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CANADIAN ARTHRITIS NETWORK | LE RÉSEAU CANADIEN DE L'ARTHRITE

2nd International Symposium on Biotechnology in Musculoskeletal Repair

Program
October 2 - 4, 2008 / Beaulieu Convention Centre, Lausanne, Switzerland

2008

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Introduction

The Second International Symposium on Biotechnology in Musculoskeletal Repair, sponsored by the AO Foundation, highlights recent progress across a wide range of clinically relevant topics in bone and cartilage de- and re- generation, therapy, and repair strategies. Technical programming encompasses advances in understanding healing, inflammation, angiogenesis, bone biology, and preclinical efficacy studies, within the context of developing new approaches to innovative patient management. Research related to modern fracture management must also focus on bone and cartilage diseases that increasingly impact aging populations through modern life styles (arthritis, osteoporosis, etc.). Peripheral research fields including haematology, rheumatology, endocrinology, clinical biochemistry, biotechnology and cell biology are increasingly exploited to provide new information that will ultimately improve patient management with fractures based on bone and cartilage diseases, or poor healing complications. Combinations of molecular and cellular diagnostics and therapeutics are increasingly exploited in this context.

The AO Foundation's Biotechnology Advisory Board, organizing this event for the second time, seeks to highlight the roles of inflammation, infection, and angiogenesis in bone and cartilage repair, specifically to better understand the key signals, players, and physiological forces driving and inhibiting tissue healing. Increasing involvement of genetics tools and molecular diagnostics with preclinical models seek to provide insight into improving strategies for the bioengineering of bone and cartilage repair. Biomarkers and bioassays provide enormous amounts of new information but with little clinical validation to date. New cell-, protein-, and gene-based biotechnology approaches to musculoskeletal repair must integrate all of these resources and capabilities to optimize therapeutic outcomes in bone and cartilage.

Experts from all over the world have been invited to report their newest achievements in research and commercial translation to enable the clinicians' quests for new patient treatments of tomorrow. Parallel breakout sessions on the first and third day will focus on important technical details and promote better networking within this research and clinical community.

Organizing Committee: The Biotechnology Advisory Board

Margarethe Hofmann, Jörg Auer, Steve Feinberg, Robert Guldberg,

David Grainger, Elliott Gruskin, Norbert Haas, Dick Heinegård,

Robin Poole , Jill Urban

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Friday, October 3, 2008

Welcome Address

CHAIR: Margarethe Hofmann, Chairperson AO Biotechnology Advisory Board, Research Advisory Council

8:30 **Paul Manson, President AO Foundation**

Bone and cartilage repair : Stem Cell Engineering and Genetics

CHAIR: Georg Duda, Charité Universitätsmedizin, Berlin Germany

8:50 **Mesenchymal stem cells and osteoblast-chondroblast differentiation.**

Jane E. Aubin, University of Toronto, Toronto, Ontario Canada

9:15 **Xenogenic Transplantation of Human Mesenchymal Stem Cells for Treatment of Critical Size Bone Defects in Sheep**

Philipp Niemeyer et al., Department of Orthopedic Surgery and Traumatology, Freiburg University Hospital, Germany, and AO Research Institute, AO Foundation, Davos, Switzerland

9:30 **Syndecan-4 Deficiency Leads to an Osteoporotic Bone Structure in vivo and an Impaired Osteoblast Functionality in vitro**

R. Stange et al. , University Hospital Muenster, Germany

09:45 **Break & Poster**

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Friday, October 3, 2008 (cont.)

Bone and cartilage repair : Stem Cell Engineering and Genetics

CHAIR: Tim Pohlemann, Universität des Saarlandes, Homburg Saar, Germany

10:15 **Targeting the HIF-1 pathway for bone repair.**

Thomas Clemens, UAB, Birmingham, Alabama USA

10:40 **Expedited strategies for the restoration of bone.**

Christopher H. Evans, Orthopedic Surgery-Brigham & Women's Hospital, Boston USA

11:05 **Chitosan-based hybrid biomaterials applied with bone marrow stimulation improves cartilage repair."**

Michael Buschmann, Ecole Polytechnique Montréal, Montreal, Canada

11:30 **A Comparative Study of Drilling Versus Microfracture for Cartilage Repair in a Rabbit Model.**

H. Chen et al. Ecole Polytechnique de Montreal, Montreal, QC, Canada. and Bio Syntech Ltd., McGill University, Montreal, QC, Canada.

11:45 **How has genetics research altered our understanding of Degenerative Disc Disease: Implications for intervertebral disc regeneration**

Kenneth MC Cheung, Department of Orthopaedics and Traumatology, University of Hong Kong, Hong Kong, China.

12:10 **Lunch & Poster**

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Friday, October 3, 2008 (cont.)

Infection and its influence on healing and repair

CHAIR: David Grainger, University of Utah, Utah, USA

13:45 **Establishing the diagnosis of low grade infection, and the problem of identifying potential hypersensitivity reactions to metal in patients with total joint prostheses.**

Thomas W. Bauer, The Cleveland Clinic Foundation, Cleveland, Ohio USA

14:10 **Biomaterial-associated infection: breaking out of the biofilm**

Sebastian A.J. Zaat, University of Amsterdam, Amsterdam, The Nederlands

14:45 **The Role of MSCRAMMs in Staphylococcal Skeletal Infections**

Magnus Hook, Texas A&M University, Texas USA

15:10 **Infection and osteoporosis: influence on healing and repair**

Michael J. Raschke, Universitätsklinikum Münster, Münster Germany

15:30 **Coffee Break & Poster**

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Friday, October 3, 2008 (cont.)

Biotechnology: From bench to bedside,

CHAIR: Steve Feinberg, University of Michigan, Ann Arbor, MI, USA

16:00 **Orthopedic Applications of Recombinant Platelet-Derived Growth Factor"**

Samuel Lynch, BioMimetic Therapeutics, Inc., Franklin, TN USA

16:25 **Synthes strategy for combination devices**

Elliott Gruskin, Biomaterials, Synthes USA, West Chester, PA, USA

16:50 **The gap between research & clinical applications of bone substitutes**

Marc Bohner, Robert Mathys, Dr. h. c. Robert Mathys Stiftung, Bettlach, Switzerland

17:10 **A successful paradigm for NIH Funding in CMF: Enhancing Translational Research by Expanding the Network between the Basic and Clinical scientist. (Successful development of an interdisciplinary collaboration)**

Steven Buchman, Medical School, University of Michigan, Ann Arbor, MI, USA

17:30 **Poster Session**

18:30 Return to the hotels

19:15 Embarkment Lausanne Ouchy, Lake Geneva

19:30 Start of the Dinner Cruise on the Lake Geneva

22:30 Return to Lausanne Ouchy

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Saturday , October 4, 2008

Welcome Address

CHAIR: Margarethe Hofmann, Chairperson AO Research Advisory Council

8:30 Norbert Südkamp, Chairperson AO Exploratory Research Board

Stimulation of bone & cartilage repair

CHAIR: Norbert Südkamp, Albert Ludwigs Universität Freiburg, Germany

08:40 **Materials for angiogenesis on demand"**

David Mooney, Harvard Engineering & Applied Science, Harvard University DEAS, Cambridge, USA

09:05 **Molecular and cellular mechanism of pathologic bone remodeling in inflammatory conditions**

Steve R. Goldring, Beth Israel Deaconess Medical Center, Department of Medicine, Boston, MA, USA

09:30 **The cellular composition of the initial fracture and soft tissue hematoma during theinflammatory phase of the healing process"**

K. Schmidt-Bleek et al., Julius Wolff Institut and Center for Musculoskeletal Surgery, Charité-Universitätsmedizin Berlin, Germany, and Department of Rheumatology and Clinical Immunology, Charité University Hospital, Germany

09:45 **Oxygen measurement in three-dimensional scaffolds for the tissue engineering of bone**

S. Otto et al., Ludwig-Maximilians-University, Munich, Germany and University of Munich (LMU), Munich, Germany

10:00 **Break & Poster**

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Stimulation of bone & cartilage repair

CHAIR: Adrian Sugar, Chairperson AO Research Fund, Morrison Hospital, Swansea, UK

10:40 **Remodeling rAAV-Coated Allografts for Musculoskeletal Repair**

Edward Schwarz, University of Rochester, Medical Center, Rochester , New York USA

11:05 **The Role of Osteoclasts in Osteoblast Regulation**

J. E. Fong et al., McGill University, Montreal and Université de Montréal, Montreal, Canada

10:20 **Mesenchymal Stem Cells for the Augmentation of the Maxillary Sinus**

S. Sauerbier et al., University Hospital Freiburg, Germany

11:35 **Development of brushite matrices able to stimulate bone remodelling**

D. Le Nihouannen et al., McGill University, Montreal, Canada, University of Würzburg, Würzburg, Germany.

11:50 **A bioactive guided bone regeneration membrane enhances BMP signalling and tunes the natriuretic hormone system into a pro osteogenic state”**

FE Weber et al., University Hospital Zurich, Zurich,

Switzerland and Inion Ltd, Cambridge UK

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Saturday , October 4, 2008 (cont.)

Stimulation of bone & cartilage repair

CHAIR: Adrian Sugar, Chairperson AO Research Fund, Morrison Hospital, Swansea, UK

12:05 **Tackling the challenging indications in orthopaedic surgery - application of scaffoldbased tissue engineering in osteochondral and spinal fusion models in the pig**

Dietmar Hutmacher, Institute of Health and Biomedical Innovation, Queensland University of Technology, Kelvin Grove, AUS

12:30 **Lunch & Poster**

14:15 – 14:45 **Presentation of Best Poster Awards**
Short overview of the posters with disposition of awards by the Jury and 5 min presentation by the three laureats each

12:30 – 14:00 LUNCH SESSION Networking in a clinically inspired research community

Chair: Jill Urban, Oxford University, Oxford UK

This Lunch Session will explore the advantages of networking among basic and clinical researchers from academia and industry with particular focus on patients' involvement in research and development. Working together with members of the Canadian Arthritis Network, who will share their experience with network creation and administration, possible opportunities for the AO Foundation and its future activities in the musculoskeletal field will be discussed.

12:30 **The Canadian Arthritis Network**

Jane E. Aubin, University of Toronto,Toronto, Ontario Canada and Scientific Director of the Institute of Musculoskeletal Health and Arthritis, Canadian Institutes of Health Research

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Saturday , October 4, 2008 (cont.)

12:30 – 14:00 **LUNCH SESSION Networking in a clinically inspired research community (cont.)**

Chair: Jill Urban, Oxford University, Oxford UK

12:50 **The German Rheumatology Network**

Ulf Müller-Ladner, Justus-Liebig Universität Giessen, Bad Nauheim, Germany

13:05 **The AO Spine Network**

Kenneth MC Cheung, The University of Hong Kong and Queen Mary Hospital, Hong Kong, SAR, China

13:20 **The AO Osteoporosis Network**

Jörg Goldhahn, Schulthess Clinic and University Zurich, Zurich Switzerland

13:35 - 14:00 **Discussion**

Saturday , October 4, 2008 (cont.)

Breakout Session 1 - Animal models ; which ones work in research dealing with bone and cartilage repair ?"

Chair: Joerg Auer, University of Zurich, Switzerland

Today it is claimed that animal "models" are required for concept validation in vivo as pre-clinical models for medical progress, but all models suffer from lack of relevance to the human condition for various reasons. The biggest problem is that animal models will never exactly mimic the approach used in human as the conditions are not the same from the physiological/anatomical, mechanical, biological and genetic points. Others are acute models in health animals when the human condition is otherwise.

Some models even deliver information which may be misinterpreted or grossly misleading. This breakout session will offer a short overview of animal modeling concepts especially for technologies and other approaches in cartilage repair and bone healing and then discuss pro and cons in this respect.

14:45 **The use of Rodent models in musculoskeletal research for fracture repair; a need to consider a standard ?**

Allen Goodship, Royal Veterinary College and Institute of Orthopaedics and Muscloskeletal Science UCL, London UK

15:00 **Experiences with sheep models in musculoskeletal research at MSRU**

Brigitte von Rechenberg , University Zurich, Switzerland

15:15 **Use of Equine Models to Evaluate Articular Cartilage Repair**

Wayne McIlwraith, Colorado State University, USA

15:30 **The use of clinical patients as animal models in musculoskeletal research.**

Joerg Auer, Equine Departement, Vetsuisse Facutly, University Zurich, Switzerland

15:45-16:30 **Discussion and adoption of appropriate animal models**

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Saturday , October 4, 2008 (cont.)

Breakout session 2 - Biological markers and diagnostic tools for early disease detection.

Chair: Dick Heinegard, Lund University, Lund Sweden and David W. Grainger, University of Utah, Utah, PA, USA

Injury, inflammation, healing and disease have distinct patterns of gene, protein and cellular markers associated with their progression. Disease, infection, medical interventions, complications, and pharmacology will influence the balance of such biological markers. Relative amounts, temporal sequences, and dynamic changes of such markers characterize the state of a disease or imbalance.

Wide accessibility to convenient, reliable, sensitive, highly precise detection tools and analysis methods for measuring even very low amounts of such molecules from tissue biopsies is desired. Highly parallel high throughput assays that produce information on many markers simultaneously is also desired. The workshop will overview progress in identifying key biomarkers for bone, cartilage, and healing progression, and also novel technologies under development to better assay genes, proteins, and cell types involved in musculoskeletal trauma and healing.

14:45 **System Biology Approaches of Cartilage Degeneration – A New Way for Understanding Pathogenesis and Markers for Disease Monitoring ?**

Thomas Aigner, Universitätsklinikum Leipzig, Leipzig, Germany

15:05 **Molecular markers in the study of cartilage destruction in joint disease**

Dick Heinegard, Lund University, Lund, Sweden

15:25 **Biological markers of bone turnover**

Patrick Garnero, National Institutes of Health and Medical Research (INSERM, research Unit 664) and Synarc; Lyon, France

15:50 - 16:30 **Discussion**

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Saturday , October 4, 2008 (cont.)

Breakout session 3 - New challenges in biotechnologies for bone and cartilage

Chair: Robert Guldberg, Georgia Institute of Technology and James Kellam, Carolinas Medical Center, Charlotte USA

Trauma, diseases and the consequences of diseases like cancer or arthritis may cause tissue defects (bone, cartilage, muscles etc.) that seriously challenge the skills of orthopedic and maxillofacial surgeons. Many surgical techniques are used in clinics including allografts, fixation devices, mechanical and physical stimulations to improve the patient's welfare. However, each of these techniques shows deficiencies and disadvantages. New treatment concepts including biotechnology are therefore continually introduced. Opportunities and challenges of different approaches will be discussed among researchers, clinicians and industrial scientists.

14:45 **What I need to do my job : Get the bone to heal!**

James Kellam, Carolinas Medical Center, Charlotte, USA

15:00 **Biomaterials Horizons - Lessons Learned and a Look to the Future**

Karen Burg, Clemson University, South Carolina, USA

15:15 **Stem cells for musculoskeletal regeneration**

Mauro Alini, AO Research Institute, Davos, Switzerland

15:30 **Tissue engineered medical devices: Are there conditions for manufacturing and approval?**

Joerg Vienken, BioSciences Department, Fresenius Medical Care, Bad Homburg, Germany

15:45 – 16:30 **Discussion**

16:40 **Closing words and Farewell**

Norbert P. Haas, President Elect AO Foundation, Director Center of Musculoskeletal Surgery, University Hospital Charité, Berlin, Germany

17:00 **End of the Meeting**

Friday, October 3, 2008

Session 1

Bone and cartilage repair : Stem Cell Engineering and Genetics

CHAIR: Georg Duda, Charité Universitätsmedizin, Berlin Germany

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Mesenchymal Stem Cells and Osteoblast-Chondroblast Differentiation

Jane E. Aubin

Dept. of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada M5S 1A8

INTRODUCTION: The fact that bone formation takes place not only during development but throughout life suggests that there is a large reservoir of cells in the body capable of osteogenesis. The nature of these cells over the lifetime of the animal, contributions from stem cell versus committed progenitor pools, and identification of developmental transition steps remain subjects of intense study. Osteoprogenitors (colony forming unit-osteoblast (CFU-O)) arise from multipotential mesenchymal stem cells (often designated CFU-fibroblast or CFU-F) that also give rise to chondroblasts, adipocytes and myoblasts and in which fate choice may be both stochastic and driven by environmental cues..

METHODS: Primary cultures of fetal (E21) rat or neonatal (P1-2) mouse calvaria and young adult mouse or rat bone marrow stromal cells were prepared and cultured as described[1]. Cells were cultured at high ($5X10^3$ cells/cm^2) or low (5 cells/cm^2) or a limiting dilution series, under conditions for osteogenic, chondrogenic, adipocytic or myogenic conditions as appropriate. In some experiments, cells were analyzed and sorted by flow cytometry or magnetic beads. At various times, RNA was extracted for PCR or cells were fixed and stained by histochemistry or immunohistochemistry for marker expression.

RESULTS: CFU-O comprise only a proportion of the CFU-F, express limited self-renewal and occur at frequencies of ~$1/10^5$ to $1/10^2$ cells in bone marrow or stromal and calvaria-derived populations respectively. Fractionation based on Hoechst dye efflux (side population or SP) or alkaline phosphatase (ALP) and/or parathyroid hormone/parathyroid hormone related protein receptor (PTH1R) expression enriches significantly for CFU-F and CFU-O, and reveals that both cell autonomous and non-autonomous developmental events occur, that the majority of immature progenitors reside in the ALP/PTH1R negative fraction and that cell autonomous clonogenic osteoprogenitors reside only in the latter pool. By other novel fractionation strategies based on positive and negative selection with lineage markers, we achieved >150-fold enrichment for a multipotential mesenchymal population with robust chondrogenic and osteogenic differentiation capacity *in vitro* and *in vivo*. Replica plating, global amplification poly(A)PCR, BrdU labelling and immunocytochemistry revealed at least seven transitional stages in osteoblast differentiation. Statistical analysis of the gene expression profiles uncovered multiple potential developmental pathways in osteoblast differentiation. A hierarchical distribution of simultaneous marker expression for multiple lineages was seen in morphologically indistinguishable CFU-F, suggesting that gene priming may contribute to mesenchymal cell fate selection. Mesenchymal stem and progenitor cell fate choice can also be altered by cytokines and hormones, e.g., leukemia inhibitory factor (LIF) and $1,25(OH)_2D_3$[2].

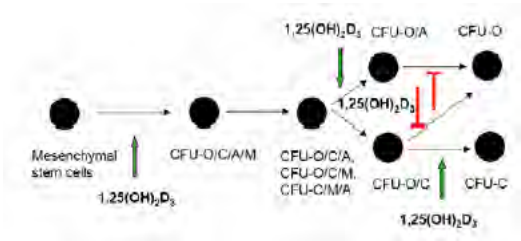

Fig. 1: Hormones, e.g. $1,25(OH)_2D_3$ alter the fate choice of mesenchymal stem and progenitor cells.[2]

DISCUSSION & CONCLUSIONS: Our data support both a deterministic/hierarchical and non-deterministic (environmental cues and modifiers) model of mesenchymal stem cells in which gene priming and non-reciprocal regulation of fate selection play roles, and offer new strategies for enriching for multipotential cells for regenerative medicine applications.

REFERENCES: [1]J.E. Aubin and J. Triffitt (2002) Mesenchymal stem cells and the osteoblast lineage. *Principles of Bone Biology*, 2nd ed. (eds J.P. Bilezikian, L.G. Raisz, and G.A. Rodan) Academic Press, pp 59-81. [2] S. Zhang, S. Uchida, T. Inoue, M. Chan, E. Mockler and J.E. Aubin (2006) Side population (SP) cells isolated from fetal rat calvaria are enriched for bone, cartilage, adipose tissue and neural progenitors. *Bone* **38**:662-670.

ACKNOWLEDGEMENTS: This work is supported by the Canadian Institutes of Health Research (FRN 483033).

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Xenogenic Transplantation of Human Mesenchymal Stem Cells for Treatment of Critical Size Bone Defects in Sheep

Philipp Niemeyer[1], Thomas Schönberger[1], Joachim Hahn[2],
Norbert P. Südkamp[1], Erich Schneider[2], Simon Pearce[2] and Stefan Milz[2]

[1] *Department of Orthopedic Surgery and Traumatology, Freiburg University Hospital, GER*

[2] *AO Research Institute, AO Foundation, Davos, CH*

INTRODUCTION: Mesenchymal stem cells (MSC) from bone marrow represent an attractive cell source for tissue engineering purposes such as the regeneration of bone. Due to a lack of expression of immunologically relevant surface antigens, this cell type might even be available for non-autologous cell transplantation. Although immunosuppressive properties have been demonstrated in vitro and xenogenic MSC show an engraftment after transplantation in immunocompetent mice, it remains unclear if HLA-mismatched MSC have a regeneration potential equal to autologous MSC.

METHODS: After isolation and cultivation on mineralized collagen as described earlier[3], xenogenic human and autologous ovine MSC were transplanted into a 3.0 cm diaphyseal tibia defect in Swiss Alpine sheep. Animals were sacrificed after 3 and 6 months. Unloaded mineralized collagen scaffolds served as a control. Radiography was performed every 2 weeks, in addition histological evaluation was performed after euthanasia (including in-situ hybridization for detection of human MSC).

Bone regeneration was analyzed using semi quantitative scoring systems on radiographic and histological levels. Furthermore, the amount of newly formed bone was quantified using the digital image software analysis program *GIMP*.

RESULTS: Autologous MSC lead to significant increase in radiological bone density in the defect after 6 weeks compared to unloaded controls ($p < 0.05$). This difference could also be confirmed by histological evaluation after euthanasia ($p < 0.01$). Compared to the autologous MSC group, the transplantation of xenogenic MSC leads to a lower rate of bone formation ($p < 0.05$). Nevertheless, an engraftment of xenogenic MSC was detected in 3 out of 7 animals using human specific in situ hybridization, while no severe systemic or histological immune response could be detected. Bone formation in the xenogenic group was higher than in the unloaded control group with statistical significance in radiological data evaluation. On semi quantitative histological level however, there was no statistically significant difference between the two groups.

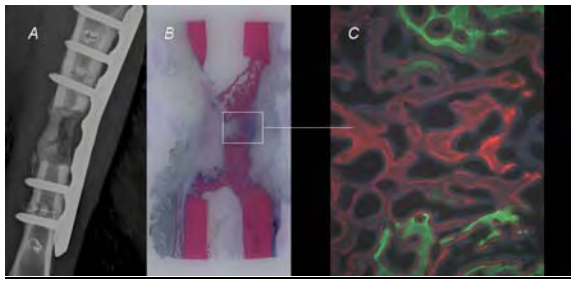

Fig.1: Sufficient bridging of the defect was found in 3 animals of the autologous MSC group (radiography at 24 weeks (A). Giemsa-Eosin staining of conventional histology (B). In vivo fluorescent sequence labeling revealed that bone formation in these defects occurred as late as 20 weeks after surgery (C).

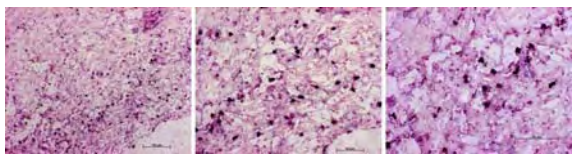

Fig.2: Human-specific in-situ hybridization revealed presence of human MSC after xenogenic transplantation in 3 of 7 animals

DISCUSSION & CONCLUSIONS: Although xenogenic MSC could be detected in a significant number of animals after transplantation, no severe immune response was detected. Nevertheless xenogenic transplantation of MSC seems to lead to a lesser rate of bone formation compared to autologous ovine MSC which performed best. Identification of the biological principle beyond this observation will be part of further studies.

REFERENCES:

[1] Le Blanc K, et al. (2003) HLA expression and immunologic properties of MSC … Exp Hematol 31:890-896.
[2] Niemeyer P, et al. (2007) Comparison of immunological properties of bone marrow stroma cells and adipose tissue-derived stem cells …. Tissue Eng 13:111-121.

ACKNOWLEDGEMENTS:

The Project was supported by the AO Research Fund (No. 04-N94) of the AO Foundation, CH and by the Albert-Ludwigs-University Freiburg, Germany.

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SYNDECAN-4 DEFICIENCY LEADS TO AN OSTEOPOROTIC BONE STRUCTURE *IN VIVO* AND AN IMPAIRED OSTEOBLAST FUNCTIONALITY *IN VITRO*

R. Stange [+], M. Timmen [+], T. Stoltenberg [+], H. Hidding [+], F. Echtermeyer *, K. Neugebauer *, T. Pap *, M. Raschke [+]

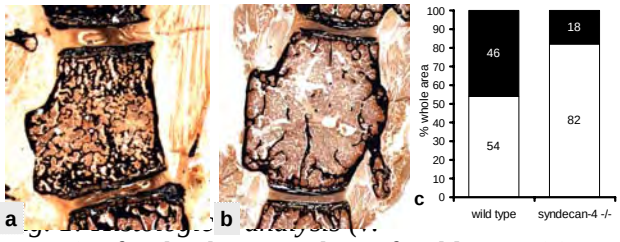
[+]*Dept. of Trauma, Hand and Reconstructive Surgery, University Hospital Muenster*
[*]*Division of Molecular Medicine of Musculoskeletal Tissue, Dept. of Orthopaedics, University Hospital Muenster, Germany*

INTRODUCTION: Members of the syndecan family of heparan sulfate proteoglycans play important roles in cell adhesion and cell communication by serving as co-receptors for both extracellular matrix molecules and growth factors together with integrins. Syndecan-4 is ubiquitously expressed and appears to be involved in cell proliferation, differentiation, adhesion and migration. It has been demonstrated that syndecan-4 is upregulated in the dermis after injury, and syndecan-4 knockout mice show a delay in wound healing[1]. During embryogenesis, syndecan-4 is important for regulation of chondrocyte differentiation and enchondral ossification. To determine the influence of syndecan-4 on bone morphology and development, wild type and syndecan-4 knockout mice were investigated.

METHODS: *Biomechanical testing*: 20 Femurs of 12 months old, female C57BL/6 mice (10 wild type and 10 syndecan-4 knockout) were dissected and torsional testing was performed with a special testing device. Maximum torque, angle at max. torque and torsional stiffness were determined. *Histomorphometry*: Spines were dissected and embedded in methylmetacrylate. After µCT-Analysis, longitudinal serial sections (5 µm) were prepared, stained (von Kossa) and histomorphometrical analysis was carried out. *Cell culture*: Osteoblast progenitor cells were isolated from the calvaria of newborn mice and cultured in αMEM +10% FCS complemented with β-glycerophosphate, L-ascorbate and dexamethasone. Osteoblastic cells were characterized by detection of osteoblast markers (alkaline phosphatase, osteocalcin) and mineralization (von Kossa staining).

RESULTS: The biomechanical testing demonstrate a significant higher ($p<0.05$ Mann Whitney) maximum torque in the wild type group compared to the syndecan-4 knockout group. The stiffness of the femur bone was higher in the wild type group although not significant.

µCT analysis as well as histomorphometry (Fig. 1) showed 30-50% less trabecular and mineralized bone in the syndecan-4 knockout group compared to the wild type group.



Kossa) of a lumbar vertebra of wild type (a) and syndecan-4 knockout mice (b); c Quantification of mineralized bone area

In vitro analysis of osteoblast differentiation revealed no differences between wild type and syndecan-4 deficient cells with regard to the synthesis of osteocalcin or the activity of alkaline phosphatase. Interestingly, after 25 days the mineralization of syndecan-4 deficient osteoblasts was reduced clearly compared to wild type mice (Fig. 2).

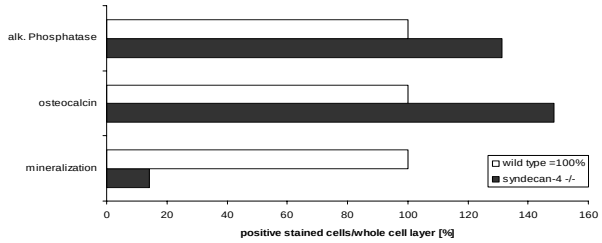


Fig. 2. Determination of differentiation and mineralization of osteoblasts in vitro

DISCUSSION: The absence of syndecan-4 leads to an osteoporotic-like phenotype of the bone with decreased mechanical properties. These findings might be explained by an impaired osteoblast mineralization during bone development of syndecan-4 deficient mice which leads to a derogated cell-matrix interaction and connection.

REFERENCES: [1]F. Echtermeyer et al. (2001) *J. Clin. Invest*. **107**:R9–R14

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Friday, October 3, 2008

Session 2a

Bone and cartilage repair : Stem Cell Engineering and Genetics

CHAIR: Tim Pohlemann, Universität des Saarlandes, Homburg Saar, Germany

Targeting the HIF-1 pathway for bone repair.

Thomas Clemens, UAB, Birmingham, Alabama USA

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Expedited strategies for the restoration of bone

C.H. Evans[1], V. Glatt[1], F-J.Liu[1], J.W.Wells[1], E. Ferreira[1], M.S.Vrahas[1], M.B.Harris[1], A. Ivkovic[1,2], R.M. Porter[1]

[1]*Department of Orthopaedic Surgery, Harvard Medical School, Boston, USA*

[2] *Department of Orthopaedic Surgery, Zagreb Medical School, Croatia*

INTRODUCTION: *We are trying to devise expedited technologies for the repair and regeneration of connective tissues that obviate the need for cell culture, bioreactors and other expensive components [1]. For bone healing, a basic strategy aims to develop intra-operative procedures in which tissue is harvested, modified and returned to the individual in one surgery. We have concentrated on the use of bone marrow, fat and muscle as tissues that can be readily harvested, and which contain osteoprogenitor cells. Gene transfer is one way in which the osteogenic differentiation of the resident progenitor cells can be initiated and sustained once the tissues are retuned to the body. Here we describe data obtained using muscle and bone marrow as sources of osteoprogenitors.*

METHODS: *Muscle:* A skin punch was used to harvest discs of skeletal muscle 5mm in diameter and 1mm thick from the gastrocnemius muscles of rats. These were transduced *in vitro* using an adenovirus carrying the human BMP-2 cDNA (Ad.BMP-2). Expression of BMP-2 was confirmed by ELISA and expression of transcripts associated with osteogenesis was confirmed by RT-PCR. The genetically modified discs were placed into 5mm femoral defects in rats [2]. Healing of the defect was monitored by weekly X-ray. After 8 weeks, rats were euthanized and defects examined by μCT, mechanical testing, DXA and histology. *Bone marrow:* Material was harvest from the intermedullary canals of human with femoral neck fractures using the reamer-irrigator-aspirator (RIA)[3]. The aspirate was centrifuged to recover the cells, whose osteogenic properties were then studied under *in vitro* conditions using standard osteogenic medium containing dexamethasone, ascorbate and β-glycerol phosphate. To determine their osteogenic properties *in vivo*, reamings were placed into 5mm femoral defects in nude rats in the presence or absence of rhBMP-2. Healing was assessed by the methods indicated above using genetically modified muscle.

RESULTS: *Muscle:* Discs of muscle maintained in culture started to express transcripts associated with osteogenesis after transduction with Ad.BMP-2. Implantation of these discs into the femoral defect led to rapid, uniform and impressive radiologic union in all rats within 10 days, with little callus formation. By 8 weeks, the defects had recovered full mechanical strength and stiffness. Bone volume and mineral content were slightly higher than normal because of the presence of trabecular bone within the medullary space.
Bone marrow Preliminary radiologic data suggest that cells and bone fragments recovered by the RIA can enhance the healing in the presence of rhBMP-2.

DISCUSSION & CONCLUSIONS: These data indicate 2 strategies for the facilitated, endogenous repair of osseous defects. Both make use of existing technologies and are achievable within a single operative session. Genetically modified muscle proved a particularly powerful osteogenic material, and preliminary studies are underway in sheep to determine whether its potency is maintained in a large animal. As it presently stands, the procedure uses a viral vector and its potential use in humans requires careful examination. The RIA provides a convenient way to harvest large amounts of highly osteogenic, autologous material and is already in human, clinical use. Preliminary data from our rat study suggest that the reamings improve the potency of rhBMP-2, also in clinical use. A way of promoting their osteogenicity that avoids the expense and inconvenience of BMPs would be very helpful.

REFERENCES: [1] C.H.Evans, G.D. Palmer, A. Pascher, et al (2007) *Tissue Eng* **13**: 1987-1993. [2]O.B. Betz, V.Betz, A. Nazarian et al (2006) *J Bone Jt Surg* **88A**:355-365. [3] R.M. Porter, F-J. Liu, Pilapil, C et al (2008) *J Orthop Res* In Press

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Chitosan-based hybrid biomaterial applied with bone marrow stimulation improves cartilage repair

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INTRODUCTION: Augmenting microfracture with biomaterials to stimulate marrow derived cartilage repair is becoming recognised as a practical, effective and safe means to improve cartilage repair outcomes. We have performed small [1] and large animal [2] studies that demonstrated improved quantity and hyaline quality of cartilage repair tissue using a hybrid biomaterial composed of a chitosan glycerol phosphate (GP) solution (BST-CarGel®), mixed with whole blood, to provide a polymer-reinforced blood clot with cartilage repair properties.

METHODS: Fresh autologous whole blood was mixed with chitosan-GP in a 3:1 volume ratio. Solidification occurred by blood coagulation and the hybrid biomaterial was then visualised in environmental SEM or applied to condylar cartilage defects in an adult sheep model. Outcomes were examined histologically.

RESULTS: Representative structure of the chitosan-GP/blood hybrid biomaterial (BST-CarGel) and 3 and 6 month repair tissue outcomes are shown in Figs 1, 2, and 3.

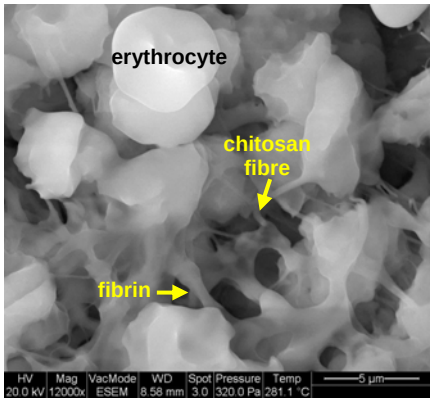

Fig. 1: This unfixed chitosan-GP/blood clot was imaged in a hydrated state using environmental SEM to visualise chitosan fibres (identified through EDS analysis) throughout the clotted blood along with other normal blood components.

ACKNOWLEDGEMENTS: This work was funded by the Canadian Arthritis Network, CIHR and BioSyntech Canada Ltd.

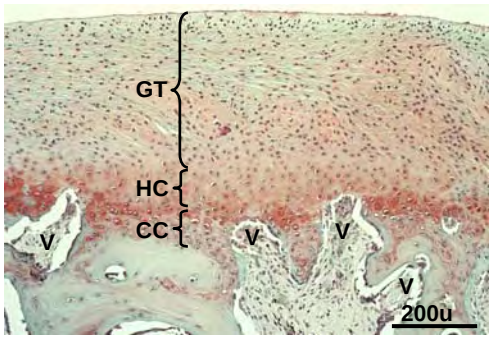

Fig. 2: Cartilage repair with chitosan-GP/blood A at 3 months post-operative reveals repair processes simulating the epiphyseal growth plate with hypertrophic cartilage (HC), calcified cartilage (CC) and invading blood vessels (V) below a differentiating granulation tissue (GT).

