibroblast cell culture. Cells exhibited rather good proliferation and partially covered the composite surface after 48h.

Conclusions: In this work a method of producing three dimensional, open-cell, composite scaffold of HA-GEL has been developed. The technique involves solvent-casting method combined with freeze drying process which has the advantages of both the methods. It was found that the properties prepared HA are close to that of trabecular bone.

5. What is the Role of MYSTIQUE Plating and Bioresorbable Technology in Anterior Cervical Fusion Procedures?

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Introduction: Anterior cervical discectomy and fusion (ACDF) is a commonly performed spinal procedure. Occasionally, due to pseudarthrosis and transition level/adjacent segment disease syndrome, patients require revision procedures which can include the arduous removal of original hardware. The MYSTIQUE Plate (Medtronic Inc.) is a resorbable graft containment plating system for cervical spine fusions composed of a co-polymer containing Poly (L-lactide) and Poly (D,L-lactide), called HYDROSORB. The flexible plate can be contoured to match patients' anatomy. Its transparent material allows surgeons to visualize the spine during surgery. The materials are designed to dissolve and resorb into the body 18-36 months after implantation. Because the device is resorbed by the body, the use of bioresorbable plating in cervical fusion may eliminate hardware removal steps during revision procedures. Furthermore, the plate is radiolucent and allows fusion status to be assessed without titanium artifact. We attempted to utilize bioresorbable technology in conjunction with bone morphogenic protein (BMP) in anterior decompression and fusion procedures to explore the role of bioresorbable technology in anterior cervical fusion.

Methods: Five female patients and one male patient between 31 and 50 years old (mean 40.7 years) underwent single level ACDF utilizing HYDROSORB, interbody grafting, bone morphogenic protein arthrodesis and MYSTIQUE resorbable anterior cervical plating. Patients were treated at either C5-C6 or C6-C7. Patients were followed for three months with periodic follow-up visits. Each patient's post-procedure outcome was assessed and radiographic evaluations of the fusion status were performed. Patients are still in follow-up.

Results: At the latest follow-up, four of the six patients appear to have normal graft incorporation and are healing well with little to no neck symptoms. One male patient (44 years old) experienced hardware failure approximately 4 months post-procedure. The patient experienced complete collapse of the disc space with kyphotic deformity at C6-7. (fig 1) Revision was done via complete removal of instrumentation, complete corpectomy at C6, and partial corpectomy at C5 and C7. The patient was then instrumented with a titanium cage and Danek-Atlantis vision plate at C5-6-7. (fig 2) A female patient's (33 years old) radiographs appear to show calcification of the MYSTIQUE plating and osteophytic spur development one level above the fusion level. The patient is asymptomatic.

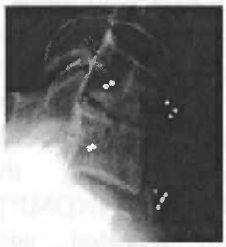

Fig 1. Male, age 44, with collapsed disc at 4 months post-operative

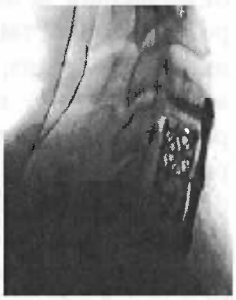

Fig 2. Revision with instrumentation of a titanium cage and Danek-Atlantis vision plate

Conclusions: Although four of six patients treated had acceptable results in this study, the potential benefits of this plate may not outweigh the risk of hardware failure. If stable fusion is not achieved prior to the degradation of the resorbable plate and screws, the construct may fail. Furthermore, the resorbable screws may dissolve more quickly than the plate, causing the plate to prematurely release from the bone and become dislodged in the patient's anatomy. If these fragments are sharp or resting near the esophagus or trachea, they can cause significant discomfort. Although successful results are possible, the MYSTIQUE system, in our limited experience, does not have the appropriate material properties for consistently successful ACDF outcomes.

6. EVALUATION OF MONOMER POLYMERIZATION BY RAMAN MICROSCOPY

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INTRODUCTION: 2-hydroxyethyl methacrylate (HEMA) is the main component of intraocular lens and is also used in a variety of other biomedical applications (e.g., dental cement). HEMA is usually polymerized by using a redox system combining a polymerization initiator of (often benzoyl peroxide-BPO) and a disubstituted amine like N,N,dimethyl-para-toluidine-(NNDMPT). However, it is well known that the amount of accelerator is used in excess to allow a polymerization rate compatible with the needs of surgeons, and some molecules of accelerator are not consumed in the polymerization process. Furthermore, side products are obtained during the degradation of BPO and NNDMPT. Irritative effects were reported with disubstituted amine and the need of new systems appears suitable. They should have a polymerization rate compatible with the classical methods and a simple methodology to assess polymerization kinetics needs to be developed. The aim of this study was (i) to develop a new couple of accelerator/initiator suitable for HEMA polymerization and (ii) to assess the polymerization kinetics by raman microscopy.

METHODS: HEMA was purified and distilled under reduced pressure (5.10^{-2} mBar, 65°C) to remove impurities. Two different couples of accelerator/initiator were used: the conventional NNDMPT/BPO (ratio 1:500) and ascorbic acid (AA)/BPO (ratio 2:1). Raman analysis was performed on a Senterra microscope with the OPUS 5.5 software (BRUKER OPTIK, Ettlingen, Germany). The excitation laser wavelength was 785 nm. The long working distance of the 20X microscope objective used gave a spot size of the order of a couple of micrometers. Values from peak intensities and bandwidths of the peaks recorded on the spectrum were used as described in the literature. Four bands were investigated: =CH2 group at 1407 cm^{-1}, C-H at 1455 cm^{-1}, C=C at 1641 cm^{-1} and C=O at 1714 cm^{-1}.

RESULTS: The intensities of the two bands corresponding to the vinyl bond (at 1407 cm^{-1} and 1641 cm^{-1}) vanished with time of polymerization, indicating the break of the double bond of the methacrylate group. The intensity of the band corresponding to the C-H increased with time, confirming the elongation of the polymeric chain. These observations were recorded independently of the initiator/accelerator system used. However, differences in kinetics of polymerization were reported since 50% of the vinyl bonds were converted after 10 minutes with the NNDMPT / BPO system whereas it took 13 minutes with the AA / BPO system.

CONCLUSION: AA/BPO appeared suitable to induce polymerization of HEMA in a time acceptable. Raman microscopy appeared an easy and powerful tool to measure the polymerization rate *in vitro*.

7. HYDROXYAPATITE IMPLANTATION AFFECTS BONE GROWTH AND FORMATION

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Introduction: the experiment was aimed at investigation of bone growth after implantation of hydroxyapatite to the proximal metadiaphysis of the tibia.

Materials and methods: for the purposes of the experiment we selected 126 male rats with the initial mass of 145-155 grams and distributed them into 3 groups as follows:
1 – Intact animals;
2 – The animals with the defects at the border of the proximal metaphysis and diaphysis of both tibia, 2.2 mm of diameter;
3 – Animals with implanted blocks of hydroxyapatite (containing 6.6% of vitreous phase of material) into the similar defects;
Upon expiration of observation terms (7, 15, 30, 60, 90 and 180 days) the animals were killed by ether overdose and hip-bones, shoulder-bones and the third lumbar vertebrae were excised for measurements and hystomorphometry. External morphometry was performed by means of a standard calipers with 0.05 mm precision.

Results and discussion: Intact animals exhibited continuous bone growth. This is an evidence of active growth processes in the skeleton.
The group with the plain defects exhibited retardation of growth processes. Maximum length of shoulder-bone in the period from the 30th to the 90th day of the experiment was lower than that of controls by 3.21%, 2.44% and 5.44% respectively. Body height of the vertebrae was lower than controls only at the 30th day; for the hipbone no significant deviations were determined.
Appositional growth rate in this group also retarded – maximum cross-sizes of the vertebrae and shoulder-bone in the period from the 7th to the 90th day of observation were lower than controls by 3.93-8.13% and 2.44-8.24 and in hipbone only maximum height was lower than control value by 5.42-8.93%. The deviations returned to baseline values by the 180th day.
Implantation of HA blocks also led to growth inhibition but to lower degree – maximum length of the shoulder bone was lower than control values by 3.06-4.20%; in hipbone and vertebra maximum length remained unchanged. Cross-sizes of shoulder-bone were lower than control values by 3.17-6.45% and insignificant changes in hipbones and vertebrae were observed.

Conclusions: Defect formed in the proximal meadiaphysis leads to retardation of growth rate in skeletal bones. Appositional growth retards in all bone types while longitudinal – only in long bones. This is probably due to epiphyseal cartilages in long bones affected in the experiment more than any other part of the bones. As far as the maximum deviations come at the terms of active regenerate rebuild we may assume that growth retardation is a systemic reaction of the skeleton to optimize regeneration. By the 180th day of observation no growth rate deviations were observed.
HA implantation also leads to growth retardation though less expressed. This may probably be explained by presence of extra calcium in the defect, which in turn decreases release calcium from the skeleton and thus optimizes regeneration processes.

8. The Role of a Gore-tex (polytetraflurocthylene) in a CHARITÉ Artificial Disc Revision: A Case Report

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Introduction: Recent enthusiasm for total disc replacement (TDR) has wavered as varying success rates have been reported. Currently, the literature cites TDR revision rates as high as 24%. Vascular injuries are of particular concern because their incidences appear to be significantly higher for TDR revisions than other anterior surgeries. This case report will highlight a potentially valuable solution: Placement of a Gore-tex surgical membrane to the anterior vertebral column at the site of the TDR implant in order to shield the neighboring vasculature from the artificial disc prosthesis.

Methods: A 37 year old woman had a history of intractable lumbosacral back pain with intermittent lower extremity radiculopathy. MRI revealed severe degenerative disc disease at L5-S1 with a right paracentral disk herniation. After the patient failed to respond to conservative treatment, she underwent subsequent discography which revealed concordant pain at L5-S1. In November 2004, the patient underwent an anterior L5-S1 total disk arthroplasty using a CHARITÉ implant. Before closing the wounds, the vascular co-surgeon who provided anterior spinal access placed a 3x4 cm Gore-tex patch anteriorly over the site of Charité insertion. This created a protective barrier between the implant and the common iliac vessels. One week after surgery, the patient experienced an episode of sudden severe, lower back pain. The spacer of the CHARITÉ prosthesis appeared to impinge the right nerve root. It was decided to explant the CHARITÉ prosthesis and revise the TDR using an anterior lumbar interbody fusion with posterior instrumented fusion. Fourteen months after initial Charité implantation the patient underwent revision surgery. A vascular surgeon performed an anterior retroperitoneal approach to the spine. The Gore-tex shield was then carefully elevated off the L5-S1 anterior vertebral bodies, thus exposing the Charité artificial disk prosthesis. The disc space was distracted and the device was removed entirely. A carbon fiber reinforced polymer cage was inserted into the anterior disc space. Pedicle screws and rods were used to stabilize the posterior elements under fluoroscopic guidance.

Results: The surgery lasted a total of 2.5 hours, and the patient lost a total of 150 cc of blood. The patient experienced no postoperative complications. One month after the revision surgery, the patient was relatively asymptomatic. Her low back pain improved significantly and her right sided radiculopathy completely resolved.

Conclusion: Historically, vascular injuries resulting from anterior spinal surgery are relatively infrequent (0-3.0%). With the advent of TDR surgery in the United States, and subsequently necessary revisions that will stem from it, rates of vascular complications have risen. The authors suggest that the incidence of injuries to adjacent vessels could be reduced if a Gore-tex membrane is placed on the anterior aspect of the TDR during the initial surgery. This case report has demonstrated that Gore-tex can be utilized in TDR surgeries to decrease and limit the severity of vascular complications in the event of subsequent revision. In our case, a CHARITÉ artificial disc revision surgery transpired without complication, and without a large amount of blood loss (150 mL). The authors believe Gore-tex may have an important prophylactic role in the case of primary artificial disc replacement.

11. BIOMECHANICS OF VERTEBROPLASTY MAY BE SENSITIVE TO INTERVERTEBRAL DISC QUALITY

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INTRODUCTION: Clinical studies on vertebroplasty have reported an increasing number of adjacent vertebral fractures after vertebroplasty [1]. The cause of adjacent bone fractures is still controversial, however, endplate loading has been widely thought to be influenced by cement properties and disc quality [3-5] (Figure 1). The objective of this study was to determine the effects of cement distribution patterns within the vertebrae as well as intervertebral disc quality on the biomechanics of vertebroplasty.

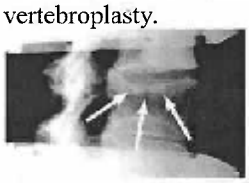

Figure 1: *Endplate compression fracture in the adjacent vertebrae of a spinal segment with top and bottom vertebrae treated with PMMA vertebroplasty [2].*

METHODS: High fidelity computer models were employed to study the effects of intervertebral disc degeneration and a localized versus disbursed filling pattern of bone cement on the biomechanics of vertebroplasty. The two different bone cements investigated were CORTOSS (Orthovita, Inc.) and PMMA.

A specimen specific voxel based finite element (FE) models of a fractured vertebral body (L4, female, 90 years) was generated from postoperative quantitative computed tomography (QCT) scans. Cement distribution patterns of both CORTOSS and PMMA were simulated on the same anatomically detailed, specimen specific finite element models. Simulations included bipedicular vertebroplasty treatment with 3 cm^3 of CORTOSS and the same volume fill with PMMA.

The effect of disc degeneration was simulated by varying the elastic property ratio between nucleus and annulus. Vertebral stiffness and intravertebral stresses and strains were evaluated for various vertebroplasty cement material properties and intervertebral disc quality levels.