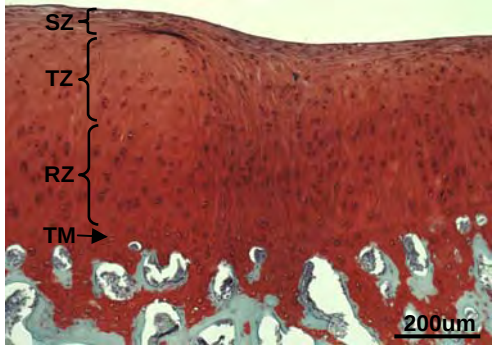

Fig. 3: At 6 months, a hyaline articular cartilage bearing a superficial zone (SZ), transitional zone (TZ), radial zone (RZ) and tidemark (TM) can be observed when chitosan-P/blood was used to stimulate marrow derived cartilage repair.

CONCLUSION: Chitosan-GP/blood improves marrow derived cartilage repair by stimulating cell recruitment, bone remodelling and transient vascularisation [3]. Clinical development of BST-CarGel® [4] has led to a multicenter randomized clinical trial comparing repair tissue quality with a microfracture control, and is nearly completed.

REFERENCES: [1]Chevrier OA&C 15 : 316 (2007). [2]Hoemann JBJS 87:2671 (2005). [3]Buschmann in *Cartilage Repair Strategies*, Humana Press 2007. [4]Shive in *Operative Techniques in Orthopaedics.* 2006.

AO Foundation

THE FIRST 50 YEARS

A Comparative Study of Drilling Versus Microfracture for Cartilage Repair in a Rabbit Model

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INTRODUCTION: Bone marrow stimulation initiates cartilage repair by inducing a fracture repair response in subchondral bone [1]. Clinical treatment algorithms recommend microfracture although drilling and abrasion are also practiced [2-3]. However no study has directly compared microfracture to drilling. The present study compares microfracture (MF) to microdrilling (MD) for cartilage repair in a rabbit model.

METHODS: Cartilage defects in trochleas and condyles were prepared bilaterally in 24 adult NZW rabbits. Marrow stimulating holes were created using customized surgical tools (*Fig. 1*). Both MF and MD were performed to a depth of 2 mm. Animals were sacrificed at 1, 14, 21 and 90 days post-operatively. Fixed joints were scanned by micro-CT followed by histological analyses.

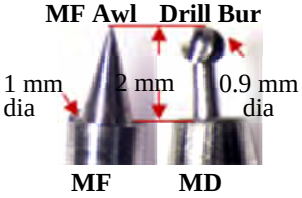

Fig.1: Customized surgical tools bearing stoppers that control hole depth.

RESULTS: MF induced acute crushing of bone resulting in compacted bone surrounding the holes on Day 1 (arrowheads in *Fig. 2A*), which was absent around drilled holes (arrows in *Fig. 2A*). While heat necrosis is often said to be produced by drilling, we found mechanical crushing by MF may produce greater osteocyte death than thermal necrosis in MD (data not shown). At intermediate times, MD displayed a more robust angiogenic and chondrogenic response than MF. Bone repair in MD holes was also greater than in MF ones (*Fig. 2B* & *Tab. 1*). Cartilage resurfacing at 90 days showed MD had larger amount of hyaline cartilage than MF (*Fig. 2C-D).*

DISCUSSION & CONCLUSIONS: Our study revealed significant differences in acute subchondral bone damage and subsequent repair responses comparing MF to MD technique. These methods may affect the patterns and connectivity of subchondral bone marrow channels. Compacted bone surrounding MF holes could inhibit vascular invasion, chondrogeneisis and new bone synthesis at intermediate stages, resulting in inadequate long term cartilage repair. Drilling technique may bear differential capacity to generate more effective repair.

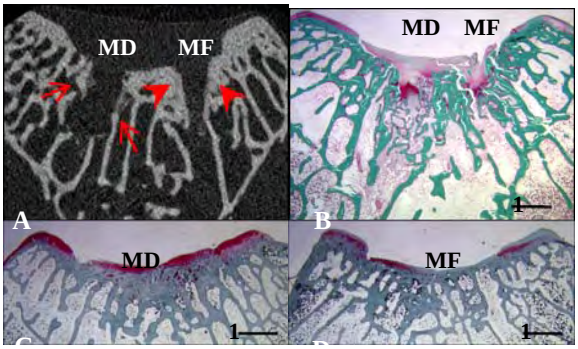

Fig.2: µCT scan image (A), Goldner's Trichrome stained MMA section (B) and Safranin O-Fast Green stained cryo-sections (C-D) from trochlea of rabbits sacrificed at 1(A), 21 (B) and 90 (C-D) days post-operatively.

*Table 1. Bone Repair in Holes at Day 14 and Day 21 Post-operative **

		MD	MF
Day 14	BV(x10^5 µm^3) #	12.9 ± 14.67	6.0 ± 5.83
	BV/TV (%)	2.7 ± 2.34	1.9 ± 1.52
Day 21	BV(x10^5 µm^3) #	25.6 ± 18.53	8.5 ± 5.01
	BV/TV (%)	5.2 ± 3.62	3.3 ± 1.98

* mean ± std (n=6). # p<0.05 for MD>MF (Day 14 and Day 21, n=12)

REFERENCES: [1] Lewis P et al. J Orthop Sports Phys Ther. 2006, 36(10):717-27. [2] Mithoefer K et al. JBJS. 2006, 88:294-304. [3] Schultz W and Gobel D. Knee Surg Sports Traumatol Arthrosc. 1999, 7(1):29-36.

How has genetics research altered our understanding of Degenerative Disc Disease: Implications for intervertebral disc regeneration

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Introduction: Low back pain is a major cause of disability and suffering world-wide, and has a lifetime prevalence of around 80%. Current methods of treatment for severe pain usually involve fusion of the affected spinal segment. While it can be successful in terms of symptom relief, the increased spinal stiffness may have consequences on adjacent segments. Motion preservation technologies are a "hot" research topic. However, there is a current mismatch between biological therapies for intervertebral disc regeneration and the etiology of back pain. Since many studies described the presence of degenerative changes in subjects without symptoms; yet surgeons rely on the correlation between symptoms and MRI findings for decisions regarding surgery. One of the reasons for the controversy is that disc degeneration is an ageing phenomenon, thus a "normal" level of degeneration for a young versus an elderly subject would be quite different. There has been no large scale population based study that critically examines the contribution of genetic factors to degenerative disc disease, and the relationship of MRI changes of degeneration to symptoms of back pain.

Method: Since 2001, the authors have been recruiting volunteers from the local population, between 18 and 55 years, and carried out MRI examinations of their lumbar spine. Disc degeneration for each level on the MRI was graded by Schneiderman's classification (0 to 3) and a total DDD score was calculated by summation of the scores for each of the 5 levels. Additional MRI changes, such as disc herniation, annular tears, Schmorl's nodes and marrow signal changes, were noted. All MRI scans were assessed by 2 observers blinded to the clinical information, any disagreement were settled by consensus. Clinical information on pain, if present, was obtained using both specific questions and questionnaires including the Oswestry Disability Index, Roland Morris Questionnaire, and the SF36. Blood were taken for DNA extraction, and predisposing genes were screened using case-association approaches including a genome wide association analysis by the use of the 500K Affymetrics chip. Additional 30 families with early onset severe disease were identified and predisposing genes were screened by linkage analysis.

Results: A total of 2000 subjects have so far been recruited. By use of microsatellite markers, and comparing their frequencies to a group of university students, we were able to demonstrate that our recruited cohort is representative of the general Southern Chinese population. As expected, there is a progressive increase in the prevalence of degeneration with age. Surprisingly, some 40% of those less than 30 years of age have some degeneration, progressively increasing to over 90% above 50 years of age. However 10% of over 50 years old have no degeneration, suggesting that factors other than age play a part in this process. There is a linear correlation between the severity of the DDD score, and the proportion of subjects with back pain, and that degeneration on MRI scans can be used as a predictor for back pain (odds ratio = 2.2, p = 0.001).

Based on the large database of population information, we adjusted for age using the following equation:

Age-adjusted DDD score
= [Ln(Mean-DDD score + 2) - mean] / S.D.

We were able to demonstrate that use of this age-adjusted DDD score allowed us to identify individuals with disc degeneration that correlated more strongly with symptoms when compared with the previously described DDD score, and that previous genetic predispositions could be reproduced using this age-adjusted score as the new phenotype.
This age-adjusted score was then used as the phenotype in the case association studies and the family linkage study. A number of new genes are identified, including genes that predispose to osteoarthritis and genes that are either structural components or involved in the regulation of the extracellular matrix. Their relative risk in causation of disease varies from 2 times to some 6 times relative to the risk of developing disease in the general population. We are in the process of replicating these in another population, some of these will be presented in detail during the presentation.

Discussion: This study suggests that there is a close correlation between low back pain and intervertebral disc degeneration detected by MRI. Thus the study of the etiology of disc degeneration is clinically relevant. Treatment approaches that can stop disc degeneration should have a good chance of preventing low back pain.

The study has also gone on to demonstrate that it is possible to predict who will get low back pain from disc degeneration by use of the Age-adjusted DDD score. This adjustment is made possible by using our large database, so that a mean level of degeneration for a particular age could be defined, and a more severe level of degeneration than can be expected for a particular age could then be identified. The adjusted score would support the concept that "age-related changes" are normal and expected, and that it is usually those that have a more severe form of degeneration for their age tend to be symptomatic. Thus Degenerative Disc Disease should be defined based on these individuals.

This understanding and definition will help scientists, as the age-adjusted score would be a better phenotype definition; and will help clinicians and patients, as age related changes are expected and does not need treatment.

Through the discovery of new genes and the understanding of their relative contribution to the etiology of disc degeneration, a new understanding of this condition is beginning to emerge.

Acknowledgment:
We thank Pei Yu for her technical assistance; The Hong Kong Jockey Club MRI Center and the Hong Kong Sanatorium and Hospital for the utilization of their MRI facilities. This work was supported by grants from the Research Grant Council of Hong Kong (HKU7230/01M) and (HKU7509/03M), from the University Grants Committee of Hong Kong (AoE/M-04/04), by grant MH44292 from the U.S. National Institute of Mental Health and from Academy of Finland (AR45982).

Friday, October 3, 2008

Session 3

Infection and its influence on healing and repair

CHAIR: David Grainger, University of Utah, Utah, USA

Diagnosing Low Grade Infection and the Problem of Potential Hypersensitivity in Patients with Total Joint Prostheses

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Introduction: Infection is one of the most important causes of arthroplasty failure, and recent observations suggest that the incidence of infection may be higher than previously thought. Cultures are the "gold standard" for diagnosis, but false negative and false positive results are well known, so many tests are used to help diagnose infection. Among those tests, culture of joint fluid, serum CRP and sed rate levels, and interpretation of frozen sections have high predictive value, but are not ideal (1). Recent studies using PCR have reported evidence of bacteria on up to 65% of culture-negative retrieved implants (2).

Development of New PCR Assays: To improve the sensitivity and specificity of broad-spectrum PCR assays, we first developed a new assay that uses two fluorescence resonance energy transfer probe sets and targets the tuf gene of *Staphylococcus(3)*. One probe set detects the genus, while the other is specific for *S. aureus*. While useful for many orthopaedic infections, that assay does not provide information about other genera. We then used post-amplification pyrosequencing combined with a broad-range PCR to distinguish Gram positive from negative bacteria based on DNA sequence rather than staining characteristics (4,5).

Evidence of Bacteria on Retrieved Implants: A combination of our Staph. species-specific PCR and a universal PCR followed by DNA sequencing was used to evaluate sonication fluid from 92 implants obtained at revision arthroplasty (6). All implants clinically thought to be aseptically loose were culture negative, but 12% had bacterial DNA by PCR.

False Positive PCR Results? Although our results suggest a lower prevalence of implants with evidence of bacteria than previous PCR studies, only a few of those 12% of cases showed subsequent evidence of clinically significant infection so we hypothesized that one potential cause of false-positive PCR results might be the detection of necrotic bacteria. In vitro studies using heat inactivated bacteria at variable dilutions followed by real-time quantitative PCR confirmed that PCR can detect necrotic organisms.

Molecular Methods to Detect Viable/Necrotic Bacteria: It would be desirable to have an index of bacterial viability in order to limit false positive PCR results due to DNA from necrotic bacteria. It is widely assumed that the half-life of mRNA is short, and a previous report suggested that the use of RT-PCR to detect mRNA for *groEL* could distinguish viable from necrotic bacteria in joint fluid. However, our in vitro studies using culture and quantitative PCR with viable and heat-inactivated E. coli and Staph. demonstrated persistent *groEL* mRNA up to 2 weeks after heat inactivation. Other studies have suggested that ethidium bromide monoazide (EMA) penetrates membranes of necrotic bacteria and interferes with polymerization during PCR. However, our recent in vitro studies suggest that EMA can also penetrate membranes of viable cells, reducing the sensitivity of PCR assays. On the other hand, our preliminary results suggest that Propidium monoazide (PMA) may effectively cross-link DNA of necrotic bacteria, preventing polymerization, and thereby reducing false positive PCR results without significantly reducing sensitivity.

Metal Ion Hypersensitivity? Recent reports of failed metal-metal total hip prostheses have illustrated patterns of inflammation that have been interpreted as representing a hypersensitivity reaction to metal ions. That histologic pattern can also be seen in cases of indolent or treated infection. The significance of lymphocytes, plasma cells, and perivascular inflammation in implant membranes needs to be defined by controlled studies.

References:

1. Bauer TW et al. J Bone Joint Surg 88:869, 2006. 2. Tunney MM, et al. J Bone Joint Surg Br 1998;80:568. 3. Sakai H et al. J Clin Micro 42:5739, 2004. 4. Kobayashi N et al. Diagn Mol Pathol 14:83, 2005. 5. Kobayashi N et al. J Orthop Res 24:1641, 2006. 6. Kobayashi N et al. Clin Orthop 466:1716, 2008.

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THE FIRST 50 YEARS

Biomaterial-associated infection: breaking out of the biofilm

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Infection Due To Bacterial Biofilm Formation And Tissue Colonization.

Biofilm formation on the surface of biomedical devices is generally recognized as a cause of biomaterial-associated infection. However, in many studies in the mouse experimental biomaterial-associated infection model, we have observed that subcutaneous tissue surrounding biomaterials is another important niche for persisting *Staphylococcus epidermidis* bacteria (1-3). This has been observed by others in rabbits as well (4). The combined presence of biomaterial and bacteria affects the local immune efficacy, resulting in survival of bacteria in the tissue, and even within macrophages (1,5). Bacteria residing in tissue surrounding implants are more difficult to eradicate with antibiotics (6) or with a cationic antimicrobial peptide (7) than bacteria on the biomaterial implants themselves.

Peri-Catheter Tissue Colonization In Humans.

We recently performed a study to assess whether bacteria were also present in tissue surrounding biomaterial in humans (8). Using a carefully controlled excision protocol, we collected catheters and surrounding tissue from deceased human patients. After removal of the skin, the catheter with surrounding tissue was axenically excised. The biopsy was cut into slices, the catheter segment was removed for sonication and quantitative bacterial culture, and the remaining tissue was cut into parts, which were individually homogenized for quantitative culture (Fig 1A). From the homogenized tissue large numbers of bacteria were cultured (Fig. 1B), predominantly staphylococci and enterococci. Presence of the bacteria within the tissue was confirmed by immunomicroscopy with antibodies specific for the cell wall lipoteichoic acid (LTA) of gram-positive bacteria (Fig. 1 C,D). These results indicate that surrounding tissue is an as yet generally overlooked reservoir for biomaterial-associated infection, in mice as well as in humans.

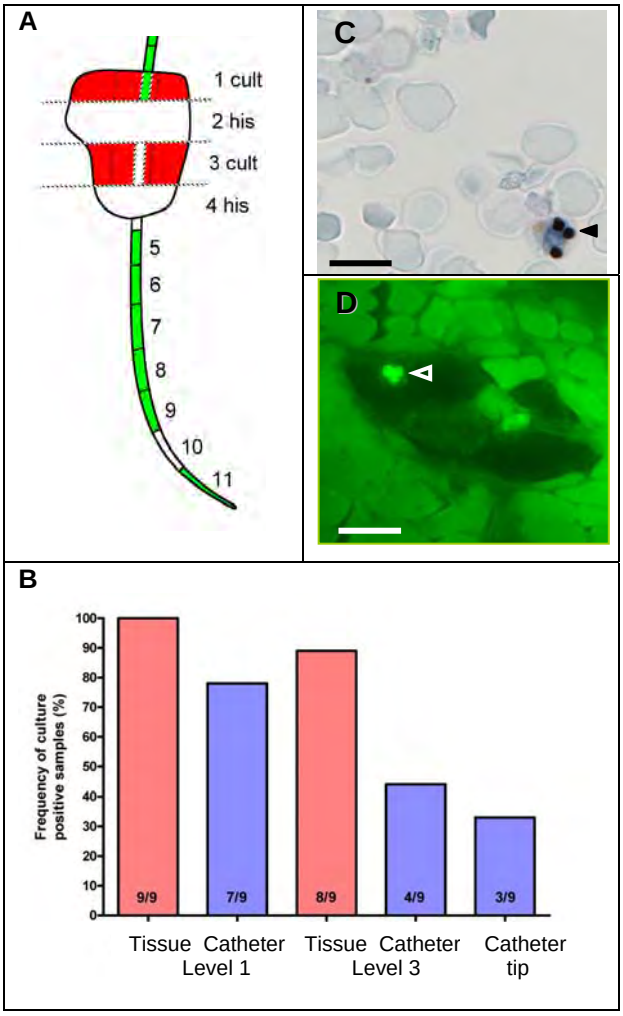

Fig.1: Bacterial colonization of catheters and surrounding tissue of deceased patients. A, catheters and surrounding tissue were excised axenically and processed for quantitative culture (cult) or histology (his); B, frequency of positive cultures of tissue and corresponding catheter segments of the 1st and 3d level of the biopsies, and of the catheter tip; C, immunostaining of bacteria in tissue sections with anti-LTA antibody; D, immunofluorescent detection of bacteria with AlexaFluor488-labelled anti-LTA. Arrows indicate bacteria. Bar represents 10 µm.

Bacterial Tissue Colonization And Intracellular Survival Due To Immune Dysregulation.

The physico-chemical nature of the biomaterial surface is decisive for the nature of the immune response to biomaterial in combination with bacteria. The tissue may respond properly, but the condition in the tissue may also become too pro-inflammatory, giving rise to a protracted pro-inflammatory phase of the foreign body response (2), or too anti-inflammatory, causing lack of intracellular killing of phagocytosed bacteria (1). Both these developments contribute to higher infection rates / prolonged persistence of bacteria.

Interestingly, these unfavorable conditions can be restored by modulation of the immune response, provided that the details of this response around the particular biomaterial are known. *Staphylococcus epidermidis*-infected mice implanted with polyvinylpyrrolidone (PVP)-grafted silicon elastomer developed a protracted pro-inflammatory response with high and persistent levels of the proinflammatory cytokine IL-1β (2). This dysregulation was corrected in mice which lacked the receptor for IL-1β (9). Conversely, infected mice with PVP-grafted polyamide showed a relatively anti-inflammatory response, resulting in large number of viable staphylococci residing inside macrophages in the tissue around the implant (1). In these mice, upregulation of the macrophage-activating cytokine interferon-gamma (IFN-γ) was suppressed due to the presence of the biomaterial. This could be corrected by treating the mice with IFN-γ, which restored the killing of bacteria (5).

Discussion and Conclusion

Thus, biomaterials not only provide bacteria a surface for adherence, but also induce a niche for bacterial survival in the tissue surrounding the biomaterial, and even within host immune cells. This novel element in the pathogenesis of biomaterial-associated infection may have consequences for the design of biomaterial surfaces and for the antibiotic strategies employed to prevent or treat infection. The biomaterial surface characteristics may be optimized to result in a biomaterial with optimal “immunocompatibility” i.e. allowing efficient activity of the local immune system to prevent colonization by micro-organisms.

References

1. Boelens, J. J., J. Dankert, J. L. Murk, et al. (2000) *Biomaterial-associated persistence of Staphylococcus epidermidis in pericatheter macrophages*. J. Infect. Dis. **181**:1337-1349.
2. Boelens, J. J., S. A. J. Zaat, J. L. Murk, et al. (2000). *Enhanced susceptibility to subcutaneous abscess formation and persistent infection around catheters is associated with sustained interleukin-1beta levels*. Infect. Immun. **68**:1692-1695.
3. Broekhuizen, C. A. N., L. de Boer, K. Schipper, et al. (2007) *Peri-implant tissue is an important niche for Staphylococcus epidermidis in experimental biomaterial-associated infection in mice*. Infect. Immun. **75**:1129-1136.
4. Vuong, C., S. Kocianova, Y. Yao, et al. (2004). *Increased colonization of indwelling medical devices by quorum-sensing mutants of Staphylococcus epidermidis in vivo*. J. Infect. Dis. **190**:1498-1505.
5. Boelens, J. J., T. van der Poll, J. Dankert, and S. A. J. Zaat (2000) *Interferon-gamma protects against biomaterial-associated Staphylococcus epidermidis infection in mice.* J. Infect. Dis. **181**:1167-1171.
6. Broekhuizen, C. A.N., L. de Boer, K. Schipper, et al. (2008). *Staphylococcus epidermidis is cleared from biomaterial implants but persists in peri-implant tissue in mice despite rifampicin/vancomycin treatment.* J. Biomed. Mater. Res. A **85**:498-505.
7. Kwakman, P. H. S., A. A. Te Velde, C. M. Vandenbroucke-Grauls, et al. (2006). *Treatment and Prevention of Staphylococcus epidermidis Experimental Biomaterial-Associated Infection by Bactericidal Peptide 2.* Antimicrob. Agents Chemother. **50**:3977-3983.
8. Broekhuizen, C.A.N., M. J. Schultz, A. C. van der Wal, et al. (2008). *Tissue around catheters is a niche for bacteria associated with medical device infection.* Crit Care Med. **36**:2395-2402.
9. Boelens, J. J., T. van der Poll, S. A.J. Zaat et al. (2000). *Interleukin-1 receptor type I gene-deficient mice are less susceptible to Staphylococcus epidermidis biomaterial-associated infection than are wild-type mice*. Infect. Immun. **68**:6924-6931.

The Role of MSCRAMMs in Staphylococcal Skeletal Infections

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INTRODUCTION: The pathogenesis of microbial infections can be characterized as a war between the attacking microbe and the defending host. Initially bacteria need to attach to a host tissue to create a beachhead for the infection. Most bacterial pathogens that infect skeletal tissues in the host use adhesins of the MSCRAMM family to adhere to proteins in the extracellular matrix in bone and cartilage. The goal of our work is to determine in submolecular detail the mechanisms of pathogen host interaction and to explore this information in the design of new strategies to prevent and treat infections.

METHODS: We use a combination of modern biomedical techniques to identify MSCRAMMs and their ligands, characterize their interaction in terms of affinity and specificity, and determine their roles in the disease process.

RESULTS & DISCUSSION: Staphylococcus aureus is a major cause of septic arthritis and osteomyelites in man. A characteristic of this opportunistic pathogen is the ability to disseminate from minor seemingly trivial skin infections to life threatening diseases. This behaviour reflects an extensive adhesive machinery with multiple MSCRAMMs. Earlier work in our laboratory showed that one MSCRAMM called CNA specifically binds collagen. Characterization of CNA revealed that the protein is covalently anchored to the cell wall of the bacteria and that the minimal ligand binding region is composed of two sub-domains each with an IgG like fold. We further postulated a ligand binding mechanism by solving a crystal structure of the minimal ligand binding region in complex with a synthetic collagen like triple helix peptide. A number of other gram positive bacteria contain cell wall anchored proteins that show significant sequence similarities with CNA and bind collagen by a similar ligand binding mechanism.

Comparison of staphylococcal strains and isogenic mutants that differ in their expression of CNA show pronounced difference in their ability to cause infection suggesting that CNA is an important virulence factor in S. aureus induced infections. Active and passive vaccination of mice with CNA result in protection against subsequent

challenge with staphylococci, demonstrating that MSCRAMMs are potential vaccine candidates.

CONCLUSION: Bacteria causing skeletal infections express adhesins mediating adherence to the host tissue. These adhesins are often of the MSCRAMM type and may play key roles in the disease process. Studies in animal models show that these MSCRAMMs are potential vaccine components.

Infection, Inflammation and osteoporosis: influence on healing and repair

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Despite improvements in operating techniques and the use of sophisticated implants in trauma and orthopaedic surgery, complications continue to occur. These may result from complex injury patterns, the shift of the population pyramid increasing the occurrence of complex fractures involving osteoporosis and more intricate operating procedures[4]. The complications range from delayed healing and non-union of fractures to extensive bone infections. Disturbances of fracture healing can be traumatically, mechanically or biologically caused. Many of the complications are directly correlated with the total duration of treatment, as well as with potential risks such as blood loss, infections, injuries to blood vessels or nerves, compartment syndrome and lasting loss of function. Depending on the duration of the hospital stay, infections resulting from hospital pathogens may also occur, sometimes with lethal consequences. Fractures of the long tubular bones bring with them the risk of development of proximal deep leg vein thromboses in 30–50% of patients as a result of the prolonged immobilisation. In up to 5% of these patients this can lead to a clinically significant pulmonary embolism. Up to 5% of fractures of the lower extremities are affected by delayed healing or remain as non-unions. These data have a significant influence on therapeutic treatment of fractures, esp. in the elderly, where comorbidities and impaired quality of bone are present.

Osteoporosis therefore remains a major public health problem in association with fragility and complex fractures. Despite the availability of preventative therapeutic agents, the incidence and its associated costs continue to rise globally.
Recent studies estimated 7, 8 million patients with osteoporosis in Germany 2003. More than 300.000 (4,3 %) of these patients sustained osteoporotic related fractures[1].
Osteoporosis can be divided into primary and secondary osteoporosis. Primary osteoporosis again can be subdivided into postmenopausal and senile osteoporosis according to age, sex and type of fracture. Secondary osteoporosis includes just 5 % of all osteoporotic patients but causes 20 % of osteoporotic related fractures. In contrast to primary osteoporosis, men and younger patients are prevalent. Different types of secondary osteoporosis are glucocorticoid-induced, metabolic-endocrine associated[2], (e.g. Cushing-syndrome, diabetes mellitus, hyperparathyreoidism, osteomalacia), tumor-associated (e.g. plasmocytoma, metastasis) and infection/inflammation associate (e.g. rheumatoid arthritis, inflammatory bowel diseases).

Emerging clinical and molecular evidence suggests that inflammation also has significant influence on bone turnover, inducing osteoporosis. Numerous pro-inflammatory cytokines like IL-1, IL-6 or TNFa have been identified as primary mediators of accelerated bone loss and osteoclastic bone resorption[3].

Yet unknown and untreated osteoporosis puts patients at risk of fractures, which may cause severe complications, substantially in the elderly. Furthermore especially these patients are senescence - related affected by inflammatory diseases and a weakened immune system.
The orthopaedic surgeon has to address not only biomechanical properties, but also the special biologic conditions of these patients.

Recent literature dealing on infection *and* osteoporosis is hardly available. We have just started to understand the interaction between bone and immune system in the elderly; looking at the demographic changes in our society these diseases will be of increasing clinical and scientific interest in the future.

Häussler B et al. Epidemiology, treatment and costs of osteoporosis in Germany--the BoneEVA Study. Osteoporos Int 2007; 18:77-84.

Kanis JA et al. A meta-analysis of prior corticosteroid use and fracture risk. J Bone Miner Res 2004; 19:893-9.

1. Mundy, GR Osteoporosis and Inflammation. Nutr Rev. 2007 Dec; 65 (12 Öt 2): S147-51
2. Einhorn TA et al. Significant advances have been made in the way surgeons treat fractures, *Clin Orthop Relat Res*, 1998;(Suppl. 355):S2–3.

Friday, October 3, 2008

Session 3

Biotechnology: From bench to bedside,

CHAIR: Steve Feinberg, University of Michigan, Ann Arbor, MI, USA

AO Foundation

THE FIRST 50 YEARS

Osteogenic Applications of rhPDGF

S.E. Lynch, C, Hart, W. Beasley, R. Pagano

BioMimetic Therapeutics, Inc. Franklin, TN, USA. *www.biomimetics.com*

INTRODUCTION: Bone repair is characterized by the recruitment and differentiation of mesenchymal stem cells (MSCs) and osteoblastic cell populations whose cellular activities are regulated by an elaborate system of growth factors and cytokines. Platelet-derived growth factor-BB (PDGF-BB) is a key early mediator of the wound healing and osteogenic cascades via potent chemotactic and mitogenic actions on these cell populations as well as its ability to promote angiogenesis (Fig. 1). The combination of rhPDGF-BB with an osteoconductive bone matrix, such as bone allograft or beta-tricalcium phosphate (β-TCP) provides both the biological stimulus and the osteoconductive framework to enhance bone tissue repair and has been shown to enhance bone regeneration in preclinical and clinical studies[1,2].

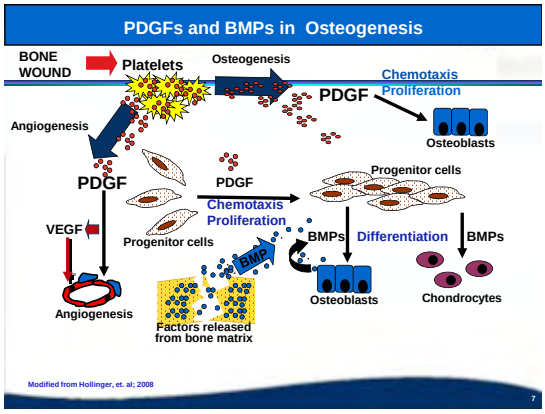

Fig. 1: PDGFs and BMPs both play critical but different roles in the healing of bone injuries. PDGFs function more "upstream", serving as one of the key initiators of the healing cascade. BMPs primary role occurs later in the healing cascade to drive the healing process towards osteogenesis.

METHODS: Pure, recombinant human (rh) PDGF-BB in combination with osteoconductive allograft or synthetic TCP matrices has been evaluated in a variety of periodontal and orthopaedic bone healing indications, including periodontal and peri-implant defects, alveolar ridge augmentation, fracture repair and foot and ankle fusion.

RESULTS: Results are available from 279 patients in four clinical trials and three anatomic sites. Clinical results were first achieved using *rhPDGF* for the treatment of bone loss associated with advanced periodontal disease. Results from a 180 patient prospective, randomized, controlled, pivotal clinical trial demonstrated that treatment with *rhPDGF+TCP* resulted in robust alveolar bone formation that was significantly greater than the results observed with the TCP alone. Human block sections have confirmed the osteogenic response to rhPDGF+allograft. To date an estimated 80,000 orofacial cases have been treated with rhPDGF[1].

In a recent 60 patient trial using rhPDGF-BB combined with β-TCP for fusions in the foot & ankle, bony fusion as measured by radiographic and clinical assessments was achieved in 87%-90%, respectively, of patients by nine months. In total, 124 of 130 treated joints were considered clinical successes, as measured by no need for revision surgery through one-year follow up. In addition, the clinicians reported that the high fusion success rate was accompanied by superior overlying soft tissue healing characterized by a complete absence of dehiscences and no deep tissue infections. Additionally, a pilot 20 patient RCT compared rhPDGF+TCP to autogenous bone graft for foot and ankle fusions. On 12 week CT scans, 69% of rhPDGF subjects showed 50% or greater osseous bridging compared to 60% autograft subjects. There was no abnormal bone formation observed[1,2].

Results of a 40 patient clinical trial assessing rhPDGF plus fixation to fixation alone in distal radius fractures will be presented at the meeting.

DISCUSSION & CONCLUSIONS: The combined clinical data provides support for the safety and effectiveness of *rhPDGF* for the treatment of bony injuries.

REFERENCES: [1]Lynch SE et al. (2008) *Tissue Engineering: Appl in Max Surg Perio, 2nd Edition.* [2] Hollinger JO., et al. (2008) *JBJS.*

ACKNOWLEDGEMENTS: These studies were supported by BioMimetic Therapeutics, Inc.

Synthes Strategy for Combination Devices

Elliott A. Gruskin

*Synthes, Biomaterials Division, West Chester, Pennsylvania, USA**Orthopaedic surgeons have an extensive array of implants to tackle clinical problems. The potential to couple implants with therapeutic modalities is gaining significant interest and industry is reacting to this new demand. Therapeutics can include growth factors to promote bone formation, analgesics, chemotherapeutics and antibiotics.*

Synthes is confronting the problem of implant-related infection. Colonization of implant surfaces with bacteria is a predecessor to infection and osteomyelitis. Implant surfaces can provide a substrate for biofilm formation wherein bacteria evade the host immune system and are less susceptible to antibiotic therapy. Consequently, the implant surface is the first battleground to defend against infections. An anti-infective implant coating is one approach to inhibit colonization of an implant surface. If biofilm formation can be prevented, then the likelihood of an implant related infection is reduced.

Synthes developed a resorbable gentamicin-impregnated coating for tibia nails. This presentation will describe several of the challenges encountered in the development of the coated nail as a case study to define a strategy for the development of similar combination devices.

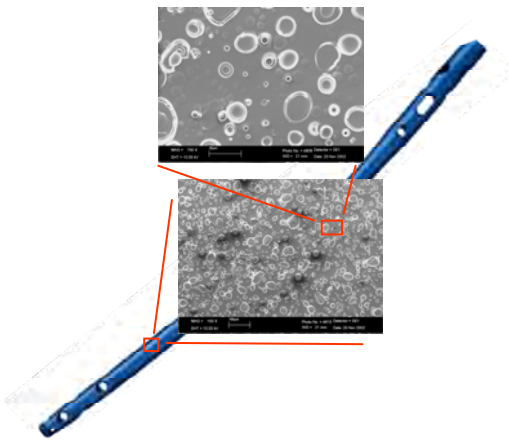

Fig. 1: The tibia nail depicted here is coated with a thin, but extremely durable film consisting of fully amorphous poly(D,L-lactide) impregnated with particles of gentamicin sulfate.

The presentation will start with a general description of the gentamicin-coating including the *in vitro* assessment of the gentamicin release kinetics. Gentamicin particles are suspended in the polymer matrix and are released by an initial burst over the first few hours followed by a more gradual release of the remainder over several days. A series of animal models were conducted in rats and rabbits using coated pins that were placed in the tibia along with an inoculation of *staph aureus*. After a period of time the pins were removed to determine the level of bacterial colonization. Radiographs and histology were also performed to assess tissue responses to the coatings and evidence for peri-implant infection. In addition, to these small animal studies a similar study was performed in sheep using a coated human humeral nail to more closely approximate the human clinical situation. In all preclinical studies the gentamicin coating provided near complete protection against bacterial colonization.