RESULTS: When the volume and material properties of both cements were set equal, vertebral stiffness augmentation with a healthy disc was more effective with the dispersed cement fill pattern with CORTOSS than with a compact cement fill pattern with PMMA (Figure 2).

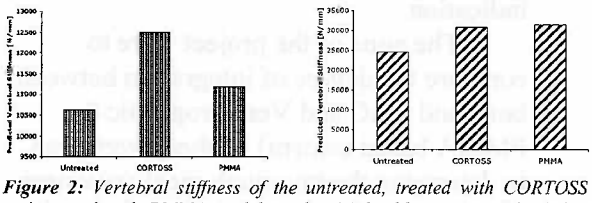

Figure 2: *Vertebral stiffness of the untreated, treated with CORTOSS and treated with PMMA models under (a) healthy inter-vertebral disc and (b) degenerated disc boundary conditions . The CORTOSS treatment improved vertebral stiffness for both cases, while the PMMA treatment was only successful under degenerated disc boundary conditions.*

Disc degeneration in PMMA vertebroplasty resulted in stress-risers in the bone elements directly above and below the bolus (Figure 3). The CORTOSS treated model however did not experience any stress concentrations with either the degenerated disc or the healthy disc.

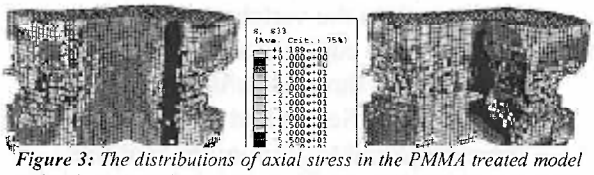

Figure 3: *The distributions of axial stress in the PMMA treated model with (a) intervertebral discs and (b) casting cement included along with the metal plates.*

DISCUSSION: Intervertebral disc degeneration played a significant role in the biomechanics of localized PMMA vertebroplasty. Vertebroplasty biomechanics of a dispersed cement fill with CORTOSS seems to be independent of disc quality. In addition, the minimal stress-risers seen with a dispersed fill may indicate a lowered risk of subsequent fractures in the adjacent untreated vertebrae after vertebral augmentation.

ACKNOWLEDGMENTS

This work was supported by Orthovita, Inc.

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12. EXPERIMENTAL STUDY OF BONE INTEGRATION BETWEEN XERASPINE™ AND SHEEP VERTEBRAE

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Xeraspine™, a hydrophilic calcium aluminate cement (CAC), has recently been CE certified for the vertebroplasty indication.

The aims of the project were to compare the degree of integration between bone and CAC and Vertebroplastic (a PMMA based cement) in sheep vertebrae, to determine the immunological responses to the materials, and to quantify the amount of aluminium in serum and in selected organs.

Five sheep were selected as experimental animals since they have vertebrae of a suitable size and consistency similar to humans. Holes (3.5 mm) were drilled through the pedicles of the vertebrae into the vertebral body. The animals were euthanized one week (two animals) and three months (three animals) after surgery. Serum and tissue samples were subjected to aluminium analyses. Formalin fixed organ samples were subjected to histological determination of aluminium deposits, and vertebrae to histopathological examination of tissue reactions to the treatments. Samples of resin embedded vertebrae were studied using SEM and TEM.

It can be concluded that:

- Aluminium found in serum is regarded as within the normal variation in sheep.
- No deposits of aluminium were found in tissue parenchyma.
- No inflammation could be detected surrounding the CAC material
- CAC integrates closely with bone.

13. A COMPARATIVE EVALUATION OF THE IN VITRO HEMOCOMPATIBILITY OF ORTHOPAEDIC CEMENTS

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INTRODUCTION: The clinical use of injectable orthopaedic cements is steadily increasing. From a materials science point of view, formulation of injectable biomaterials is problematic, since the material should form *in situ* with suitable mechanical, biological, and chemical properties. This is especially difficult for chemically inert materials since the material should be stable in the same environment as it forms. Injectable orthopaedic cements originate from two classes of materials, resins and ceramics. During setting and hardening, the materials are still chemically active and, depending on the indication, may come in contact with blood, which may cause thromboembolism. The current study aimed at evaluating the hemocompatibility of some orthopaedic cements in a model system with circulating human whole blood.

MATERIALS AND METHODS: This work investigated three orthopaedic cements: A polymethylmetacrylate (PMMA) indicated for vertebroplasty, and two calcium-based ceramic cements, a calcium phosphate (CP) based material and a calcium aluminate based material (CAC) using a closed circuit Chandler loop model with the inner surfaces of the PVC tubing coated by heparin. In this model the test materials were exposed to fresh human whole blood. A special procedure was developed to evaluate solidifying pastes in the Chandler loop model. This procedure covers a section of the inner wall of the tubing with a thin layer of non-cured cement paste. Thereafter the tubing was filled with fresh whole blood with addition of 0.5 IU/ml heparin and the loop was closed. The loops were rotated at 32 rpm in a 37°C water bath for 4 h and the cements were curing in contact with the circulating blood. Blood samples were collected and supplemented with EDTA for cell count analysis after 60 min and 4 h. After 4 h blood from the loops was centrifuged to generate plasma for analysis of TAT, C3a and TCC complement marker. After the incubation, the blood and the materials surfaces were investigated with special attention to formation of clots...

RESULTS: The CP cement did not cure to a solid piece but was retrieved as fine particles in the blood. PMMA and CAC cured as adherent pieces of material that was not dissolved by the blood. There was only limited consumption of platelets in the tubings with PMMA and CAC, whereas the platelet number fell dramatically in the tubings with CP, coinciding with extensive clotting. There was no visible clotting with PMMA and CAC.

CONCLUSIONS: It is concluded that the PMMA and the CAC based materials induce blood clotting to a much lower degree than the CP based material in these tests.

14. COMPRESSIVE STRENGTH DEVELOPMENT OF XERASPINE™ DUE TO AGING IN PBS

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INTRODUCTION: Compressive vertebral fractures caused by low-energy trauma are common in osteoporotic patients. Conventional treatments include analgesics, bed rest and bracing. Over the last decade, vertebroplasty has developed into an established method of treatment for compressed vertebral fractures in osteoporotic patients. For vertebroplasty, the injectable biomaterial should have optimised injection characteristics, high X-ray opacity, favourable bone integration and yield immediate stability, hardening without extensive exothermal reaction and strength enough to allow early and active rehabilitation. Today the injectable biomaterials used for vertebroplasty originates from the resin chemistry, mainly PMMA. Within industry and academia other formulation routes are currently also being evaluated, e.g. calcium phosphate cements (CPC). A novel route is to use another inorganic cement system based on calcium aluminate (CAC). Calcium aluminate is delivered as powder and liquid components. Upon mixing the two components an injectable paste is formed. The setting reaction is described as a dissolution precipitation process, where the calcium aluminate powder is first dissolved in water and precipitates as hydrates, i.e. under the bonding of water. The system has several unique features such as high strength, possibility to achieve high radio opacity, and chemical inertness in body fluids after hardening.
The objective with the investigation was to describe the compressive strength development of a CAC based material, designed for vertebroplasty, in comparison to a typical PMMA during aging in phosphate buffered saline (PBS).

MATERIALS AND METHODS: A material based on the calcium aluminate chemistry, Xeraspine™, was compared to a PMMA based material with barium sulphate as radio-opacity additive. Xeraspine contains calcium aluminate and zirconium dioxide powder that is machine mixed with a liquid. The zirconium dioxide is added to achieve extra radio-opacity. The liquid contains mainly water and small amounts of additives to control rheology during injection and setting time.
The compressive strength development of the two materials was measured after aging in (PBS) at 37°C for time periods ranging from 24 hours to 3 months. At least 8 samples per time period were measured. The CAC microstructure and crystalline constituents were also followed for the same time period using SEM and XRD.
RESULTS: The compressive strength level was about the same for both CAC and PMMA, i.e. about 90 MPa for CAC and 80 MPa for PMMA after 24 hours. The values for PMMA did not change much over time. For CAC an increase in strength to above 130 MPa was measured after 3 days of aging. For all other time points the strength was about 90 MPa. The CAC microstructure developed over time with the formation of CAC hydrates and reduction of calcium aluminate.
CONCLUSIONS

- The CAC material did not degrade due to aging in PBS and showed an equal strength level after 3 months as after 24 hours.
- The CAC material had a comparable compressive strength as a typical PMMA cement intended for vertebroplasty.

19. Dense HA bioceramics with a high compressive strength

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INTRODUCTION: It is well known that bioceramics made of pure hydroxyapatite (HA) possess poor mechanical properties. Various approaches have been already elaborated in the attempts to reduce this drawback. In this study, we tried to produce dense bioceramics with the highest possible compressive strength.

METHODS: Powders of stoichiometric HA were prepared by mixing aqueous solutions of calcium nitrate and ammonium phosphate at pH ~ 10. The suspension was centrifuged, washed out by distilled water followed by spray drying at 120 °C. Afterwards, a dry HA powder was split into several parts and each part was calcined for 2 h at different temperatures: 300, 500, 700, 900 and 1100 °C. Dense cylinders of 10 mm high and 12.9 mm in diameter were compacted from both the initial and calcined HA powders in a hydraulic press, using the standard equipment for pellets preparation in IR-spectroscopy. Next, the green bodies were sintered at 1200 °C for 4 hours. After cooling down, the cylinders were weighted and their dimensions were measured. Finally, the compression tests were performed using an Instron 5587 machine. The influence of several sample preparation parameters (the presence of a polyvinyl alcohol binder and a vegetable oil as a lubricant), as well as the compaction load of 1000, 2000, 3000, 4000, 5000 kg were investigated.

RESULTS: Sintering of dense cylinders always resulted in mass decreasing and sample shrinkage. The shrinking degree of the sintered cylinders prepared from an un-sintered HA (~ 50-60%) was much greater than that of the cylinders prepared from the calcined powders. The shrinking degree correlated well to the calcining temperature of the initial HA powders and appeared to be ~ 2-4% for t_c=1100 °C, ~ 8-10% for t_c=900 °C, ~ 15-20% for t_c=700 °C, ~ 30-35% for t_c=500 °C and ~ 45-50% for t_c=300 °C. A similar tendency was found for the mass decreasing during sintering; the numerical values were: ~ 1-2% for the cylinders prepared from HA powders calcined at t_c=1100 °C, ~ 3-4% for those calcined at t_c=900 °C, ~ 5-6% for those calcined at t_c=700 °C, ~ 9-10% for those calcined at t_c=500 °C, ~ 12-14% for those calcined at t_c=300 °C and ~ 40-50% for the cylinders made of uncalcined HA. The correlation between the bulk density of the cylinders and their compression strength was not so straightforward: the highest density was found for the sintered cylinders prepared from HA powders calcined at t_c=700 °C, while the sintered cylinders prepared from HA powders calcined at t_c=300 °C appeared to possess the highest compressive strength. The presence of both an oily lubricant and a binder simplified the cylinder preparation but did not influence the bulk density of unsintered cylinders. However, by using both additives, the compressive strength of the sintered cylinders could be increased by ~ 5-10% and the cylinders could sustain the compression load exceeding 1000 kg.

CONCLUSIONS: The results revealed that bulk density and compressive strength did not correlate well enough: although, in general, a high density is desirable, the cylinders with the highest density did not possess the highest strength. To prepare dense HA bioceramics with the highest compressive strength, the following conditions were found to be favourable:
1. A high compaction pressure.
2. Using calcined HA powders for the sample manufacturing.
3. Using a binder and an oily lubricant at the cylinder manufacturing stage.

22. Calcium phosphate biomaterials for a local-delivery of bisphosphonate: chemical association vs *in vitro* activity.

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Introduction:
Calcium phosphate (CaP) biomaterials such as calcium deficient apatite (CDA) or β-tricalcium phosphate (β-TCP) have been used as bone substitutes. At present, we investigate the capacity of these CaP to release locally drugs. Bisphosphonates (BP) are antiresorptive agents largely used in clinical treatment in bone disorders. Our laboratory has reported that the reaction of CaP powders with aqueous zoledronate can result in different association modes between the two components (1).
Through a *in vitro* osteoclastic model (2), this study was designed to evaluate the *in vitro* bioactivity of two innovative bone drug delivery system (DDS), based on different association of zoledronate on PCa (CDA or β-TCP) matrices.

Materials & Methods:
Chemical:
7.5mg and 28mg of zoledronate were respectively loaded on 100mg of CDA and on 200mg of β-TCP. Powder samples were then compacted to obtain 10mm diameter pellets.

In vitro cell experiments:
5.10^6 of total rabbit bone cells were seeded on dentin slices in the presence of BP-loaded materials. Pellets of materials were prepared by mixing zoledronate-loaded CaP powder in zoledronate-free CaP powder in different ratio. Pellets of pure vehicle were used as control. The resorption activity of osteoclasts, determined by an image analyser, was estimated by the total resorbed surface and the total number of pits on dentin slices.

Results:
We observed a dose-dependent effect of both systems on inhibition of resorption activity, according to the quantity of zoledronate loaded on CaP.

Discussion:
These data demonstrate that both DDS were effective for release of Zoledronate at doses which inhibiting osteoclastic resorption.
This study shows that the two vehicle materials (CDA and β-TCP) are good candidate for releasing BP molecules which will allow to develop with porous ceramic forms (β-TCP) or injectable suspension (CDA). However, we have to modulate initial dose to obtain comparable results.

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Acknowledgements: C Faucheux, G Grimandi, A Soueidan

26. Cryopreservation of Human Mesenchymal Stem Cells – Effect of Different Cryoprotectants

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INTRODUCTION: Cryopreservation is a critical step in the logistics, or cell supply chain, for stem cell therapy. The common practice of freezing stem cells in 10% DMSO with high concentration of bovine calf serum (BCS) is not desirable in many cases. Firstly, it has been found that cell survival rate may be low, especially for embryonic stem cells. Secondly, it is not clear whether the cells survived are what are desired, as often the starting cells are a mixed population. Thirdly, for even the cells survived, it is not clear whether their functions as stem cells are still present after cryopreservation. Finally, the presence of serum is highly undesirable as many clinical practices would prefer bovine serum free culture.

An important factor affecting the fate of stem cells during cryopreservation is the type, concentration and the addition protocols of cryoprotecting chemicals (CPA) (Xu, 2003). In this work we examined the effects of representative cryoprotectants on the survival of stem cells. The stem cells studied here is the human Mesenchymal stem cells for its close relevance to muskoskeletal tissue engineering and stem cell therapy.

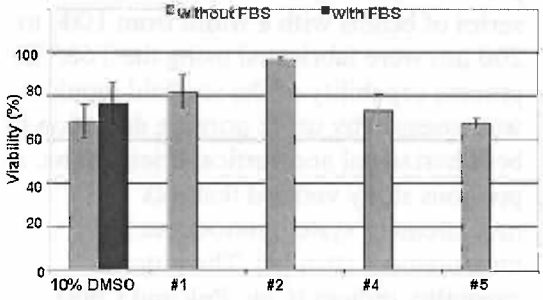
METHODS: 6 types of cryopreservation solutions were prepared and tested. The table below showed the main compound matrix.

CPA Solution	#1	#2	#3	#4	#5	#6
DMSO	+	+	+	+	+	-
Glycerol	-	-	-	-	-	+
Trahelose	-	+	-	+	-	-
PEG	+	+	-	-	-	-
CEW	-	-	+	-	-	-

\+ for present, - not present

Fresh human mesenchymal stem cells (hMSC) were suspended in different CPA solutions and were frozen at 1 °C/min. Frozen cells were thawn in 37°C water bath, and cell viability was assessed with cell membrane integrity test (e.g. 4% Trypan blue in PBS)

RESULTS: It is found that different CPAs have profound effect on cell survival as shown in the figure below.



CONCLUSIONS: Human MSC can be preserved with high survival rate without the use of FCS. Well-defined CPA solution can successfully preserve the viability of hMSC.

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ACKNOWLEDGEMENTS: This work was partially sponsored by the UK BBSRC/EPSRC Stem Cell Science and Technology Initiative (BBSRC BB/D014751/1). We would like to thank Professor Jim Triffitt and Dr Zhidao Xia for providing us with the tested hMSC.

27. Process Capability of Scaffold Mould Using a 3D Printing Technique

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INTRODUCTION The development of 'capable' manufacturing processes for the production of reproducible scaffolds with consistent properties is an important challenge for tissue engineering [1]. This paper reports a preliminary evaluation of scaffold mould process capability for an indirect 3D fabrication of collagen based scaffolds.

MATERIALS AND METHODS

Scaffold moulds were created using a commercial solid free fabrication system T66 (Solidscape Inc.). To determine the process capability of the scaffold mould, a series of beams with a width from 1000 to 200 μm were fabricated using the T66.The process capability of the scaffold mould was assessed by using printing deviation in both horizontal and vertical orientations. A previous study verified that this measurement system introduces little measurement error [2]. The process capability indices (Cpk, Ppk and Cpm) were calculated to provide a numerical summary that compares the behaviour of printing process to the engineering specification.

RESULTS AND DISCUSSION

The results of the process capability analysis are shown in Figure 1. It was clear from the data the scaffold mould printing process is statistical control, and the dimension deviations are normally distributed. The process under normal cooling condition has low Cpk, Ppk and Cpm value of 0.45, 0.55, and 0.4, respectively. Indicated the process under normal cooling condition is not a capable process to manufacture scaffold mould with required dimension specification. However, printing process capability improved greatly under improved cooling condition, with moderate Cpk and Ppk value 1.56 and 1.66, respectively. Such process approximately fabricate only 2.4 in 1 million printed features fall outside the specification limit, indicate a satisfactory process to manufacture scaffold moulds within the specifications.

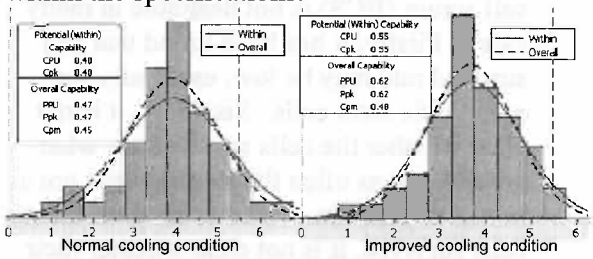

Figure 1. Scaffold mould printing process capability analysis for T66 under normal and improved cooling conditions.

CONCLUSIONS This preliminary work has determined the capability of the scaffold mould process using a indirect 3D fabrication process The results show that the normal process is not capable of meeting the specifications. Printing under improved cooling condition exhibited a satisfactory process to produce scaffold within the specifications. The results highlighted the necessary for regenerative medicine engineering to the optimise and improve the process that take account of the regulatory environment.

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ACKNOWLEDGEMENTS This work was financially supported by The Wellcome Trust under University Translation Award Scheme and EPSRC Innovative Manufacturing Grand Challenge in Regenerative Medicine - remedi.

28. Engineered Collagen-Hydroxyapatite Scaffolds for Bone Tissue

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INTRODUCTION: A well defined and controlled architecture that facilitates cellular infiltration and transport of nutrients and waste products is essential [1]. This paper reports the strategy for the fabrication of collagen-hydroxyapatite composite scaffolds using a 3D printing technique to achieve such a controlled architecture. The *in vitro* performance of the scaffold in terms of cells' viability, migration and proliferation behaviour using human mesenchymal stem cells (hMSC) is reported.

MATERIALS AND METHODS: Collagen type I (Sigma-Aldrich) and stochiometric HA powder (Biotal, UK; Sigma-Aldrich) were used to make composite scaffold. The manufacturing fabrication process involves fabrication of a sacrificial negative mould, casting of the collagen/HA dispersion, remove the negative mould and dehydration process [2].
The scaffolds have been characterised by micro-CT, and associated mechanical properties were investigated by dynamic mechanical analysis (DMA). The cell viability/proliferation was evaluated in vitro using human mesenchymal stem cells (hMSCs).

RESULTS AND DISCUSSIONS: Micro-CT examination revealed the resultant scaffolds have a porosity of 92%, with pore sizes distributed in the range of 100~300 μm. The *in vitro* evaluation revealed that the micro-channels were well preserved during cell culture (Figure 1) the hMSCc cells can migrate into the scaffold, preferably through the micro-channels to proliferate and differentiate. A combination of Alizarin red and Alcian blue staining revealed bone and cartilage-like tissue formed after 8 weeks culture. his was confirmed by ALP staining.

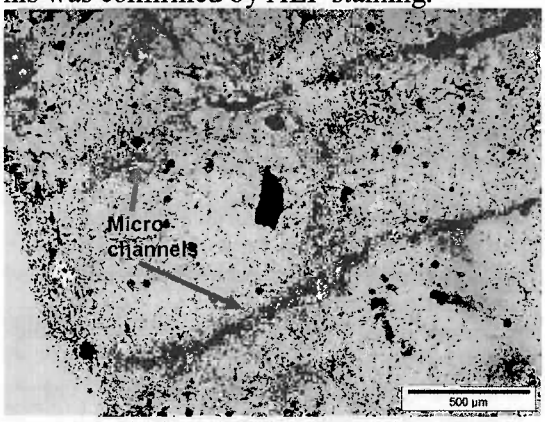

Figure 1: Toluidine blue staining revealed that micro-channels were well preserved after 13 weeks culture and cell preferably to migrate along these channels.

CONCLUSIONS: A collagen based composite scaffold, feature predefined internal architecture and interconnected pores network, has beensuccessfully fabricated using a 3D printing technique. *In vitro* evaluation demonstrated hMSCs can migrate deep into scaffold, and proliferate and differentiate there. As the manufacturing process is carried out under temperatures below physiological temperature, the technique has the potential to integrate growth factors into the scaffold fabrication.

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ACKNOWLEDGEMENT: This work was financially supported by The Wellcome Trust under University Translation Award Scheme (grant no: 074486).

31. HYDROXYAPATITE-COATED LIPOSOMES FOR POTENTIAL USE AS LOCAL DRUG DELIVERY DEVICES.

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Introduction: Liposomes have been extensively investigated for drug delivery applicatios. The high biocompatibility and osteoconductivity of hydroxyapatite (HA), Ca10(PO4)6(OH)2 means it is widely used as a bone substitute in the form of coatings, particles, porous granules and beads. Here we report on the processing and drug release of HA-coated liposomes, which have been prepared by the constant composition method1-3. The constant ionic activities were controlled by the simultaneous addition of reagent solutions, which contained calcium, phosphate and hydroxyl groups.
Materials and Methods: An anti-inflammatory drug Indomethacin (IMC) was chosen as a model drug. Drug-loaded liposomes were prepared from the lipid film of dimyristoyl phosphocholine(DMPC), dimyristoyl phosphatidic acid (DMPA) and cholesterol (1/1/2 in molar), followed by the modified thin film method. The constant composition experiment was then performed.(4) Liposomes were added to a stable supersaturated solution of calcium phosphate (Ca/P=1.67) to induce surface precipitation of HA. The drop in pH and Ca2+ concentration of the reaction solution triggered the simultaneous addition of titrants from autoburettes (ABU Triburete, Radiometer Copenhagen). The titrants: (1) [CaCl2] = 1.17×10-2M, (2) [KH2PO4] = 7.02×10-3M + KOH = 1.3×10-2M. IMC-loaded liposomes and the HA-coated liposomes were analyzed by dynamic light scattering (DLS) and by zeta-potential measurement. The formation of HA is confirmed by Fourier-transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) pattern. Transmission electron microscopy (TEM) was used to characterize the morphology. *In-vitro* drug release experiments of IMC-loaded liposomes, HA-coated liposome were carried out in acetic acid buffer solution (pH 4.0) or PBS (pH 7.4), at 80rpm and 37□. The amount of IMC released at certain time was determined by UV-Vis spectroscopy (Jasco V-530) at 320nm.
Results and discussion: DMPA in the liposome walls provides a negatively charged phosphatidic acid headgroup, which aids in the localization of ions around the liposome. In the case of negatively charged liposomes, the Ca2+ is electrostatically attracted by the exterior charge. The formation of an electric double layer effectively increases the local concentration of the calcium and phosphate above the saturation point and causes crystallization to preferentially occur near the surface of the liposomes.(1,3) DLS measured an average liposome size of 163.8nm which increased to 585.4nm after HA precipitation. Liposomes held a zeta-potential of -56.5±2.6mv and decreased to -4.9 ±1.9mv for the HA-coated liposomes. TEM image (Fig.1) shows a core-shell structure of the liposome-HA with particle sizes ranging from 200-400nm. The XRD patterns of HA coated PLGA microspheres revealed the characteristic peaks (25.8, 31.5, 32.1) of HA, corresponding to (002), (211) and (112) planes. FTIR spectra exhibited bands at 961, 1045 and 1092 cm-1 (stretching vibration of PO43-) and 570, 602 cm-1 (deformation vibration of PO43-) derived from HA. The bands at 3570 and 633 cm-1 derived from the stretching and liberational modes of OH- are very week, indicating the HA coating in liposome-HA was poorly crystalline.

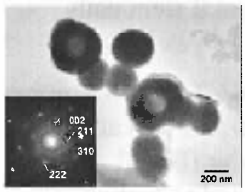

Fig.1 TEM image;

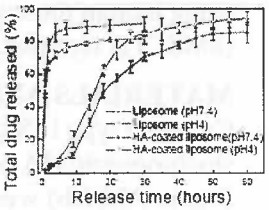

Fig.2. in-vitro drug release profile

The in-vitro drug release profiles are shown in Fig.2. 70% of the drug is released after approximately 5 hours from the liposome, but coating with HA changes this time to over 20 hours. Moreover, it has been observed that for uncoated liposomes, indomethacin is released at a greater rate at pH=7.4 than at pH=4. However, coating with HA reduced the rate at pH=7.4 compared to pH=4. This behaviour arises because indomethacin is more soluble under basic conditions, but HA is more soluble under acidic conditions. This behaviour shows the potential for environmental control for the release of drugs from HA-coated liposomes.
Conclusion: The HA-coated liposomes were synthesized by the constant composition method and characterized by FTIR, DLS, Zeta-potential, XRD and TEM. The HA in liposome-HA was poorly crystalline, and slightly negatively charged. A sustained drug release from the HA coated liposome can be achieved.

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33. Percutaneous Injectable Synthetic Calcium Sulfate for the Enhancement of Percutaneous Spinal Fusion

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INTRODUCTION:. Percutaneous spinal fusion is a minimally invasive posterior spinal stabilization with minimal dissection and soft tissue retraction, no muscle stripping and complete preservation of posterior elements. Although the application of bone grafts in order to achieve a solid fusion is well recognized, many surgeons do not use bone grafts due to the difficulty of their percutaneous application. We present a case of percutaneous spinal fusion with application of injectable synthetic calcium sulfate graft.

METHODS:. A 46-year-old male underwent percutaneous L4-S1 posterolateral spinal fusion for L5 spondylolysis using a Sextant percutaneous spinal fusion device (Medtronic, USA). Under fluoroscopic control the fusion area was prepared with a long, thin curette in order to remove the periosteum and bring the synthetic bone substitute into contact with the bone. A synthetic calcium sulfate bone substitute (Stimulan kit, Biocomposites, UK) was introduced into the surgical site using a syringe with a radioopaque extension tube.