Having demonstrated the safety and efficacy of the gentamicin coating in animals a coated tibia nail was developed and cleared for clinical use. Initial limited clinical use in Europe started, but regulatory approvals in the US will require a clinical trial. The development process included substantial challenges in product development including process validation and analytical demands. On-going challenges, both process related and from regulators, resulted in a challenging development cycle exposing several important conclusions:

- Combination devices require significant preclinical testing and substantial process and quality demands
- The development cycle of the device is more rapid than that for the combination device. Consequently, second generation versions of the device can reach the market before it is possible to launch the first version of the combination device.
- The regulatory environment is constantly evolving

The gap between research and clinical application of bone substitutes

M. Bohner

Dr Robert Mathys Foundation, Bischmattstrasse 12, 2544 Bettlach, CH.

The American Society of Biomaterials is organizing a meeting on "Translational Research". This new term refers to the branch of medical research that attempts to more directly connect basic research to patient. The need to organize a large meeting on such a topic is the expression of the increasing complexity of product development, from research to product certification. The goal of this communication is to present the perspective of a researcher who has been involved in industrial research for the last 10 years.

Raw materials and product design – There are many obstacles that can prevent excellent research results to be transformed into a product. Very often, problems result from a bad choice of raw materials or difficulties arising from the combination of raw materials (e.g. polymer – ceramic composites). When choosing raw materials or designing a product, the following questions should be answered: What is the raw material availability (licensing, patents, producers)? Purity? Certification status (e.g. topical, oral, parenteral use)? Ability to be sterilized (particularly in composites polymer-ceramic or polymer-metal)? Long-term stability (shelf-life, conservation temperature)? Cost? Flowability (for a powder)? If the answer to one of these questions is negative, a different solution must be searched for. In fact, the final product might have to be formulated or presented to best accommodate these various problems.

Handling – When these questions have been clarified and a prototype can be proposed which might potentially lead to a large clinical benefit, only products with an easy handling have a chance to be successful. For example, an injectable calcium phosphate cement must have an easy and reliable mixing, the paste must be well injectable (no syringe plugging) and the cement must harden in contact to body fluids.

Social, economic and political hurdles – Assuming that production and handling are satisfactory, many other aspects might prevent a successful market introduction. Potential obstacles can be:

- Political – for example, in some countries, only products that have been tested in vivo in the country itself can be approved
- Strategical – for example, the industrial project partner might not be interested in launching new products that might decrease the market share of one of his/her own products
- Regulatory – for example, the requirements set for product certification vary from country to country which can lead to large variations of the product types available in different countries
- Socio-economic – for example, in most countries, the cost of a surgical operation is not considered globally but is decomposed into several parts such as the share of the patient, the social security, and the company in which the patient works
- Religious – for example, certain product (e.g. allogenic products) are forbidden in certain countries.
- Temporal – the first synthetic bone substitutes were commercially available in the early 1980's, but the market was not ready (HIV or BSE were not known at that time).
- Intellectual property (IP) – in some countries, the IP rights belongs to the universities in which the research work was done, independently of the source of financing
- Financial – aspects to consider are the global market size, country size, desired and expected product price, agreement between hospital and company, insurance costs, etc…
- Risk of law suits – a law suit in the largest market in the world (US) can lead to bankruptcy

To summarize, the large number of aspects to consider during product development prevents a rapid translational research and hence product innovation. The increasing need for security and regulations is not improving the situation.

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A Successful Paradigm For NIH Funding In CMF: Enhancing Translational Research By Expanding The Network Between The Basic And Clinical Scientist. (Successful Development of an Interdisciplinary Collaboration)

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INTRODUCTION: Advances in biotechnology and surgical instrumentation have afforded a unique opportunity for collaborative efforts combining knowledge from both basic and clinical investigation to conduct translational research. Successful translational research that aims to bring discoveries from the bench to the bedside will require the amalgamation and coordination of specialized expertise among a diverse group of investigators in different disciplines that can integrate their capabilities to define, test, and validate the specific outcome metrics that can accurately measure the success of therapeutic strategies to treat clinical problems. This new paradigm of translational research has shown success in garnering NIH funding and has the potential to enhance the conventional model of isolated individual investigations by fostering the development of networks of basic and clinical scientists in order to by bring novel and more effective therapeutic strategies into clinical settings.

METHODS: An R01 grant application was sought by the PI, a clinically active Cranio-Maxillofacial Plastic Surgeon, aimed at a remedy for the pernicious effect of radiation on bone regeneration and fracture healing. Utilizing the expertise of a PhD in Orthopaedic Bioengineering as a co-investigator, the PI's laboratory engaged in the creation and gradual enhancement of novel experimental models and the corresponding investigations into the generation and maintenance of bone growth. Further networking and collaboration with an AO-BAB funded DDS PhD co-investigator added key preliminary data for the utilization of tissue engineering therapeutics by exploiting the anabolic actions of PTH to augment stem cell auto-transplantation in clinically relevant radiation induced injury in the rat mandible.

RESULTS: The central hypothesis to be tested in the R01 proposal was that the deleterious effects of radiation on bone formation could be mitigated to allow functional restoration and successful regeneration of the mandible. To test this hypothesis a novel rodent model of Distraction Osteogenesis and fracture repair would be utilized to generate specific metrics of diminished bone quality within the regenerate of irradiated distracted mandibles. A series of pharmacologic and tissue engineering strategies would then be employed to assuage the adverse impact of radiation induced injury on new bone formation and healing in order to optimize reconstruction and repair. Successful funding for the R01 was awarded by the National Cancer Institute upon the first submission after review by the Musculoskeletal Tissue Engineering Study Section whose central theme of applications is translational research at the interface between basic cellular processes, materials sciences and modeling on the one hand, and clinical treatment on the other, with an emphasis on pre-clinical biological questions.

DISCUSSION & CONCLUSIONS: Extra-mural federal funding is becoming much more challenging in the United States. Funding agencies are showing great interest in the translation of basic scientific findings into clinically useful applications. Expanding the network between the basic scientist, engineer, and the clinician-scientist has proven to be a successful paradigm to secure NIH funding in the field of Cranio- maxillofacial surgery. This strategy may also prove to be a winning model in attempts to secure funding for translational research in other disciplines. Multidisciplinary networks such as AO-BAB have fostered the link between the basic scientist and the clinician, additional collaborations and the strategic use of technology may aide in further expansion of such networks with researchers in both universities and other consortiums throughout the world.

ACKNOWLEDGEMENTS: I would like to acknowledge my co-investigators, Steven Goldstein PhD and **Paul Krebsbach** DDS PhD.

Saturday, October 4, 2008

Session 4a

Stimulation of bone & cartilage repair

CHAIR: Norbert Südkamp, Albert Ludwigs Universität Freiburg, Germany

MATERIALS FOR ANGIOGENESIS ON DEMAND

David Mooney
Harvard University, Cambridge, MA USA

Networks of new blood vessels are essential to provide nutrients, circulating factors, and stem cells in virtually all tissue regeneration efforts, and technologies to drive neovascularization may also find utility in the treatment of ischemic diseases. Most attempts to drive this process go directly from biological discovery (e.g., identification of growth factors or cell types) to therapy, and poorly mimic the complex interplay of multiple factors and cells that regulate angiogenesis and vasculogenesis. This likely underlies the limited success to date of both growth factor and cell transplantation approaches to neovascularization (Rosenzweig 2006; Henry et al., 2003).

Engineering successful neovascularization strategies will likely require specific quantitative information of the role of various factors and cells in angiogenesis, and the development of sophisticated delivery approaches that provide appropriate spatiotemporal regulation over this signaling. The ability of materials to regulate the presentation and environment of cells and morphogens suggests they may be useful in driving this process. 3-D in vitro models are first used to develop design criteria (e.g., role of morphogen concentrations and gradients) for the materials (Chen et al., 2007). Subsequently, materials based on these design criteria (e.g., inclusion of cell adhesion peptides and/or morphogens) allow one to program both resident endothelial cells and transplanted progenitor populations to regulate the extent, maturation, and functionality of engineered networks of blood vessels (Richardson et al., 2001; Silva et al., 2008). This approach to locally regulate angiogenesis may have a wide array of potential applications, which include promoting bone regeneration (Huang et al., 2005; Kaigler et al., 2006).

References:

Chen RR. Silva EA. Yuen WW. Brock AA, Fischbach C. Lin AS. Guldberg RE. Mooney DJ. Integrated approach to designing growth factor delivery systems. **FASEB J**. 21:3896-903, 2007.

Henry TD, Annex BH, McKendall GR, et al. The VIVA trial: Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis. **Circulation**, 107(10):1359, 2003.

Huang YC, Kaigler D, Rice KG, Krebsbach PH, Mooney DJ. Combined angiogenic and osteogenic factor delivery enhances bone marrow stromal cell-driven bone regeneration. ***J Bone Miner Res***. 20(5):848-57, 2005.

Kaigler D, Wang Z, Horger DJ, Krebsbach PH. VEGF scaffolds enhance angiogenesis and bone regeneration in irradiated osseous defects. ***J Bone Miner Res*** *21:735-44, 2006.*

Richardson TP, Peters MC, Ennett A, Mooney DJ. Polymeric system for dual growth factor delivery. ***Nature Biotechnology***, 19:1029-1034, 2001

Rosenzweig, A. Cardiac cell therapy--mixed results from mixed cells. **N Engl J Med** 355:1274, 2006.

Silva EA, Kim ES, Kong HJ, Mooney DJ. Material-based deployment enhances efficacy of endothelial progenitor cells. **Proc Natl Acad Sci** USA 105:14347-52, 2008

Molecular and Cellular Mechanisms of Pathologic Bone Remodeling in Inflammatory Conditions

SR Goldring, Hospital for Special Surgery, Weill Medical College of Cornell University, New York, NY, USA.

We have utilized human and animal models of pathologic bone resorption to gain insights into the cellular and molecular mechanisms involved in pathologic bone remodeling. Particular attention has focused on rheumatoid arthritis (RA) and osteolysis associated with failed orthopedic implants. Analysis of samples from the bone-pannus interface from patients with RA and juvenile rheumatoid arthritis reveals that the multinucleated cells in resorption lacunae express the phenotypic features of osteoclasts.[1] The osteoclastic bone resorption is induced by potent cytokines and immunomodulatory factors that are produced in the inflamed synovium, including receptor activator of NF-kB ligand (RANKL) that is required for osteoclast differentiation and activity. To demonstrate that osteoclasts are essential for the development of focal bone erosions, we induced inflammatory arthritis in RANKL-null mice using a novel serum transfer model.[2] In the absence of osteoclasts, we could detect no significant bone erosions, despite the formation of extensive synovial inflammation. In addition to the localized increase in bone resorption in inflamed joints, bone formation and repair are impaired. Recent evidence suggests that the defect in bone formation results at least in part from the production of molecules such as Dickkopf-1 (DKK-1), which is a potent inhibitor of the Wnt bone formation regulatory system[3,4]. Analysis of tissues from sites of bone erosion in RA specimens also reveals that cells expressing the full morphological and functional properties of mature osteoclasts are restricted to the immediate bone surface.[5,] This finding suggests that, in addition to cytokines, components of the bone matrix play an essential role in determining the genetic profile and functional properties of fully differentiated resorbing osteoclasts. This hypothesis is supported by analysis of retrieved tissues from patients with peri-implant osteolysis.[6] We have employed expression profiling with an *in vitro* model of matrix-dependent osteoclast differentiation to identify the molecular pathways by which bone matrix-interactions induce terminal osteoclast differentiation and activation.[5] In preliminary studies we have identified unique genes and transcriptional pathways that are induced by interaction of osteoclast precursors with specific components of the mineralized bone matrix. The authenticity of the gene profiles as markers of osteoclast differentiation and activation have been provisionally validated using an *in vivo* animal bone implantation model and by examination of tissues from patients with specific forms of pathologic osteoclast-mediated bone resorption. The ultimate goal of our studies is to identify new molecular targets for inhibiting osteoclast-mediated bone loss in disorders of pathologic bone loss.

REFERENCES

1. **Gravallese et al. Am J Pathol. 1998; 152:943-951**
2. **Pettit et al. Am J Pathol. 2001; 159:1689-1699**
3. **Diarra et al. Nat Med. 2007; 13:156-163**
4. **Goldring SR, Goldring MB. Nat Med. 2007;13:133-4**
5. **McHugh et al. Adv Exp Med Biol. 2007; 602:107-11**
6. **Shen et al. Arthritis Res Ther. 2006; 8:R70**

AO Foundation

THE FIRST 50 YEARS

The cellular composition of the initial fracture and soft tissue hematoma during the inflammatory phase of the healing process

K. Schmidt-Bleek[1], H. Schell[1], P. Kolar[2], C. Perka[1], F. Buttgereit[2], G. Duda[1], J. Lienau[1]
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INTRODUCTION:
Bone healing involves a cascade of events that begins with the formation of a hematoma and an inflammatory response. It was shown that the fracture hematoma is essential for the initiation of the healing cascade [1], but the cellular composition of the hematoma remains largely unknown. The aim of this study was to characterize and quantify immune cell populations present in the initial fracture hematoma. Since bone is one of the few tissues in the body capable of healing without the formation of scar tissue, it was further intended to compare the results with those gained from cell characterization of a soft tissue hematoma.

METHODS:
A mid-shaft osteotomy of the tibia was performed in skeletally mature female sheep (n=6) and stabilized with an external fixator. In addition, a soft tissue trauma was induced in the *M. gracilis* of the same hind limb. The hematoma were harvested 1 and 4 hours postoperatively. Preoperatively, peripheral blood was taken as a reference. The cells were prepared for FACS-analysis and labelled with CD45, CD5, CD2, CD8, CD4, CD21, CD14, WC1, CD25. For statistic evaluation, the Wilcoxon test was used (SPSS 14.0). A p-value of less than 0.05 was taken as a significant difference.

RESULTS:
Cell numbers increased in the fracture and soft tissue hematoma from 1 to 4 hours. However, the fraction of dead cells was highest in the four hour soft tissue hematoma.
Comparing the amount of granulocytes present in the fracture and soft tissue hematoma 1 hour post osteotomy, a significantly lower percentage ($p=0.031$) was found in the fracture hematoma.
The CD21 positive cells showed an increase from 1 to 4 hours in the fracture hematoma, whilst in the soft tissue hematoma the corresponding cells declined. In the 4 hour hematoma, a distinctly higher percentage of CD21 positive cells ($p=0.063$) was found in fracture compared to soft tissue hematoma.
A higher percentage of the T-helper cell population was detected in the fracture hematoma compared to the soft tissue hematoma at one and four hours. Considering the CD4:CD8 ratio the larger T-helper cell percentage in the fracture hematoma was confirmed ($p=0.063$).

DISCUSSION AND CONCLUSIONS:
For the first time the cellular composition in the early fracture hematoma has been investigated under standardized conditions. Changes in the cellular composition of the fracture hematoma over a time course have been found as well as differences to the cellular composition of a comparable soft tissue hematoma. In the fracture hematoma the cell death was less pronounced, the granulocyte percentage was lower and the T-helper cell percentage was higher in the T-cell population compared to the soft tissue hematoma. These differences in the cellular composition between fracture and soft tissue hematoma might be based on a uniquely coordinated inflammatory process in the fracture hematoma. The divergences in the immune cell populations might result in a special sequel of signalling molecules in the fracture hematoma contributing to the healing outcome and may be an explanation for the regenerative capacity of fractured bone.

REFERENCES:
[1] Park S-H. et al., J Orthop Res 20, 2002

ACKNOWLEDGEMENTS:
This study was supported by the German Research Foundation (DFG SFB760).

Oxygen measurement in three-dimensional scaffolds for the tissue engineering of bone

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Abstract

Tissue engineering provides the potential for unlimited bone substitute material. So far clinical applications of scaffold-based tissue engineering of bone exceeding a critical size failed. It has been hypothesized that uneven oxygen and nutrient supply are the limiting factors for cell survival within cell-seeded three dimensional (3D) constructs.

Therefore we continuously measured the oxygen concentration within the centre of cell-seeded scaffolds using needle type oxygen microsensors and analysed the effect on cell survival under static and dynamic cell culture conditions. The oxygen measurements were used to optimize dynamic 3D cell culture conditions regarding the perfusion speeds.

Our results show that under static conditions central oxygen concentrations drop to 0% after as little as 5 days leading to cell death in the centre of the scaffold. Dynamic cell culture conditions significantly increase central oxygen concentrations thus promoting cell survival in central areas of the constructs.

We conclude that oxygen is a key factor when optimizing three dimensional culture conditions. These limitations have to be overcome in order to guarantee an effective (pre-) clinical use of tissue engineered bone grafts.

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THE FIRST 50 YEARS

Saturday, October 4, 2008

Session 4b

Stimulation of bone & cartilage repair

CHAIR: Adrian Sugar, Chairperson AO Research Fund, Morrison Hospital, Swansea, UK

AO Foundation

THE FIRST 50 YEARS

Remodeling rAAV-Coated Allografts for Musculoskeletal Repair

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Abstract

In this paper, we review our progress towards developing strategies to engineer improved structural grafting of bone. We have previously established a murine femoral model in which allografts can be revitalized via recombinant adeno-associated virus (rAAV) gene transfer. Specifically, allografts coated with rAAV expressing either the constitutively active BMP type I receptor Alk2 (caAlk2, which is now known to be the mutation that causes fibrodysplasia ossificans progressiva (FOP)), or the angiogenic factor VEGF combined with the osteoclastogenic factor RANKL, have remarkable osteogenic, angiogenic, and remodeling effects that have not been previously documented in healing allografts. Using histomorphometric and micro computed tomography imaging we show that rAAV-mediated delivery of caAlk2 induces significant osteoinduction manifested by a voluminous mineralized callus on the surface of the allograft, which resembles the healing response of an autograft. We also demonstrate that the rAAV mediated gene transfer of the combination of VEGF and RANKL can induce significant vascularization and remodeling of processed structural allografts. By contrast, rAAV-LacZ coated allograft controls appeared similar to necrotic allografts and lacked significant mineralized callus, neovascularization, and remodeling. Therefore, the rAAV-mediated gene delivery offers a novel acellular approach in tissue engineering of structural bone substitutes that can potentially have clinical applications in challenging indications.

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THE FIRST 50 YEARS

The Role of Osteoclasts in Osteoblast Regulation

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INTRODUCTION: Many diseases associated with bone loss, such as osteoporosis and periodontitis, are due to imbalance between bone formation by osteoblasts and bone destruction by osteoclasts, which in turn depends on the coupling between osteoclasts and osteoblasts [1]. Osteoblasts are known to produce critical osteoclast regulators, M-CSF and RANKL [2]. However the effects of osteoclasts on osteoblasts are not well understood [3]. The aim of this project is to examine if osteoclasts affect osteoblast differentiation.

METHODS: Bone marrow cells were collected from C57BL6/J mice (tibia & femurs) and cultured during 7 days in the presence of ascorbic acid (AA, 50 μg/ml) alone or with a combination of ascorbic acid and receptor activator for nuclear factor κB ligand (RANKL, 50 ng/ml). After 7 days of culture, alkaline phosphatase (ALP) stain and tartrate-resistant acid phosphatase (TRAP) stain were used as markers for osteoblast and osteoclast differentiation respectively. Differentiated osteoblastic cells formed specific nodules of ALP positive cells. Image analysis (ImageJ, NIH) was further employed to quantify the percentage of area covered by ALP positive cells, the size of osteoblastic nodules and density of ALP staining.

RESULTS: After 7 days of mouse bone marrow cell culture, in the untreated culture condition, few ALP positive cells were observed and no TRAP positive cells were present. In AA culture condition, a large number of ALP positive cells were observed covering 18 ± 6% of the well but, as for the untreated condition, no TRAP positive cells were present. With a co-treatment of AA and RANKL, a large number of ALP positive cells were observed in addition to the giant cells between and underneath these ALP positive cells (Fig. 1). These giant cells were revealed to be multinucleated and TRAP positive. Stimulation of osteoclastogenesis resulted in the formation of 100 ± 50 osteoclasts/cm^2, generally imbedded within osteoblastic nodules, which covered 14 ± 5% of the well. However, presence of osteoclast in the culture did not induce difference in cell density. We analyzed if the presence of osteoclasts locally affected the size or appearance of individual osteoblastic nodules. Image analysis of nodules formed after treatment with AA or AA and RANKL demonstrated that osteoclasts were generally present in larger nodules. Interestingly, when nodules of similar size were compared, the density of ALP staining was significantly higher in the nodules containing osteoclasts (Table 1).

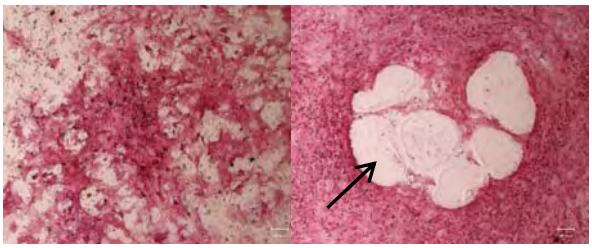

Figure 1: Mouse bone marrow cell cultures treated for 7 days with ascorbic acid (left) and ascorbic acid + RANKL (right). Note osteoclast presence arrow).

Relative Size of Nodules (% of field)		Average ALP Stain Density of Nodules	
Without osteoclasts	**With osteoclasts**	**Without osteoclasts**	**With osteoclasts**
82.84 ± 4.14	**91.13 ± 2.06**	**89.49 ± 4.48**	**139.50 ± 4.94**

Table 1: Effect of the presence of osteoclasts on both size and staining density of ALP-positive nodules.

DISCUSSION & CONCLUSIONS: Our data demonstrate that osteoclasts locally stimulate osteoblast differentiation and further suggest that osteoclasts produce local factors that stimulate ALP expression by osteoblasts. This finding may lead to new approaches for the treatment of bone diseases associated with imbalances in bone remodeling.

REFERENCES: [1] M. Zaidi (2007) *Nat Med* **13**:791-801. [2] T.J. Martin, N.A. Sims (2005) *Trends Mol Med* **11**:76-81. [3] R.J. Galvin, J.W. Cullison, L.V. Avioli, P.A. Osdoby (1994) *J Bone Miner Res* **9**:1167-78.

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Mesenchymal Stem Cells for the Augmentation of the Maxillary Sinus

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INTRODUCTION: Autologous, allogenic and alloplastic materials for the reconstruction of bone and soft tissue have specific applications in the cranio-maxillofacial area [1, 2]. The research for biomaterials and tissue engineering procedures aims at new synthetic and autologeous materials. Cultivated skin and mucosa are already in clinical routine use in head and neck reconstruction. There have been many draw backs in the research of bone substitute materials because of the oxygen-sensitivity of osteoblasts[3]. We suggest that mesenchymal stem cells (MSCs) are the better source because the are able to proliferate under low oxygen tension and differentiate when the oxygen level rises[4]. Depending on the micro-environment MSCs have the ability to differentiate into osteoblasts[5]. In animal experiments stem cell application in combination with a bio material (BioOss) show lamellar bone formation and bone invasion into the micropores. So far there has been no successful clinical application to the best of our knowledge of processed stem cell-derived bone for augmentation of the edentulous posterior maxilla.

METHODS: In a pilot study, the augmentation of the posterior maxilla was carried out using stem cells from bone marrow aspirate concentrate (BMAC) on a bovine bone matrix (BioOss®, Geistlich Wolhusen, Switzerland). The mesenchymal stem cells were purified from the aspirate by an especially developed cetrifuge. The phenotype was proven by plastic adherence and flowcytometrical-analysis of relevant markers as CD 44, CD 73, CD 90, CD 166, CD 34 and CD 45. Pluripotency was evaluated by a differentiation assay. Biopsies were harvested with a trephine burr when dental implants were inserted 3 months after sinus augmentation.

RESULTS: The results suggest that stem cell derived osteoblasts form lamellar bone within 3 months which allows reliable implant insertion. 50 maxillary sinus have been treated so far with this procedure. No dental implant was lost over the observation period. Histomorphometric analysis showed new bone formation in the transplant. The FACS-analysis revealed cells which were positive for CD 44, CD 73, CD 90, CD 166 and negative for CD 34 and CD 45. Plastic adherent cells showed the same FACS-characteristics as the afore mentioned fraction of the BMAC. The cells could be differentiated into adipocytes, chondroblasts and osteoblasts.

DISCUSSION & CONCLUSIONS: The successful clinical application and the histological results of this pilot study show the feasibility of this procedure. FACS-analysis and in-vitro tests prove that actually mesenchymal stem cells had been transplanted.

REFERENCES: [1] Sauerbier, S., Gutwald, R., Wiedmann-Al-Ahmad, M., Lauer, G., Schmelzeisen, R. (2006) Clinical Application of tissue engineered Transplants - Part I: Mucosa. Clinical Oral Implants Research, **17**:625-32. [2] Schmelzeisen, R., Schimming, R., Sittinger, M. (2003) Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation- a preliminary report. Journal of Cranio-Maxillo-Facial Surgery, **31**:34-39, [3] Zizelmann, C., Schoen, R., Metzger, M.C., Schmelzeisen, R., Schramm, A., Gellrich, N.C. (2007) Bone formation after sinus augmentation with engineered bone. Clinical Oral Implants Research, **18**:69-73. [4] Lepperdinger, G., Fehrer, C., Laschober, G., Brunauer, R. (2006) Mesenchymal Stem Cell Aging. Nova Acta Leopoldina NF 95, **352**:151. [5] Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S. & Marshak, D.R. (1999) Multilineage potential of adult human mesenchymal stem cells. Science, **284**:143-147.

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AO Foundation

THE FIRST 50 YEARS

Development of brushite matrices able to stimulate bone remodelling

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INTRODUCTION: Calcium phosphate (CaP) biomaterials are the main alternative to biological bone grafts and can be employed as a matrix for localised delivery of therapeutic components [1]. Although CaP cements are resorbed, they lack osteogenic potential [2]. A recent study demonstrated that RANKL and VEGF are required for bone allograft remodelling [3]. Thus we have investigated the capacity of brushite cement to act as a release matrix for RANKL. The stability of system was further tested and various regimens to improve stability were compared.

METHODS: Brushite cement was prepared by mixing β-tricalcium phosphate and monocalcium phosphate hydrate with a citric acid solution. A 3% sodium alginate solution was cross linked with $CaCl_2$ solution and both brushite cement and sodium alginate gel were set in the form of discs 3 mm diameter. Different volumes of a RANKL (50 µg/ml) or RANKL-trehalose (300 mM) were coated onto brushite or injected into alginate. To test the stability of our system, formulations were stored for various times points (from 30 minutes to 5 weeks), at two different temperatures (at 4ºC and at -20ºC) and the effect of light exposure humidity and oxygen were assessed.

To assess biological activity of RANKL-loaded biomaterials formulation, osteoclastogenesis from RAW264.7 cells was tested. After 5 to 7 days of culture the number of multinucleated TRAP positive cells was assessed and the actin ring formation was characterized.

RESULTS: Sodium alginate injected with RANKL was ineffective in inducing osteoclast formation. Osteoclastogenesis was only observed with at least 800 ng of RANKL coated onto brushite cement. Stability of RANKL solution coated onto brushite cement cylinders and the effect of trehalose addition to the coated RANKL formulation were evaluated. Addition of trehalose slightly increased the stability of RANKL after 30 minutes storage but increased by 5 fold the bioactivity of the protein after 1 day's storage. Effect of trehalose in RANKL-coated brushite cement formulations was further investigated during 4 consecutive cell cultures (Fig. 1). The percentage of TRAP positive cells induced by RANKL-coated brushite cement continuously decreased from 162 ± 4% after the first 7 days culture period to 15 ± 2% of positive control after 28 cumulative days of culture. In contrast, the presence of trehalose significantly increased the osteoclastic formation to 75 ± 2% after 28 cumulative days of culture.

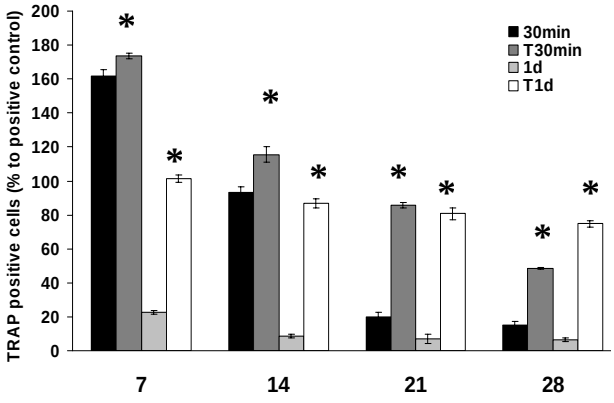

Figure1: Percentage of TRAP positive cells normalized to positive control induced during several culture periods ($p < 0.01$).

The bioactivity of RANKL-trehalose loaded cement stored for 3 and 5 weeks decreased by 40% compared with the same formulations stored for 1 day. However there was no further deterioration of the RANKL bioactivity after 3 weeks. Exclusion of light had no effect and the stability of our system. However, reducing storage temperature to -20°C and the absence of oxygen were found to improve by 20% the long term stability of RANKL.

DISCUSSION & CONCLUSIONS: The results show that brushite cement loaded with RANKL solution directly stimulates osteoclast formation. RANKL stability was improved by the presence trehalose and modification of storage parameters. This formulation is suitable for the delivery of key molecules critical for bone remodeling process.

REFERENCES: [1] U. Gbureck, E. Vorndran, F.A. Müller, J.E. (2007) *J Control Release* **26**:173-80.[2] J. Jansen (2005) *Orthop Clin North Am* **361**:89-95.[3] H. Ito (2005) *Nature Medicine* **11**:291-297.

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AO Foundation

THE FIRST 50 YEARS

A bioactive guided bone regeneration membrane enhances BMP signalling and tunes the natriuretic hormone system into a pro osteogenic state

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INTRODUCTION: Medical devices derived from biomaterials of the 3rd generation are biocompatible, biodegradable and bioactive. Recently we developed and characterized a 3rd generation guided bone regeneration membrane and showed that the plasticizer N-methyl-pyrrolidone (NMP) is bioactive, since it enhances bone formation *in vivo (Fig. 1)*. The bone morphogenetic protein (BMP) triggered pathway is certainly central for bone formation and repair, but the skeleton is also affected by additional growth factors and hormones. Dwarfism, manifested in the human disease Acromesomelic dysplasia Maroteaux type is linked to mutations in the natriuretic peptide receptor 2[1]. Over-expression and knock-out experiments in mice showed that also other elements of this system can affect the skeleton by decreasing or increasing bone formation. In order to get a wider view on the effect of NMP exposure to cells of the osteoblastic lineage, a micro array experiment was performed, which showed that the natriuretic hormone system is affected by NMP.

METHODS: MC3T3-E1 pre-osteoblastic cells were tested for different cell maturation responses: ALP (Alkaline phosphatase activity) and Alizarin Red mineralization assay. Micro array experiments were performed with C2C12 cells. The effect of NMP was determined *in vivo* in a guided bone regeneration model.

RESULTS: NMP enhances bone regeneration in a guided bone regeneration model in vivo (Fig.1) In vitro, NMP increased ALP activity and mineralization of MC3T3-E1 cells concentration dependent. In combination with rhBMP-2 NMP showed a synergistic effect on ALP activity, mineralization p38 and Smad 1,5,7 phosphory-lation. Micro array experiments performed with the multi potent mesenchymal stem cell revealed that 4 h exposure of C2C12 cells to 5mM of NMP halved the expression of natriuretic peptide receptor type 3 (npr3) and increase in the expression of the natriuretic peptide precursor type B (BNP) 1.5 fold. These results were confirmed by low density arrays and RT-pcr.

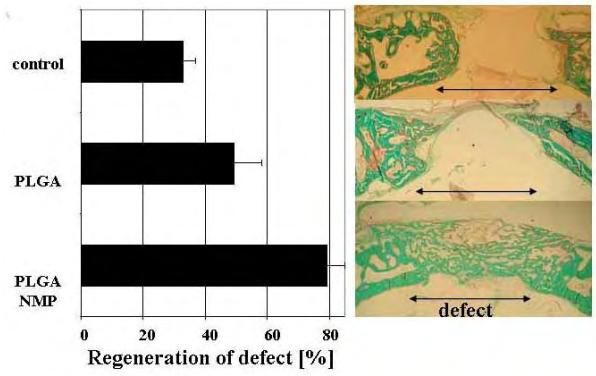

Figure 1: Enhancing of bone repair by guided bone regeneration and NMP: Defects of 6 mm in diameter created in the calvarial bone were treated with PLGA membrane, NMP-PLGA membrane or left untreated (control). The left panel shows the percentages of the area where bone regeneration in the defect has occurred.

DISCUSSION & CONCLUSIONS: The results show that NMP enhances bone regeneration in vivo. In part, this effect can be attributed to the synergistic effect of NMP and BMP since NMP enhances the kinase activity of the BMP-BMP-receptor complex.

Interestingly, NMP shows also effect in the absence of BMP. The plasticizer NMP decreases npr-3 expression and increases BNP expression, mimicking the knock-out and over-expression of those genes in mice. Therefore, the direct effect of NMP on the transcription of 2 elements of the natriuretic peptide hormone system in a pro osteogenic way could at least partially account for the accelerated bone healing seen under the influence of NMP *in vivo*.

REFERENCES: [1]Bartels CF, Bükülmez H, Padayatti P, et al .Mutations in the transmembrane natriuretic peptide receptor NPR-B impair skeletal growth and cause acromesomelic dysplasia, type Maroteaux. Am J Hum Genet. 2004 75(1):27-34

ACKNOWLEDGEMENTS: This study was in part supported by Inion OY, Tampere Finland and by a grant from the AO Foundation (Davos, Switzerland) through the Biotechnology Advisory Board.

Saturday, October 4, 2008

Lunch Session

Networking in a clinically inspired research community

CHAIR: Jill Urban, Oxford University, Oxford UK

The Canadian Arthritis Network

Jane E. Aubin, University of Toronto, Toronto, Ontario Canada and Scientific Director of the Institute of Musculoskeletal Health and Arthritis, Canadian Institutes of Health Research

The German Rheumatology Competence Network

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Starting in 1999, the Competence Network has created a multidisciplinary national consortium devoted to excellence in basic, clinical and health care research in the field of rheumatology in Germany. One of the major aims has been to build up and further develop close and sustainable collaboration among and between researchers and clinicians. Today, the Competence Network Rheumatology is an internationally recognised research structure and has put Germany in a favourable position in the competition for European research grants. Crucial elements of this progress were the development of horizontal and vertical networking structures, a central network management, funding of individual research projects, research coordination, joint data bases and biobanking, a uniform communication platform and intensive PR work.

While in the 2nd funding period, from 2003 – 2004, individual projects were funded with results outlined in last years report, from 2005 federal funding was reduced to projects dealing with the completion and the maintenance of material banks, projects of the health services research section and the funding of the central structures. However, since the researchers participating in the network were able to raise funds from other resources such as the German Research Foundation or EU grants, the collaboration was maintained and extended even without grants given by the Network. Based upon a decision in the coordination committee and the existence of externally reviewed and funded projects, new groups were integrated. An important stimulus for research was set by the funding programme of the DGRh in 2005.