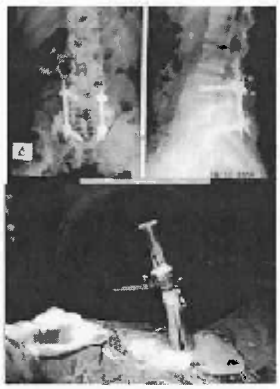

Figure 1:
(A) postoperative plain radiographs.

(B) percutaneous injection of calcium sulphate

The synthetic bone substitute is a high purity calcium sulfate, therefore eliminating risk of disease transmission. The material sets within ten minutes and is radioopaque. The material is therefore easily detected under fluoroscopic control enabling accurate placement.

RESULTS: At three months postoperatively the patient was pain free and has subsequently returned to normal activities. Plain radiographs and Computed Tomography scans suggested a successful solid spinal fusion with complete resorption of calcium sulfate and its replacement with newly formed bone.

CONCLUSIONS: Percutaneous spinal fusion is a minimally invasive posterior spinal fusion technique with minimal paraspinous tissue trauma without compromising the quality of spinal fixation. The handling characteristics and clinical performance of injectable synthetic calcium sulphate indicates that the material is ideal for percutaneous application in addition to providing a safe and effective graft material for the enhancement of percutaneous spinal fusion.

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34. Characterization of a biological bone substitute for vertebroplasty

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INTRODUCTION: Today vertebral fractures are generally treated with painkillers and bed rest. In some cases vertebroplasty or kyphoplasty are performed. They are both minimal invasive methods where PMMA is injected into the fractured vertebra and becomes a permanent implant. To avoid the risks connected with use of PMMA in vertebroplasty, such as adjacent fractures and risk of nerve damage, a new biological bone substitute was investigated.

MATERIALS & METHODS: The biological bone substitute, Cerament™ SpineSupport, is formed by mixing a powder, consisting of synthetic calcium sulfate (60%) and hydroxyapatite (40%), with an aqueous solution that includes radio-contrast agent. The mixing is performed for 30s in a combined mixing and injecting device, which assures sterility and makes the handling easier. The material has a consistence similar to that of toothpaste, but sets with time and becomes hard.
To characterize the material, setting time in blood (37°C) (ASTM C266-03) was recorded, the maximum temperature during setting was measured and the strength of the material was observed by compression until failure (ASTM F451-99a). Extrusion test through thin needles with inner diameters between 0.140mm and 1.003mm were performed, to prove a good material spread within the narrow trabecular system of cancellous bone. Data are presented as mean ± standard deviation of 8 samples, except for the setting time in blood where 3 samples were used.

RESULTS: The biological bone substitute could be extruded through needles with inner diameters of 0.191-0.495mm for 12 minutes. The material was completely hardened at 41.0 ± 3.6min in blood (37°C) and the temperature rise during setting was 2°C. The compressive strength of the material was 29.6 ± 5.0 MPa.

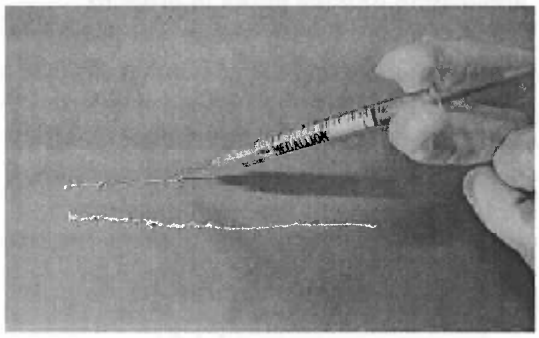

Fig. 1: Extrusion through a needle with inner diameter of 0.27mm.

DISCUSSIONS & CONCLUSIONS: Cerament™ SpineSupport consists of calcium sulfate, which resorbs with time, and hydroxyapatite, which is osteoconductive and assures integration of in-growing new bone. Further, the low temperature rise during setting excludes the risk of nerve damage in the event of posterior material leakage. Combined with its good injectability, Cerament™ SpineSupport has high potential as a biological alternative for use in vertebroplasty. Since its compressive strength is closer to bone than that of PMMA, the risk of adjacent fractures would be lower.

35. Scaffold Characterisation for Human Mesenchymal Stem Cell Culturing

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INTRODUCTION: As tissue engineering advances, there is a greater need to understand the internal cell-cell and cell-matrix interactions when growing cells in three dimensions (3D) (Abbot et al., 2003). Current methods of analysis are often invasive and laborious, whereas in this study a non-invasive method, utilising a nonlinear optical imaging system, has been used to characterise scaffolds and human mesenchymal stem cells (hMSCs) embedded in collagen type I, fibrin and chitosan/gelatin.

METHODS: Collagen type I, fibrin and chitosan/gelatin were selected for the scaffolding material based on the following criteria: biocompatible, porous, inexpensive, ease of use and the ability to obtain uniform cell seeding. Various material concentrations and cell seeding densities were tested and monitored over time using a nonlinear optical imaging system. This system has a tunable (700-1050nm) Ti:Sapphire laser source (Mira, Coherent), a MicroRadiance 2100 MPMD (BioRad/Zeiss) and a SpectraCube (ASI Ltd. Germany) as the spectral analysis system. Images were loaded into Imaris 4.0 (Bitplane Ag, Zurich, Switzerland) for processing and analysis.

RESULTS: Fibrin was rejected as a suitable biomaterial as the cells did not spread and some died. The structure of chitosan/gelatin was initially imaged by observing a blue endogenous autofluorescence, but as this was not consistently produced, the chitosan was labelled with FITC. Chitosan/gelatin was not able to support the healthy growth and spreading of hMSCs as the cells clumped together forming a large cluster rather than interacting with the scaffold (Fig. 1A). Collagen consistently produced a natural endogenous emission (Fig. 1B).

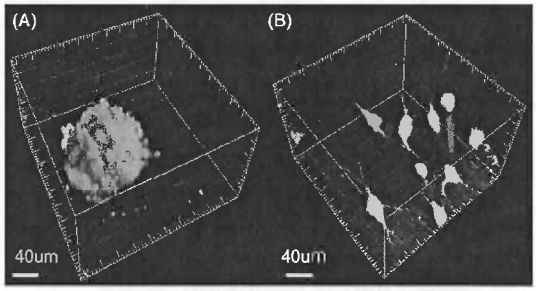

Fig. 1: Green represents live cells stained with Calcein-AM, red represents dead cells stained with ethidium homodimer-1 and blue is the endogenous autofluorescence emitted by the collagen. (A) Unhealthy cell cluster seeded in chitosan/gelatin (day 4). (B) Healthy hMSCs in collagen type I gel (day 2).

Cells in the collagen remained viable and spread along the collagen fibres resulting in a decrease of their sphericity.

CONCLUSIONS: A novel method was used to characterise 3D scaffolds, cell viability, cell morphology, cell-cell and cell-matrix interaction over time using nonlinear optical imaging. Of the biomaterials tested, collagen type I was found to best support the cell growth. Neither fibrin nor chitosan/gelatin were successful in supporting cell viability over time. Preliminary data was acquired showing a relationship between cell sphericity and time.

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ACKNOWLEDGEMENTS: This work is funded by BBSRC/EPSRC: grant number Gr BB/D014751/1)

38. Addition of polymeric resorbable phases to calcium phosphate bone cements

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INTRODUCTION: Calcium phosphate bone cements (CPC) are generally defined as apatite or brushite-based. Brushite cements have been linked to fast resorption rates, whereas apatitic cements are much less soluble taking a longer duration to fully resorb in-vivo [1]. It is postulated that the introduction of bioresorbable polymeric additives would provide some short term structural reinforcement and facilitate the generation of porosity within the cement during resorption, hence creating pathways for bone ingrowth.
The aim of this study was to examine the effect of incorporating polyglycolic acid (PGA) into α-TCP based cements.
MATERIALS & METHODS: Cement formulation: 61% α-TCP, 26% $CaHPO_4$, 10% $CaCO_3$, 3% HA and 4% Na_2PO_4 solution. Liquid to powder ratio was 0.35:1.
PGA-trimethylene carbonate (TMC) rod stock (Smith & Nephew plc, UK) of Ø1±0.2mm was pelletised, then milled using a Rondol Mill (Rondol Technology Ltd, UK) and sieved between 106μm and 425μm. Up 20%wt loading of granules were added to the powder (G). Fibres were manually cut into 2-4mm lengths from drawn PGA fibre (Smith & Nephew plc, UK) of Ø0.2±0.1mm. Up to 10%wt fibre loading was examined (F).
Compressive strength of cement samples was determined according to ISO 5833. Setting times were monitored using the Gillmore needle apparatus according ASTM 266. The injectability was determined by applying a load of 100N to a syringe filled with cement paste, which was forced through an orifice of Ø2.3±0.01mm.
RESULTS & DISCUSSION: Granule addition resulted in similar modes of failure to that of the control samples. For fibre additions the cement failed in a less catastrophic manner however there was a tendency of fibre pullout (Figure 1b).

Fig 1: Fractured CPC with a) granules; b) fibres.

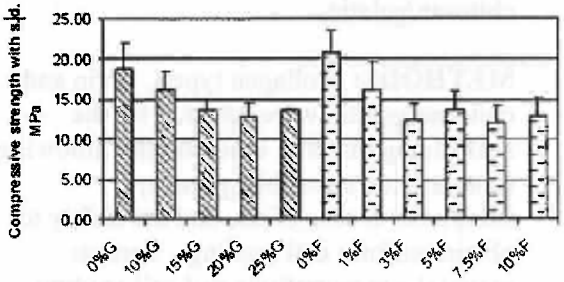

Fig 2: Compressive strength of CPC at the various levels of granule and fibre loading.

By adding 10%wt granules no significant difference was found in the compressive strength, however further addition resulted in a significant difference. Respectively the addition of fibres to the cement blend resulted in a significant difference. There was also a significant difference between the Young's modulii of the samples. Workability of the cements decreased with increases in loading percentages.
CONCLUSIONS: The inclusion of PGA additives resulted in a significant loss in compressive strength. However considering the potential improvements to resorbability and pore forming ability this system warrants further investigation. In the case of the fibres further work will investigate inclusion of a fibre bonding agent to control the degree of pullout.
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39. Oxygen monitoring in regenerating tissue

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INTRODUCTION: One of the great challenges in regenerative m edicine is to achieve a controlled delivery of oxygen and nutrients to the cells in the tissue construct. Here we present an electrochemical technique for measuring regional PO_2 and perfusion that helps identifying regions of poor oxygenation, possibly as a result of a failure of angiogenesis. In avascular tissues, this technique can be used to monitor the precarious oxygen concentrations arising from the balance between the rate of supply and the rate of cellular consumption. The needle microelectrodes used in this study have been used *in vivo* previously in a range of tissues such as skeletal muscle [1], bone [2] and intervertebral discs [3].

METHODS: The technique used in this work, amperometry, measures the current resulting from substrate reduction when an electrode is held at a steady potential of the appropriate magnitude. The measured current is directly proportional to the substrate concentration in the tissue. A non- metabolised agent nitrous oxide (N_2O) was used as a tracer substrate to provide information on transport of small soluble molecules into the tissue, whereas the measurement of oxygen (O_2) gave information on both transport and metabolic activity of the cells. Both gases can be detected on silver needle electrodes, which can be built in a broad range of lengths and gauge sizes. In short, needle micro-electrodes were constructed by epoxy embedding a silver-wire into a stainless steel hypodermic needle and connecting the wire to screened electrical cable (Fig. 1). These give results within 1-2 minutes when inserted into tissues.

To examine use of this technique *in vitro*, intervertebral discs were cultured and perfused with N_2O; the resulting profiles were measured across the disc at several moments in time.

RESULTS:

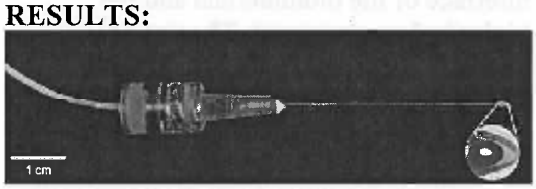

Fig. 1: Purpose-built microelectrode, with the exposed silver wire at the electrode surface (inset).

Nitrous oxide was introduced into the medium perfusing an intervertebral disc at time zero and its change in concentration with time and distance from the disc-perfusing surface measured using a needle microelectrode inserted into the disc.

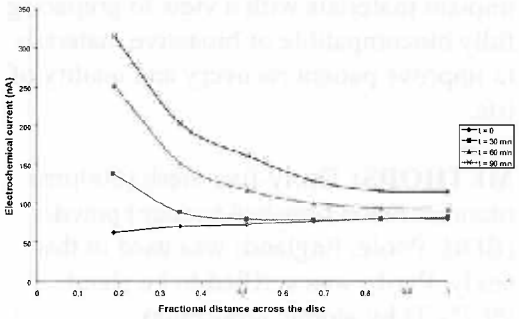

Fig. 2: N_2O concentrations in a perfused intervertebral disc vary spatially and in time, as predicted from theory.

CONCLUSIONS: Needle microelectrodes have proven to be an excellent tool to monitor perfusion and local PO_2 in several tissue types. Their minimally-invasive and non-destructive property opens possibilities for measurement of oxygenation non-destructively in regenerating tissues *in vivo* and *in vitro*.

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40. The Mechanism of the adsorption of Fibrinogen on CP Titanium

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3.

INTRODUCTION: Protein adsorption to the surface of medical implants is an essential aspect of the cascade of biochemical reactions taking place at the interface of the biomaterials and the biological environment. The use of titanium and titanium alloys as medical implants has been widely reported. It has been observed[1] that the first event to occur at the tissue –material interface which dictates biocompatibility is the non-covalent adsorption of plasma proteins from blood. Once proteins have adsorbed to the surface of the implant, host cells adsorption becomes governed by this protein overlay. The nature of this adsorption process is of interest in order to better understand the biointegration of implant materials with a view to preparing fully biocompatible or bioactive materials to improve patient recovery and quality of life.

METHODS: Thirty-five mesh (500μm) titanium metal (crushed sponge) powder (BDH, Poole, England) was used in this study. Purity was verified to be about 99.7% Ti by atomic absorption spectroscopy (Buck Scientific 200A). Samples of fibrinogen (Sigma, Japan) solution were incubated with treated or untreated Ti and the unabsorbed amount of fibrinogen (fbn) were determined using Bradford reagent (Biorad, Richmond, CA) and measuring absorbance at 595nm in a Spectronic 20 UV – Visible Spectrophotometer (Bausch & Lomb Co., NY, USA). Concentration was calculated according to a standard solution of bovine serum albumin (Sigma, Japan). The maximum amount of adsorbed fibrinogen and the fibrinogen-Ti association constant were calculated according to the slope and the x-intercept, respectively, of the linear curve:

$$F/B = 1/K\alpha N + 1/NF$$

Where B= bound fbn; F=free fbn; Kα=association constant; and N= maximum amount of fbn adsorbed.

RESULTS: Fig.1 shows the adsorption isotherm of fbn to Ti. Increasing the concentration of fbn from 0.2 to 0.8 mg/ml resulted in increased adsorption to Ti. The adsorption continued till equilibrium was established indicating a monolayer adsorption.

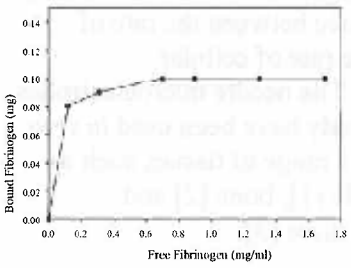

Fig. 1: Adsorption Isotherm of Fibrinogen to Titanium

Ti treated with Ca ions and incubation at physiological pH showed the best adsorption of fbn. The maximum amount of fbn adsorbed to Ti was 0.0853mg/1g. The Ti-fbn association constant was 11.3ml/mg. A Scatchard plot of adsorption data showed a lack of linearity.

CONCLUSIONS: Electrostatic attraction constitutes the main mechanism in the adsorption of fbn to Ti. At pH 7.0 to 7.4 fbn binds to Ca ions bound electrostatically to Ti.

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41. Modelling of oxygen, glucose, lactic acid and pH in engineered cartilage

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INTRODUCTION: Nutrient supply and metabolic waste removal are important issues for avascular constructs such as tissue-engineered cartilage. However there has been relatively little work on factors influencing these transport processes, particularly in regard to the complex interdependence between concentrations of substrates and rates of metabolism. For example, both oxygen and glucose consumption rates are dependent on both oxygen tension and glucose concentration while accumulation of lactic acid, the metabolic product of glucose metabolism will lead to a fall in pH. Low pH, in turn, can affect oxygen and glucose consumption.
Most modelling of metabolite gradients through cartilage deals, for example, with oxygen and glucose separately while the effect of pH has generally been ignored. One reason is that data on interactions between metabolism, substrate levels and the physico-chemical environment is sparse. We have recently measured the relationship of (i) oxygen consumption rates to oxygen tension, glucose concentration and pH (ii) glucose consumption rates to oxygen tension, glucose concentration and pH (iii) the dependence of pH on lactic acid concentrations and tissue buffering. This information makes it possible to model the interdependence of oxygen, glucose, lactic acid and pH profiles through cartilage constructs. Here we present these profiles and examine the effects of cell density and culture conditions.

METHODS: One-dimensional diffusion models were developed for three culture conditions, i.e. static culture, perfusion and suspension systems. Equations were solved using either finite difference or finite element methods. Profiles of oxygen, glucose, lactic acid and pH in constructs were calculated using measured rates. Limiting construct sizes, cell densities and ratio of medium volumes/construct volumes were defined

RESULTS: For constructs incubated under static culture, concentrations of oxygen, glucose and pH in the construct were calculated to change with time and the distance from the surface of the construct. Results indicated that the maximum construct size and cell density which could be supported before nutrients were depleted from the construct centre was very limited. A function predicting the relationship between construct dimensions and the maximum viable cell density was developed. For perfusion cultures, constructs could support a significantly greater cell density than under static conditions, while for batch suspension cultures, volume of medium also influenced the maximum cell density which could be supported

CONCLUSION: This study provides models which defines the limits of some important tissue engineering parameters such as construct size and cell density and hence provides useful guidance for design of tissue-engineered cartilage

ACKNOWLEGEMENTS: This work was funded by BBSRC.

42. Spectrometric analysis of non-stoichiometric hydroxyapatite nanocrystals

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INTRODUCTION: Nanocrystalline apatites are involved in vertebrates and have been suggested to play a crucial role in the biological activity of biomaterials. Despite this wide spread occurrence, fine structural details related to the calcium deficiency and the existence of non-apatitic environments are not well known. In the present work, we present results obtained with a series of spectrometric techniques.

METHODS: Nanocrystalline apatites were obtained by the double decomposition method. The calcium content was determined by complexometry using EDTA and the phosphate by spectrometry of the yellow phospho-vanado-molybdic complexometry. X-ray diffraction patterns were obtained using a Inel CP120 curved counter with a Co K□ radiation. FTIR spectra were recorded on a Perkin-Elmed spectrometer. Finally, EELS spectra were obtained with a FEI Tecnai G2 F30 S-Twin microscope equipped.with a Gatan EELS filter.

RESULTS: The following samples have been processed:

Sample	Ca/P	HPO_4	Maturation time
OCP	1.3333	33%	-
nanoAp1	1.40	22.0%	0
nanoAp2	1.45	18.4%	3 days
nanoAp3	1.50	13.0%	15 days
nanoAp4	1.534	9.8%	2 months
OHAP	1.667	0%	

Table 1: The sample used for the study.

X-ray diffractogrammes confirm the formation of poorly crystalline apatite, although the 100 OCP peak was not present on any of the diffractogrammes. All peak width decrease with the maturation time indicating an increase of crystallinity.

IR spectra correspond to poorly crystalline apatites. In addition to phosphate and water bands, on may notice HPO_4^{2-} bands and a clear OH^- bands in most mature samples. The analysis of the η_4PO_4 domain (500-650 cm^{-1}) allows the identification of 6 bands assigned respectively to OH^- ions, apatitic PO_4^{3-}, non apatitic PO_4^{3-}, apatitic HPO_4^{2-}, non apatitic HPO_4^{2-}. The non-apatitic bands correspond to ions located in a hydrated layer at the surface of the crystals. During maturation, the non-apatitic species decrease and the OH^- content increases. These variations appear consistent with the increase of the Ca/P ratio and an evolution of the samples towards stoichiometric apatite.

A the nanometric scale, the EELS OHAP spectrum is characterised by two relatively thin which have been respectively assigned to L_3 $2p^{3/2}$ □$3d^{3/2}$, $3d^{5/2}$ and L_2 $2p^{1/2}$ □$3d^{3/2}$ transitions. The spectrum of apatite nanocrystals appears rather different and depend strongly on the maturation time. For the most mature samples the spectra resemble that of HA with two bands however one may notice a clear band broadening, especially on the high energy side of the L_2 peak and a dissymmetry on the low energy side of the L_3 peak. The relative height of the main L_2 band seems to decrease with respect to the intensity of the main L_3 band as the maturation time increases. As the maturation time increases the instensity of these bands progressively decrease.

CONCLUSIONS: In the present work, calcium deficient hydroxyapatite nanocrystals have been investigated with a series of spectrometric techniques;

ACKNOWLEDGEMENTS: EFB wish to thank the Fédération Biomatériaux Nord-Pas de Calais France for financial support.

43. Hydroxyapatite – catgut composite as a prospect bone implant

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INTRODUCTION: A natural tissue is composed of collagen matrix filled with needle shaped hydroxyapatite (HAp) crystals. HAp-based implants for bone replacement are preferable than titanium ones as more biocompatible and non-toxic[1]. Nevertheless, they are still not widely used due to lack of proper mechanical properties comparing to natural bone[2]. The current research is gaining to solve this problem by imitating natural bone composite growth: we used catgut surgery threads as strings and precipitate matrix filler with HAp.

METHODS: We synthesized bio composites by precipitating amorphous calcium phosphate on gelatin covered catgut threads. Strained threads were held in 10% gelatin solution, afterwards they were used as a matrix for precipitated ACP (conditions: pH = 11, cold solutions of $CaCl_2$ and NaH_2PO_4 at T = 4 °C):

$$\mathbf{3CaCl_2 + Na_2HPO_4 + 2NaOH =}$$
$$\mathbf{= Ca_3(PO_4)_2 + 6NaCl + 2H_2O}$$

Then you need to wash rapidly filtered powders on catgut matrix at least 3 times with cold de-ionized water and treat it cryogenically during 24 hours. To synthesize HAp/catgut matrix further we pressed composites samples at 100°C during 5 min at P=200 MPa.

RESULTS: The samples of amorphous calcium phosphate before pressing were analyzed using diffraction analysis. We waive the ordinary way of HAp synthesis directly applied to catgut matrix assuming to improve its strength as outcome of phase transition from amorphous phosphate to HAp during it's compressing. We put the catgut threads in one direction to attain a mechanical anisotropy of the composite matrix. A further mechanical probe on composites strength proved their higher strength parameters than the known calcium phosphates cements and ceramics. The most challenged results were the found higher transverse plasticity and elasticity achieved with catgut filament.

CONCLUSIONS: This research allowed suggesting a method to synthesize Hap/catgut bio composites with high yield point – up to 150 MPa. The resulted catgut matrix being able to dissolve in tissue dates to be fixed mechanical parameters implants for a defined period. This phase would allow the bone tissue to regenerate and fill emptied channels freed of catgut filament. These synthetic models demonstrate highest plasticity properties as well.

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44. Spherical α-TCP Obtained by Suspension Induction-Plasma Spraying

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

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

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

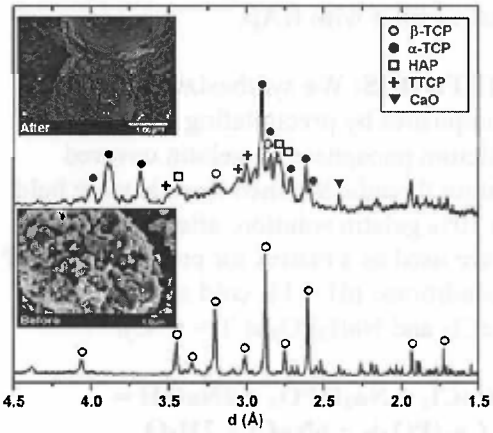

Fig. 1: XRD spectra and SEM of powders before and after the SIPS process.

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

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45. Enhancing PMMA Bone Cement with Radiopacifier for Vertebroplasty

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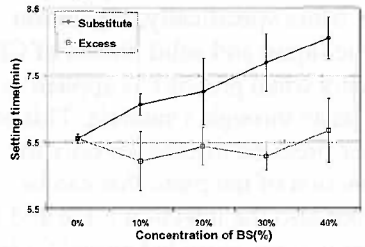
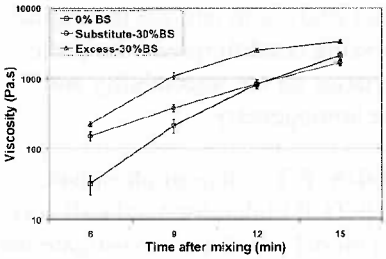
INTRODUCTION: In vertebroplasty, physicians often modify the formulation of cements. Some physicians add radiopacifier to PMMA powder—excess. Others substititute some of the PMMA powder a radiopacifier substitute [1]. Both the excess and substitute techniques are designed to increase the visibility and minimize the risk of leakage and inadvertent extravasations.
Higher visibility and proper viscosity are important for safe injection; maximum temperature (heat generation) and residual monomer have to be controlled to reduce damage to adjacent tissue. The higher initial viscosity and working time of the cement were more amenable for safe vertebroplasty [2]. The relation between the thermal and rheological properties of acrylic bone cement, with excess or substitute barium sulphate (BS), is studied in this work.

METHODS: Acrylic bone cements with 10, 20, 30, and 40% BS in excess and as substitute were prepared. Thermal properties (heat production, setting time, and residual monomer) were measured using differential scanning calorimetry (DSC) in different modes—isothermal, heating, or cooling cycles. Using a controlled-stress rheometer with couette geometry (gap = 2000 μm) and a time-sweep procedure (frequency = 1 Hz, strain = 0.5%), the rheological properties of cement (e.g., dynamic viscosity, storage modulus) were studied.

RESULTS AND DISCUSSION: The heat production of cement decreased significantly with the addition of radiopacifier in both formulations. Cement with excess BS showed lower heat generation than substitute cement (e.g., pure cement with 135.2 J/g and 102.9 J/g for substitute and 81 J/g for excess in the case with 20% BS). Setting time in the isothermal case increased with substitute BS cement (Fig. 1a). Initial viscosity increased by adding radiopacifier/filler, but the rate of increase in substitute case was lower than in the excess formulation. Viscosity for the substitute case showed a higher initial viscosity compared with cement without BS and a lower viscosity during curing compared with the excess formulation (Fig.1b), resulting in a longer working time.



(a)



(b)

FIGURE 1. (a) Setting time for excess and substitute BS; (b) variation of viscosity.

The concentration of residual monomer in substitute cement is minimal, and the excess formulation shows higher amounts compared with 0% BS (e.g., the residual monomers for pure cement, substitute, and excess 40% BS cement are 2.64%, 1.73%, and 3.98%, respectively).