The specific **scientific aims** of the Network for the time period 2005 – 2007 have been

- to monitor the outcomes of current health care
- to develop evidence-based guidelines for diagnosis and therapy
- to identify markers allowing a prognostic classification of rheumatic diseases
- to identify markers predicting success of therapy
- to develop concepts for cell-based, curative therapies

Large patient databases were continued and further developed in order to monitor longitudinally disease outcomes and health care provision. The German national databases for adults and children with inflammatory rheumatic diseases are internationally unique. They provide sound information on the processes and outcomes of rheumatologic health care in the era of new therapies and the ongoing changes in the health care system. The databases were transformed to IT data entry, and funds from other sources (industry, arthritis centres) were successfully acquired. The continuation in 2008 is secured. Cohorts built up in the second funding period were followed-up and completed. Sound methodology has been guaranteed by central biometrical advice.

The evidence based guideline on the management of early rheumatoid arthritis was updated and released in a second edition in 2007. Guideline development and integration of EBM into medical education and training will be continued in collaboration with the "Rheuma Academy" an institute for continuing medical education set up by the German Society of Rheumatology (DGRh).

In summary, the network has initiated all crucial steps for a sustained structure after the 3rd funding period. The integration in the DGRh secures the network on a national level; the various EU activities on an international level. The involvement of the industry forum will secure the basic finances with expected increase over the years and a successful fundraising should raise substantially amounts of funds for research projects in the long run.

ACKNOWLEDGEMENTS: Funded by the German Ministry of Research and Education and the German Society of Rheumatology

The AO Spine Network

Kenneth MC Cheung, The University of Hong Kong and Queen Mary Hospital, Hong Kong, SAR, China

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THE FIRST 50 YEARS

Saturday, October 4, 2008

Breakout Session 1

Animal models ; which ones work in research dealing with bone and cartilage repair?"

CHAIR: Joerg Auer, University of Zurich, Switzerland

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THE FIRST 50 YEARS

The use of Rodent models in musculoskeletal research for fracture repair; a need to consider a standard?

Allen Goodship

Royal Veterinary College and Institute of Orthopaedics and Muscloskeletal Science UCL
London UK.

Musculoskeletal diseases in general and acute trauma in both young and the elderly in particular represent a huge financial burden for the modern World. The demographics of ageing populations will further exacerbate the prevalence of these diseases and associated impact on quality of life and socioeconomic cost.
The research agenda defined through the Bone and Joint Decade includes trauma and osteoporosis. Clinical experience and evidence based analyses are now required to inform the research agenda. The progression of research to provide new understanding and translation to clinical practice in this field involves a wide range of research methods. Within the portfolio of these methods the use of *in vivo* models still represents an essential component. However, it is of paramount importance that these models are used appropriately and their use constantly reviewed in line with the 3R's of refinement, reduction and replacement.

In vivo models are used to test biomedical hypotheses rather than to replicate human disease per se. Therefore the design of the model and the experiments performed should relate to a testable and relevant hypothesis as part of a structured approach to advancement of the field of research and improvement of clinical practice

The process of bone modelling, remodelling and fracture repair is controlled by both biological and biomechanical signals. In the repair process the mechanical environment modulates the biological cascades and associated connective tissue differentiation. In order to address questions relating to the biology of fracture repair the molecular tools available relate largely to rodent species in particular the rat and mouse.

In developing the devices used to stabilise fractures and provide mechanical stimulation the requirements are for large animal models.

The elucidation of biological processes involved in bone repair has progressed significantly though the use of rodent models using simple fixation techniques such as intramedullary pins. The temporal sequence of gene expression and protein synthesis during repair has been determined with such models. However, in these models there is a limitation as a consequence of the lack of control of mechanical stability. In many models the heterogeneous nature of the fracture callus makes consistent planes of section difficult. The importance of interactions between mechanical conditions and biological responses can be addressed through a controlled fixation system to refine these rodent fracture models. In addition a standardised plane of section for comparative histology and histomorphometry can be obtained.

Rodent murine models are now being used extensively in relation understanding genetics and the genotype used may influence the results obtained significantly. For example, recent work has shown significant differences in the response of the skeleton to loading between different commonly used murine genetic strains. The material properties of the bone matrix in these strains may also differ by some 200%. It is therefore important to be aware of these factors when comparing data across laboratories.

As understanding of mechanobiology increases it is becoming apparent that some form of standard model is required to enable different laboratories to compare data and to advance both basic and translational research to contribute to development of new clinical treatment strategies.

Rodent models play an important role in the portfolio of research methodology, but have some significant limitations which must be both recognised and addressed in any comprehensive research programme.

Experiences with sheep models in musculoskeletal research at the MSRU

Brigitte von Rechenberg, Prof. Dr. med.vet. Dipl. ECVS

Musculoskeletal Research Unit, Equine Department, Vetsuisse Faculty, University of Zurich, Switzerland

The use of sheep as animal models in musculoskeletal research is based on several decisions, of which some of them are related to scientific background, others to animal welfare aspects or more prosaic to costs.

As for scientific background, sheep are suitable as experimental animals especially due to their morphology and lamellar structure of bone similar to humans. Their harversian system, physiologic remodeling rate and capability for primary bone healing are comparable to humans. In addition, their size, weight and, therefore, load on the skeletal system is similar and lend itself for testing of all kind orthopedic implants. Last but not least, they have a similarly functioning immune system as humans rendering them also suitable for testing biocompatibility issues for degradable and non-degradable biomaterials. Sheep are docile in nature and also relatively easy to handle for researchers. They can be kept in groups according to their character and depending on the experimental design may be allowed to roam free on pasture, both important issues in relation to animal welfare. Due to their herd nature, being kept in flocks together with peers is more important than free ambulation, which makes them superior experimental animals compared to dogs and horses, even if the experimental design warrants restriction. The psychological wellbeing of dogs and horses is much more impaired if they need to be confined to stalls compared to sheep. Compared to dogs, horses or even primates, costs related to maintenance are less, although if appropriate herd and health management is applied, the first impression may be misleading.

Although sheep have proven to be suitable for research in bone, they are not without problems and complications are common, mostly related to spontaneous fractures either through a stress riser such as a simple drill hole, or above or below a plate fixed to the bone. Complication rates are seldom openly communicated, but vary between 0- 100%, with an average between 18-40%. However, from an animal welfare viewpoint, any complication rate above 2-5% is ethically unacceptable, since major suffering is associated with these complications.

Complications related to spontaneous (re)fractures normally are related to *i)* stress risers, *ii)* implant size and placement, and lastly *iii)* improper postoperative care. Stress risers are usually due to the very nature of brittle sheep bone in combination with placement of drill holes for screws or other implants in cortical bone without additional protection. If drill holes are placed off center, or through trajectories important for mechanical load, the result can be disastrous, such that long spiral, multi-fragmented fractures occur. Sheep bone fractures easily in the longitudinal axis, since through its brittleness it is not very resistant against torsion or shear forces. Implant size is also a problem, since - as typical for ruminants – body weight and dimension of long bones resp. size of implants often don't match. For eg. The 3.5mm system in screws perfectly fits for the dimension of sheep long bones, but they are often too weak for their body weight. If full weight bearing is applied, implants may break too easily. If the 4.5mm system is used instead, the stress rising through too large a hole may end up in catastrophic failure. Furthermore, not all long bones are similarly suitable for studies of fracture repair. Apart from size and form in relation to the implants, coverage with soft tissue and number of parallel bones (eg. metacarpals, metatarsals), animal behavior such as getting up, lying down, matching of claws and stall floor, etc. have to be considered as well. Maintenance at least for long bone fractures, includes protective bandages or casts and suitable suspension systems that prevent animals from lying down while at the same time not compromising their mental and physical wellbeing.

If orthopedic research should become global, such that experiments conducted can be truly compared between studies, it is imperative to look more closely at some of the sheep models used, analyze why these complications occur and try to establish models that are safe for the animals and good for research. In addition, data banks for sheep models should be established and centralized, where models are screened for suitability for their original research question, outcome and complication analysis. Only then, results can be truly compared for research purposes.

REFERENCES: [1-4]

1. Nunamaker DM. Experimental models of fracture repair. Clin Orthop Relat Res. 1998 Oct(355 Suppl):S56-65.
2. Auer JA, Goodship A, Arnoczky S, Pearce S, Price J, Claes L, et al. Refining animal models in fracture research: seeking consensus in optimising both animal welfare and scientific validity for appropriate biomedical use. BMC Musculoskelet Disord. 2007;8:72.
3. Fini M, Pierini G, Giavaresi G, Biagini G, Mattioli Belmonte MM, Nicoli Aldini N, et al. The ovariectomised sheep as a model for testing biomaterials and prosthetic devices in osteopenic bone: a preliminary study on iliac crest biopsies. Int J Artif Organs. 2000 Apr;23(4):275-81.
4. Steffen T, Stoll T, Arvinte T, Schenk RK. Porous tricalcium phosphate and transforming growth factor used for anterior spine surgery. Eur Spine J. 2001 Oct;10 Suppl 2:S132-40.

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THE FIRST 50 YEARS

Use of Equine Models to Evaluate Articular Cartilage Repair

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INTRODUCTION: The repair of articular cartilage defects is often inadequate and regeneration is not achieved. Animal models of articular cartilage defects are critical pre-clinical means of assessing repair techniques. The key issue in the selection of the appropriate model is to match the model to the question being investigated and the hypothesis being tested. [1] Equine models of articular cartilage repair have recently been recognized to have specific advantages for translation to human articular cartilage resurfacing.

METHODS: Study 1: Histologic measurements of the thickness of non-calcified and calcified cartilage, as well as subchondral bone plate in three locations on the femoral trochlea and two locations on the medial femoral condyle on six human knees, as well as six equine, six goat, six sheep and six rabbit stifle joints were made. [2]

Study 2: After initial work in equine cadavers a 1 cm^2 full-thickness articular cartilage defect was developed on the central weight-bearing area of the medial femoral condyle. This defect was created arthroscopically and tested in four separate studies. [3-6]

Study 3: A second model has been developed on the medial trochlear ridge of the femur and tissue engineering studies made with two 15 mm defects. The defects can be created with or without calcified cartilage present. [7-8]

RESULTS: Study 1: Average articular cartilage thickness over five locations was 2.2-2.5 mm for humans, 0.3 mm for rabbit, 0.4-0.5 mm for sheep, 0.6-1.3 mm for dog, 0.7-1.5 mm for goat and 1.5-2.0 mm for horse.

Study 2: One cm^2 defects could be consistently made on the weight-bearing area of the medial femoral condyle. A technique was developed where the calcified cartilage layer could be selectively retained or removed and significant differences in healing were apparent with this technique. Separate studies demonstrated increased repair tissue filling with microfracture at 12 months[3], significant upregulation of type II collagen expression at 8 weeks in association with microfracture[4], improved healing with removal of the calcified cartilage layer[5], and improved quality of repair tissue with additive gene therapy using IGF-1/IL-1ra with an adenoviral vector administered intra-articularly. [6]

Study 3: The trochlear model has been used to demonstrate superiority of an autologous chondrocyte implantation (ACI) technique[7], as well as, more recently, improved repair with morselized cartilage fragments. [8]

DISCUSSION & CONCLUSIONS: The horse provides the closest approximation to humans in terms of articular cartilage thickness and this is considered relevant to pre-clinical studies in cartilage healing. The equine medial femoral condylar defect can be created arthroscopically and selective removal of calcified cartilage (or retention) achieved. Horses can be followed clinically, as well as with conventional outcome parameters and athletic exercise performed using a high-speed treadmill. A critical sized defect can be created and more tissue for examination attained. The femoral trochlear model allows two defects per femoropatellar articulation to be created. With the ability to make critically sized defects in multiple locations, the requirement for horse numbers (and consequent costs) can be reduced.

REFERENCES: [1Morisset], S. Frisbie, DD. Robbins, PD. Nixon, AJ. McIlwraith, CW. (2007) *Clin Orthop* **462**:221-228. [2] Frisbie, DD. Cross, MW. McIlwraith, CW. (2006) *Vet Comp Orthop Traumatol* **19**: 142-146. [3] Frisbie, DD. Trotter, GW. Powers, BE. Et al. (1999) *Vet Surg* **28**:242-255 [4] Frisbie, DD. Oxford, JT. Southwood, L. Trotter, GW. Rodkey, WG. Steadman, JR. Goodnight, JL. McIlwraith, CW. (2003) *Clin Orthop* **407**:215-227. [5] Frisbie, DD. Morisset, S. Ho, CP. Rodkey, WG. Steadman, JR. McIlwraith, CW. (2006) *Am J Sports Med* **11**:1824-1831. [6] O'Driscoll, SW. (2001) *Clin Orthop* **391S**:387S-401S. [7] Frisbie, DD. Bowman, SM. Calhoun, HA. DiCarlo, EF. Kawcak, CE. McIlwraith, CW. (2008) *Osteoarthritis and Cart* DOI **16:**667-679. [8]Frisbie, DD. Lu, Y. Colhoun, HA. Kawcak, CE. Binette, F. McIlwraith, CW. (2005) *Proceeding Orth Res Soc* 1355

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THE FIRST 50 YEARS

The use of clinical patients as animal models in musculoskeletal research

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Equine Department, Vetsuisse Faculty, University Zurich, Switzerland

INTRODUCTION: Implants, biomaterials etc. are usually tested in animals prior to their application in human patients. Even though the results are promising, their efficacy in a clinical situation was not tested. Controlled studies using animal models are expensive and more and more controversial. Some extreme animal rights activists even propose murder to safe the life of research animals. Implantation of materials, devices or implants that were previously successfully tested through *in vitro* studies or in small animals into clinical animal patients such as horses, cattle or dogs, may serve as an alternate and worth while step in the validation of new implants and biomaterials, prior to their safe application in human patients.

METHODS: Implants and Biomaterials that were previously successfully tested in controlled studies on sheep were applied in spontaneous clinical cases in domestic large animal species (horses and cattle). Such applications will be discussed using a study on the efficacy of the PcFix[1] and bioresorbable implants in subchondral bone cysts.[2] The implants and biomaterials were applied to the clinical situation according to the needs of each specific patient. All observations were recorded and continually analyzed in relation to earlier patients and the respective results of the treatment. If necessary adjustments in the treatment regimen were implemented to try to improve the results. Complications were recorded and discussed with the manufacturers to find ways to improve the implant design and avoid future complications.

RESULTS: Applying this approach, the PcFix was redesigned prior to its application in human patients. The implant was used only in fractures of the humerus and after the development of the LCP it was abandoned. Nevertheless the development process provided valuable input for the development of the LCP.

The study with the bioresorbable bone cements eventually culminated in the availability of a vauable implant that is presently clinically applied in human distal radius fractures with good results. The same material is also successfully applied in the management of subchondral bone cysts in horses.

DISCUSSION & CONCLUSIONS: There are different parameters that need to be kept in mind if "pre-human" clinical trials on domestic clinical large animal patients are conducted. i: The implant / material has to be proven in controlled animal studies (eg. sheep) to be effective and safe; ii: The implant / material has to fit the clinical case (size, strength etc.)

The following advantages of such research can be listed: i: The implant/material is tested in clinical situation; ii: Animals may test the limits of the implant/material; iii: Win-win situation for developer and owner of the animal; iiii: It represents a cost-effective approach with usable results if conducted properly.

On the negative side i: a longer testing phase of the implant/material, and ii: a delayed application in humans must be mentioned.

The "take home message" is: Pre-human clinical studies of new implants/materials in animal patients are: reasonable, cost-effective and worth while.

REFERENCES: [1] Auer J.A., Lischer C., Kaegi B., Iselin U., Fürst T., Matter G.: Application of the Point Contact Fixator in large animals. Injury, AO/ASIF Scientific Supplement, Vol. 26, Suppl. 2, S-B37-S-B46 (1995). [2] Fürst A., Kaegi B., Rechenberg B.v., Auer J. A. : Die Behandlung von 5 Pferden mit subchondralen zystoiden Defekten im Fesselbein. Pferdeheilkunde, 1997, 13, 3 April-März, 147-161.

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AO Foundation

THE FIRST 50 YEARS

Saturday, October 4, 2008

Breakout Session 2

Biological markers and diagnostic tools for early disease detection.

Chair: Dick Heinegard, Lund University, Lund Sweden and
David W. Grainger, University of Utah, Utah, PA, USA

System Biology approaches of cartilage degeneration – A New Way for Understanding pathogenesis and markers for disease monitoring

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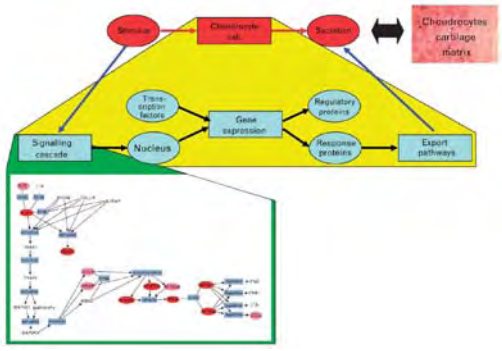
One of the most striking features of OA cartilage is the high phenotypic pleomorphism displayed by osteoarthritic chondrocytes. In OA cartilage many different processes run in a parallel or anti-parallel fashion and there are many reasons for this: In addition to individual variation and experimental measurement errors, causes the heterogeneity of gene expression pattern and cellular behaviour found in between and within different OA samples. Most likely this is one important reason that despite many observations on chondrocyte behavior so far no unifying picture of cellular changes specific to OA has emerged.
This contribution is about the key question, how to integrate a high complexity of processes and data to a unifying picture of disease processes and progression relevant for osteoarthritis.

Systems Biology tries to model biological systems in a comprehensive and quantitative way such that testable predictions can be made from such models. Typically systems are networks connecting variables (genes, proteins, promoters, enhancers, transcription factors, …) of the system. Biological networks are important for describing various aspects of biological processes. Metabolic networks represent processes converting substrate into product compounds, signalling cascades transmit signals from external ligands bound to respective receptors to activated transcription factors, transcription networks regulate the expression of target genes depending on the availability and activity of transcription factors. A sketch of such a hierarchical system for chondrocyte cells relevant for osteoarthritis is shown in Figure 1. Systems Biology networks tries to integrate all these types of networks into an integrative model which allows to analyse the underlying biological system. This includes modes of physiological behaviour, the sensitivity of the system in response to certain stimuli or perturbations, and differences in the behaviour of the system in case of disorders or diseases. The network models specifying the amount and state of all molecular compounds together with their interactions promise to derive not only the static topology but also the dynamic behaviour of the modelled biological unit. Thus, these systems models also provide causal network explanations of actual experimental high-throughput measurements.

Moreover, a second goal of these models is to integrate the massive amounts of the available data into human comprehensible models in order to master the inherent complexity of biological systems and the associated experimental data and facts.

To look at the biology of the whole system by systems biology approaches appears to be a promising way in the future to develop new understanding of the pathogenesis of osteoarthritis and new markers/marker profiles for diagnosing and prognosing the disease: thus, though at the moment these attempts are still in their infancy, there appears no alternative to trying to tackle the whole system. The way appears to be “modelling” of reality, instead of metaphysics - something realized by philosophy and exact natural sciences since centuries, more specifically since Plato, Kant and Einstein.

Fig. 1: Chondrocyte model: The figure shows a schematic view of a chondrocyte cell in a Petri net representation (a formal graph model consisting of system states and their transitions) with three layers of detail: Systems Biology promises to investigate various in vivo and in vitro systems and measurements of gene expression as well as secreted proteins in order to develop a predictive quantitative model of the involved processes.



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Molecular markers in the study of cartilage destruction in joint disease

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A hallmark of progressive joint disease is a progressive breakdown of the joint tissues, including the articular cartilage and underlying bone. In this process protein fragments are released from the tissue as a result of loosing the domain anchoring them to other molecules in the matrix.

The released fragments can be assayed by immunoassay and provide an indication of the activity of the ongoing process.

Over recent years assays for collagen fragments, COMP and other molecules have been applied to demonstrate that indeed the fragments can provide information on disease activity, prognosis both in rheumatoid arthritis and in osteoarthritis. Also, changing levels can be used to document therapeutic effects. A major shortcoming has been that the normal homeostasis and adaptation of the tissues to changing loads has contributed a background level of fragments, such that the procedures have been insensitive to low disease activity and therefore single data points from a given patient has been of little informative value unless the disease process has been intense. However, using cohorts of patients it has been possible to provide proof of concept.

Novel openings for specific and sensitive disease monitoring is based on accumulating evidence for degradation of extracellular matrix molecules at specific cleavage sites, following the original observations of one unique specific cleavage by collagenases of triple helical collagen II and of five unique sites in aggrecan by aggrecanases (ADAMTS-4 and 5). For collagen as well as aggrecan, the normal and pathological turnover is accomplished by the same enzymes and the same sites are cleaved. New information on degradation of other matrix constituents indicates that there are processes involving enzymes that appear more unique to a pathological process and leading to unique cleavage sites. Data are now being unraveled to demonstrate that release of fragments containing these cleavage sites offers new very sensitive measurements of disease conditions with no overlap to the normal situation.

To understand the repair process that invariably is associated with breakdown, information needs to be gained about repair responses and the assembly of the constituent building blocks into larger structural assemblies such as the collagen fibrillar networks. Dysregulation of any of these constituents can hamper the process of tissue repair and be detected as increased release of particular matrix constituent present in excess.

Some of the fragments of matrix molecules released will contain active domains that may bind to cells and factors of various defense systems. One example is that fragments of several matrix proteins can bind to factors of particularly the classical pathway of the complement system, where some can activate and others can inhibit the response. They therefore are potentially regulating the innate immune system and the inflammation that is almost invariably part of any joint disease and may be a factor in propagating the disease.

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Biochemical Markers in Osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) is a prevalent, age-related disease characterized by degraded cartilage, mild to moderate synovial inflammation, and altered bone structure. Plain radiography, the reference technique for assessing the severity of joint destruction, provides direct information on bones but not on cartilage, and has limited sensitivity. Magnetic resonance imaging (MRI) provides direct information on the different joint structures and is currently being optimized for use in OA. Conventional laboratory tests, such as serum C-reactive protein, show inflammation in some patients but provide little information on joint damage. These limitations have led to considerable interest in exploring the potential of specific biological markers that reflect dynamic variations in joint tissue remodeling (1, 2).

NEW BIOCHEMICAL MARKERS: New soluble biochemical markers include 1) for bone: the regulators of osteoclastic and osteoblastic activity (OPG/RANK-L/Dkk-1) activity, the non-collagen protein periostin, and non-enzymatic post-translational modifications of type I collagen such as i somerization of C-telopeptide (CTX-I). Changes in isomerisation, an index of bone matrix maturation, can be assessed by measuring the urinary ratio between native () and isomerized CTX-I (CTX-I) 2) for cartilage; markers of type II collagen synthesis (PIIANP) and degradation (CTX-II, Helix-II and Col21), MMPs and aggreganase-mediated fragments of aggrecan, degradation products of type IX collagen and fibronectin and 3) for synovial tissue; glucosyl-galactosyl-pyridinoline a crosslinking molecule specific of synovial collagen and nitrosylated type III collagen N-telopeptide (IIINys). Besides providing useful information on the physiopathological pathways of joint damage, they have been suggested to have important role for the clinical investigation of patients with OA.

CLINICAL USES OF BIOCHEMICAL MARKERS IN OA: Progression in OA shows considerable variation across individuals, and the predictive capacity of clinical indices is poor. Obtaining biochemical markers that could identify rapid progressors would be particularly useful for the selection of patients to be included in clinical trials of disease-modifying therapy. Abnormalities in subchondral bone metabolism are believed to be an early event of OA initiation and progression. We recently found that patients with knee OA have lower circulating levels of Dkk-1 which have been shown to be associated with more rapid progression in hip OA. Structural abnormalities in the collagen matrix have been reported in subchondral OA bone. In patients with osteogenesis imperfecta, a disease characterized by bone type I collagen defects, we recently found an increased urinary CTX-I ratio and increased type II collagen degradation (urinary CTX-II). Thus, alterations in bone matrix maturation may be associated with increased cartilage damage. Several prospective epidemiological or clinical trials have shown consistently shown that subjects with CTX-II levels above the upper limit of controls had a 2–3-fold increased risk of progression at the knee or the hip, as assessed by radiography, arthroscopy and more recently MRI of cartilage loss. Because of the complex involvement of bone, cartilage, and synovial tissue in OA joint damage, it is likely however that only a combination of several markers will adequately predict disease progression. Combining two markers of type II collagen degradation, Helix-II and CTX-II, which reflect different aspects of cartilage degradation, is more effective than using one marker alone to predict OA progression. In patients with knee OA, we found that the combination of CTX-II with a marker of synthesis (PIIANP) was also more efficient than one marker alone for identifying patients with knee OA whose joint will deteriorate were

elevated. An optimal combination of biochemical markers, together with imaging and genetic markers and clinical risk factors, is likely to provide a useful tool set for identifying OA patients at increased risk of disease progression. One of the main issues that currently impair efficient development of disease-modifying OA drugs is the low sensitivity of plain radiography, requiring long-term studies to show a significant difference between placebo and active-drug-treated patients. Biological markers may prove capable of providing earlier information compared to a demonstration of slowing joint space narrowing by x-ray. In two large randomized phase III trials of the bisphosphonate risedronate in patients with knee OA, a dose-dependent decrease of urinary CTX-II was also reported. Although risedronate did not demonstrate a statistically significant reduction of radiological progression compared to placebo, there was an association between the level of CTX-II measured before and 6 months after treatment and joint space narrowing at 2 years.

CONCLUSIONS: Biochemical markers of bone, cartilage and synovial tissues are increasingly tissue-specific. The panel of new markers is likely to expand with the use of proteomic-based technologies. An optimal combination of biochemical markers, together with clinical, imaging, and genetic parameters, is likely to be useful for identifying OA patients at increased risk for disease progression and to speed the development of new therapies in arthritis.

REFERENCES:

1- Garnero P. New biochemical markers of cartilage turnover in osteoarthritis: recent developments and remaining challenges. BoneKEy, 2007;4:7-18; 2: Garnero P. New biochemical markers of bone turnover. BoneKEy, 2008;5:84-102.

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Saturday, October 4, 2008

Breakout Session 3

New challenges in biotechnologies for bone and cartilage

CHAIR: Robert Guldberg, Georgia Institute of Technology and
James Kellam, Carolinas Medical Center, Charlotte USA

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Where are we and where are we going?

A Clinician's View of Biotechnology in Orthopaedic Trauma

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The potential for the use of biotechnology to aid in bone and cartilage healing opens many new avenues for the orthopaedic traumatologist. However, the present progress and techniques are still controversial. Two areas of need are regeneration of articular cartilage and the formation of bone. This talk will deal with bone regeneration.

The gold standard for bone regeneration in a post-traumatic or post-infective defect is autogenous cancellous bone graft. This provides osteoconductivity, osteoinductivity and osteogenesis however has imitations secondary to procurement and supply. Recent attempts to use bone substitutes such as allograft, polymers etc, as scaffolds alone or in association with biological active substances such bone morphogenic protein or stem cells has been confusing and poorly developed and failed to address the clinical issues faced by surgeons.

The first problem is the failure to recognize the requirement for a viable vascularized recipient site (angiogenesis) if any of these grafts are to be successful. The second issue is how much graft or scaffold and how strong. Strength is not the issue as internal fixation is universally used so allowing the development of scaffold or materials that might better resemble cortical or cancellous bone in structure, shape and morphology so that they can vascularize rapidly to support the osteogenic cells.

Third, when and how do we deliver the proteins and cells that stimulate bone growth? Present techniques involve the use of massive amounts of bone graft or substitute with the application of bone morphogenic protein in a non biological sense. Clinically, it is not known how to apply the bone morphogenic protein, how much substitute or graft should be placed in a defect, how should this be placed, or how resorbable ?

The AO Research Clinical Priorities Program on Large Bone Defect Healing is attempting to address this through a 4-prong approach. Recognition of the need for angiogenesis is undertaken through a program by Mauro Alini on endothelial progenitor cells and in conjunction with Michael Menger to evaluate the vascular angiogenesis into materials. Dr. Karen Berg and Elliott Greshkin are looking at new bio materials and how they should constructed to facilitate cell ingrowth and what form and shape they should take. George Duda is investigating the mechanical properties of fracture healing particularly at the cellular and protein level. Chris Evans is working on the genetic engineering aspects for bone growth. These four areas in combination will hopefully address the unknown issues of how to prepare the bed to allow angiogenesis to occur in an appropriate scaffold placed in the ideal mechanical environment so biologically active proteins and activated cells have the best possible chance to restore cortical bone.

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The Effects of Conditioned Media on the Osteogenic and Chondrogenic Differentiation of Mesenchymal Stem Cells

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INTRODUCTION: Chondrocytes and osteoblasts share a complex relationship during the development of the human skeletal system which, in part, is attributed to the complicated cytokine and growth factor signaling pathways that exist between the two cell populations[1]. Paracrine signaling may be modulated *in vitro* to find optimal culturing conditions and to tune mesenchymal stem cell (MSC) differentiation for tissue engineering applications, more specifically for osteochondral tissue engineering. The objectives of this study were to determine the effect of chondrocyte conditioned media on MSC differentiation to an osteoblast phenotype and to determine the effect of osteoblast conditioned media (CM) on MSC differentiation to a chondrocyte phenotype.

METHODS: Murine MSCs were cultured in two different groups: a chondrogenic group and an osteogenic group. The cells in the chondrogenic group were encapsulated in alginate beads (a common polysaccharide used for cartilage tissue engineering) and cultured in chondrogenic differentiation medium. The cells in the osteogenic group were cultured on porous scaffolds, made of 75/25 poly-L-lactide/polycaprolactone with 10 weight % hydroxyapatite, and cultured in osteogenic medium. Samples from the chondrogenic group were immersed in medium conditioned by the osteogenic group, and samples from the osteogenic group were immersed in medium conditioned by the chondrogenic group at concentrations of 25% and 50%. Control samples included cells grown on 2-D plates, cells grown on a scaffold or in alginate without differentiation medium, cells grown on a scaffold or in alginate with differentiation medium, and cells grown on a scaffold or in alginate with the appropriate differentiation medium mixed with 25% or 50% of the "opposite" differentiation media. Several different differentiation markers were then analyzed.

RESULTS: By Day 21, the osteogenic samples that were administered 25% and 50% chondrocyte conditioned medium showed higher alkaline phosphatase (ALP) activity than the controls that were not administered CM, with the 50% samples showing the highest activity. Additionally, the cells that were given chondrocyte CM had higher osteocalcin expression than the controls on both Days 14 and 21. Finally, the bone sialoprotein expression was higher in the samples given chondrocyte conditioned medium by Day 21.

The chondrogenic samples that were administered osteoblast conditioned medium at a concentration of 50% showed a slightly higher glycosaminoglycan production than the controls. The aggrecan expression was significantly higher in the samples given 50% CM, with a small increase in the samples given 25% CM as compared to the controls. Finally, the cells given 50% CM had a higher expression of Sox9 on Days 14 and 21. The results also suggested that the cells that were encapsulated in alginate but not given differentiation media had similar levels of differentiation as those in samples that were administered differentiation medium, suggesting the material played a large role in the chondrogenic differentiation.

DISCUSSION & CONCLUSIONS: Results from this study showed that CM from differentiating chondrocytes can enhance the differentiation of MSCs toward osteoblasts and that CM from differentiating osteoblasts can enhance the differentiation of MSCs toward chondrocytes. These effects are likely due to soluble factors that are released by the cells such as TGF-β1 and BMP-1. Results from this work may assist in optimizing culturing conditions of MSCs and may contribute to the development of clinically viable tissue engineered osteochondral devices. The results provide rationalization for development and implantation of immature tissue engineered osteochondral constructs that facilitate interaction of the two different cells types.

REFERENCES: [1] Lacombe-Gleize S., Gregoire M., Demignot S., Hecquet C., and Adolphe M. (1995) *In Vitro Cell Dev Biol Anim* **31**:649-652.

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STEM CELLS FOR MUSCULOSKELETAL REGENERATION

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The use of human stem cells to regenerate damaged tissue within the musculoskeletal system has been already applied to treat several of them, such as bone and intervertebral disc. Mesenchymal stem cell based approaches for treatment of other tissues, like cartilage, meniscus, ligament and tendon are at the later stages of development.
Although, some encouraging results have been observed, the clinical application of mesenchymal stem cells is far from being a routine approach in current medical treatments. Several biological and clinical issues have still to be improved.
Presently, we are tackling two major problems, one related to the heterogeneity of mesenchymal stem cell isolation procedures and the second, which is more clinically relevant, concern the use of mesenchymal stem cells for repairing large bone defect. Mesenchymal stem cells are normally isolated by adherence to plastic or by using antibodies that recognize specific cell surface molecules (i.e. CD 90). With the first approach, other cells will also adhere to the well of the plate and indeed only few percent of all the adherent cells could be considered mesenchymal stem cells. The use of a CD antibody is also confronted with problems related to the specificity of the antibody, when used to fish out a homogeneous cell population from a heterogeneous mixture of phenotypically different cells. In order to improve the isolation of committed (osteogenic or chondrogenic) mesenchymal stem cells, we have developed a GFP-vector that once transduced into the cells (Adenovirus) will produce green fluorescence upon differentiation towards a specific phenotype (osteogenic or chondrogenic). So, we will be able to isolate those committed cells and further study their behavior. We could test if a more committed and homogeneous cells population would be more efficient (to form bone or cartilage) than those presently used, which are dispersed and fill the presence of different type of cells. Autologous bone grafting is the current golden standard for the repair of large bone defects, despite drawbacks such as limited availability of grafting material and donor site morbidity. Possible alternatives like allografts or xenografts also have serious limitations; the risk of infections, possible immune reactions and ethical issues. Due to these problems, researchers in the area of bone repair have explored alternative solutions. Calcium and phosphate based materials as well as polymer scaffolds have shown some interesting osteoconductive properties. Nevertheless, the lack of osteoinductive potential prevents the healing of large bone defects treated only with such alloplastic materials. Many studies have shown that the lack of osteoinductive potential of such scaffolds can be partly overcome by seeding mesenchymal stem cells (MSC) onto the scaffold prior to implantation. However, a major problem still remains, namely the insufficient vascularization of the central part of these large grafts (>4cm). Thus, one of the most limiting aspects in obtaining tissue-engineered bone suitable for repairing large bone defects is the inadequate bone vascularisation. We have therefore addressed the enhancement of endothelial progenitor cells (EPC) as one of the key mechanisms in autologous bone grafting. The means by which these progenitors for neovascularisation can be isolated and characterized have recently been described. However, one of the major obstacles preventing the clinical application is the time needed to expand the EPC in vitro in order to obtain the required cell numbers. Out of the hundreds of common culture media compositions specifically designed to effectively culture cells of endothelial lineage none seems to be powerful enough for the desired purpose. Furthermore, these media are not autologous, which would be ideal for clinical use. We have therefore investigated the “Platelet-released growth factors” (PRGF) cocktail as a possible autologous source for EPC expansion. Our results show that PRGF is a highly efficient growth medium for EPC in vitro expansion. Moreover PRGF maintains the endothelial differentiation capacity of EPC. Immunostaining and PCR analysis showed persistence of angiogenic markers on CD34+ and CD133+ cells up to 21 days of culture. In addition, the capacity to form a cellular network after expansion in PRGF medium indicates that the EPC/PRGF association could have a positive influence on the formation of a vascular network within bone tissue engineered constructs.