CONCLUSIONS: The substitute formulation has a lower initiator, and thus the viscosity increases slowly, resulting in higher settings and, for the physicians, a longer working time for the cement. Also, lower heat production makes the substitute formulation more appealing. Future studies will focus on the interaction between leakage and formulations.

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46. Enhancement of the Injectability of Calcium Phosphate Bone Substitute

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INTRODUCTION: Calcium phosphate bone cements (CPBCs) are increasingly being used to treat bone fractures. Nevertheless, CPBCs are often poorly injectable. More specifically, separation between the liquid and solid phases of CPBC pastes occurs when pressure is applied to inject the paste through a cannula. This so-called filter-pressing affects not only the volume fraction of the paste that can be extruded but also the injection force and the homogeneity of the extruded paste.[1,2] The aim of this study is to address the impact of the processing conditions and the paste characteristics on the injectability and extrudate homogeneity.

METHODS: β-Tricalcium phosphate powder (β-TCP Fluka, Switzerland) was used as a model powder to investigate the injectability of calcium phosphate (CaP) pastes. The powders used were analyzed by X-ray diffraction (XRD), scanning electron microscopy (SEM). Particle size distribution (PSD) was also measured. Injectability test was applied using a hydraulic press. The force required to inject the paste and the extrudate percentage were measured. The injectability was assessed in terms of particle size distribution, particle shape, liquid to powder ratio (LPR), injection rate, syringe size (5, 10, and 20 mL) and with or without a cannula. Extruded paste samples were taken every 30 s, and the LPR was measured and compared with LPR of the initial paste to examine extrudate homogeneity.

RESULTS: The phase separation happened from the beginning of each injection process (Fig. 1), resulting in a limited injectability. Sixty percent could, for example, be extruded, with an acceptable force for the 40% LPR paste after the syringe was plugged and the force curve increased suddenly. This percentage could be increased gradually by increasing the LPR, reaching 100% with 60% LPR paste. A higher injection rate also increased it (up to 78.83%). A small syringe size (5 mL) enhanced the injectability by about 5%. Interestingly, injecting the paste without a cannula only improved the injected fraction by 2.5%. The most effective parameter controlling the injectability was found to be the particle size distribution. Fully injectable paste could be achieved by using a well-designed powder using the plasma process.

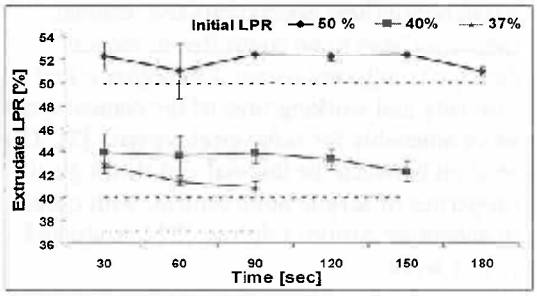

FIGURE 1. The homogeneity of the extruded paste for different liquid to powder ratios (LPRs) over the course of the experiment.

CONCLUSIONS: The phase separation is an important phenomenon, limiting the minimally invasive use of bone substitutes. A high-injection rate and small syringe size favor good injectability. The particle distribution and shape contribute to a better injectability. Finally, according to this study, if we can maintain a homogenous extrudate during the entire injection process, a fully injectable paste with an acceptable injection force can be achieved. To reach this target the powder design should be investigated in future work.

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49. Two methods to characterize calcium phosphate putties

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INTRODUCTION: There are several forms of calcium phosphate bone substitutes: granules, blocks, cements and the so-called putties. Despite the fact that the first studies on putties are from the early 1990's [1] and that such products are widespread in the US, very few scientific studies are available on the topic. One particular point of interest is the characterization of the rheological properties. Here, two simple test setups were developed to characterize putties consisting of β-tricalcium phosphate (β-TCP) granules and a gel: a "compression-tension test" (C&T test) and a "cohesion test". Factors of interest were the β-TCP granule size distribution, the water content, as well as the intrinsic viscosity (IV) and the concentration (C) of the polymer in the gel.

METHODS: The paste consisted of a mixture of 0.94g β-TCP granules, sodium hyaluronate powder; and 0.6 to 1.0mL deionized water. IV and C varied in the range of 1.3–3.0m^3/kg and 0.00–0.17g, respectively. Various β-TCP granule fractions were used and combined: 0.125-0.18, 0.18-0.25, and 0.50-0.71mm in diameter. To prepare the paste for the C&T test, the water was added to the two other ingredients, kneaded with a spatula for one minute, left to rest during one minute, and placed into a 5mL truncated cylindrical form (Ø12.5mm). The resulting cylindrical paste was pushed out of the syringe, placed vertically on a metallic (lower) plate and submitted to a compressive load via a flat (upper) plate at 20mm/min. The test was stopped when the distance between the plates amounted 2.0mm. The force was recorded as a function of the upper plate displacement. Subsequently, the upper plate was raised at 20mm/min. Again, the force was recorded as a function of the upper plate displacement. The compressive force recorded at a 4mm interplate distance was defined as the "hardness" and the maximum force measured during the tensile test was defined as the "stickiness". For the cohesion test, the preparation was the same except that the paste was directly inserted into a metallic cavity which was subsequently linked to a scale and dipped into a beaker filled with deionized water. The weight of the metallic cavity filled with the paste was recorded over time [2]. The time measured to obtain a 0.3g weight loss was recorded. At least three repeats were performed for each test and composition.

RESULTS: Well formable and viscous pastes were obtained within 2 minutes of preparation. The paste properties varied with their composition: harder, stickier, and more cohesive pastes (= lower weight loss) were obtained with a lower water content, at a longer time after the start of mixing, and with a higher IV and C. Very few changes were noticed with a modification of β-TCP granule size distribution. The reproducibility of the cohesion test was much better than that of the C&T test. An excellent correlation was found between the cohesion results and the IV values of the polymer.

CONCLUSIONS: Putties can be characterized by the two methods presented here, even though the cohesion test provides more accurate and reproducible results.

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ACKNOWLEDGEMENTS: This study was financially supported by Mathys Ltd Bettlach, Switzerland.

50. Lumbar Spondylolisthesis Treated by Bi-posterolateral Fusion Combined with Coralline Spinal Interbody Fusion

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INTRODUCTION: Although combined with rigid internal fixation, the conventional posterior fusion methods like bi-posterolateral fusion could only provide two point support and the anterior fusion like interbody fusion provide one point support to the spine, so these methods were not the idea ways to establish the spine stability. Bi-posterolateral fusion combined with coralline interbody fusion, a three point stability spinal fusion system provide three point support simultaneously, still not be proved in clinic application. A preliminary study of 21 patients that underwent bi-posterolateral fusion combined with coralline interbody fusion is reported. The objective of this study is to explore the clinic results of lumbar spondylolisthesis treated by bi-posterolateral fusion with coralline spinal interbody fusion.

METHODS: Twenty-one patients of spondylolisthesis, average age was 48.5, L_4 grade I° 5 cases, L_4 grade II° 3 cases, L_5 grade I° 7 cases, L5 grade II° 3 cases, both L_4 with L_5 grade II° 3 cases were treated by bi-posterolateral fusion with coralline spinal tinerbody fusion, as well as posterior approach and spinal pedicle screw fixation.

RESULTS: The average follow up was 19.1 month. A-P, bi-oblique, flexion and extension X-ray were taken 2 week,6 month and 12 month .The clinic effectiveness of patients were evaluated excellent 71.4%(15/21), good 23.8%(5/21), and fair 0.5%(1/21). The anterior and posterior disc height was measured before and after operation. The initial anterior and posterior disc height was 8.35±2.73mm, and 6.32±1.63mm, the degenerative disc height was decreased 40% compared with up or lower adjacent normal disc height. After instrumentation and fusion, the anterior and posterior disc height increased significantly to 11.56±3.38mm and 8.50±2.10mm ($P<0.05$). The vertebra posterior ream slipping distance before and after operation were 4.66~14.22mm, average 8.88±2.32mm and 0~5.12mm, average 1.92±3.37mm ($P<0.01$). The fusion rate was 95.2% (20/21).

CONCLUSION: High fusion rate and excellent clinic results were obtained by combination of posterolateral and anterior interbody fusion, so the technique of bi-posterolateral fusion combined with interbody fusion proved three point supports can provide more stable fusion then conventional spinal fusion. **51. Application of absorbable screw in treatment of the olecranon fractures**

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51. Application of absorbable screw in treatment of the olecranon fractures

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INTRODUCTION: To evaluate the results of olecranon fracture treated with absorbable screw internal fixation.

METHODS: 38 patients diagnosed as olecranon fractures were treated with open reduction and internal fixation. In treatment group, 16 patients were fixed using absorbable screw, 7 fractures were transverse, 6 were diagonal, 3 were comminuted. They were all closed fractures. In operation, fixed the fracture fragments with 2 K-wires temporarily, drilled 2 holes from either side of olecranon apophysis to anterior ulnar cortex with 3.5mm aiguille, threaded screws with 4.5mm diameter. Post-operative immobilization using a plaster in 90 degrees of elbow flexion. Light active motion was begun after 3 weeks. 2~3 times per day, then increase the intensity gradually. In the control group, 22 patients were fixed with tention band wiring. 12 fractures were transverse, 3 were diagonal, 7 were comminuted. The patients were open fracture in 3 cases and closed fracture in 19 cases. In operation, two parallel Kirschner wires cross the fracture through olecranon carina, drilling a hole on the dorsal unlar cortex 3~4 cm away from the fracture, then inserted through the hole and the K-wires by using a strand of steel wire. For comminuted displaced fractures, small K-wires may be required for stabilization of minor fragments before using tention band wiring. Post-operative, immobilized the elbow for 1 week , then began the active exercise.

RESULTS: The follow-up period ranged from 8 to 49 months(average 24.8 months). All the fractures obtained solid union. The outcome was evaluated with Weseley rating system, the elbow function was excellent to good in 87.5% in the experimental group, no pain and apophysis on posterior of the elbow. proximal radioulnar joint fixed by screw was found in 1 patient, and the function of rotation recovered after 5 months. Another 1 case which associated with fracture of middle radius and ulnar, was found the internal fixation invalidated through roentgenogram 2 weeks after operation. The fracture displaced again. We treated it with internal fixation using tention band wiring.. The elbow function was good after 6 months . The function was excellent to good in 90.9% in the control group. 7 patients(31.8%) complained there were apophysis on posterior of the elbow and pain when motion. Roentgenogram revealed the K-wires backing out in 2 cases(9.1%) . All the patients in this group had taken the internal fixations out after 8~15 months post-operative. The excellent to good rate of the two group have no statistical significant difference ($P>0.05$).

CONCLUSION: Open reduction and internal fixation with absorbable screw is an effective way for treatment of olecranon fracture. As it can decompound completely, patients need not to undergo operation again, and can return to the original job earlier.

52. In Vitro Experimental Study on Chitosanas Carrier for Sustained Release of Recombinant Human Bone Morphogenetic Protein-2

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INTRODUCTION: To assess the feasibility of chitosan scaffold as the carrier for controlled release of recombinant human bone morphogenetic protein-2 (rhBMP-2) and evaluate the release kinetics of rhBMP-2 through in vitro release test.

METHODS: The chitosan scaffolds with rhBMP-2 sustained release system were made by emulsion freeze-dried method, and were compared with the empty chitosan scaffolds as control group. The samples were observed with scanning electron microscope. The samples were immerged into phosphate buffer solution(PBS). The released rhBMP-2 content from the samples was dynamically observed using high performance liquid chromatography at various set times. The curve of cumulative rhBMP-2 release kinetics was designed.

RESULTS: The surface and section of the scaffold was loose, multipore and spongelike. An initial burst release (33.2%) of the incorporated rhBMP-2 was observed during the first day; rhBMP-2 had been released rapidly during the 2nd to 7th day; released slowly during the 8th to 14th day; followed by a sustained release for the 15th to 30th day, which reached 81.3% at the 30th day.

CONCLUSIONS: It can be concluded that the loose chitosan showed its characteristics as a sustained delivery system for rhBMP2 and may be used as a sustained release carrier for rhBMP2. It provided initial elements for rhBMP2 continually inducing bone formation in bone tissue engineering and is suitable for rapid advancement into clinical work.

53. Biomechanical and clinical study of atlantoaxial instability in posterior atlantoaxial transarticular screw fixation

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INTRODUCTION: To determine the biomechanical stability of C1-2 and clinical effects after instrumentation with two screw trajectory in posterior atlantoaxial transarticular screw fixation.

METHODS: The three-dimensional motions of C1 relative to C2were measured in 8 human cadaveric specimens,which was by turns under 4 models:nomal,□odontoid fractures, with Ao or modified posterior atlantoaxial transarticular screw fixation respectively.The16atlantoaxial instability patients were tested with posterior C1-C2 transarticular screws and the autogenous granulated cancellous bone graft, in which the screw entry point was the center of lower edge on axis processes articularis inferior.

RESULTS: Two inserting methods of posterior atlantoaxial transarticular screw fixation significantly decreased motion in all directions. There was generally no significant difference in the amount of motion among the two screw trajectory. During a follow-up of 5-48 months, atlantoaxial instability was restored satisfactorily in 16patients with no complication and bone fusion was obtained.

CONCLUSION: The bony landmark of the new entry point was definite, which can provide the long length of screw trajectory and the rigid fixation with low complication rate.

54. Tissue Regeneration of Severely Infected Bone Exposure Wound by Combining Vacuum Sealing Drainage with Flap Transplantation

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INTRODUCTION: To investigate the effect of combining vacuum sealing drainage with flap transplantation on the management of severely infected bone exposure.