Tissue engineered medical devices:

Are there conditions for manufacturing and approval?

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The European medical technology Industry defines human tissues as constituent parts of the human body, such as: bones, skin, heart valves, cornea, tendons, etc. Included are also human cells intended for grafting, tissue repair and cell lines from cell cultures. Excluded from this definition are solid organs for traditional transplantation, blood and blood products, gametes, reproductive tissues and embryos and tissues stored in genomic banks for obtaining genetic information (DNA).
The number of human tissue products is increasing and ranges from minimally manipulated products, such as cornea, to manipulated products of high compelxity such as cultured and modified cell lines and tissues with or without systemic effect. Complexity even increases, once these products are combined with biomaterials serving either as scaffolds in bioreactors or used as plasters.
The complexity of these products requires specific rules and regulations, mainly for their isolation procedures, the assembly in specific devices and the control of system performance and functionality. Unfortunately, there is still a lack of consistent consensus among the various national regulatory authorities which renders it difficult to get global approval and to provide tissue-engineered products for medical application on a world-wide basis.

1. Standards, Guidelines & Concerns

Guidelines for tissue-engineered products are available in some European countries although they are not consistent which each other. This holds true e.g., for France, Spain, and the UK. Further, a final decision has not yet been taken as to whether devices which contain tissue-engineered compounds or cells have to be considered as "medical devices" or
"medicinal products". In some countries, a third term is being applied. Here, human tissue-containing devices are considered to be "biodeviceuticals" or "biological products" (e.g., FDA). This still missing consensus in the regulatory framework leaves industry in the position to apply a worst case scenario. Preparational work for product approval is done under the premise that these products are considered to be a "medicinal product", which has been indirectly presribed in the EU´s Council Directive 93/42EEC from June 14, 1993 amended in 2001 (6). This directive describes in detail Medical Devices, their properties and regulatory background. It is stated explicitely in this directive that it does not apply to "....tissue or cells of human origin nor to products incorporating or derived from tissues or cells of human origin."
However, big effort is currently undertaken within the European Union in order to finalize the regulatory framework.

2. Safety Issues

It is easily understood that safety issues in human tissue derived products for medical application are of paramount importance. The observation that porcine retrovirus can be found in animal organs intended to be used as xenotransplants has been a drawback for the application of animal tissue and organs. The FDA extended its precautions in this context even to human tissue or cells which have been cultured with animal-derived feederlayer cells.
The increasing potential of human stem cell application puts forward another issue of controversy. The most important and useful property of stem cells is that of self renewal. Through this property striking parallels can be found between stem cells and cancer cells and it is thus important that industrial applications in medical therapies should be carefully controlled.
Saftey issues also apply to industrial aspects of patent application. Many developmental projects are undertaken with the help and cooperation of unversities and their research institutions. It is important that the intelectual property is clearly defined and that patent-owners are identified. Otherwise problems may arise as exemplified recently at Harvard University where a scientist was accused to have stolen cell lines and sold to industry.
Safety of tissue engineered products also depends on quality control and management. The performance of biological cells, once they are incorporated in a device, needs to be carefully defined even for longterm medical application. A “performance window” has, thus to be defined and information on this property to be indi-cated in the leaflet accompanying the device.
Safety also relates to product approval and its administrative aspects. The case of a drug developed against Gaucher disease, which had failed approval by the FDA but received approval by the "European Agency for the Evaluation of Medicinal Products

(EMEA, London)" offers a broad spectrum of speculation around politics involved in scientific affairs and renders industrial economical guestimates about product development and return on investment (ROI) difficult.

3. Economic issues

Tissue-engineered products are highTech devices which require huge early investments both in terms of basic scientific investigations and in manufacturing conditions and thus, reflect enormous financial risks. The return on investment may be considerably delayed if regulatory and saftey issues are not carefully monitored and controlled.

4. Conclusion

Tissue-engineered products exhibit a huge economic potential for both, medical and therapeutical application, given that regulatory -, safety - and legal aspects are under control and carefully monitored.

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Poster Session

No	Topic	Autor(s) /Affiliation
1	**N-Acylated Glucosamine Derivatives for the Treatment of Arthritis and Osteoporosis**	T. Anastassiades[1], K. Rees-Milton[1], M. Grynpas[2] *[1] Arthritis Centre, Dept of Medicine, Queen's University, Kingston, ON,, K7L 3N6 [2] Dept of Pathology, Mount Sinai Hospital, University of Toronto, Toronto, Canada*
2	**Activation of P2X7 Nucleotide Receptors Induces Actin Reorganization in Osteoclasts**	S. Armstrong, A. Pereverzev, Eun-ji Kwon, S. J. Dixon, S. M. Sims *CIHR Group in Skeletal Development and Remodeling, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Ontario, Canada N6A 5C1*
3	**Bone Sialoprotein-Mediated Mineral Formation: Role of Phosphorylation & Development of a Fusion Peptide for Bone Repair**	G.S. Baht, G.K. Hunter, H.A. Goldberg *CIHR Group in Skeletal Development and Remodeling, Dentistry and Department of Biochemistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, ON, Canada.*
4	**Titanium (IV) Ions Induced Bone Resorption Due to Osteoclast Recruitment and Activation: A Human *in vitro* Study**	*Dieter Cadosch[1,2], Erwin Chan[1], Oliver P. Gautschi[1,2], James Meagher[1], René Zellweger[2] and Luis Filgueira[1]* *[1]School of Anatomy and Human Biology, University of Western Australia, Crawley, Australia; [2]Department of Orthopaedic and Trauma Surgery, Royal Perth Hospital, Perth, Australia*
5	**The Efficacy of Low Intensity Pulsed Ultrasound Treatment on Experimental Spinal Fusion Implanted with Tissue Engineered Composite**	C.W. Chan[1,2], G. Li[1], C.F.F. Hui[1], K.M. Lee[3], L. Qin[1], Y.Y. Hu[4], W.M. Pan[4], K.S. Leung[1], J.C.Y Cheng[1,2] *[1] Department of Orthopaedics and Traumatology, [2]The Li Ka Shing Institute of Health Sciences, [3]Lee Hysan Clinical Research Laboratories, The Chinese University of Hong Kong, Hong Kong, China, [4]The Institute of Orthopaedics and Traumatology, Xian, China*

No	Topic	Autor(s) /Affiliation
6	**Regulation of Bone Remodeling by P2X7 Nucleotide Receptors in Osteoblasts and Osteoclasts**	S. J. Dixon, N. Panupinthu, J. Korčok, M. W. Grol, S. M. Sims *CIHR Group in Skeletal Development and Remodeling, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Ontario, Canada N6A 5C1*
7	**Bone Repair Mediated by Bone Sialoprotein**	H. A. Goldberg, G. Baht, J. Gordon, V. Pitelka, H. Chen, D. Holdsworth and G. K. Hunter *CHIR Group in Skeletal Development and Remodeling, Schulich Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada*
8	**Angiogenic Potential of the Early Fracture Haematoma is Increased by Mechanical Stimulation**	A.Groothuis[1, 2], G. Kasper[1, 2], U. Lehnigk[1, 2, 3], H. Bail[1], K. van Scherpenzeel[1], G. N. Duda[1, 2, 3] *[1]Center for Muskuloskeletal Surgery, Charité – Universitätsmedizin Berlin, Germany; [2] Julius Wolff Institute, Charité– Universitätsmedizin Berlin, Germany* *[3] Berlin-Brandenburg Center for Regenerative Therapies, Berlin, Germany*
9	**Bioavailability of PAPP-A, the Local Regulator of IGF-I (Insulin-like Growth Factor-I), in the Intervertebral Disc**	H.E. Gruber, G. Hoelscher, J. Ingram, B. Loeffler, M. Kuremsky, N. Zinchenko, E.N. Hanley, Jr. *Department of Orthopaedic Surgery, Carolinas Medical Center, PO Box 32861, Charlotte, N.C., U.S.A*
10	**Osteochondral Tissue Engineering by Implication of Perfusion and Cyclic Compression on Mesenchymal Cell-Loaded Hybrid Grafts**	C. Haasper[1], M. Colditz[1], S. Budde[1], T. Tschernig[2], M. Wehmeier[3], E. Hesse[1], C. Hurschler[4], C. Krettek[1], M. Jagodzinski[1] *[1] Trauma Department, Hannover Medical School (MHH), Hannover, Germany. [2.] Department of Functional and Applied Anatomy, MHH. [3.] Clinical Chemistry, MHH. [4.] Department of Orthopedics, MHH.*

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11	**In vivo Gait Analysis in Murine Femoral Fracture Model**	**T. Histing[1,2,3] A. Kristen[1,3], C. Roth[4], JH. Holstein[1,2,3], P. Garcia[1,2,3], MD. Menger[2,3] and T. Pohlemann[1,3]** *[1]Department of Trauma, Hand and Reconstructive Surgery, [2]Institute for Clinical & Experimental Surgery, University of Saarland, Homburg/ Saar, Germany; [3]Collaborative Research Center, AO Foundation, Switzerland, [4]Departemt of Diagnostic and Interventional Neuroradiology, University of Saarland, Homburg/ Saar, Germany*
12	**BMP-7 Gene Therapy for Mitigation of Post-traumatic Osteoarthritis in Sheep**	Mark Hurtig*, Frederic David^, Darilyn Fraser^, Betina Lapostolle¶, Antonio Cruz*, Laurent Fischer¶ **Canadian Arthritis Network Core Facility, University of Guelph, Guelph, ON, Canada, N1G 2W1, ^Biological R&D Merial Ltd., Athens GA, ¶ Biological R&D, Merial SAS, 254 rue Marcel Mérieux, 69007 Lyon, France*
13	**Mesenchymal Stem Cell Ageing is Associated with Alterations in Antioxidant Defense and Cytoskeleton Dynamics**	G. Kaspar[1,2,3], L. Mao[2,3], S. Geissler[1,2], J. Trippens[1], J. Kuehnisch[4] , C. Perka[1,2] , G. N. Duda[1,2] & J. Klose[2,3] *[1] Julius Wolff Institute and Center for Muskuloskeletal Surgery, Charité-Universitätsmedizin, [2] Berlin-Brandenburg Center for Regenerative Therapies, [3] Institute for Human Genetics, Charité – Universitätsmedizin Berlin, Germany, [4] Institute for Medical Genetics, Charité – Universitätsmedizin Berlin, Germany*
14	**Solidification Mechanisms of Chitosan-Glycerol Phosphate/Blood Implant for Articular Cartilage Repair**	C. Marchand 1, G.-E. Rivard 2, J. Sun 3 C. Hoemann 1,4 *[1]Institute of Biomeical Engineering & [4]Dept Chemical Engineering, École Polytechnique, Montréal, Québec, Canada. [2]Hematology-Oncology, Hôpital Sainte-Justine, Montréal, Québec, Canada, [3] Bio Syntech, Laval, Québec, Canada.*

No	Topic	Autor(s) /Affiliation
15	**Intercellular Propagation of Signal Induced by Mechanical Stimulation of a Single Bone Marrow Cell**	Maria O.M.*, Komarova S.V.* **McGill University, Faculty of Dentistry, 3460, Rue University, H3A 1A4, Montreal, Quebec, Canada*
16	**Investigating the Effects of OA on Rodent Knee Vasculature Using Contrast Enhanced Micro-Computed Tomography**	D.D. McErlain[1,2], V. Pitelka[3], J.L. Henry[5], D.W. Holdsworth[1,2,4] *[1] Imaging Research Laboratories, Robarts Research Institute; [2] Department of Medical Biophysics ;[3]Department of Physiology and Pharmacology; [4]Department of Diagnostic Radiology & Nuclear Medicine; University of Western Ontario, London, Canada.[5] Michael G. DeGroote Institute for Pain Research and Care, McMaster University, Hamilton, Canada.*
17	**Normal Human Dermal Fibroblasts Differentiate into Contractile Myofibroblasts after Contact with Bioactive Surfaces**	Metzger W.[1], Grenner N.[1], Strehlow R.[2], Pohlemann T.[1], Oberringer M.[1] *[1]Clinic of Trauma-, Hand- and Reconstructive Surgery, Saarland University, Kirrberger Straße, Building 57, 66421 Homburg, Germany, [2]Fraunhofer Institute for Biomedical Engineering IBMT, Department for Molecular Bioanalytics and Bioelectronics, 14476 Potsdam-Golm, Germany*
18	**Transforming Growth Factor β_1 does not Attenuate the Hypoxia Induced Response of Fibroblasts and Endothelial Cells in a New in Vitro Co-culture Wound Healing Model**	Oberringer M., Meins C., Bubel M., Pohlemann T. *Clinic of Trauma-, Hand- and Reconstructive Surgery, Saarland University, Kirrberger Straße, Building 57, 66421 Homburg, Germany*
19	**Fetal Spine Cells: A Potential Cell Source for Intervertebral disc Regeneration?**	Aurelie Quintin, *Laboratory of Biomechanical Orthopedics EPFL-HOSR, Station 15, 1015 Lausanne, Switzerland*
20	**Impact of Peroxynitrite on Intervertebral Disc Cells**	L. Poveda[1], K. Wuertz[1], M. Hottiger[2], N. Boos[1] *[1] Spine Research Unit, University Hospital Balgrist, Zurich, CH, [2]Institute for Veterinary Biochemistry and Molecular Biology, University of Zurich, Zurich, CH*

No	Topic	Autor(s) /Affiliation
21	**Impact of the Spatial RANKL/OPG Distribution on BMU Branching**	M.D. Ryser[1], N. Nigam[1], S.V. Komarova[2] *[1]Department of Mathematics and Statistics, [2] Faculty of Dentistry, McGill University, Canada*
22	**Collagen-Hydroxyapatite Scaffolds for Bone Engineering**	J. Smedeck[1], F. Otto[1], K. Pöpperl[1], J. Kuschnierz[1], J. Haberstroh[2], R. Gutwald[1], R. Schmelzeisen[1], E. Sachlos[3], S. Sauerbier[1] *[1] Department of Oral and Maxillofacial Surgery, University Hospital Freiburg, Germany, [2] Section of Experimental Surgery, University Hospital Freiburg, Germany, [3] Harvard School of Engineering and Applied Sciences, Auguste Lab, Cambridge, MA, USA*
23	**P2X7 Receptor Activation Causes Isoform-Specific Translocation of Protein Kinase C in Osteoclasts**	Stephen M. Sims, Souzan Armstrong, Alexey Pereverzev, S. Jeffrey Dixon *CIHR Group in Skeletal Development and Remodeling, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Ontario, Canada N6A 5C1*
24	**Bone Tissue Engineering using Synthetic Fibrin-Like Hydrogels**	Franz E. Weber[1], Mathias P. Lutolf[2], Simone Rizzi[1,3], Jeff Hubbell[2], Martin Ehrbar,[1] *[1] Bioengineering, Dept. of Cranio-Maxillofacial Surgery, University Hospital, Zurich Switzerland, [2]Institute of Bioengineering, Ecole Polytechnique Fédéral de Lausanne (EPFL), Switzerland, [3]School of Life Sciences, University of Technology (QUT), Brisbane, Australia*
25	**Mesenchymal Stem Cell Response to a pH Challenge with Applications to Intervertebral Disc Repair**	K. Wuertz[1,2], K. Godburn[2], J. Iatridis[2] *[1]Spine Research Unit, University Zurich, Switzerland [2]University of Vermont, Burlington VT, USA*
26	**Rat Bone Marrow Stromal Cells Differentiation and Proliferation: Effects of Continuous and Pulsatile Parathyroid Hormone Treatments**	C. Yang[1], H. Frei[2], H. M. Burt[1] and F. M. Rossi[2] *[1]Faculty of Pharmaceutical Sciences, [2]Biomedical Research Centre, University of British Columbia, Vancouver, Canada*

No	Topic	Autor(s) /Affiliation
27	**Investigation of Three Different Labelling Techniques to Visualize Vital Cells**	Th. Ziebart[1], M.O. Klein[1], J. Adler[1], H. Goetz[2], M. Grez[3], W. Wagner[1], H. Duschner[2], B. Al-Nawas[1] *[1]Oral and Maxillofacial Surgery, University of Mainz, [2]Applied Structure and Microanalysis, University of Mainz [3]Applied Virology and Gene Therapy, Georg Speyer Haus Frankfurt*
28	**Mesenchymal Stem Cells for the Augmentation of the Maxillary Sinus**	S. Sauerbier[1] , J. Kuschnierz[1], H. Nagursky[1], R. Gutwald[1], R. Schmelzeisen[1] *[1] Department of Oral and Maxillofacial Surgery, University Hospital Freiburg, Germany*
29	**Comparatitive Gene Transfer Analysis in Human Mesenchymal Stem Cells using 15 Novel Optimized nonviral transfection systems.**	[+1]Elsler, S; [1]Schetting S; [1]Kohn D; [1]Madry H; [1]Cucchiarini M *[+1]Laboratory for Experimental Orthopaedics, Department of Orthopaedic Surgery, Saarland University Medical Center, Homburg, Germany*
30	**Hydroxyapatite, BMP and Wnt signaling are required in cell based ectopic bone formation**	J. Eyckmans [1,3], J.Schrooten[2,3], F.P. Luyten[1,3], *[1] Laboratory for Skeletal Development and Joint Disorders, Katholieke Universiteit Leuven, O&N1, Herestraat 49, bus 813, 3000 Leuven, Belgium. [2] Department of Metallurgy and Materials Engineering, Kasteelpark Arenberg 44, bus 2450, 3001 Leuven, Belgium. [3.]Prometheus: Division of Skeletal Tissue Engineering, O&N 1, Herestraat 49, bus 813, 3000 Leuven, Belgium.*
31	**Nanotechnology for Medical Application: Potential, Challenges and Results**	H. Hofmann, A.Fink Jat. Salaklang, V. Bernau, *Powder Technology Laboratory, Institute of Materials, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland*
32	**Use of Cell Therapy to Enhance the Bone Tendon Healing, Validation of a Small Animal Model and Results with the Use of Chondrocytes in Rat.**	G Nourissat MD [1], A Diop PhD [2], N Maurel PhD [2], M Gosset DMD PhD [1], F Berenbaum MD PhD [1] *[1]UMR 7079, Bat A, 5ième étage, 7 quai claude Bernard, 75252 Paris cedex 5, UPMC; [2]ENSAM, EPBRO – Paris, France*

No	Topic	Autor(s) /Affiliation
33	**Microvascular Bone Reconstruction with Induction of Ectopic Bone Formation by Multipotent Human Adipose Stem Cells - Case Report**	S. Miettinen[1], B. Lindroos[1], K. Mesimäki[2], J. Törnwall[2], R. Suuronen[1,3,4] *[1]REGEA, Institute for Regenerative Medicine, University of Tampere and Tampere University Hospital, Biokatu 12, 33520 Tampere, Finland. [2]Department of Oral and Maxillofacial Diseases, Helsinki University Central Hospital, Helsinki, Finland. [3]Department of Eye, Ear and Oral Diseases, Tampere University Hospital, Finland. [4]Department of Biomedical Engineering, Tampere University of Technology, Finland.*
34	**Integrin α2: Deficiency Causes Impaired Bone Stability *in vivo* and Reduced Mineralization of the Extracellular Matrix *in vitro***	Timmen, M.[+]; Wieskötter, B.[+]; Hidding, H.[+]; Everding, J.[+]; Peters, M.[*]; Eckes, B.[**]; Pap, T.[*]; Raschke, M.[+] and Stange, R.[+] *[+]Dept. of Trauma, Hand and Reconstructive Surgery, University Hospital Muenster, Germany* *[*]Division of Molecular Medicine of Musculoskeletal Tissue, Department of Orthopedics, University Hospital Muenster;[**]Department of Dermatology, University of Cologne, Cologne, Germany*
35	**Generation of human tendon- and ligament-derived cell lines, via immortalization with hTERT, for tissue engineering applications**	D. Docheva [1], D. Padula [1, 2], S. Poppe [1], P. Weishaupt [3], C. Popov [1], M. Schieker [1] *[1] Experimental Surgery and Regenerative Medicine, Department of Surgery, Ludwig-Maximilians-University (LMU), Nussbaumstr. 20, D-80336 Munich, Germany* *[2] Precision- and Micro-Engineering, Engineering Physics, Faculty 06, University of Applied Sciences, Lothstr. 34, D-80335 Munich, Germany.* *[3] Department of Restorative Dentistry and Periodontology, Dental School of the LMU, Goethestr. 70, D-80336 Munich, Germany*
36	**Mechanism of Decreased Expression of Type X Collagen in Human Mesenchymal Stem Cells from Osteoarthritis Patients Cultured on Nitrogen-Rich Plasma Polymers: Implication of Cyclooxygenase-1**	[1]Mwale, F; [1]Wang, HT; [2]Girard-Lauriault, P-L; [2]Wertheimer, MR; [1]Antoniou, J; [1]Petit, A *[1]Division of Orthopaedic Surgery, McGill University, Montréal, QC, Canada.; [2]Department of Engineering Physics, École Polytechnique, Montréal, QC, Canada*

No	Topic	Autor(s) /Affiliation
37	**Early Vascularization and new bone formation in a critical bone defect is improved by Co-culture of Endothelial progenitor cells (EPC) and mesenchymal stem cells (MSC) in Rats**	Christopher Kähling[1], Caroline Seebach[1], Dirk Henrich[1] , Ingo Marzi[1] [1] *Department of Traumatology, University of Frankfurt, Germany*
38	**Extracorporal Bone Generation**	N Rosenberg *The Ruth and Bruce Rappaport Faculty of Medicine, Technion – Israel Institute of Technology, Haifa, Israel*
39	**Time-dependent VEGF expression modulates ectopic bone formation mediated by muscle-derived stem cellsTIME-**	[1]Y. Chun, [1]A. Usas, [1]A. Ho, [1]H. Peng, [1]J. Huard *[1]Stem Cell Research Center, Children's Hospital of Pittsburgh and Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA*
40	**Human osteoblasts induce proliferation and neo-vessel formation of human umbilical vein endothelial cells in a long-term 3D co-culture on polyurethane scaffolds**	A.Hofmann[1], U. Ritz[1], S. Verrier[2], D. Eglin[2], M. Alini[2], S. Fuchs[3], C.J. Kirkpatrick[3], P.M. Rommens[1] [1] Department of Trauma Surgery, Johannes Gutenberg University School of Medicine, Germany, [2] AO-Research Institute, Davos, Switzerland, [3] Institute of Pathology, Johannes Gutenberg University School of Medicine, Germany
41	**Controlled release of antibiotics from Bone Cement.**	Jackson JK, Tredwell S, Leung F, Duncan C, and Burt HM. *Faculty of Pharmaceutical Sciences UBC, Dept of Pediatric Orthopedics (Children's Hospital) the Dept of Orthopedics VGH, Vancouver. BC. Canada.*

N-Acylated Glucosamine Derivatives for the Treatment of Arthritis and Osteoporosis

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INTRODUCTION: Chemical N-acylation of glucosamine (GlcN) resulted in compounds (GlcNAcyl) with distinct properties. N-butyrylGlcN (ANABU™, Bu) had stimulatory effects on chondrocyte growth, matrix synthesis and gene expression, compared to GlcN. Initially Bu was considered to be primarily chondro-protective. We demonstrated striking preservation of articular cartilage, by oral feeding of Bu, in a rat model for inflammatory arthritis, Fig 1, [1].

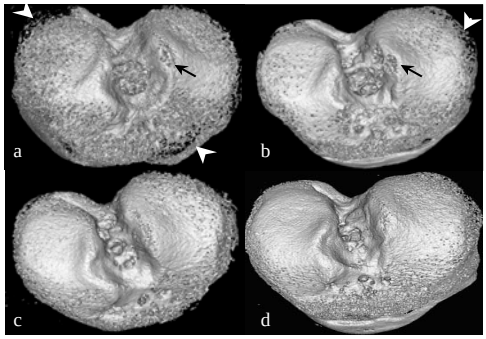

Fig 1: Micro-CT of rat tibia plateaus showing severe subchondral bone erosions (e.g. arrows) in untreated rats with inflammatory arthritis (a). Oral treatment with low and high dose Bu: (b,) (c), respectively, show progressively reduced erosions and bone loss. (d) smooth surface of control.

For the inflammatory rat model we also demonstrated preservation of subchondral bone architecture and increased connectivity, as a result of Bu treatment. We asked, therefore, if the primary effect of Bu has is on bone. This was tested in an ovariectomised (OVX) rat model.

METHODS: The groups of mature female rats were: (1) Glcucose (Glc)-fed, non-OVX (control); (2) Bu-fed, non-OVX; (3) Glc-fed, OVX; (4) Bu-fed, OVX. Bone mineral densities (BMD) of femurs and spines were measured every 2 months. At 6 months animals were euthanized and the bones evaluated for bio-mechanical properties. RNA was extracted from the livers and subjected to microarray analysis.

RESULTS: Ovariectomy resulted in lower femoral and spinal BMDs. Bu-fed OVX rats (Group 4) demonstrated maintenance of femoral head and total femur BMDs. Trends of the BMD for the spines were similar. Mechanical properties in 3 Point Bending showed a significant increase in the ultimate load and displacement and energy to failure of Group 4 over Group 3. Normalized data supported this trend as the ultimate strain and the toughness of (Group 4 were > 3). Also, femoral neck fracture showed an increase in the ultimate displacement and energy to failure, with a decrease in the stiffness (Group 4 >3). The microarray results showed significant up-regulation for secreted phosphoprotein 24 (spp24) (Group 4 > controls). Spp24 has strong homologies to cystatin and BMP-2 binding regions and is expressed only in the liver and bone.

DISCUSSION & CONCLUSIONS: Orally administered ANABU™, preserves BMD and bone biomechanical properties in the OVX rat, an animal model for post-menopausal osteoporosis. This constitutes an additional application to the use GlcNAcyls in arthritis [2]. The effect on bone may be mediated, in part, by regulation of the little studied spp24. Based on other data, we speculate that spp24 is part of a fetuin-Ca complex and is deposited, as well as being synthesized, in bone.

REFERENCES: [1] Wang SX, Cherian A, Dumitriu M et al. Disease-modifying effects of N-butyryl glucosamine in rats with streptococcal cell wall-induced arthritis model in rats. J Rheumatol (2007) **34**:712-20. [2] T. Anastassiades"Treatment of Arthritis and Compositions Therefore" US patent 6,479,469 B2. [3] Terry DE, Rees-Milton K, Smith P, Carran J, et al. N-acylation of glucosamine modulates chondrocyte growth, proteoglycan synthesis, and gene expression. J Rheumatol (2005) **32**:1775-86.

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Activation of P2X7 Nucleotide Receptors Induces Actin Reorganization in Osteoclasts

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INTRODUCTION: Nucleotides such as ATP can act as extracellular signalling molecules via interactions with P2 purinergic receptors. Osteoclasts express functional P2X7 receptors – calcium-permeable channels activated by high concentrations of extracellular ATP [1]. Nucleotides, released from bone cells into the extracellular environment in response to mechanical or inflammatory stimuli, provide localized and transient signals that regulate bone formation and resorption [2]. Genetic disruption of *P2rx7* leads to increased bone resorption [3] and reduced response of the skeleton to mechanical strain [4]. Podosomes are small punctate adhesion structures, each consisting of a core of filamentous actin (F-actin) and actin-associated proteins surrounded by integrins and integrin-associated proteins [5]. In osteoclasts, podosomes organize into a belt at the cell periphery (*Fig. 1*). Since integrity of the actin cytoskeleton is critical for osteoclastic resorption, we investigated the effect of P2X7 activation on actin organization.

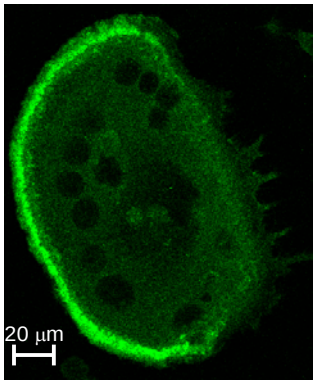

Fig. 1: Fluorescence image of a single live osteoclast-like cell, expressing EGFP-actin. Podosome belt is apparent in the cell periphery at left. Multiple nuclei are visible as dark round structures in the cell interior

METHODS: RAW 264.7 cells were differentiated into multinucleated osteoclast-like cells and transiently transfected with a construct encoding EGFP-actin. Live-cell confocal imaging was then used to monitor actin dynamics. Osteoclasts were also isolated from neonatal rats, treated, fixed and F-actin revealed with fluorescently labelled phalloidin. Lastly, microinjection of living rat osteoclasts with Alexa-488-labelled actin provided a complementary approach to monitor dynamic changes in the actin cytoskeleton.

RESULTS: In unstimulated rat osteoclasts, the majority of cells exhibited an F-actin belt at the cell periphery. This was evident in fixed osteoclasts stained with phalloidin, as well as in live osteoclasts microinjected with fluorescently labelled actin. Live cell fluorescence imaging revealed dynamic reorganization of actin in the pseudopodia of migrating osteoclasts. Stimulation of osteoclasts with benzoyl-benzoyl-ATP (BzATP, a potent P2X7 agonist, 150 μM, 10 min) disrupted the F-actin belt in ~75% of rat osteoclasts. In contrast, stimulation of cells with a low concentration of ATP (100 μM, sufficient to activate P2X4 and P2Y2, but not P2X7 receptors) did not cause apparent changes in actin distribution. In live osteoclast-like cells, BzATP caused dramatic actin reorganization within 5-10 min. In contrast, addition of vehicle did not affect organization of the actin belt. Disruption of the actin belt on stimulation of P2X7 was largely reversible within 30 min, even in the continued presence of BzATP.

DISCUSSION & CONCLUSIONS: This study reveals for the first time that activation of P2X7 nucleotide receptors induces dramatic reorganization of the actin cytoskeleton in osteoclasts. This finding reveals a new mechanism by which P2X7 receptors might suppress the resorptive activity of osteoclasts.

REFERENCES: [1]Naemsch L.N., Dixon S.J., Sims S.M. (2001) *J Biol Chem* **276**:39107-14. [2]Dixon S.J., Sims S.M. (2000) *Drug Dev Res* **49**:187-200. [3]Ke H.Z., Qi H., Weidema A.F., et al. (2003) *Mol Endocrinol* **17**:1356-67. [4]Li J., Liu D., Ke H.Z., et al. (2005) *J Biol Chem* **280**:42952-59. [5]Jurdic P., Saltel F., Chabadel A., et al. (2006) *Eur J Cell Biol* **85**:195-202.

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Bone Sialoprotein-Mediated Mineral Formation: Role of Phosphorylation & Development of a Fusion Peptide for Bone Repair

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INTRODUCTION: In osteoarthritis, bone loss secondary to joint degradation may lead to improper integration of orthopedic implants, especially in revision implants, which can in turn lead to chronic pain and morbidity. Bone sialoprotein (BSP) is a phosphoprotein capable of nucleating hydroxyapatite (HA) *in vitro*[1] (an activity enhanced by interaction with collagen[2]) and inducing bone repair *in vivo*[3]. Native, bone-extracted BSP (nBSP) is a more potent HA nucleator than the recombinant form (rBSP)[4]. This enhanced activity is likely due to post-translational modifications, specifically phosphorylations, on nBSP. The study presented here investigates the role of phosphorylations in BSP-mediated HA nucleation and verifies the collagen-binding and HA-nucleation activities of a fusion peptide to be tested as a therapeutic in bone regeneration.

METHODS: HA nucleation was assessed by determining the lowest concentration of protein able to induce crystal formation in the agarose gel steady-state mineral formation assay. Phosphorylation of reagents was carried out with protein kinase CK2 and verified by mass spectrometry. Collagen-binding activity was assayed using an ELISA-type method. A novel fusion peptide of BSP containing the collagen binding domain (CBD) and the second HA-nucleating domain (HAND) was generated by subcloning the corresponding sequences into a pET-28a expression vector. All proteins and peptides studied were prokaryotically expressed rat sequences with the exception of native rat bone-extracted BSP (nBSP).

RESULTS: Nucleation potency of nBSP was compared to that of rBSP. nBSP was 100 times more potent than rBSP, an enhancement lost upon treatment with calf intestinal phosphatase (CIP). CK2 treatment of rBSP added approximately 4 phosphates and resulted in a ten-fold increase in nucleation potency. CK2 treatment of rBSP(1-100) and rBSP(134-206) resulted in the addition of 2 and 1 phosphates, respectively. Whereas nucleation potency was unaltered in the CK2-treated rBSP(1-100), an increase in potency was observed for CK2-treated rBSP(134-206). This gain in nucleation potency for CK2-treated rBSP and rBSP(134-206) was lost upon treatment with CIP. A fusion peptide of BSP containing the CBD and the sequence within rBSP(134-206) responsible for HA nucleation was generated. This peptide had an affinity for collagen (K_d = 13 nM) and HA-nucleating activity (0.100 nmol) (Fig. 1).

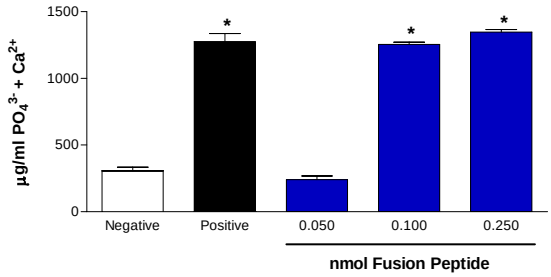

Fig. 1: Mineral formation activity of BSP-derived peptide determined using the steady-state assay system.

DISCUSSION & CONCLUSIONS: nBSP is two orders of magnitude more potent a nucleator than rBSP, an activity that is lost upon dephosphorylation. Phosphorylation of rBSP increases its HA-nucleating potency, which appears to be dependent on modifications within the rBSP 134-206 sequence. A novel BSP-derived peptide containing the CBD and the second HAND was successfully generated. This reagent had affinity for collagen and HA-nucleation activity and thus has potential for use as a therapeutic reagent for bone repair and to promote enhanced osseous integration of implants.

REFERENCES: [1]G.K. Hunter and H.A. Goldberg (1993) *PNAS* **90**:8562-8565. [2]G.S. Baht, G.K. Hunter, and H.A. Goldberg (2008) *Matrix Biol.* (In Press: doi:10.1016/j.matbio.2008.06.004). [3]J. Wang *et al.* (2006) *Calcif Tissue Int* **79**:179-189. [4]C.E. Tye *et al. JBC* **278**:7949-7955.

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Titanium (IV) Ions Induced Bone Resorption Due to Osteoclast Recruitment and Activation: A Human *in vitro* Study

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INTRODUCTION: There is increasing evidence that titanium ions are released from orthopedic implants resulting in 1µM concentration in surrounding tissues and blood, and playing a role in aseptic loosening. Our aim was to investigate, whether Ti(IV) ions induce recruitment and differentiation of monocytes into osteo-resorptive multinucleated cells and influence the activation and function of *in vitro* generated osteoclasts.

METHODS: Human monocytes and *in vitro* generated osteoclasts were exposed to 1µM Ti(IV) ions for ten days. Thereafter, transcription of specific osteoclast genes, including tartrate-resistant acid phosphatase (TRAP) and cathepsin K (CATK), and chemokines (CCL17 and 22) were measured using quantitative reverse transcription polymerase chain reactions. The effects of Ti(IV) on osteoclastic activity and differentiation were also evaluated by measuring the enzymatic activity of TRAP using ELF97 as a fluorescent substrate, and the amount of secreted chemokines using ELISA assay. Additionally, functional evidence of osteoclastic bone resorptive activity was determined by a lacunar resorption assay system using cell cultures on dentin slides.

RESULTS: In total, cells derived from 22 healthy individuals were studied. After Ti(IV) treatment blood monocytes from five donors (22.7%) showed a "responsive" pattern characterized by increased gene expression of osteoclast markers, TRAP activity and bone resorptive ability. The treated monocytes of the "responsive" cohort showed an increased expression of TRAP and to a lesser extent CATK. Detection and quantification of intracellular TRAP activity, by using ELF97 for fluorescence microscopy and flow cytometry, revealed a significant increase of TRAP-positive cells in Ti(IV) treated monocytes of the same "responsive" individuals. Ti(IV) treated monocytes of the same cohort became functional bone resorbing cells, as shown on dentin slide cultures, increasing significantly their osteo-resorptive activity to similar levels of osteoclasts *in vitro*. *(Fig. 1)*. Additionally, Ti(IV) treated osteoclasts showed significantly increased CCL17 and 22 expression and secretion.

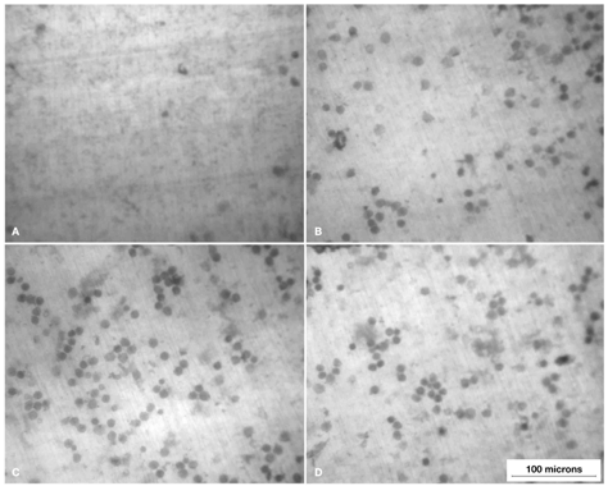

Fig.1: Effects of Ti(IV) on bone resorption. The images show a representative example of resorption pits (dark spots) on dentine slides after 21 days incubation at different culture conditions. ***A:*** *Untreated Monocytes* ***B:*** *Monocytes + Ti(IV)* ***C:*** *Untreated Osteoclasts* ***D:*** *Osteoclasts + Ti(IV).*

DISCUSSION & CONCLUSIONS: The present study provides strong support for the hypothesis that Ti(IV) ions, released by bio-corrosion from metal implants, induce differentiation of monocytes towards mature, functional osteoclasts in approximately 20% of individuals. Additionally, Ti (IV) ions activate the expression and secretion of chemokines in mature osteoclasts. These results suggest enhanced recruitment of osteoclast precursors from the blood circulation and induced osteoclastic differentiation, which may well contribute to the pathomechanism of aseptic loosening.

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The Efficacy of Low Intensity Pulsed Ultrasound Treatment on Experimental Spinal Fusion Implanted with Tissue Engineered Composite

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INTRODUCTION: Experimental posterior spinal fusion with autograft was found to be enhanced by low intensity pulsed ultrasound (LIPUS) [1]. Studies on stem cell-bioceramics composite applied as bone substitute has been reported recently [2,3,4]. The effect of LIPUS on the bioengineered composite in posterior spinal fusion model was investigated in the current study.

METHODS: Bone marrow was aspirated from rabbit femur. The mesenchymal stem (MSC) cells were isolated by adherence of plastic culture-ware. The number of MSCs expanded and differentiated into osteogenic cells in the presence of osteogenic supplements (dexamethasone, ascorbic acid and beta-glycerophosphate) plus basic fibroblast growth factor. The $5X10^6$ cells were impregnated on beta-tricalcium phosphate block (TCP). The tissue engineered stem cell-TCP composite was implanted on L5 and L6 transverse processes of the same animal in posterior spinal fusion with decortication as previous established model (3,4). Low intensity pulsed ultrasound (intensity of 30mW/cm^2, frequency of 1.5 MHz in 200μs pulse bursts at 1.0 kHz repetition rate) was applied for 20min daily on the back of rabbit, starting from 3 day post-operation and lasted for 7 weeks (LIPUS group, n=6). The untreated animals served as the control group. The spinal segments were harvested at week 7 assessed by manual palpation and scanned by microCT for radiological bony fusion. The volume of transverse processes was measured by peripheral quantitative computed tomogprahy (pQCT).

RESULTS: In the LIPUS group, 67% of spinal segment was found rigid by manual palpation Vs 0% in control group. The three dimensional microCT image of LIUPS group also showed bony fusion between two processes beneath TCP block (Fig. 1) but there was inter-transverse process gap in the control group. In the pQCT result, the volume of transverse processes in LIPUS group was 21.9% greater than the control group (p<0.05).

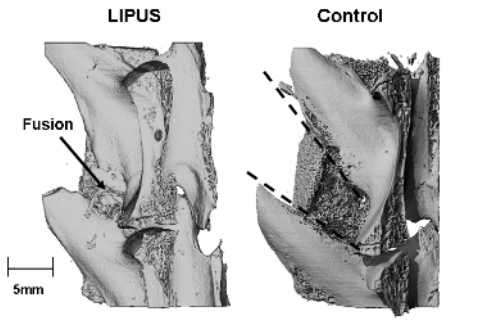

Fig. 1. 3D reconstructive microCT images of LIPUS and control groups

DISCUSSION & CONCLUSIONS: LIPUS exerted micro-mechanical stress on fusion bed in spinal fusion model. It stimulated proliferation and differentiation of mesenchymal stem cells into osteogenic cells which were more favorable to enhance bone formation in spinal fusion (2). Thus LIPUS treatment is shown to be able to promote spinal fusion with implanted tissue engineered stem cell-tricalcium phosphate composite.

REFERENCES: [1]P.A. Glazer, M.R. Heilmann, J.C.Lotz, et al (1998) *Spine* **23**:1142-8. [2]C.W. Chan, K.H.K. Wong, K.M. Lee, et al (2007) *Key Eng Mater* **330-332**:1137-40. [3]C.W. Chan, K.M. Lee, L. Qin et al (2007) *Key Eng Mater* **330-332**:1149-52. [4]C.W. Chan, L. Qin, K.M. Lee et al (2007) *Key Eng Mater* **334-335**: 1201-3.

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Regulation of Bone Remodeling by P2X7 Nucleotide Receptors in Osteoblasts and Osteoclasts

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INTRODUCTION: ATP and other nucleotides are released from cells in response to mechanical stimuli. Studies by us and others have shown that extracellular nucleotides interact with osteoblasts and osteoclasts through multiple subtypes of P2 cell-surface nucleotide receptors (*Fig. 1*). The P2X receptor family are ligand-gated cation channels, whereas the P2Y family are G protein-coupled receptors [1]. The P2X7 receptor knockout (*P2rx7*$^{-/-}$) mouse exhibits a unique skeletal phenotype – diminished periosteal bone formation, excessive trabecular bone resorption [2], and impaired response to mechanical loading [3]. Our current studies focus on the signaling pathways and roles of P2X7 receptors in bone.

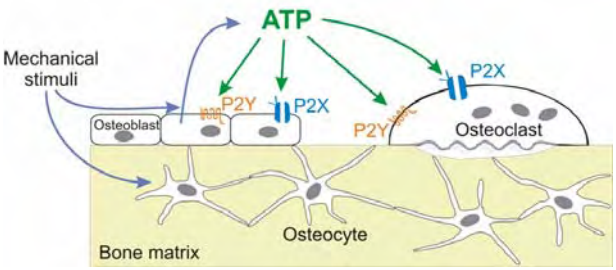

Fig. 1: Nucleotides are released from cells of the osteoblast lineage in response to mechanical stimulation. Once in the extracellular environment, ATP may act in an autocrine or paracrine manner to stimulate bone remodeling through activation of P2 receptors expressed on the surface of skeletal cells.

METHODS: Osteoblast-enriched cultures were obtained by sequential collagenase digestion of calvariae of newborn rats and mice (*P2rx7*$^{-/-}$ and wild type). After reaching confluence, cultures were supplemented with ascorbic acid and β-glycerophosphate to induce nodule formation. Osteoclasts were isolated from long bones of newborn wild-type and *P2rx7*$^{-/-}$ mice.

RESULTS: In differentiated osteoblast-enriched cultures, cells expressing P2X7 receptors were associated with bone nodules. Osteoblast differentiation and mineralization in *P2rx7*$^{-/-}$ cultures were diminished compared to wild type. Furthermore, benzoylbenzoyl-ATP (BzATP, P2X7 agonist) promoted differentiation and mineralization in rat calvarial cell cultures. P2X7 receptors coupled to production of the potent lipid mediators lysophosphatidic acid (LPA) and prostaglandin E_2. Moreover, either an LPA receptor antagonist or cyclooxygenase inhibitors abolished the stimulatory effects of P2X7 receptor activation on osteogenesis *in vitro*. Next, osteoclast apoptosis was assessed based on nuclear morphology. In the absence of exogenous nucleotides, higher numbers of wild-type than *P2rx7*$^{-/-}$ osteoclasts exhibited apoptosis 6 hours following isolation. Consistent with this observation, fewer wild-type osteoclasts survived at 12 hours. Interestingly, BzATP had no additional effect on either apoptosis or survival. Brilliant blue G (P2X7 antagonist) decreased apoptosis and increased survival of wild-type osteoclasts.

DISCUSSION & CONCLUSIONS: These investigations have led to the discovery of a novel signaling axis that couples P2X7 receptors on osteoblasts through phospholipases to production of LPA, which in turn stimulates osteogenesis. In contrast, activation of P2X7 receptors on osteoclasts leads to cell death through apoptosis. Thus, nucleotides – released in response to mechanical stimulation – may serve as autocrine/paracrine regulators of bone cell function, providing a biological basis for mechanotransduction in the skeleton. Moreover, the P2X7 receptor is a potential target for the development of drugs with combined anabolic and antiresorptive effects.

REFERENCES: [1] Burnstock G. (2007) *Physiol Rev* **87**:659-797. [2] Ke H.Z., Qi H., Weidema A.F., et al. (2003) *Mol Endocrinol* **17**:1356-67. [3] Li J., Liu D., Ke H.Z., et al. (2005) *J Biol Chem* **280**:42952-59.

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Bone Repair Mediated by Bone Sialoprotein

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INTRODUCTION: In bone, the organic matrix consists of type I collagen and a variety of proteins, including bone sialoprotein (BSP). BSP is an Arg-Gly-Asp (RGD) containing adhesive protein with expression essentially restricted to mineralized tissues. BSP has numerous postulated functions including promoting mineralization, osteoprogenitor cell differentiation and angiogenesis, properties relevant to bone formation and repair. Recent studies have shown the efficacy of BSP in promoting bone repair *in vivo* [1]. The purpose of our studies is to delineate the mechanism by which BSP promotes osteoblast differentiation and matrix mineralization.

METHODS: *Cell Culture:* BSP expression was specifically increased and decreased by CMV-mediated adenoviral overexpression in primary osteoblasts and RNA interference in the MC3T3E1 cell line, respectively. Alternatively, osteoblast cultures were supplemented with recombinant BSP protein. Markers for osteoblast differentiation were determined by real-time PCR, western blot, enzyme analysis and mineral deposition.
BSP-Collagen Interaction: A solid-phase binding assay and affinity chromatography were used to characterize the BSP-collagen interaction. A hydroxyapatite (HA) nucleation assay involving steady state agarose or collagen gels was used to determine the potency of BSP in promoting mineralization.

RESULTS: The overexpression of BSP increased osteoblast-related gene expression as well as nodule formation and calcium incorporation by osteoblastic cells in culture. Similarly, supplementation of osteoblastic cultures with recombinant BSP increased several markers of osteogenic differentiation. Conversely, suppression of BSP expression by small hairpin RNA-encoding plasmids inhibited expression of osteoblast markers and nodule formation. We also examined the signaling pathways that are activated by BSP. Infection with adCMV-BSP resulted in increased expression of the integrin subunits α_v, ß$_3$ and ß$_5$ activation of focal adhesion kinase (FAK) and ERK, effects that were not observed in cells infected with control virus or BSP with an altered RGD sequence.

We have previously shown that a specific sequence in rat BSP (19-46) binds collagen [2] and that the glutamic-acid rich sequences of BSP (located within 43-100 and 134-206) mediate the nucleation of HA [3]. Our current studies show that BSP-binding affinities to triple-helical and fibrillar type I collagen are similar (K_D ~13 nM), that collagen telopeptides are not required for binding, and binding affinities to heat-denatured collagen is lower (K_D ~44 nM). Using immobilized collagen linked to beads, the majority (~80%) of collagen-bound BSP was eluted by acetonitrile, whereas 1 M NaCl released only control levels of bound BSP. This indicates that hydrophobic interactions are responsible for binding of BSP to collagen. Using a HA-nucleation assay, we demonstrate that BSP is ten-fold more potent a nucleator of HA in reconstituted fibrillar collagen gels than in agarose gels (under conditions where there is no mineralization in the control gels), whereas nucleation potency was not altered for a non-collagen-binding, HA-nucleating peptide [BSP (134-206)]. This suggests a co-operative effect of BSP and collagen in mineral formation.

DISCUSSION & CONCLUSIONS: These results demonstrate that BSP may serve multiple functions in promoting bone formation and repair: as a matrix-associated signal directly promoting osteoblast differentiation resulting in the increased production of a mineralized matrix, and as a nucleator of hydroxyapatite that would allow for stabilization of the collagenous matrix. These results support the concept of bone-repair therapeutic peptides based solely on the collagen-binding and HA-nucleating properties of BSP. Ongoing *in vivo* studies are being utilized to characterize the efficacy of BSP and its functional domains in promoting bone regeneration and repair.

REFERENCES: [1] J Wang, HY Zhou, E Salih, et al. *Calcif Tissue Int* (2006) **79:** 179-89. [2] CE Tye, GK Hunter, HA Goldberg (2005) *J Biol Chem* **280**:13487-92. [3]CE Tye, KR Rattray, KJ Warner, et al (2003) *J Biol Chem* **278:** 7949-55.

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Angiogenic potential of the early fracture haematoma is increased by mechanical stimulation

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INTRODUCTION:
Mechanical stimulation and angiogenesis play important roles in the bone healing process. The increased secretion of matrix metalloproteases (MMPs) and paracrine stimulation of angiogenesis by mechanical loading of mesenchymal stem cells have been described previously [1]. However, the full interaction between angiogenesis and mechanical loading in the *in vivo* situation remains unclear. This study aimed to investigate the angiogenic potential of the human fracture haematoma itself and how this changes with mechanical stimulation, as well as identify molecules potentially involved.

METHODS:
Fracture haematomas were isolated during surgery within 72 hours post-trauma. They were embedded in a fibrin matrix, immersed in cell culture medium and mechanically stimulated in a bioreactor simulating the in vivo-conditions in the early phase of bone healing (3 days, 1 Hz, 10 kPa). Conditioned media (CM) were collected and analysed with zymography (collagen, gelatine, casein and reverse gels) for detection and identification of proteases and their inhibitors and ELISA kits for their quantification. The pro-angiogenic potential of the stimulated haematomas was evaluated by measuring the length of the capillary network formed by endothelial cells (HMEC-1 cell line) cultivated on Matrigel™ in the presence of the CM in an angiogenesis assay. Endothelial cells proliferation was measured using an MTS test at the end of the angiogenesis assay. Pro-angiogenic properties of the haematomas themselves were analysed in parallel using the same techniques.

RESULTS:
Both MMP-2, active and proform, and MMP-9 proform were detected in haematomas by gelatine zymography, but no significant stimulation of angiogenesis could be shown by application of the haematoma itself to cell cultures. HMEC proliferation was not affected by the application of the haematoma.
Zymography and ELISA assays showed a regulation of molecules involved in angiogenesis, such as TIMP-2. In vitro angiogenesis assays showed increased tube formation when stimulated with CM from mechanically stimulated haematomas in bioreactor. Proliferation of HMECs in CM was not affected by mechanical stimulation.

DISCUSSION AND CONCLUSIONS:
In summary, extracellular pro-angiogenic MMPs are present in the early human fracture haematoma and mechanical loading enhances its pro-angiogenic potential. Their regulation could be a bridge between mechanical stimulation and angiogenesis. Such a link could provide new therapeutic strategies for bone healing by stimulating blood vessel formation.

REFERENCES:
[1] G. Kasper et al., Stem Cells, 2007 Apr; 25(4): 903-10

ACKNOWLEDGEMENTS:
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Bioavailability of PAPP-A, the Local Regulator of IGF-I (Insulin-like Growth Factor-I), in the Intervertebral Disc

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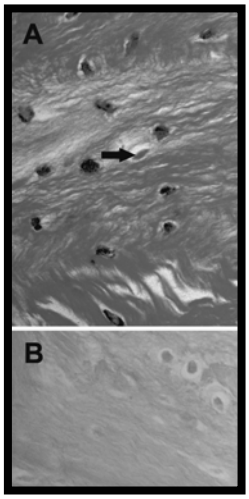
INTRODUCTION: Little is known about cellular repair processes during disc degeneration. We recently found that there was a significantly greater expression of PAPP-A in Thompson grade III and IV discs compared to healthier grade I/II discs (Spine 32:1181, 2007). We think that this is an important finding reflecting a cellular repair process involving modulation of the IGFBPs (insulin-like growth factor binding proteins) which are potent inhibitors of cell proliferation and matrix synthesis. PAPP-A cleaves IGF-binding proteins-2, 4 and 5, thereby increasing the bioavailability of IGF-I to nearby cells. Objectives: 1) To test for the presence of PAPP-A in human discs using immuno-histochemistry (IHC); 2) To test for gene expression of PAPP-A in the human disc in vivo and in vitro; 3) To determine whether cultured human annulus cells express/produce detectable amounts of PAPP-A, and 4) To test for expression of insulin-like growth factor binding proteins (IGFBP)-2,-4 and -5 in vivo and in vitro by human disc cells.

METHODS: Studies were approved by our human subjects review board. Human discs were obtained from surgical specimens and control donors. IHC utilized anti-human pregnancy-associated plasma protein A (DAKO) at a 1:25 dilution. The proportion of cells with immunolocalization was measured. Disc cells from the annulus were cultured in replicates in a collagen sponge 3D growth microenvironment for 9 days (2,3). Cells grew an additional 5 days without changing media and at which time experiments were terminated, conditioned media collected, and PAPP-A detected with an ELISA assay (Ultra-Sensitive PAPP-A kit, Diagnostic Systems Laboratories; assay sensitivity 0.06 µIU/mL.) Gene expression studies utilized laser capture microdissected cells, or cultured cells, assayed with the Affymetrix human U133 X3P microarray (1). Data are means ± SD (n).

RESULTS: Positive cytoplasmic localization of PAPP-A was present in most cells of the outer annulus (OA) (Fig. 1). A significant positive correlation was found between the % positive cells and disc grade. Human annulus cells produced and secreted abundant PAPP-A into the culture media (Table 1)

Fig. 2



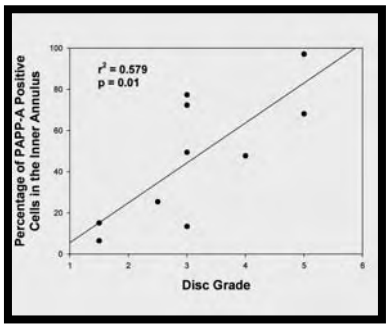


Fig. 1

Fig. 1: *A. Positive localization of PAPP-A is present in most OA cells. A few negative cells are present (arrow). B. Negative control.*

Fig. 2*: A significant positive correlation was present between disc grade and the percentage of PAPP-A positive cells in the inner annulus grade (r^2 = 0.579; p = 0.01).*

Table 1. PAPP-A Production (µlU/ml) by Human Annulus Cells in 3D Culture

Grade	Age	PAPP-A Levels (µlU/ml)
2	45	23.33
3	38.5 ± 7.9 (4)	24.34 ± 6.55 (4)
4	41.6 ± 15.8 (2)	19.18 ± 15.79 (3)

Molecular studies confirmed the expression of PAPP-A (NM_002581.1) and IGFBP-2, -4, and -5 (NM_000597.1, NM_001552.1, NM_0005991, respectively) in vivo and in vitro.

DISCUSSION & CONCLUSIONS: Results confirm PAPP-A expression/production by human disc cells in vivo and in vitro, and confirm expression of IGFBP-2, -4, and -5. These novel data are consistent with the hypothesis that increased expression of PAPP-A in more degenerate discs (which may be activated by cytokines such as TNF-α) may reflect cellular repair responses designed to cleave IGFBP-2, -4 and -5, thereby uncovering IGF-I and increasing proliferation and matrix synthesis cues signaled by the resultant increased IGF-I bioavailability.

In vivo Gait analysis in murine femoral fracture model

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INTRODUCTION

Gait analysis is used as a powerful technique for evaluating locomotion such as motion and load patterns in humans and large laboratory animals. In small animal models the gait analysis is only limited useful. A murine fracture model has not been described yet. Therefore the aim of the study was to define and describe parameters of gait analysis in murine fracture model.

METHODS

Ten CD-1 mice (32 ± 3 g) were divided into two groups. All mice underwent prior training to become accustomed to walking in the running wheel. In 5 mice, a standardized closed midshaft fracture was produced using a 3-point bending device. For fracture stabilization a common pin (10 mm) was used. 5 mice served as control group, one femoral pin (10 mm) was inserted without producing a fracture as femoral marker. Both groups were additionally marked by one tibial pin (5 mm). An x-ray permeable running wheel was used for gait analysis. Multiple running cycles were digitized with 30 images/ s by a digital angiography x-ray system to identify the markers. Fourteen days after surgery the following gait parameters were determined: the minimum and maximum tibio-femoral angle, the stride frequency, the stride time, the stride length and the stride velocity. Eighteen representative strides per mouse were analyzed. All measurements were done using the Java based open source program ImageJ.

RESULTS

The control group showed a significantly higher maximum (96.6 ± 1.2° vs. 74.7 ± 1.2°; $p \leq 0.05$) and minimum (41.6 ± 1.4° vs. 51.4 ± 1.2°; $p \leq 0.05$) tibio-femoral angle compared to the fracture group. Measuring the stride frequency and stride time both groups showed comparable results without significant differences (stride frequency: 4.8 ± 0.3 vs. 4.6 ± 0.3 strides/ s; stride time: 0.3 ± 0.1 s). In the control group stride length was significantly larger (9.2 ± 0.2 cm) than in the fracture group (5 ± 0.1 cm). By consequence, a significantly higher stride velocity was observed in the control group (42.7 ± 5.3 cm/ s vs. 23.6 ± 0.4 cm/ s).

CONCLUSION

In the present study we describe a novel approach to quantify the fixation techniques and the fracture healing process using gait analysis. This study was conducted to measure different characteristic parameters to investigate the stability served by different methods of fracture stabilization. Further the herein presented results will help to design future studies with standardized mechanical conditions for analyzing mechanisms of fracture healing.

Osteochondral tissue engineering by implication of perfusion and cyclic compression on mesenchymal cell-loaded hybrid grafts

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INTRODUCTION: Osteochondral lesions are often seen in trauma and orthopedics but available treatment strategies are limited in success. Regenerative medicine may provide novel concepts for curing osteochondral lesions[1, 2]. The purpose of this study was to establish a system of human bone marrow stromal cells (hBMSCs) cultured in a 3-D collagen I-bone hybrid matrix to test the effects of perfusion and cyclic compression on cell differentiation and mechanical properties using a custom-made biomechanoreactor[3].

METHODS: Human BMSC were harvested from the iliac crest during routine trauma surgeries. Briefly, density centrifugation was used to obtain a cell pellet that was resuspended in culture media supplemented with 10% fetal calf serum, 200 U/ml penicilline/streptomycin, 2.5 µg/ml amphotericin B, 3 ng/ml FGF-2 buffered with Hepes buffer, subsequently plated in 75 cm^2 culture flasks and incubated at 37°C and 5% CO_2 in humidified atmosphere. Cells of the third passage were used for the experiments. hBMSCs were mixed with a collagen I matrix and consolidated onto cancellous bone slices (Fig. 1). The osteochondral matrices consisted of commercially available products: CaReS® (rat collagen I, Arthrokinetics, Esslingen, Germany) and Tutobone® (bovine spongiosa, Tutogen Medical GmbH, Neunkirchen a. Br., Germany). Continous pressure and vacuum forces were applied in a specially developed glass kit. Constructs were stimulated by perfusion or cyclic compression for 7, 14, 21, and 28 days. Cell viability, expression of collagen II and X, the amount of glycosaminoglycan (GAG) and DNA as well as mechanical properties were analyzed at each time point.

RESULTS: hBMSCs were viable for 88 ± 8.9% during the entire experimental period. Levels for GAG and DNA did not change. Perfusion induced collagen II and cyclic compression increased collagen X expression, indicating the activation of distinct gene expression patterns. Matrix stiffness was significantly increased after 28 days of cyclic compression (Fig. 2).

Fig. 1: Compressed osteochondral construct (22 mm x 11 mm)

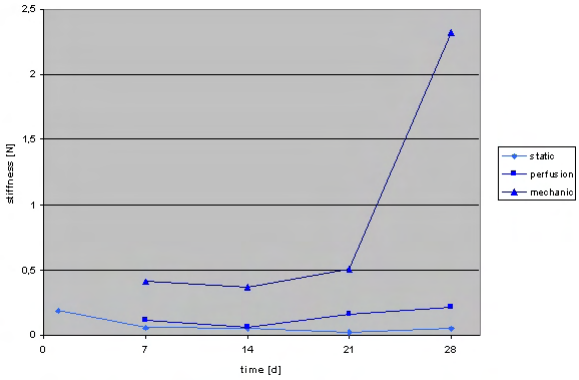

Fig. 2: Biomechanical assay results for stiffness.

CONCLUSIONS: Cyclic compression of cell-loaded collagen I-bone hybrid constructs enhanced chondrocyte differentiation and matrix stiffness[4]. This system is a promising tool to develop novel concepts to cure osteochondral lesions[3].

REFERENCES: [1] A.I. Caplan, S.P. Bruder (2001) *Trends Mol Med* **7**:259-264. [2] M.J. Zuscik, M.J. Hilton, X. Zhang, D. Chen, R.J. O'Keefe (2008) *J Clin Invest.***118**:429-438. [3] C. Haasper, J. Zeichen, R. Meister, C. Krettek, M. Jagodzinski (2008) *Injury S* **39**:66-76. [4] C. Haasper, M. Colditz, L. Kirsch, J. Zeichen, C. Krettek, C. Hurschler, M. Jagodzinski (2007) *Osteoarthritis Cartilage* **15**:B78.

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BMP-7 Gene Therapy for Mitigation of Post-traumatic Osteoarthritis in Sheep

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INTRODUCTION Purified hrBMP-7 protein has been an effective treatment in experimental models of osteoarthritis and cartilage repair, and its success may be attributable to its homeostatic role in the synovial environment[1]. These experiments have used multiple injections, scaffolds or osmotic pumps to sustain levels of BMP-7 in synovial fluid over several days; however, there are many problems associated with purified protein production and delivery. An alternative approach is gene therapy using vector or physical transfection systems to drive expression of proteins at the appropriate location. This experiment describes the use of an adenoviral vector successfully expressing recombinant murine BMP-7 for amelioration of post-traumatic osteoarthritis. The objective of this experiment was to demonstrate gene expression at a therapeutic concentrations in synovial fluid and demonstrate protection against post-traumatic osteoarthritis.

METHODS: Ex vivo and in vivo gene expression and dose titration pilot studies were done to optimize expression of rmBMP-7 in synoviocytes, HEK cells and chondrocytes. BMP-7 concentration in synovial fluid was measured by ELISA (R&D systems) with appropriate standard curves to ensure cross-reaction with the murine BMP-7. To investigate the chondroprotective potential of BMP-7 in a model of post-traumatic osteoarthritis, 16 sheep underwent a bilateral minimally-invasive arthrotomies to create contusive impact injuries on the weight bearing surface of the medial femoral condyle. One week later 12 sheep received 10^9 Ad5-BMP-7 virus particles in one knee joint by intra-articular injection. Four animals received an equal volume of saline and acted as controls. Synovial fluid was aspirated from all joints to assess inflammation and BMP-7 concentration at intervals during the study, and the sheep were sacrificed 90 days after knee injury.

Post-sacrifice assessments included microCT imaging of the knees, macroscopic assessments, digitized India ink staining for assessment of cartilage damage, histological scoring (OARSI) and immunostaining for TUNEL, and the collagen degradation fragment Coll 2 ¾ short (Ibex Pharmaeuticals, Montreal).

RESULTS: Ex vivo transfection demonstrated that BMP-7 concentrations up to 3000 pg/mL could be achieved at 96 hours with MOIs of 10 to 200 in HEK cells while synoviocytes had slightly lower concentrations expressed at 50-200 MOI. Sheep had minimal post-injection flare and synovial fluid leukocytosis after injection of the vectors. Peak levels of synovial fluid BMP-7 (1000 pg) occurred 14 to 21 days post transfection and remained elevated beyond day 28. Ad5-BMP-7 treated joints had less abnormal cartilage area ($p<.001$, T-test) and improved histological scores ($p<.004$ T-test) compared to control group joints. Immunostaining for TUNEL and the Coll 2 ¾ short collagen epitope was also reduced in Ad5-BMP-7 treated joints. No ectopic mineralization was detected osteophytes were a rare finding.

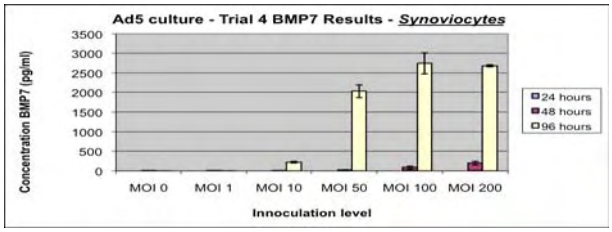

Fig. 1: Effect of MOI on BMP-7 expression kinetics in ovine synovial cells.

DISCUSSION & CONCLUSIONS: These data demonstrate protection against post-traumatic osteoarthritis in a large animal model using a relatively low number of viral vector particles. This may have helped limit the inflammatory response to the vector that in turn allowed prolonged expression of the gene product. Additional studies that investigate additional factors governing the rate and amount of gene expression in this system are ongoing.

REFERENCES: [1] S. Chubinskaya et al. OP-1/BMP-7 in cartilage repair. Int Orthop. 2007 Dec;31(6):773-81.

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Mesenchymal stem cell ageing is associated with alterations in antioxidant defense and cytoskeleton dynamics

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INTRODUCTION:

Mesenchymal stem cells (MSCs) are involved in the regeneration of several tissues, such as bone, cartilage, tendon and skeletal muscle [1, 2]. Thus, these cells have elicited great hopes for the development of novel cell based therapies for the augmentation of the physiological regeneration processes, e.g. in bone defect healing. This regeneration potential decreases with age, raising questions about the efficiency of autologous approaches in elderly patients [3, 4]. To elucidate the mechanisms and cellular consequences of MSCs ageing, this study investigated the functional and proteomic changes occurring in MSCs derived from young (yMSCs) and old (oMSCs) Sprague-Dawley rats.

METHODS:

MSCs were isolated from bone marrow and selected by plastic adherence. Their phenotype was characterised by flow cytometry (CD44+; CD73+, CD90+ and CD45-). The numbers of colony forming units (CFUs) were determined four days after isolation. The migration capacity was measured in a modified Boyden chamber assay. Senecent cells were identified by their ß-galactosidase activity. The osteogenic differentiation potential was analysed by measurement of alkaline phosphatase activity (ALP) and matrix mineralization (Alizarin Red) after their incubation in osteogenic medium. Adiopogenic differentiation was visualized by Sudan IV staining after stimulation. Cell proliferation capacity was investigated by short term proliferation assays as well as determination of the population doubling time over a culture period of more than 10 weeks. The proteomes of yMSCs and oMSCs were analysed using high resolution 2D-PAGE followed by mass spectrometric protein identification as described by Klose and colleagues [5]. Antioxidant activities were assayed using a commercially available kit according to the manufacturer's instructions. The actin cytoskeleton was stained with conjugated phalloidin and imaged using confocal microscopy.

RESULTS:

Both yMSCs and oMSCSs were homogeneous and similar in their expression pattern of surface marker proteins (CD44+, CD73+, CD90+ and CD45-). We further observed that MSC concentration in bone marrow declines with the age of rats. In addition, their migratory capacity and susceptibility towards senescence were altered. In contrast, the differentiation potential of rat MSCs was not dependent on the age of the animals. High resolution 2D electrophoresis of the MSC cultured either in expansion or osteogenic media revealed that age-related molecular changes to be associated with cytoskeleton organization and antioxidant defence. Observably, MSC populations from old animals contain more actin filaments and harboured a reduced total antioxidant power. Interesting age-dependent proteins identified in this study are members of the calponin protein family as well as galectin-3.

DISCUSSION AND CONCLUSIONS:

These data imply that MSC-based therapeutic approaches for the elderly should focus on attracting cells to the site of injury and oxidative stress protection, rather than stimulating differentiation.

REFERENCES:

[1] Shirley et al. (2005), *J Orthop Res* 23(5):1013-1021;
[2] Pittenger et al. (1999) *Science* 284:143-147;
[3] Meyer et al. (2001) *BMC Musculoskelet Disord.* 2:2;
[4] Rauscher et al. (2003) *Circulation* 108(4):457-463;
[5] Klose et al. (1999) Methods Mol Biol. 112: 147-172

ACKNOWLEDGEMENTS:

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Solidification Mechanisms of Chitosan-Glycerol Phosphate/Blood Implant for Articular Cartilage Repair

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INTRODUCTION: Chitosan-glycerol phosphate is a unique chitosan polymer solution that is mixed with whole blood and solidified over microfractured or drilled articular cartilage defects in order to elicit a more hyaline repair cartilage [1]. Rapid *in situ* solidification could improve clinical ease-of-use. Therefore, we investigated the mechanisms of chitosan-GP/blood implant solidification and evaluated the effect of added clotting factors.