METHODS: Twelve patients with severely infected bone exposure wound were treated by the vacuum sealing drainage technique after debridement. Wound closure was performed with flap transfer or transplantation after 7 to 10 days.

RESULTS: Twelve patients underwent this procedure, this results in infection under control, the wound with marked proliferation of granulation tissue except the region of bone exposure and the outcome of bacterial culture is negative. The wound closure was all succeed with skin transplantation, local flap grafting, or flap transplantation after 7 to 10 days.

CONCLUSIONS: Vacuum sealing drainage can protect the wound against infection, refrain from traditional dressing, stimulate the growth of granulation tissue and facilitate the flap transplantation. So combining vacuum sealing drainage with flap transplantation is a effective method in treatment of severely infected bone exposure.

55. Surgical Reconstruction with Internal Fixation for intra-articular calcaneal fractures with malunion

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INTRODUCTION: To discuss three methods to treat intra-articular calcaneal fractures with malunion, and to investigate the indications and the significances of the operative treatment.

METHODS: From June 2002 to March 2004, 28 intra-articular calcaneal fractures with malunion patients (26 males and 2 females, 30 feet) suffered operations. The average age of these patients was 39.2 years old (range,23-59years).The malunion happened at left feet in 15 patients, right feet in 11,and bilateral feet in 2.The mean duration between injury and operation was 3.3 months (range 2-5months). All cases used improved "L" incisions at lateral of heels.13 patients(14cases)did open reduction and internal fixation; 9 patients (10 cases) operated calcaneal osteotomy to reserve subtalar joint and correct deformity with apophysis excision, then used internal fixations;6 patients (6 cases) used correct deformity with apophysis excision, calcaneal cumulus reconstruction and subtalar arthrodesis.The calcaneal lateral and axial X-ray and CT films were got to measure the Böhler angle, Gissane angle, calcaneal width and axial length, and height of calcaneal bodythalamic portion before and after operation. There were significant differences at all index before and after operation ($P<0.01$).

RESULTS: 29 feet in 30 feet were primarily healing, one infective foot delayed healed after treatment. The follow-up period was from 10 months to 29 months (average 15.6 months). According to American Orthopaedic Foot and Ankle Society (AOFAS) Maryland score's standard:7 cases were excellent, 19 cases were good, 3 cases were fair, and only 1 case was poor, The score was 32 preoperatively, 83 postoperatively, the average improvement was 51, The excellent and good rate was 86.7%.

CONCLUSIONS: The operations of intra-articular calcaneal fractures with malunion should be chosen according to the pathological character of deformity to get significant curative effects.

56. The Preliminary Study of Navel Injectable Biomaterial to Repair Cartilage --High Viscous Chitosan/Glycerol Phosphate -Demineralized Bone Matrix

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INTRODUCTION: Cartilage defects suffering from joint traumatic injures, degenerative changes and tumours etc. are very common (in clinic) which lead to osteoarthritis. We try to prepare a novel injectable biomaterial, high viscous Chitosan/Phosphate gel-Demineralized Bone Matrix (HV_C/GP- DBM), to repair cartilage defects.

METHODS: (1) Preparation of High Viscous Chitosan/Phosphate and its Properties: Prepare HV_C/GP and C/GP by two different methods respectively. Examine the rheological properties of HV_C/GP and C/GP with a Gemini rheometer to evaluate and compare their thermosensity including of the solution viscosity, gelation temperature and gelation time. Place the gels into continuous flow thermostated cells under the same conditions used in the release studies. At predetermined time intervals(at 1st, 2nd, 5th, 10th, 25th day), dry and weigh them. Examine the freeze-dried simples using a scanning electron microscope. **(2)** High Viscous Chitosan/Glycerol Phosphate - Demineralized Bone Matrix to Repair Cartilage Effects in Rabbits: Inject the implantation into articular cartilage defects in habbits' knees. The rabbits were then sacrificed at 2, 4, 8and 12weeks respectively. The obtained specimens were examined histologically and stained immunohistochemically for type || collagen.

RESULTS: The improved techniques of preparation of C/GP result in 10 fold increase in dynamic viscosity, about 2°C decreace in gelation temperature and increase in but no remarkable difference in gelation time. The micrographs revealed that the HV_C/GP gels are porous and pores between 5 and 10μm that is smaller than C/GP. Specimens harvested from HV_C/GP-DBM composite implantation demonstrated hyaline cartilage and a complete subchondral bone formation. The newly formed tissue with subchondral bone had perfect fusion with surrounding normal cartilage.

CONCLUSION: 1) The improved technics of preparation of C/GP result in increase in dynamic viscosity, but not impair the thermosensity in significance. 2) The degradable time in vitro of HV/C/GP is prolonged. 3) The micrographs revealed that the HV_C/GP gels are porous and pores is smaller than C/GP. In conclusion, the increase of viscosity by means of the improvement of preparetion of C/GP could increase the maneuverability and expand the application scope of C/GP . HV_C/GP is a potentially useful injectable biomaterial. 4) HV_C/GP-DBM can repair articular cartilage defects and it's a promising injectable biomaterial to repair articular cartilage defects.

57. Manufacture and application of implantation artificial skull

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INTRODUCTION: To investigate how to form the implantation artificial skull by high polymer material and medical used bionic material and its application in repair of skull defect.

METHODS: To manufacture the implantation artificial skull according to the common position of skull defect through modern advanced varying temperature, high pressure and ion spraying process. Then applied in 1000 cases in ten years and analyzed the operation process and its composition.

RESULT: All the materials are safe in the long-term used in clinical. The combination process is physical methods, no chemical changes. The animal experiences are followed the international biological material monitor standard. The implantation artificial skull had impact strength of 189.23 kg-cm/cm2, Brinell hardness of 44.2, time of heating for softening of 1 minute, flexibility cm of 28° and crack flexibility of 46°. No monomer is precipitated after intensified polymerization. There were 48 cases had suffered from complications, including 10 cases with intracranial hematoma, 15 cases with wound infection and 23 cases with subcutaneous hydrops. All the cases were cured after re-operation.

CONCLUSION: The implantation artificial skull is safe and no poisonous, easy to molding, sturdy and durable, both infection rate and complications rate are low. The bony union with the skull can be formed. It is worth popularizing for clinical use.

58. Repair of Rabbit Growth Plate Defects with Tissue-engineering Cartilage: Compound of Autologous Bone Marrow Mesenchymal Stem Cells and Biological Scaffold

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INTRODUCTION: The objectives of this study are: (1) To explore the behavior of MSCs which were isolated from rabbit bone marrow in vitro and investigate the feasibility of MSCs as seed cells of tissue engineering. (2) To study the feasibility and application value of MSCs as seed cells in tissue engineering, which were induced to differentiate into chondrocyte. (3) To construct tissue-engineering cartilage, MSCS were seeded on the biological scaffold, poly-DL-lactic acid and cultured under three-dimension condition. (4) To investigate the feasibility of repairing the growth plate defects with the compound of tissue engineering cartilage.

METHODS: MSCs were extracted from rabbit bone marrow and purified, then cultured in vitro, induced to differentiate into chondrocytes first under serum-free medium contain TGF-β_1 and dexamethsone in monolayer culture, then under three- dimension condition with PDLLA and IGF-1. The compound was implanted into the growth plate defects of proximal tibial of 8-weeks rabbits. After 4, 8 and 16 weeks, gross observation, X-ray film, histological examinations were performed to evaluate the efficiency.

RESULTS: MSCs which were in low abundance proliferated when cultured in vitro and cell survival rates were 90% ~95%. After being induced to differentiate into chondrocyte, there was much toluidine blue metachromasia matrix around the cell by toluidine blue staining. The rabbit tibia had no marked deformities after 4 weeks of operation, and histologic examination revealed that the defects were filled with cartilage. After 16 weeks, the experiment group had minor deformities, and histologic examination showed near closure of growth plates. On the contrary, the control side showed severe deformities and growth plates were closed.

CONCLUSION: The success in MSCs culture in vitro will be useful for repairing cartilage and skeletal tissue defects by tissue engineering technique in the future. MSCs can be induced to differentiate into chondrocytes under three-dimension condition and serum-free medium. PDLLA was a sponge-like porous structure and MSCs showed high level of proliferation. PDLLA was a good carrier for cartilage tissue engineering. Transplantation of cultured cartilage into growth plate defects may replace growth plate tissues, maintain normal growth of limbs and prevent developmental deformity. And it is a promising way for clinic treatment of growth plate defects.

59. Clinical Evaluation of Vacuum Sealing Drainage Effect in The Treatment of Severe Soft Tissue Defect of The Lower Limbs

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INTRODUCTION: To evaluate the results of the vacuum-assisted wound closure applied in patients presenting with open skin and soft tissue defect in the lower extremity;

METHOD: Randomized retrospective clinical study was done on 242 cases with open lower-extremity soft tissue defects using VSD device.

RESULTS: After 5-7days of the application of VSD device, the wounds generally were clean, and had no signs of infection or bacterial colonization. All wound dimensions were decreased , the granulation in the surface of wound were all fresh, second stage closure or skin grafting was done successfully to close the wound.

CONCLUSION: VSD appeared to be the effective treatment of open soft tissue injuries. It is viable, safe and may accelerate the healing of large wound.

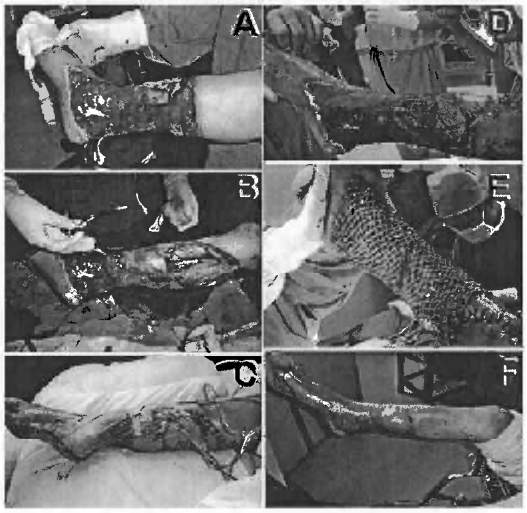

Fig. 1: (A) Right lower calf and ankle extensive soft tissue defect. (B) Gastrocnemius muscle flap. (C) VSD application on Rt lower limb. (D) One week after VSD application. (E) Net shaped skin graft post VSD application, Reapplication of VSD after skin graft. (F) Three weeks after skin graft.

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60. Effect on the osteoblastic differentiation from rabbit adipose-derived stem cells transfected by adenoviral vector mediated hBMP-2 gene in vitro

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INTRODUCTION: To investigate the proliferation of rabbit adipose-derived stem cell(ADSCs) transfected by adenoviral vector mediated hBMP-2 gene and its osteoblastic differentiation potential.

METHODS: When had constructed the Ad-hBMP-2 plasmid vetor, we could get the virals which had the hBMP-2 gene and transfected the ADSCs with it. Two groups is the hBMP-2 transfected group and the control group. The cells were cultured on complete osteoblastic medium.The morphology and localized regions of mineralization of the cells were observed ; The expression of alkaline phosphatase(ALP), hBMP-2,Collagenl,Osteocalcin and OPN were measured by the cytochemisty, RT-PCR, immunohistochemistry and Western blot.

RESULTS: The hBMP-2 transfected ADSCs became the polygon form and its proliferation and localized regions of mineralization of the cells became well. Its cytochemisty of ALP also showed better. The RT-PCR outcome of hBMP-2 on the transfected group was better than the control after transtected for 7 day and 14 day. The RT-PCR, Western blot and immunohistochemistry of Collagenl,Osteocalcin and OPN also was better than the control after transtected for 7 day and 14 day.

CONCLUSION: ADSCs transfected by the adenoviral vector mediated hBMP-2 gene have stronger potential to differentiate into osteoblasts. They therefore can serve as seed cells in bone tissue engineering.

61. Osteoclastic responses to phase pure hydroxyapatite (HA) and silicon (Si)-substituted HA

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Hydroxyapatite (HA) has a similar crystallographic structure to bone, making it a strong candidate as a bone graft in orthopaedic reconstruction surgery. An ideal bone graft for many applicants would be a material that could both be resorbed and induce bone formation, allowing for complete replacement by the "new" bone. Recent studies have indicated that the incorporation of silicon (Si) into the HA lattice increases osteoblast proliferation and differentiation (*Botelho, et al. 2004*). This effect was suggested to be due the increase in the metabolic activity of osteoblasts (*Gibson, et al. 1999*). Despite recent reports on the effects of HA-Si *in vivo* (*Patel, et al. 2003*) and *in vitro* (*Botelho, et al. 2006*) its effects on the extent of osteoclastic differentiation and bone resorption, compared to the HA alone, remains unclear. The aims of the present study, was therefore to elucidate the direct effects if HA-Si discs on osteoclast morphology, attachment, formation and lacunar resorption as compared to HA alone and a normal substrate.

Human peripheral blood mononuclear cells (PBMC) were obtained from buffy coats samples (n=4) from Blood Transfusion Centre and were cultured on sterile dentine slices (used as a normal substrate), HA and 1.5% wt HA-Si discs for 21 days in the presence of soluble receptor activator for nuclear κB ligand (RANKL) and macrophage colony stimulating factor (M-CSF); essential factors required to generate osteoclasts *in vitro*. The cell morphology and lacunar resorption formed on the discs were then compared using Scanning Electron Microscopy (SEM) and Focused Ion Beam (FIB) Microscopy. Moreover the number of tartrate-resistant acid phosphatase (TRAP) positive cells formed on discs (n=3) was also determined.