METHODS: Chitosan-GP was mixed at a 1 to 3 ratio with non-activated whole peripheral blood [1]. Clotting tests were carried out with a Thromboelastograph® (TEG) [2] with or without tissue plasminogen activator (tPA) and/or clotting factors. Serum was analyzed by TAT-ELISA for IIa generation [2], and by Western for platelet activation (Platelet factor 4, PF4), and FXIII activation. *In vivo* solidification of chitosan-GP/blood, with and without clotting factors, was analyzed in microdrilled cartilage defects of skeletally mature rabbits (N=36) [3].

RESULTS: Unlike whole blood, chitosan-GP/blood clots solidified in an atypical two-step manner, with significantly higher initial viscosity and similar final clot tensile strength (Fig.1). Addition of tPA partly depressed chitosan-GP/blood clot tensile strength compared to whole blood (Fig.1). Chitosan-GP/blood clot showed minor platelet activation followed by a secondary and more gradual increase in clot tensile strength, thrombin generation, burst platelet activation, FXIII activation and demonstrated more sustained levels of PF4 compared to whole blood (Fig. 2). Clotting factors [IIa, Tissue Factor (TF), TF-rhFVIIa, rhFVIIa] accelerated chitosan-GP/blood solidification *in vitro* ($p<0.05$). Co-delivery of IIa and layering of TF-rhFVIIa over drilled cartilage defects accelerated chitosan-GP/blood implant solidification *in vivo* ($p<0.05$) (Fig.3).

DISCUSSION & CONCLUSIONS: Chitosan-GP/blood implants solidify by coagulation. Clotting factors can be used to shorten the *in situ* solidification time of chitosan-GP/blood implants in microdrilled cartilage defects.

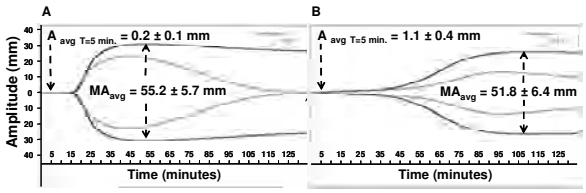

Fig. 1: Representative TEG trace of whole blood (A) and chitosan-GP/blood (B) ± tPA.

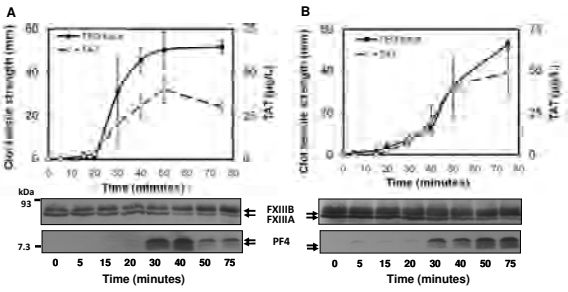

Fig. 2: Clot tensile strength (black line) and TAT generation (dotted line) for whole blood (A) and chitosan-GP/Blood (B). FXIII activation (middle panel) and platelet activation (PF4, bottom panel).

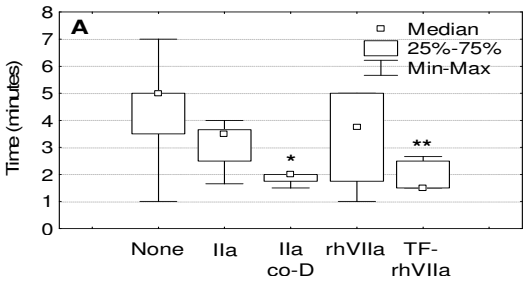

Fig. 3: Effect of clotting factors on in situ solidification time of chitosan-GP/blood implant.

REFERENCES: [1] C.D. Hoemann et al (2005) *J Bone Joint Surg Am* **87**(12):2671-86.,[2] G.E. Rivard et al *J Thromb Haemost* **3**:2039-43, [3] C.D. Hoemann et al (2007) *OA&C* **15**(1): 78-89.

ACKNOWLEDGEMENTS: Angélique Hoefer, Francine Derome. **Support** CIHR, FRSQ, CAN.

Intercellular propagation of signal induced by mechanical stimulation of a single bone marrow cell

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INTRODUCTION: Skeletal repair is influenced by mechanical loading. Mechanical loading inhibits bone resorption by osteoclasts and increases bone formation by osteoblasts, whereas unloading leads to bone loss (1). Thus osteoclasts and osteoblasts respond to mechanical stimulation in coordinated manner (2). The objective of this study was to assess how the mechanical stimulation of a single bone cell can be transmitted and perceived by other bone cells.

METHODS: Mouse bone marrow cells were cultured for 6-12 days and loaded with calcium-sensitive dye Fura-2-AM. Single cell was mechanically stimulated by gentle touch with a glass micropipette, and real-time changes in cytosolic free calcium concentration ($[Ca^{2+}]_i$) in stimulated cell and its neighbors were monitored using fluorescence microscopy (*Fig.1)*

RESULTS: Mechanical stimulation of a mononuclear, attached, well spread cell, likely osteoclast or osteoblast precursor, resulted in an increase in $[Ca^{2+}]_i$. In 11 out of 14 experiments mechanical stimulation of a single cell also led to subsequent rise in $[Ca^{2+}]_i$ in unconnected neighboring cells positioned at distance up to 140 µm away (*Fig.2*). In 3 experiments without secondary responses, change in $[Ca^{2+}]_i$ in primary cells were transient and of lower amplitude. In contrast, in 11 experiments with secondary responders, primary cells exhibited sustained calcium elevation to higher levels. Mechanical stimulation of a single cell often resulted in signal propagation to multiple secondary cells with 1 to 22 second delay from the primary response. The amplitude and duration of the responses of secondary cells was inversely proportional to delay and distance from the original cell, supporting the soluble mediator-release theory (*Fig.3* and *Fig.4*).

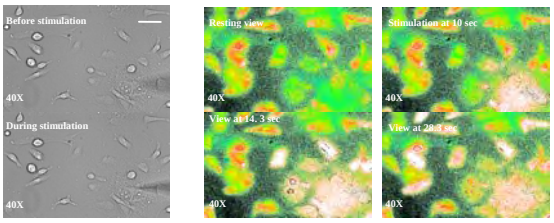

Fig.1: Bright field images showing the primary cell stimulation and snapshots of the fluorescent imaging during the experiment.

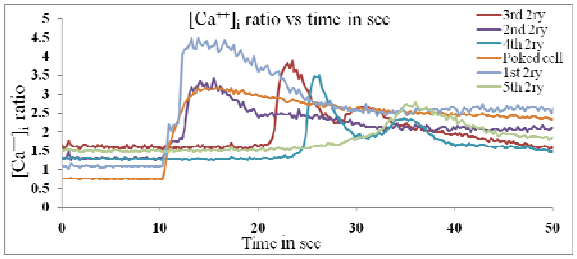

Fig.2: Mechanical stimulation produced secondary responses in unconnected neighbouring cells positioned at distance up to 140 µm away.

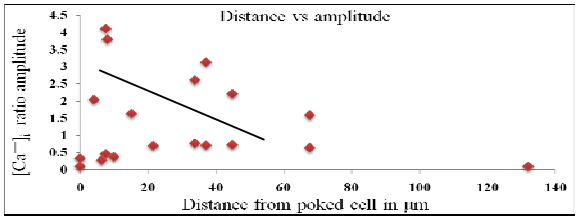

Fig.3: The more distant the secondary responder, the lower its response-amplitude

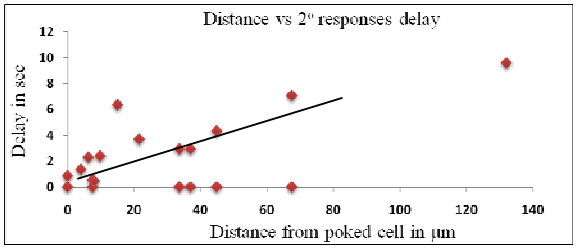

Fig.4: The more distant the secondary responder, the longer its response-delay

DISCUSSION & CONCLUSIONS: we have found that mechanical stimulation of a single bone marrow cell results in a release of a soluble mediator which transmits the signal to unconnected neighboring cells. This phenomenon may be important in coordination of the responses of different cells to mechanical stimulation.

REFERENCES: [1] R. L. Duncan, C. H. Turner (1995), *Mechanotransduction and the Functional Response of Bone to Mechanical Strain*, Calcif Tissue Int. 57:344-358. [2]Abdelilah Arredouani (2004), *Diversification of Function and Pharmacology in Intracellular Calcium Signaling*, Cellscience, Vol. 1 No. 1.

ACKNOWLEDGEMENTS: This work was supported by CIHR, CAN & NERSC.

Investigating the Effects of OA on Rodent Knee Vasculature Using Contrast Enhanced Micro-Computed Tomography

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INTRODUCTION: Previous work investigating angiogenesis in models of Osteoarthritis (OA) has been both, 2-dimensional, and destructive in nature[1]. Modern micro-computed tomography systems allow for non-destructive, three-dimensional (3D) visualisation of bone and soft tissue. The purpose of this study was to determine if we could accurately measure the changes to the vascular anatomy within bone, occurring in a rodent model of OA, using contrast enhanced micro-CT.

METHODS: Male Sprague-Dawley rats underwent anterior cruciate ligament transection with partial menisectomy of the right hind limb (ACLX)[2]. After OA induction, the animals (n = 5, per group) were trans-cardially perfused with Microfil at 2, 4, 8, and 20 weeks post-surgery. Microfil is a lead-silicone based contrast agent that polymerizes inside the blood vessels and organs. The knees were disarticulated and scanned with a micro-CT system designed for small specimens. Both the operated and the un-operated knees of the same animal were scanned, along with a Sham operated group (n = 5).

The ex vivo imaging protocol produces a three-dimensional micro-CT image with isotropic voxel dimensions of 0.028 mm x 0.028 mm x 0.028 mm. Images were viewed in various formats such as multi-planar reformations (MPR), maximum intensity projections (MIP), and surface renderings to determine the size, shape, and path of blood vessels within the knee joint. The medial surface of the knee was semi-automatically segmented from the bone in order to calculate the vessel volume fraction (VVF) of the soft tissue surrounding the knee.

RESULTS: Qualitative differences in knee vasculature were observed between OA and non-OA micro-CT data. Nutrient arteries and veins, branching from the major popliteal artery/vein, were witnessed entering both the subchondral and cortical bone at various locations of the femur and tibia (Figure 1). Vessels around the patellar tendon and synovium in the surgical group increased in number and appeared to lack organisation. Quantitative differences in VVF will be discussed.

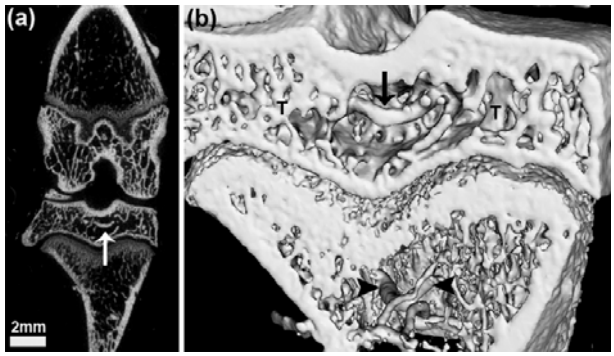

Fig. 1: Blood vessel perfusion in the rodent knee using contrast enhanced micro-CT: Multi-planar reformatted (a) and surface rendering (b) of the right knee. Note the visualisation of vessels within the trabecular bone (T) of the tibial epiphysis (arrows – a,b), and metaphysis (arrowheads - b).

DISCUSSION & CONCLUSIONS: The contrast enhanced micro-CT protocol provided sufficient spatial resolution to effectively visualize and quantify 3D vascular anatomy and its changes during early and late-stage OA.

REFERENCES: [1] P.I. Mapp, P.S. Avery, D.F. Williams, and J. Bowyer (2008) *Osteoarthritis and Cartilage* **16**:61-69. [2] C.T. Appleton, D.D. McErlain, V. Pitelka, et al (2007) *Arthritis Res Ther.* **9**(1):R13.

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Normal human dermal fibroblasts differentiate into contractile myofibroblasts after contact with bioactive surfaces

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INTRODUCTION: The aim of the EU funded research project „CellProm" is the induction of cell differentiation by contact with surfaces modified in the nanometer range. Transforming Growth Factor beta 1 (TGF β_1) is known to induce the myofibroblastic (MF) differentiation [1, 2] of normal human dermal fibroblasts (NHDF) and was immobilized covalently to functionalized slides and nanobeads, respectively. The inducing properties of the generated bioactive surfaces were evaluated in cell culture experiments.

METHODS: Aldehyde (2 manufacturers), epoxy- and amino functionalized slides served as substrates for the immobilization of TGF β_1 (c = 5 µg/ml). The nanobeads were coated with TGF β_1 and BSA (control), respectively (c = 50 µg/ml) and were subsequently spotted on glass slides. The success of the coupling was evaluated immunologically.
NHDF were seeded at a cell density of 63 cells/mm^2 on the substrates and were cultured for 2 days. The MF marker protein α-smooth-muscle actin (α-SMA) was immunostained and the amount of α-SMA-positive cells was determined microscopically in relation to all cells (differentiation rate). A t-test was performed to evaluate statistical significance ($p < 0.05$).

RESULTS: A successful immobilization of TGF β_1 could be shown in the case of aldehyde- and epoxy functionalized slides and the nanobeads, respectively. The stability of the adsorbed nanobeads on the slides was given. Compared to the uncoated control area on the same slides the MF differentiation rate was increased by 6.4% (manufacturer 1, n = 6) and by 9.4% (manufacturer 2, n = 5) on TGF β_1-coated areas of the aldehyhde functionalized slides. Immobilization of TGF β_1 to the epoxy functionalized slides led to an increase in MF differentiation rate of 5.4%. No increase in MF differentiation rate could be detected using amino functionalized slides (n = 6). On TGF β_1-nanobeads the MF differentiation rate was increased by 6.3% (n = 8) compared to BSA-nanobeads. Compared to the bead-free glass slide the MF differentiation rate was increased by 8.6% on BSA-nanobeads (n = 8), maybe due to the influence of the beads` topology itself [3].

DISCUSSION & CONCLUSIONS: It is possible to immobilize TGF β_1 to aldehyde- and epoxy functionalized slides as shown by subsequent immunological detection. The maintenance of the activity is proven by the increased MF differentiation rate on the coated areas. TGF β_1 can be immobilized to reactive nanoparticles leading to an increased MF differentiation rate, too. The results show, that coated nanobeads are an excellent tool to achieve cell differentiation in vitro.

The distinct spatial and temporal application of several relevant factors using coated nanobeads is one of our goals in the future.

REFERENCES: [1] A. Desmouliere, A. Geinoz, F. Gabbiani, G. Gabbiani (1993) *J Cell Biol* **122**, 103-11. [2] J.J. Tomasek, G. Gabbiani, B. Hinz, C. Chaponnier, R.A. Brown (2002) *Nat Rev Mol Cell Biol* **3**: 349-63. [3] W. Metzger, N. Grenner, S.E. Motsch, R. Strehlow, T. Pohlemann, M. Oberringer (2007) *Tissue Eng* **13** (11): 2751-60

ACKNOWLEDGEMENTS: Parts of this study were supported by the Sixth Framework Program of the European Commission, Project No. FP6-500039-2, "CellPROM".

Transforming growth factor β_1 does not attenuate the hypoxia induced response of fibroblasts and endothelial cells in a new in vitro co-culture wound healing model

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INTRODUCTION: The coordinated interaction of endothelial cells and fibroblasts is a prerequisite for adequate soft tissue healing. Endothelial cells assure neoangiogenesis, whereas tissue fibroblasts mainly provide extracellular matrix proteins and are responsible for tissue tension. The differentiated form of the fibroblast, the myofibroblast (MF) might support the reconstitution of microvessels during early wound healing [1].
In the present study, we investigated the interaction of endothelial cells, fibroblasts and MF in vitro applying a newly developed co-culture setup: the "co-culture scratch-wound migration assay" (CCSWMA) [2]. This assay served to monitor cellular parameters like migration, proliferation and the differentiation from fibroblasts to MF as a response to hypoxia, which is typical for early wound healing.
We additionally focused on a possible mediation of the hypoxia response by transforming growth factor β_1 (TGFβ_1), which is one of the most important modulators of soft tissue healing.

METHODS: Scratch wounds on cell monolayers were induced in parallel on seven slides per experiment using co-cultured human dermal microvascular endothelial cells (HDMEC) and normal human dermal fibroblasts (NHDF) (n=10). Mono-cultures (HDMEC: n=3, NHDF: n=3) served as controls. Cell migration was determined by wound size measurement, and multicolour immunocytochemical staining applying antibodies for α-smooth-muscle actin (MF), von Willebrand factor (HDMEC) and extra domain A fibronectin (NHDF) served to monitor proliferation and differentiation from NHDF to MF after 0 h, 24 h and 48 h. Furthermore cells suffering from 24 h hypoxia (pO_2<5mmHg) were compared to normoxic cells and to cells with additional TGFβ_1-supplementation (c = 1 ng/ml). ANOVA and post-hoc analysis were used to evaluate statistical relevance of the differences ($p < 0.05$).
In parallel endothelin-1 (ET-1) secretion by HDMEC and its uptake by NHDF were measured by ELISA (n=6). The localization of the ET-1 release was visualized by immunocytochemical staining.

RESULTS: 24 h hypoxia induced a significant migration and proliferation decrease among mono- and co-cultured HDMEC, whereas the slight migration and proliferation decrease was not significant among NHDF. TGFβ_1-supplementation did not yield in a significant attenuation of the hypoxia induced proliferation and migration decrease in this in vitro-setup.
Nevertheless, we noticed, that the MF population was even able to increase during hypoxia in co-culture with HDMEC. The data acquired by ELISA support the assumption, that this response is mediated in part by ET-1.

DISCUSSION & CONCLUSIONS: Summarizing the data concerning TGFβ_1 and ET-1, we present a model illustrating the cellular interaction during early and late proliferation phase of human wound healing [3].
The CCSWMA is an appropriate in vitro-tool to investigate the effect of mediators of soft tissue healing and of potential therapeutic agents. Modifications of the assay involving other cell types will serve to investigate general processes of cartilage or bone related angiointegration in the future.

REFERENCES: [1]D.W. Powell, R.C. Mifflin, J.D. Valentic, S.E. Crowe, J.I. Saada, A.B. West (1999) *Am J Physiol* **277**, C1-C19. [2]M. Oberringer, C. Meins, M. Bubel, T. Pohlemann (2007) *Biol Cell* **99(4)**, 197-207. [3]M. Oberringer, C. Meins, M. Bubel, T. Pohlemann (2008) *J Mol Histol* **39**, 37-47.

FETAL SPINE CELLS : A POTENTIAL CELL SOUCE FOR INTERVERTEBRAL DISC REGENERATION?

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INTRODUCTION: Degeneration of the intervertebral disc (IVDD) is thought to be one main cause 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 investigate the feasibility of using fetal disc cells for disc tissue engineering.

METHOD: Fetal spinal column tissues (1 cm) were obtained 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). Consistency of the cell culture was assessed by measuring expression of galectin-3 and HIF-1 by flow cytometry at passages 4 and 6. Sulphated glycosaminoglycan (sGAG) production by fetal spine cells entrapped in alginate beads was identified by alcian blue staining and measured by DMMB assay and normalized to DNA content. mRNA levels of aggrecan, type I and II collagen were measured after 2, 7, 14 and 28 days of culture.

RESULTS: Isolated fetal cells proliferated more rapidly than adult NP cells and showed consistent culture regarding galectin-3 and HIF-1 expression at passages 4 and 6. Compared to monolayer culture, fetal spine cells in alginate beads portrayed a rounded morphology with a ring of pericellular matrix identified by alcian blue staining. However, some cells did not produce any matrix. Production of matrix was confirmed by an increase in GAG/DNA ratio during culture. mRNA level of aggrecan and type II collagen largely increased during the 28 day culture period whereas mRNA level of type I collagen remained stable and of type X collagen decrased.

DISCUSSION: Fetal spine cells can be cultured *in vitro* with minimal requirements and the consistency necessary for a potential clinical application. When cultured in a 3-D environment, they synthesize sGAG, which are responsible for the high water content in NP matrix, and they show chondrocyte-like gene expression. It will be of interest to investigate the regeneration capacity of the fetal spine cells in an appropriate animal model.

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Impact of peroxynitrite on intervertebral disc cells

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INTRODUCTION: Disc degeneration is an age-related process that can start as early as the second decade of life and a variety of inflammatory mediators have been implicated in degeneration and/or pain induction such as cytokines. Special attention has recently been drawn towards reactive oxygen and nitrogen species for their role in disc abnormalities and sensation of dorsal root ganglion neurons. Peroxynitrite is an important tissue-damaging species generated at sites of inflammation and degeneration. It is a highly reactive agent formed *in vivo* from the interaction of superoxide and NO.

The aim of this study was to examine the effects of oxidative/nitrosative stress caused by peroxynitrite on human nucleus pulposus cells.

METHODS: Degenerated human intervertebral disc tissue was analyzed for nitrosylation by immunofluorescence. In addition, human nucleus pulposus cells were isolated from intervertebral discs, expanded and stimulated with a stable peroxynitrite donor (SIN-1). Nitrosylation, NF-κB nuclear translocation and cell viability were analyzed by immunofluorescence. In addition, gene expression of TNF-α, IL-1β, IL-6, IL-8 and IL-10 were analyzed by real-time RT-PCR. Statistical analysis was performed with a significance level of $p<0.05$, using a Student's t-Test.

RESULTS: Degenerated intervertebral disc tissue showed nitrosylation, especially in the nucleus pulposus (Figure 1).

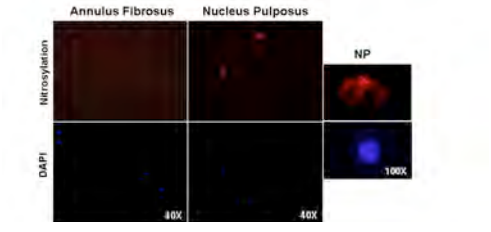

Fig. 1: Nitrosylation in human disc tissue.

Upon stimulation with SIN-1, sustained nuclear translocation of NF-κB/p65 (Figure 2) and stimulation of IL-1β, IL-6 and IL-8 expression (Figure 3) was noted in isolated human nucleus pulposus cells.

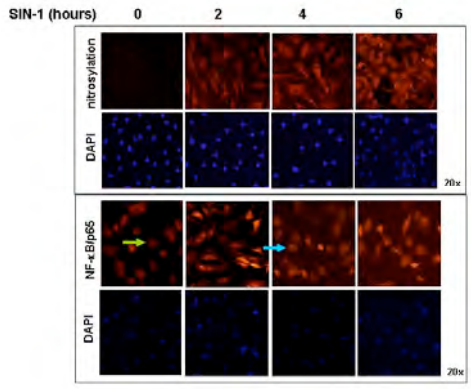

Fig. 2: Nitrosylation and NFkB translocation in human nucleus pulposus cells upon stimulation with SIN-1 (different time points).

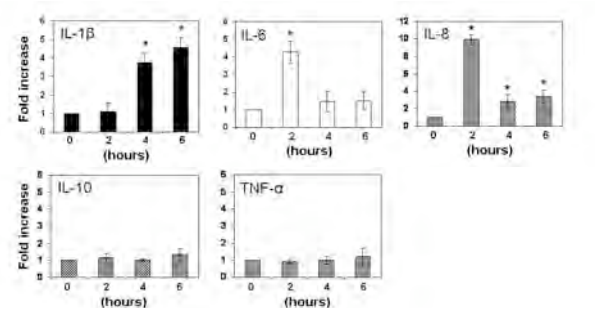

Fig. 3: Changes in gene expression in human nucleus pulposus cells upon stimulation with SIN-1 (different time points).

DISCUSSION & CONCLUSIONS: This study provides evidence that peroxynitrite may play a role in disc degeneration and may induce development of discogenic back pain due to increased synthesis of proinflammatory cytokines. Nuclear translocation of NF-κB was identified as the potential underlying pathway. Therefore, neutralizing peroxynitrite and its derivatives (e.g. via the use of antioxidants) may be a novel treatment option for discogenic back pain.

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Impact of the spatial RANKL/OPG distribution on BMU branching

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INTRODUCTION: During the process of bone remodeling, bone-resorbing osteoclasts (OC) and matrix-producing osteoblasts (OB) are organized in a Bone Multicellular Unit (BMU). OC resorb a cavity in front (cutting cone) and OB are recruited in the back of the BMU (closing cone). In the communication between the cells of the BMU, the following two chemicals play an important role: RANKL, expressed by osteocytes adjacent to fractures and along the principal strain axis, is a key factor for OC attraction and stimulation; its inhibitor, osteoprotegerin OPG, is produced by mature OB and diffuses into the bone matrix. Using a mathematical model, we investigated the impact of the spatial distributions of RANKL and OPG on the steering mechanisms of BMUs.

METHODS: Based on a temporal model by Komarova et al [1-2], we developed a mathematical model describing the spatio-temporal evolution of a single BMU. The model simultaneously describes the population dynamics of OC and OB as well as the kinetics of RANKL and OPG. The various mathematical terms accounting for mobility and interactions lead to a system of non-linear partial differential equations with time delays. Due to their complexity, these equations have to be solved numerically, using a 4^{th} order Runge-Kutta method in time and a 2^{nd} order finite difference scheme in space. Twenty-one parameters as well as the initial and boundary conditions allow us to simulate different situations that arise in microfracture remodeling. In particular, choosing initial conditions corresponding to different fracture patterns we can investigate the branching mechanisms of BMUs.

RESULTS: The model successfully recapitulates the spatio-temporal dynamics of a single BMU as observed in *in vivo* cross sections, including a self-contained progression of the cutting cone and a subsequent bone formation of the closing cone. Using different initial conditions, we simulated forward branching at acute angles (see Fig. 1, red line) and backward branching at obtuse angles (see Fig. 2, red line). Both figures show three snapshots as the cutting cone (active OC, white) moves along the fracture. We found that in the case of forward branching, division of the cutting cone results in a complete remodeling of both branches (Fig. 1). Interestingly, in the case of backward branching, the cutting cone along the upper branch disappears at t=160, resulting in an incomplete remodeling of the backward branch (Fig. 2). This phenomenon is due to the OPG field produced by OB in the back of the BMU: it inhibits RANKL and hence the OC stimulation of the backward branch.

Fig. 1: Simulated BMU remodeling of a fracture with forward branching. Cutting cone (OC concentration in white) moves along the fracture (red). Snapshots taken at t=30,90,160 days.

Fig. 2: Simulated BMU remodeling of a fracture with backward branching. t=30,90,160 days.

DISCUSSION & CONCLUSIONS: The branching dynamics observed in our model are in good agreement with experimental results obtained using three-dimensional microcomputed tomography [3], which demonstrated that BMUs only branch at acute angles. Our model suggests that the absence of backward branching is due to the spatial distribution of the field of RANKL, which is produced by osteocytes and thus located in front of the BMU, and the field of OPG, which is produced by osteoblasts in the back of the BMU. Furthermore, this mechanism naturally prevents the BMU from turning around and remodeling recently renewed bone tissue. Thus, our model provides a powerful tool for the *in silico* analysis of the impact of cytokines and growth factors on the process of bone remodeling. It also has the potential to be used for the analysis of the impact of biomaterials on local bone remodeling.

REFERENCES: [1] S.V. Komarova et al (2003) *Bone* ***33****(2):206-15* [2] S.V. Komarova (2005) *Endocrinology* ***146****(8):3589-95* [3] D.M. Cooper et al (2006) A*nat Rec A Discov Mol Cell Evol Biol* ***288****(7):806-16*

Collagen-Hydroxyapatite Scaffolds for Bone Engineering

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INTRODUCTION: The insertion of dental implants is only possible after bone augmentation procedures with autologous, alloplastic or composite materials. For reconstruction the gold standard is still the autologous bone harvested from the iliac crest, the mandible or the maxilla. Disadvantages are the limited availability of bone and the necessity of an additional surgical procedure which always implies the risk of donor site morbidity. Tissue-engineering procedures for hard tissue augmentations of the maxilla offer significant advantages compared with conventional grafts, as there is minimal or no donor site morbidity.

METHODS: For an animal trial in the sheep model Collagen-Hydroxyapatite Scaffolds were produced by solid-freeform fabrication[1]. For the test side they were vitalized with osteoblast-like cells. The scaffold without cells served as control. The cells had been harvested from the mandible of the animals in general anaesthesia 6 weeks prior to an augmentation procedure during which the constructs were placed into the maxillary sinus of 9 adult sheep. One side of each animal served as test the other side as control (split-mouth-model). Dental implants were inserted imultaneously to the sinus augementation. Animals were sacrificed after 8, 16 and 24 weeks. Volumerendering of CT-data of the augmented area was performed. Thin sections of the implants and the surrounding bone substitute were stained with Pararosanilin-Azur II and evaluated by histomorphometical analysis.

RESULTS: New bone formation was found in the augmented area and in contact with the dental implants and increased over time. There was a significant more bone formation after 7 weeks in the test side ($p < 0,05$). Bone turnover decreased from the 3rd to the 23rd week ($p < 0.05$). The scaffolds lost most of their initial volume.

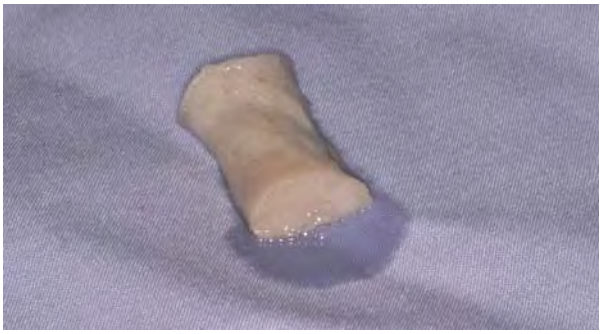

Fig. 1: Collagen-Hydroxyapatite scaffold after vitalization with osteoblast-like cells.

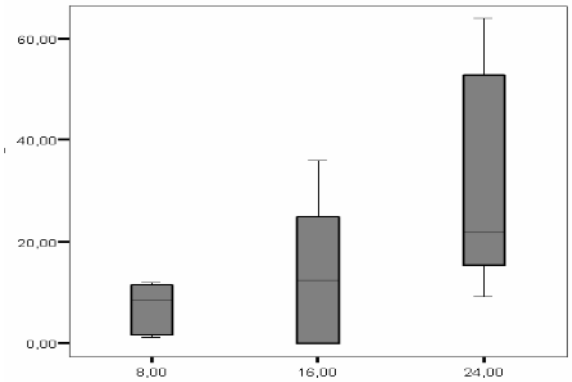

Fig. 2: New bone formation (y-axis [% of area]) increases over time (x-axis [weeks]) in both groups. This graph shows the test side as example.

DISCUSSION & CONCLUSIONS: This bone substitute material was tested *in-vivo* for the first time. It was shown that the application was feasible and that new bone formation could be induced. Future research must address volume preservation of the construct.

REFERENCES: [1] D.A. Wahl, E. Sachlos, C. Lui, J.T. Czernuska (2007) *Controlling the process of collagen-hydroxyapatite scaffolds for bone engneering*, J Mater Sci Mater Med **18**:201-9.

ACKNOWLEDGEMENTS: This animal trial was granted by the AO-Research Fund.

P2X7 Receptor Activation Causes Isoform-Specific Translocation of Protein Kinase C in Osteoclasts

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INTRODUCTION: Nucleotides, released from cells in response to mechanical or inflammatory stimuli, signal through P2 nucleotide receptors in many cell types. Osteoclasts express functional P2X7 receptors – calcium-permeable channels activated by high concentrations of extracellular ATP. Genetic disruption of P2X7 leads to increased resorption and reduced skeletal response to mechanical stimuli. However, little is known about the signaling pathways stimulated by P2X7 receptors in osteoclasts. P2X7 receptor channels permit calcium influx leading to elevation of cytosolic Ca^{2+} [1] (*Fig. 1*). Moreover, P2X7 receptors induce activation of NF-κB and cause its translocation to the nuclei [2, 3]. Our objective was to investigate whether P2X7 receptors couple to activation of protein kinase C (PKC) in osteoclasts as evidenced by translocation to membranes or nuclei.

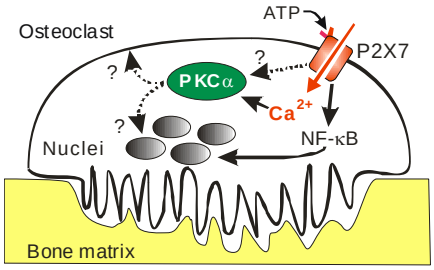

Fig. 1: Activation of P2X7 receptors results in opening of channels that permit entry of calcium and also causes nuclear translocation of the key transcription factor NF-κB. We examined whether P2X7 caused translocation of PKC in osteoclasts.

METHODS: RAW264.7 cells were differentiated into multinucleated osteoclast-like cells and live-cell confocal imaging was used to localize EGFP-tagged PKC. P2X7 receptors were activated by addition of benzoylbenzoyl-ATP (BzATP).

RESULTS: We transiently expressed PKCα, βI and δ as well as EGFP alone, which served as a control, in RAW-derived osteoclasts. PKCα-EGFP was uniformly located in the cytoplasm in unstimulated osteoclast-like cells, though notably not in the nuclei. To account for the morphology of the cells, it was necessary to examine the localization of PKC in 3-D by recording Z-stacks of each cell. Treatment with BzATP (150 μM) caused prompt translocation of PKCα-EGFP to the plasma membrane, with fluorescence apparent as an annular ring at the periphery when assessed midway in the cell. Inspection of the X-Z profile revealed that PKCα-EGFP was largely localized to the upper membrane of the cell (basolateral membrane), with less in the membrane adjacent to substrate. UTP (150 μM) or ATP (10 μM), which activate several P2 receptors other than P2X7, failed to induce translocation of PKCα; whereas, ATP (3 mM, sufficient to activate P2X7) caused translocation. Translocation was inhibited by the selective P2X7 antagonist Brilliant Blue G, consistent with involvement of P2X7 receptors. To investigate a role for Ca^{2+}, we simultaneously monitored PKCα location and Ca^{2+} concentration. BzATP induced transient rise of Ca^{2+}, and removal of bath Ca^{2+} prevented translocation of PKCα induced by BzATP (but not phorbol ester). We examined the isoform specificity of this response, and found that BzATP induced translocation of the Ca^{2+}-dependent isoforms PKCα and PKCβI, but not Ca^{2+}-independent PKCδ.

DISCUSSION & CONCLUSIONS: These findings establish that activation of P2X7 receptors induces calcium-dependent translocation of PKC to the basolateral membrane domain of osteoclasts, an aspect of spatiotemporal signaling not previously recognized. Specific subcellular localization may be critical for positioning PKC near its appropriate substrates.

REFERENCES: [1] Naemsch L.N., Dixon S.J., Sims S.M. (2001) *J Biol Chem* **276**:39107-14. [2] Korcok J., Raimundo L.N., Ke H.Z. et al. (2004) *J Bone Min Res* 19:642-51. [3] Armstrong S., Korcok J., Sims S.M., Dixon S.J. (2007) *Purinergic Signal.* 3:59-69.