Our findings indicated that the PBMCs cultured on Si-substituted HA and HA were capable of differentiating into osteoclast-like cells, an observation which is in agreement with that reported by (*Botelho et al. 2006*). More intriguing was the fact that osteoclast-like cells formed on HA-Si were more in clusters and larger in size than those formed on control HA. Furthermore the number of TRAP+ multinucleated cells formed on HA-Si was significantly higher as compared to those formed on HA alone (44.2 ± 2.9 vs. 32.1 ± 3.6, $p<0.05$). SEM and FIB examination of the cells cultured on HA-Si indicated the presence of active osteoclasts resorbing the surface of discs revealing the interface between osteoclasts and HA/Si.

62. Near surface damage characterisation of biomedical grade Y-TZP

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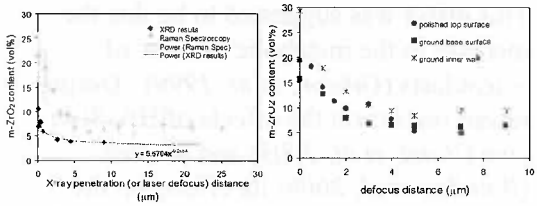
Introduction: Y-TZP has been employed as a femoral head material because of its exceptional mechanical properties and fine grain structure. However, concerns over in-vivo performance associated with the low temperature degradation (LTD) phenomena has been a continuing concern. Laboratory-based X-ray diffraction (XRD) has been widely used to measure the near-surface phase composition, but is unable to be used effectively on non-flat surfaces. In this report, we explore the feasibility of using Raman spectroscopy to quantify the near surface composition of Y-TZP, by a comparative study using grazing-incidence, high-resolution parallel-beam x-ray diffraction.

Experimental methods: Samples of Zyranox® Y-TZP were investigated in this study. The ground surfaces had a standard finish as a compression face of a bending test bar made at Morgan Advanced Ceramics and had been measured with both XRD and Raman spectroscopy. Surfaces on machined bore, machined flat and the polished spherical bearing from a prosthetic femoral ball, which had been simulated in body fluid for 11.5 years were measured with Raman spectroscopy. The x-ray powder diffraction was performed at beamline ID31 of the ESRF at Grenoble. By use of a grazing incidence angle, fixed for each set of measurements, the structure can be probed as a function of depth below the surface. Details of the instrument and data collection strategy are published elsewhere. The Raman spectroscopy was performed on an inVia Raman microscope (Renishaw) with a laser power of 5 mW. The instrument configuration was optimised for best performance of confocal measurement.

Results: The monoclinic fraction as a function of the x-ray penetration depth on the ground surface has been clearly shown; a similar depth profile along the defocus distance of the laser has also been detected by using Raman spectroscopy (fig. 1a). However, the overall phase content measured by Raman spectroscopy is higher than that measured by x-ray diffraction. The difference between these two methods indicates that a further calibration is needed for quantitative use of the intensity of the Raman peaks. By using Raman spectroscopy on a femoral ball, the depth profiles of the monoclinic phase can be determined from all the flat or non-flat, ground or polished surfaces. The phase contents display a significant differentiation among different surfaces. These preliminary results indicate that confocal Raman spectroscopy could become a method to quantify



the residual damage in the near surface of Y-TZP. Further validation is necessary before it can be judged if this could form a quality assurance method for Y-TZP implantation products.

Figure 1 Depth profile of monoclinic zirconia content under the surfaces of Y-TZP measured by x-ray diffraction and Raman spectroscopy

Conclusions: High resolution parallel beam x-ray powder diffraction has unambiguously been the most powerful and accurate method to characterise the near surface damage of Y-TZP. However, it needs the brilliance of a synchrotron radiation source and cannot be used on curved surfaces. Compared to the x-ray measurements, confocal measurements have shown the potential of Raman spectroscopy to quantify the near surface composition on various surfaces. This technique may have potential for use in quality assurance of finished biomedical products.

63. Comparative study between coralline hydroxyapatite and coverslips culture with mesenchymal stem cells

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INTRODUCTION: The aim of this study was to compare the culture expanded human mesenchymal stem cells (hMSCs) adherent to coralline hydroxyapatite(CHA) specimens(Hainan, China) and coverslips.

METHODS: Natural coral was obtained from Hainan Province, China. The material was carefully molded into 1.5 mm (thickness) ×5 mm × 5 mm (square) slices. Enhanced green fluoresecent protein (EGFP) labeled hMSCs were used for cellular assay for the coral slices. The hMSCs were seeded at 2×10^5 cells/slices on coral; same number of cells was also seeded on 10 mm diameter glass coverslips as control in 24 well culture plates. 24 hours after seeding, both coral and glass coverslips were moved to new 24 well culture plates to remove cells those did not attached on the slices. Cells were cultured in 10% FBS in αMEM. AlamarBlue assay was carried out at day 4,8,12,16 for cell proliferation and LIVE/DEAD stain was used for cell viability.

RESULTS: The cell number on coral was not as higher as those on glass coverslips at day 4. However, cell number on coral continuously increased during the culture period and reached the same level as those on coverslips at day 8 and day 12. At day 16, while the cell number on coverslips decreased with more dead cells, the cell number on coral remained stable.

Conclusion The natural coral from Hainan, China shows no cytotoxicicty to hMSCs *in vitro*. However, further study are warranted to enhance hMSC attachment at the early stage and to promote osteogenic differentiation.

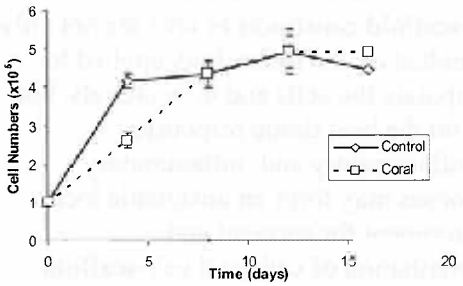

Figure 1. Alamar blue assay of hMSC proliferation on Hainan coral.

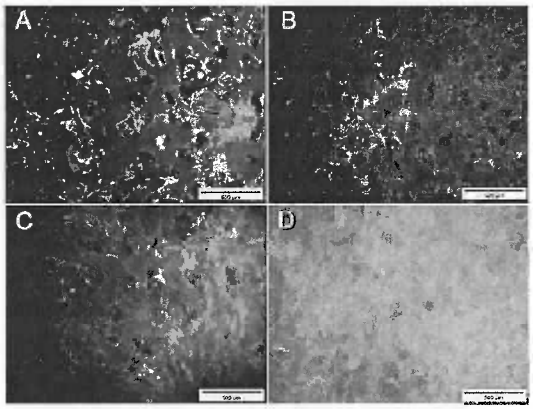

Figure 2. The hMSCs on coral at day 4 (A), 8 (B), 12 (C) and 16 (D).

ACKNOWLEDGEMENT: This study is a part of a D Phil project of Tongji Medical College, Huazhong University of Science and Technology.

65. Tissue Responses to Mesenchymal Stem Cells and Biomaterial Implants

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INTRODUCTION: Cell therapy and tissue engineering hold the promise to provide major therapeutic modalities in the 21st century. Mesenchymal stem cells have been widely studied for their potential application in cellular therapy. In particular, they have been used in construct with biomaterials/scaffolds for musculoskeletal tissue regeneration. However, the fate of these cells and the cell-scaffold constructs *in vivo* are not only dependent on the technology applied to manipulate the cells and biomaterials, but also on the host tissue responses. Proinflammatory and inflammatory responses may form an unsuitable local environment for survival and differentiation of cells and cell-scaffold constructs. In the present study, we have systematically analysed tissue responses to human mesenchymal stem cells and a variety of biomaterials after *in vivo* implantation.

METHODS: Human mesenchymal stem cells (hMSCs) were isolated and genetically labelled with enhanced green fluorescent protein (EGFP). The cells were either cultured *in vitro* for 17 days or incorporated with a variety of biomaterials, namely, poly(epsilon-caprolactone), hydroxyapatite, true bone ceramics and collagen-hydroxyapatite *in vivo* for 2-4 weeks before being implanted intromuscularly or subcutaneously into nonobese diabetic/severe combined immunodefecient (NOD/scid) mice or CB 17 scid beige (CB17 sb) mice up to four months. The specimens were harvested at different time pionts for histology, immunohistochemistry, and ultrastructural studies.

RESULTS: The EGFP labelled hMSCs could survive *in vivo* for up to four months. However, the survival cells were only a smal propotion of implanted cells, and osteogenesis only happenned in a very limited extend. Except for host fibroblastic cells, macrophages were the majority cells that infiltrated into the implants. Macrophages expressed F4/80, CR3 and other markers. Implanted hMSCs were surrounded by macrophages. Macrophages also formed multinuclear giant cells or foreign body giant cells in response to biomaterial implantation and calcified tissue formed by implanted hMSCs.

CONCLUSION: The ability of macrophages to recognise self and non-self chemicals, cells and tissue and to effectively respond to foreign invasion is through evolutionary time. These responses may provoke a physiological environment that is unsuitable for extensive osteogenesis *in vivo*; however, elimination of these cells may result in other problems as macrophage activities are an important part of biomaterial degradation and tissue regeneration. Strategies to target macrophage responses will be a major challenge for tissue regeneration by cytotherapy and tissue engineering.

67. CAMC – a new imaging device for pedicle approach

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We present the application of CAMC, a novel augmented reality system introduced by Navab[1], and our initial experience with imaging of the pedicle approach. Augmented reality is a technique that superimposes acquired patient data e.g. intra-operative imaging data in-situ. This means the imaging data is merged spatially registered with the real view of the region of interest.

We set up a camera augmented mobile c-arm[1] and used it to perform transpedicular needle placement under video control. After initial phantom studies we carried cadaver studies in different levels of the lumbar and thoracic spine using a percutaneous pedicle approach and evaluated the results by CT-control and dissection. The system hardware uses a prototypic isocentric-3D-fluoroscope with an additionally attached CCD-camera. The optical axis of video and fluoroscope are aligned via a double mirror construction. After taking a single x-ray-shot, the image is merged with the video image

The described CAMC-system enables robust 2-dimensional AR-visualization of everyday surgical tasks with a modified mobile c-arm. During spinal interventions via pedicle approach flouroscopy time and thus radiation dose can be considerably reduced, as a single-shot x-ray image is sufficient as soon as the pedicle axis is recognized. CAMC enhances the surgeons vision creating an x-ray-view, while reducing radiation time. The surgical workflow is not compromised and the user-interface provides intuitive control of the system.

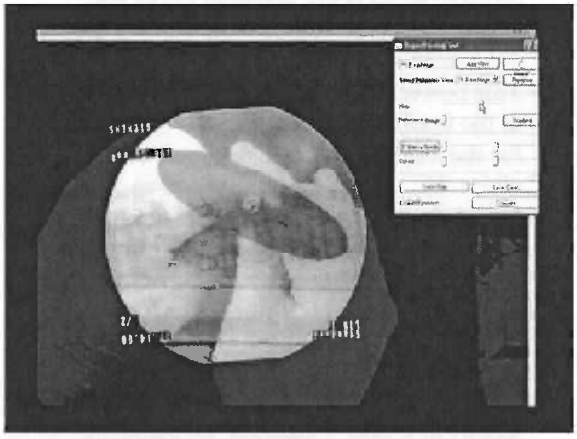

Figure: merged x-ray/video-image during transpedicular needle placement

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68. Injectable Biomaterials for the Regeneration of Intervertebral Disc

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Introduction: Millions of people in our society are affected by back pain. The main cause for the back pain is considered to be the degenerative changes in the intervertebral disc (IVD). The IVD is a complex tissue and plays an important role in spinal weight transmission. It consists two regions: annulus fibrosus (AF) and nucleus pulposus (NP). Both contain a matrix of proteoglycans (PG), collagen fibres and water. Some evidence indicates that IVD degeneration originates from the loss of NP. This poster reviews the current developments in the scaffold-based regenerative medicine for the treatment of disc degeneration.

Methods and hypothesis: One of the approaches to regenerate disc is to inject cells mixed in a supporting biomaterial (e.g. hydrogel) into the NP where they can be induced to produce an extracellular matrix (ECM) rich in aggrecan[1]. For a system to be injectable, it should be able to flow through a needle or trocar. Therefore, by necessity, most of these systems should exist, preliminarily in liquid state and must be cytocompatible to be able encapsulate cells.

Discussion: An ideal biomaterial should have the ability to retain the cells and their ECM components produced by the cells. A limited number studies have been conducted on injectable scaffolds for disc regeneration (Table 1). The carrier gel must have an optimum viscosity that allows placement of the cellular carriers and transfer of nutrients and waste products through the gel. As NP tissue is a load-bearing tissue, and their cells respond to mechanical stimulation, adequate mechanical properties of scaffolds and culture environment are required. The injectable scaffold should be able to withstand the mechanical load. Most of the currently available hydrogels lack mechanical strength which can pose difficulties in handling and high mechanical loading. Hence, injectable biomaterials should be designed with combination of stability, injectablity and biocompatibility characteristics[2].

Table 1. Injectable scaffolds for disc regeneration

Biomaterial	Inference
Gelatin[3]	Adult porcine NP cells expressed types I and II collagen and aggrecan.
Demineralised bone[3]	Promoted attachment of NP cells that were metabolically active and expressed genes for major ECM components.
Chitosan gel[1]	Suitable for encapsulation of adult bovine NP cells and restore function during early stages of regeneration.

These biomaterials have immense potential to be used as injectable therapy for NP regeneration. However, further characterisation of biomaterials for cell growth, synthesis of ECM and biomechanical properties in an *in vitro* bioreactor system is required.

Conclusion: Injectable scaffold-based regenerative medicine is a promising therapy to repair or reverse degeneration by replacing with healthier NP matrix, thereby improving disc function to alleviate back pain.

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To all delegates, thank you for attending and for helping to make this meeting a success.

My notes