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Bone tissue engineering using synthetic fibrin-like hydrogels

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INTRDUCTION: Synthetic extracellular matrices (ECM) represent interesting alternatives for biopolymers as they can be specifically designed with respect to their mechanical properties, susceptibility to degradation and presentation of cell adhesion ligands or morphogens. Here we present modularly designed poly(ethylene glycol) (PEG)-based hydrogels that are formed by Factor XIII (FXIII) catalysis. The new class of biomaterials enables the formation of matrices containing multiple tethered bioactive molecules and cell-responsive enzymatic substrates in a simple one step reaction. In the context of bone tissue engineering we have designed the system to contain building blocks that include enzymatically coupled cell adhesion ligands, the osteogenic growth factor BMP-2 and slow released VEGF.

METHODS: Hydrogel networks were formed in TBS (50mM TrisHCl, pH7.6; 50mM $CaCl_2$) by stoichiometrically balanced 8-PEG-MMP-Lys and 8-PEG-Gln solutions upon addition of activated FXIII. Cells and bioactive molecules were added to the precursor solution prior to gelation. Bone healing experiments were performed in critical-size defects of the rat cranium were harvested after five weeks of implantation and analyzed by microcomputed tomography and histology.

RESULTS: The enzymatic incorporation of TG-Gln-RGD peptides was almost quantitative and resulted in a dose-dependent spreading of MC3T3-E1 cells in 2D cultures. Cell spreading and migration in 3D cultures was strongly dependent on the incorporated TG-Gln-RGD concentration but also the matrix responsiveness to matrix metalloproteases. Furthermore 3D-cultured mouse pre-osteoblastic cells and human mesenchymal stem cells differentiated in response to BMP-2 entrapped within the gel or administered to the culture medium. When implanted in rat cranial defects, FXIII crosslinked hydrogels were remodeled by proteolytically invading cells. When BMP was entrapped within the matrix bone formation was induced on the hydrogel tissue interface as has also been observed with chemically crosslinked PEG hydrogels described in Rizzi et al. [3]

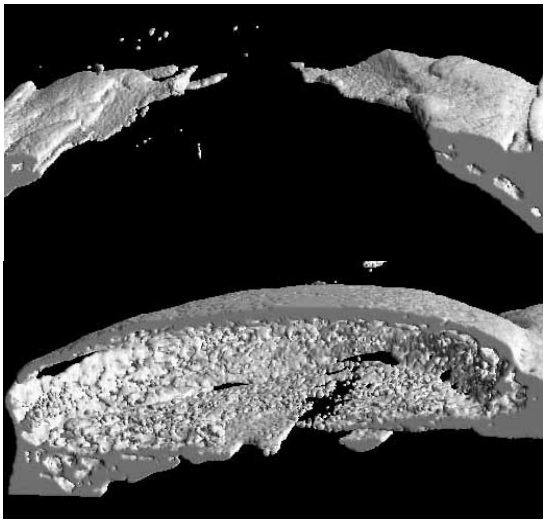

Fig. 1: bone repair in critical size defects. Defects were not healed in absence (upper panel) but were bridged in presence of BMP2 (lower panel).

DISCUSSION & CONCLUSIONS: In contrast to materials from natural sources these novel artificial ECMs allow the nearly independent control of properties including matrix stiffness, protease susceptibility and presentation of biological cues. The tailoring of these properties in a wide range enables us to rationally control cell behavior in both in vitro and in vivo contexts. These matrices could be useful tools for experimental cell biology as well as for in vivo applications such as bone tissue regeneration.

REFERENCES:

[1]Ehrbar, M., Rizzi, S.C., Hlushchuk, R., Djonov, V., Zisch, A.H., Hubbell, J.A., Weber, F.E., Lutolf, M.P..Enzymatic formation of modular cell-instructive fibrin analogues for tissue engineering. Biomaterials 28, 3856, 2007.

[2]Rizzi, S.C., Ehrbar, M., Halstenberg, S., Raeber, G.P., Schmökel, H.G., Hagenmüller, H., Müller, R., Weber, F.E**.,** Hubbell, J.A. Recombinant Protein-co-PEG networks as Cell-Adhesive and Proteolytically Degradable Hydrogel matrices. Biofunctional Characteristics Biomacromolecules 7, 3019, 2006.

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Mesenchymal stem cell response to a pH challenge with applications to intervertebral disc repair

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INTRODUCTION: The use of mesenchymal stem cells (MSCs) for treatment of degenerative disc disease offers promise but is complicated by the harsh cellular microenvironment of the intervertebral disc (IVD), one main characteristic being its acidic pH. However, the behavior of MSCs to varying pH environments remains largely uninvestigated and is the purpose of this study. The first hypothesis is that gene expression, proliferation and viability will be influenced by pH in a dose-dependent way, either with a progressive change or a threshold. The second hypothesis is that MSCs from younger donors will have a greater ability to adapt to changing pH levels than those from older donors. The clinical relevance is to determine how different acidity levels affect MSC behavior in order to establish if certain pH values exist when there is optimal likelihood of successful biological repair.

METHODS: MSCs were isolated from femoral bone marrow of either young rats (n=6, 1 month old) or mature rats (n=6, 4-5 months old), expanded and then cultured for five days under four different pH conditions: pH 7.4 (= body fluid), pH 7.1 (= healthy IVD), pH 6.8 (= mildly degenerated IVD) and pH 6.5 (= severely degenerated IVD). MSCs were analyzed for gene expression, viability and proliferation. Values were normalized to values at pH 7.4 to normalize within subject. Statistical analysis was performed with a significance level of $p<0.05$, using ANOVA and Student's t-Test to evaluate effects of pH and age.

RESULTS: The pH conditions strongly influenced gene expression in a dose dependent manner, with a significant inhibition of mRNA levels for aggrecan (Figure 1, progressive), collagen-1 (threshold pH 6.8) and TIMP3 (threshold pH 6.8) expression under acidic conditions relative to pH 7.4; MMP2 did not show dose dependent response pattern to pH. Cell proliferation was also strongly influenced by pH, with proliferation being highest for pH 7.4 and 7.1 and significantly decreased at more acidic pH conditions. These findings were confirmed with DNA content (threshold pH 6.8). Cell viability decreased with acidity, showing a decreased number of living cells and an increased number of dead cells from pH 7.4 to pH 6.5. Morphological changes were also noted as MSCs showed a loss in cytoskeleton size under acidic pH conditions. For all dependent variables, minor differences were seen between the two different age groups, with MSCs from young rats generally maintaining higher biosynthetic activity.

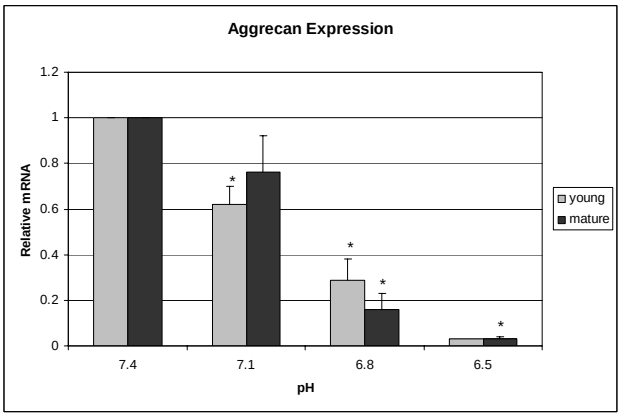

Fig. 1: Aggrecan mRNA expression under different pH levels, relative to pH 7.4 (real-time RT-PCR).

DISCUSSION & CONCLUSIONS: MSCs showed a pH dose response that was either progressive or had a threshold at pH 6.8, consistent with hypothesis 1. However, only minor differences between the two age groups were observed, in contrast to hypothesis 2, but MSCs from young rats generally maintaining higher biosynthetic activity. With a decrease in pH, gene expression of aggrecan, collagen-1 and TIMP3 as well as proliferation, viability and morphological functionality were inhibited. As the lower pH levels are typical of increasingly degenerated IVDs, our findings demonstrate the importance of early intervention when planning to use MSCs for regenerative treatments. Findings suggest a likelihood of failure for MSC based repair in severely degenerated IVDs using native MSCs. However, the pluripotential nature of MSCs may allow treatment of severely degenerated IVDs through predifferentiation steps that may allow MSCs to maintain their morphology, and biosynthesis rates under a low pH challenge. Whether MSCs will be an appropriate tool for IVD repair will depend on ability of MSCs survive, function and differentiate appropriately under the acidic pH conditions found in vivo.

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Rat Bone Marrow Stromal Cells Differentiation and Proliferation: Effects of Continuous and Pulsatile Parathyroid Hormone Treatments

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INTRODUCTION: Bone marrow stromal cells (MSC) are multipotential cells capable of differentiation along multi-lineages including osteogenic. Whether MSC differentiate into committed progenitors or proliferate as stem cells is evidently regulated by growth factors, cytokines and hormones. Parathyroid hormone (PTH) exerts both anabolic and catabolic effects on bone depending on the exposure pattern and duration [1]. Results from this study represent a first step in understanding the complex regulatory effects of PTH on rat MSC differentiation and proliferation.

METHODS: MSC were seeded at a density of $5x10^3$ cells/cm^2 and cultured in osteogenic medium (15% FBS, ascorbic acid, β-glycerophosphate, & dexamethasone, DEX). For the pulsatile treatment group (PTH-P), cells were exposed to PTH (10nM) for the first 6h of each 48-h incubation cycle, and then cultured in the absence of PTH for the remainder of the cycle [1]. In the continuous treatment group (PTH-C), cells were exposed to PTH (10nM) daily. The control group received no PTH treatment. For CFU-F assay, MSC were treated with PTH (100nM) as above for 6 days and then re-plated in medium supplemented with 15% FBS only for another 8 days.

RESULTS: Continuous PTH treatment was found to promote osteogenic differentiation of multipotential rat MSC illustrated by up-regulation of PTH receptor (PTHr) and alkaline phosphatase (ALP) gene expressions (Fig. 1A & B), increase in ALP activity (Fig. 1C) and number of bone nodules (Fig. 1D). In contrast, pulsatile PTH treatment was found to suppress osteogenic differentiation (Fig. 1A to D). However, PTH treatments had no effect on MSC proliferation demonstrated by total cell count (Fig. 1E). Moreover, continuous and pulsatile treatments increased the number of CFU-F (Fig. 1F).

DISCUSSION & CONCLUSIONS: Osteogenic differentiation of rat MSC was found to increase with continuous PTH treatment, but on the contrary decrease with pulsatile PTH exposure. These results are in contrast to the findings using rat calvariae osteoblasts, where it has been consistently shown that continuous PTH inhibits, while pulsatile PTH stimulates osteoblast differentiation [1]. The disparity might be attributed to the different cell population used (osteoblast vs. MSC), and the absence of DEX in the rat calvariae studies.

More interestingly, preliminary evidence suggests both PTH treatments might be able to *maintain* or even *increase* the stem cell population found in rat bone marrow illustrated by increase in CFU-F and limiting dilution experiments (data not shown).

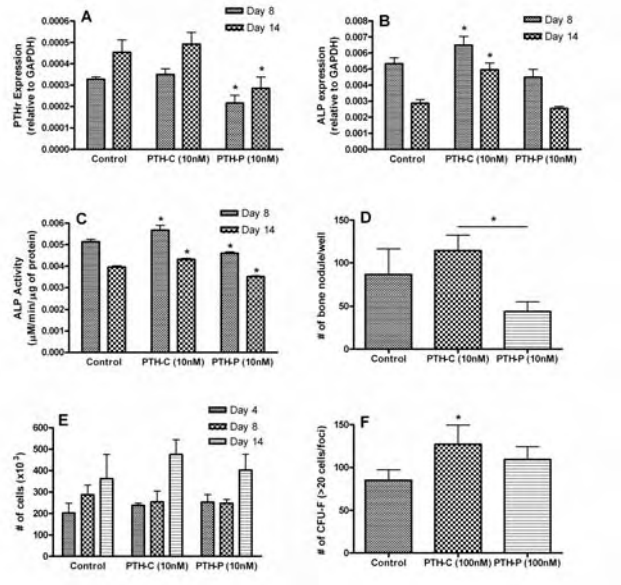

Fig. 1: Effect of continuous (PTH-C) and pulsatile (PTH-P) PTH treatments on rat MSC differentiation and proliferation. (A) PTH receptor gene expression, (B) ALP gene expression, (C) ALP enzyme activity, (D) total # of bone nodules after 14 days, (E) total cell count, and (F) total # of CFU-F after 6 days of PTH treatments. $^*P < 0.05$ compared to control, if not otherwise noted.

REFERENCES: [1] T. Ishizuya, S. Yokose, M. Hori, et al (1997) *J Clin Invest* 99(12):2961-2970.

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Investigation of three different labelling techniques to visualize vital cells

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INTRODUCTION: Analysis of cell localisation and morphology provides important information on cell-to-material interactions and bio-compatibility. On solid, non translucent surfaces, conventional scanning electron microscopy and immunohistochemical staining procedures require fixed and non vital cells. The tagging of vital cells allows monitoring of dynamic cell attributes like adhesion and contact guidance as well as long-term colonisation dynamics. Aim of the study was to compare three different approaches to tag vital cells: conventional vital staining, cell transfection and lentiviral transduction.

METHODS: After isolation and characterization, the following cell types were investigated: mononuclear cells (MNC), endothelial progenitor cells (EPC), human umbilical venous cells (HUVEC) and osteogenic cells (OC). Vital staining was performed with green cell tracker (Molecular Probes Invitrogen), according to the manufacturers staining protocol. Transfection of green fluorescent protein (GFP) containing plasmids was performed with SuperFect® (Qiagen). For the lentiviral transduction a lentivirus vector containing spleen-focus-forming-virus-promoter (SFFVp) and GFP as target gene. Different viral concentrations were tested. For all methods, the following parameters were investigated: efficiency and duration of fluorescence signal via FACS-analysis as well as vitality of the cells via MTT testing. Data were assessed after 24hrs, 72hrs, 7d, 14d and 21d. Furthermore, the parameter "handling" was established to reflect the simplicity and complexity of the method, respectively. As an example of use, the stained cells were cultured on surface modified titanium (Ti) disks and visualized by Confocal Laser Scanning Microscopy (CLSM).

RESULTS: For our laboratory, all staining procedures proved to be practicable and showed characteristic advantages and disadvantages. Conventional vital staining showed a high efficiency (~100%) and was easy to handle (table 1). Transfection showed a low efficiency (~10% transient transfection in non-dividing cells, e.g. EPC). For vital staining and transfection, cell division resulted in a significant decrease of the intensity of the fluorescent signal (dilution effect). Transduction required labour-intensive techniques and showed virus-dose dependent efficiency rates of up to 80-90% (Fig 1). Transduced cells exhibited stable fluorescent signals over the whole observation period. Fig. 2 shows GFP transduced cells on a commercial Ti surface after 7d of cultivation. All three methods resulted in vital cells over 21d.

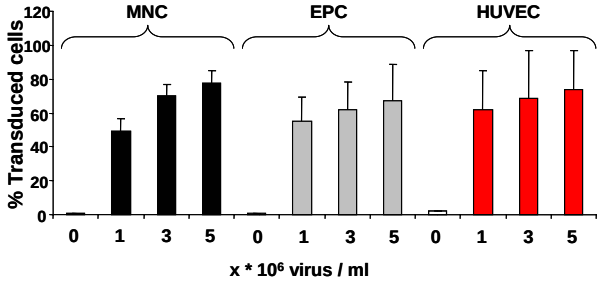

Fig. 1: Transduction efficiency of different cell types and virus loads, measured by FACS analysis.

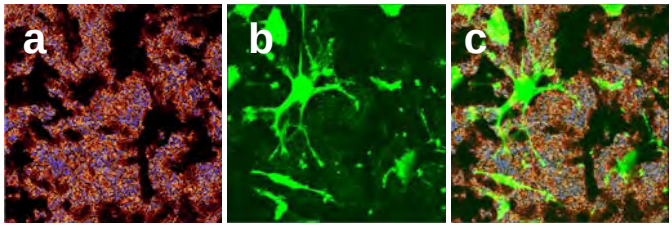

Fig. 2a: CLSM reflection mode of a commercial SLA® titanium implant surface; Fig. 2b: GFP transduced osteogenic cells on the surface. 2c: Merged image. Original magnification 40x.

	conv.	Transfection	Transduction
Efficiancy	+	-	+
Vitality	+	+	+
Handling	+	+/-	-
Duration	-	-	+

Table 1. Comparison of the assessed labelling techniques (conv.: conventional vital staining)

DISCUSSION & CONCLUSIONS: For short observation periods of up to 7d, conventional vital staining proved to be a reliable method. For longer observation periods, especially of dividing cells, transduction showed to be the best method, as the stable integration of the target gene into the host genome avoids a dilution effect, as seen for vital staining and transfection.

Mesenchymal Stem Cells for the Augmentation of the Maxillary Sinus

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INTRODUCTION: Autologous, allogenic and alloplastic materials for the reconstruction of bone and soft tissue have specific applications in the cranio-maxillofacial area [1, 2]. The research for biomaterials and tissue engineering procedures aims at new synthetic and autologeous materials. Cultivated skin and mucosa are already in clinical routine use in head and neck reconstruction. There have been many draw backs in the research of bone substitute materials because of the oxygen-sensitivity of osteoblasts[3]. We suggest that mesenchymal stem cells (MSCs) are the better source because the are able to proliferate under low oxygen tension and differentiate when the oxygen level rises[4]. Depending on the micro-environment MSCs have the ability to differentiate into osteoblasts[5]. In animal experiments stem cell application in combination with a bio material (BioOss) show lamellar bone formation and bone invasion into the micropores. So far there has been no successful clinical application to the best of our knowledge of processed stem cell-derived bone for augmentation of the edentulous posterior maxilla.

METHODS: In a pilot study, the augmentation of the posterior maxilla was carried out using stem cells from bone marrow aspirate concentrate (BMAC) on a bovine bone matrix (BioOss®, Geistlich Wolhusen, Switzerland). The mesenchymal stem cells were purified from the aspirate by an especially developed cetrifuge. The phenotype was proven by plastic adherence and flowcytometrical-analysis of relevant markers as CD 44, CD 73, CD 90, CD 166, CD 34 and CD 45. Pluripotency was evaluated by a differentiation assay. Biopsies were harvested with a trephine burr when dental implants were inserted 3 months after sinus augmentation.

RESULTS: The results suggest that stem cell derived osteoblasts form lamellar bone within 3 months which allows reliable implant insertion. 50 maxillary sinus have been treated so far with this procedure. No dental implant was lost over the observation period. Histomorphometric analysis showed new bone formation in the transplant. The FACS-analysis revealed cells which were positive for CD 44, CD 73, CD 90, CD 166 and negative for CD 34 and CD 45. Plastic adherent cells showed the same FACS-characteristics as the afore mentioned fraction of the BMAC. The cells could be differentiated into adipocytes, chondroblasts and osteoblasts.

DISCUSSION & CONCLUSIONS: The successful clinical application and the histological results of this pilot study show the feasibility of this procedure. FACS-analysis and in-vitro tests prove that actually mesenchymal stem cells had been transplanted.

REFERENCES: [1] Sauerbier, S., Gutwald, R., Wiedmann-Al-Ahmad, M., Lauer, G., Schmelzeisen, R. (2006) Clinical Application of tissue engineered Transplants - Part I: Mucosa. Clinical Oral Implants Research, **17**:625-32. [2] Schmelzeisen, R., Schimming, R., Sittinger, M. (2003) Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation- a preliminary report. Journal of Cranio-Maxillo-Facial Surgery, **31**:34-39, [3] Zizelmann, C., Schoen, R., Metzger, M.C., Schmelzeisen, R., Schramm, A., Gellrich, N.C. (2007) Bone formation after sinus augmentation with engineered bone. Clinical Oral Implants Research, **18**:69-73. [4] Lepperdinger, G., Fehrer, C., Laschober, G., Brunauer, R. (2006) Mesenchymal Stem Cell Aging. Nova Acta Leopoldina NF 95, **352**:151. [5] Pittenger, M.F., Mackay, A.M., Beck, S.C., Jaiswal, R.K., Douglas, R., Mosca, J.D., Moorman, M.A., Simonetti, D.W., Craig, S. & Marshak, D.R. (1999) Multilineage potential of adult human mesenchymal stem cells. Science, **284**:143-147.

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COMPARATIVE GENE TRANSFER ANALYSIS IN HUMAN MESENCHYMAL STEM CELLS USING 15 NOVEL OPTIMIZED NON-VIRAL TRANSFECTION SYSTEMS

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INTRODUCTION:

Genetically modified bone marrow-derived mesenchymal stem cells (MSCs) may provide suitable platforms to apply therapeutic genes in articular cartilage lesions. Here, we evaluated the efficacy of 15 non-viral gene transfer vectors to carry candidate genes in human MSCs.

METHODS:

The plasmid expression vectors employed included pCMVLacZ carrying the *E. coli* beta-galactosidase (β-gal) gene, pCMVLuc carrying the Firefly luciferase gene and pCMVhIGF-I carrying a human insulin-like growth factor I (hIGF-I) cDNA [1-3]. Bone marrow aspirates were obtained from the femurs of normal donors during knee endoprothesis and MSCs were isolated and expanded in culture using standard protocols [4-6]. Briefly, aspirates were layered onto Histopaque-1077 density gradient (Sigma), centrifuged, and the nucleated cell fraction at the interface was collected, washed, and resuspended in human MSC (hMSC) growth medium (MesenCult® MSC Basal Human; StemCell Technologies Inc.) containing MSC Stimulatory Supplements (StemCell Technologies Inc.). Cells were characterized for stem cell surface markers and multilineage potential [4]. Prior to transfection, fresh growth medium was added to the cultures. Cells (passage 1-2) were transfected in 96-well plates (5 x 10^3/well) at 60-70% confluence according to the manufacturer's instructions using FuGENE 6 (Fg; Roche Applied Sciences; 0.25 µg DNA/0.5 µl reagent), Metafectene (Mf; Biontex; 0.125 µg DNA/0.625 µl reagent), Lipofectamine 2000 (Lp; Invitrogen; 0.2 µg DNA/0.5 µl reagent), Dreamfect (Df; Oz Bioscience; 0.125 µg DNA/0.5 µl reagent), Gene Jammer (GJ; Stratagene; 0.25 µg DNA/0.75 µl reagent), Effectene (Ef; Qiagen; 0.05 µg DNA/1.25 µl reagent), Turbofectin (Tf; OriGene; 0.05 µg DNA/0.15 µl reagent), TransIT-LT1 (Ms; Mirus Bio Corporation; 0.1 µg DNA/0.28 µl reagent), Lipofectamin with PLUS (Lp+P; Invitrogen; 0.1 µg DNA/0.3 µl reagent), Gene Juice (GJu; Novagen; 0.06 µg DNA/0.2 µl reagent), Dreamfect Gold (DfG; Oz Bioscience; 0.25 µg DNA/1 µl reagent), Transpass D2 (TP; New England Biolabs; 0.1 µg DNA/0.2 µl reagent), Jet Pei (JP; Polyplus Transfection; 0.25 µg DNA/0.5 µl reagent), Ecotransfect (Ec; Oz Bioscience; 0.2 µg DNA/0.4 µl reagent), DMRIE-C (DC; Invitrogen; 0.06 µg DNA/0.18 µl reagent) [1,7]. In addition to standard protocols, hyaluronidase (4 U/ml) (Sigma) was added before and during transfection in each set of experiments [1]. Two days after transfection, transgene expression was determined using a luciferase assay (Promega). The amounts of total proteins were measured using the BCA kit (Thermo Scientific). Transfection efficiencies were determined as relative light unit (RLU) per mg of total proteins. Evaluation of the cytotoxicity in transfected cells was performed using the Cytotoxicity Detection Kit (LDH) (Roche Applied Sciences) by measuring the release of lactate dehydrogenase activity from damaged cells. Data are given as % cytotoxicity [(exp. value - low control/high control - low control) x100] where "low control" corresponds to untransfected cells and "high control" to cells placed in the lysis buffer provided in the kit. Each condition was performed in duplicate in 3 independent experiments. Data are expressed as mean ± SD. The Mann-Whitney Rank Sum Test was employed where appropriate with $P < 0.05$ considered statistically significant. In another test sequence cells were similarly transfected using Gene Jammer (GJ), the pCMVLacZ and pCMVhIGF-I as control. 48 hours after transfection cells were counted and brought to pellet culture (2x10e5 cells) [8]. Transfected cells were maintained for 21 days in pellet cultures in hMSC growth medium with MSC Stimulatory Supplement. Transgene expression was determined by X-Gal staining.

RESULTS:

Optimal luciferase activity was achieved with Gene Jammer (GJ), an effect that was even more marked in the presence of hyaluronidase (Fig. 1). In pCMVLuc-GJ-transfected (treated) cells, activity was of 11,053.46 ± 7,872.31 RLU/mg total protein without hyaluronidase (versus 1,206.83 ± 710.23 RLU/mg total protein in control cells; $P = 0.009$) and 15,356.92 ± 11,001.37 RLU/mg total protein with hyaluronidase (versus 1,373.13 ± 1,021.66 RLU/mg total protein in control cells; $P = 0.026$).

In the presence of hyaluronidase, luciferase activity increased (1.39-fold), although statistical significance was not attained ($P = 0.454$). The values obtained for treated cells using the other transfection systems were ranging between 1,382.13 ± 719.21 (DC) and 6,798.43 ± 2,315.98 RLU/mg total protein (Ec) without hyaluronidase and between 1,080.60 ± 625.87 (Fg) and 7,437.32 ± 3,307.38 RLU/mg total protein (Ms) with hyaluronidase. With these other methods, control values were between 260.50 ± 348.19 (Fg) and 5,768.35 ± 4,533.35 RLU/mg total protein (TP) with or without hyaluronidase.

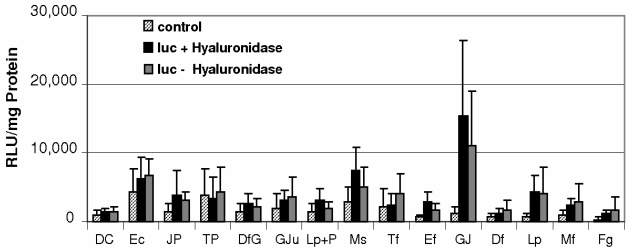

Fig. 1. Detection of luciferase activity.

Remarkably, Gene Jammer was among the less toxic gene transfer methods (13.76% ± 3.35%), whereas the other evaluated systems were ranging between the levels of detection (Ec, Ms) and 47.41% ± 2.19% (DfG) (Fig. 2, Data show dead cells as percent of total cells per well).

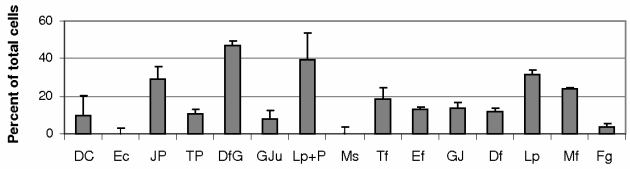

Fig. 2. Detection of cytotoxicity.

Of relevance for applications *in vivo*, transgene expression was also noted in pellet cultures 21 days post transfection, as seen by X-Gal staining of hMSCs transfected with pCMVlacZ (Fig. 3).

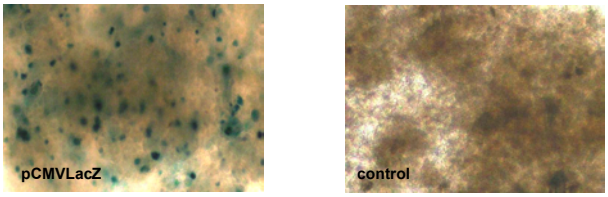

Fig. 3. X-Gal staining of pellet cultures (GJ; 21 days). Magnification x20

DISCUSSION:

Our data show that successful transgene expression can be achieved in hMSCs by using non-viral gene transfer methods. Safe treatments for articular cartilage lesions might be established using genetic modification of hMSCs via non-viral gene transfer of therapeutic candidates. 3-D pellet cultures may be relevant and prove beneficial for *in vivo* implantation approaches.

REFERENCES:

[1] Madry *et al.*, *Gene Ther* **2000**, 7(4):286-91; [2] Madry *et al.*, *Gene Ther* **2001**, 8(19):1443-9; [3] Madry *et al.*, *J Gene Med* **2003**, 5(6):502-9; [4] Pittenger *et al.*, *Science* **1999**, 284(5411):143-7; [5] Palmer *et al.*, *Mol Ther* **2005**, 12(2):219-28; [6] Solchaga *et al.*, *J Cell Physiol* **2005**, 203(2):398-409; [7] Orth *et al.*, *Mol Biotechnol* **2007**, 38(2):137-44; [8] Johnstone et al., *Exp Cell Res* **1998**, 238(1):265-72

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Hydroxyapatite, BMP and Wnt signaling are required in cell based ectopic bone formation

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INTRODUCTION: Ectopic bone formation models are of use in the field of bone tissue engineering to evaluate osteoinductive properties of cell-scaffold constructs. In our laboratory, we have explored the in vivo ectopic bone formation capacity of human periosteum derived cells (HPDCs) on different scaffolds made of titanium, hydrogels and hydroxyapatite. Interestingly, bone tissue was only found in Collagraft™ (Neucoll Inc, Campbell, CA; 65% hydroxyapatite, 35% beta tri-calcium phosphate and bovine collagen type I fibrils) carriers seeded with HPDCs after subcutaneous implantation in the back of nude mice. This observation prompted us to investigate the mechanism of action that triggers osteoinduction by HPDCs.

METHODS: HPDCs (P3-P5) were transduced with adenovirus-GFP (Ad-GFP), adenovirus-noggin (Ad-Nog) or adenovirus-Frzb (Ad-Frzb), subsequently seeded into cylindrical 27 mm^3 Collagraft™ carrier structures and implanted subcutaneously in the back of female NMRI nu/nu mice. After 2 days, 1, 2, 4 and 8 weeks the implants were collected and processed for RNA extraction or histology. The amount of bone in the implants was quantified by histomorphometry. Human cells were visualized with in situ hybridization for human specific-genomic alu repeats. Proliferation was assessed by immunohistochemistry for human-specific Ki67. Activation of BMP and Wnt signaling was evaluated by Western blot and immunohistochemistry for phosphorylated Smad 1/5/8 and β-catenin respectively. Expression of BMP and Wnt target genes and BMP ligands were measured by Taqman® PCR.

RESULTS: Between four and eight weeks after implantation bone spicules are formed in the Collagraft™ scaffolds through a process of intramembranous ossification. In our scaffolds a minimum of one million HPDCs is needed to induce the ectopic bone formation process. Cell tracking indicated that 70% of the cells in the spicules are of human origin, and 30% is host derived. Interestingly, bone deposition occurs juxta-proximal of the hydroxyapatite granules suggesting that hydroxyapatite may induce the process of ectopic osteogenesis by HPDCs. Indeed, removal of the mineral granules by EDTA/PBS decalcification prior to cell seeding and implantation leads to loss of bone formation. In addition, inhibition of endogenous BMP and Wnt signaling through transduction of HPDCs with ad-Nog and ad-Frzb results also in abrogation of bone formation. The number of proliferating human cells two weeks after implantation is significantly lower in the scaffolds which are decalcified or seeded with ad-Nog or ad-Frzb-transduced HPDCs. Expression of ID3 a BMP target gene, is only reduced in the ad-Noggin condition but not in the ad-Frzb condition or when mineral granules are removed. Protein expression of β-catenin and gene expression of its target gene Axin2 is comparable in all conditions.

DISCUSSION & CONCLUSIONS: Our data indicate that both hydroxyapatite/tri-calciumphosphate granules and endogenous BMP/Wnt signaling support proliferation of HPDCs in Collagraft™. In this experimental setup, abrogation of Wnt signaling does not affect Smad-dependent BMP signal transduction. Moreover, β-catenin expression is unaffected suggesting that other downstream Wnt signaling pathways may be involved. Further investigation regarding the interaction between hydroxyapatite and BMP/Wnt signaling is required to understand the mechanism of action of spontaneous bone formation in hydroxyapatite-based scaffolds by HPDCs.

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Nanotechnology for medical application: Potential, Challenges and Results

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INTRODUCTION: Nanotechnology for medical application is still a challenging research field especially for medical application because novel physical, chemical and biological properties of nanostructured materials have to be taken in account at the same time. These new combinations of properties, only existing in nanostructured materials and partially still not known in detail, opens the possibility for the development of new diagnostic and therapeutic tools. One of the highest potential for an application in near future shows nanosized iron oxide particles. This material is well known in his bulky form, but changes totally his physical and biological behaviour below 20 nm: it is changing from a ferromagnetic to a super-paramagnetic material with good biocompatibility and a large variety of application like Contrast agent for liver imaging (MRI), Molecular imaging (MRI, CT), Drug delivery, Hyperthermia, Tissue soldering, Separation (cell, proteins), or Biosensors.
Such applications have exploited two major advantages of iron oxides: their low toxicity to human beings and their high magnetization.

HYPERTHERMIA[1]: If exposed to an alternating magnetic field, the harmless iron oxide particles become powerful heat sources by transforming the energy from the magnetic field into heat. Magnetic hyperthermia involves the generation of temperatures up to 43°–48°C with superparamagnetic iron oxides as particles. The treatment works by rendering cells more sensitive to radiation therapy or chemotherapy. The use of nanoparticles incorporated in an implant allows the deposition of iron oxides to be restricted to the tumour area (eg, by intratumoral injection), thereby avoiding adverse systemic effects. The heating of magnetic oxides in an alternating-current magnetic field is due mainly to loss processes during reorientation of the magnetization. A large specific heating power is desirable to reduce the material amount to be administered to the patient. In combination with bone cement, stabilisation of the spine and tumour treatment is possible at the same time.

TISSUE FUSION[2]: An electromagnetically based tissue fusion system using superparamagnetic iron oxide nanoparticles (SPIONs) is actually under development. The nanoparticle soldering technique has its advantages as the electromagnetic field is not absorbed and therefore changed by the tissue itself, which leads to a selective and minimal trauma during soldering. Further it is possible to create three-dimensional scaffold containing particle gradients allowing selective dosage of heat deployment. First fusion of rabbit aortas shows that the rupture force obtained with SPION soldered aortas (3078 ± 852 mN (mean ±SD)) is higher then in r conventionally thread sutured rabbit aortas (2590 ± 950 mN).

DRUG DELIVERY[3]: One of the promising applications of SPIONs in the future could be focused on the musculoskeletal system in humans and animals. Functionalisation of these particles with a fluorescent molecule was used as example for magnetic drug targeting of the particles with drugs and studies the changes of particle performance and biocompatibility after intraarticular injection in an experimental animal model with sheep. PVA coated SPIONs were shown to be a promising delivery system for magnetic drug targeting in synovial membrane tissue as they were taken up intracellularly *in vitro* and *in vivo*. Furthermore, the particles and the fluorescent dye remained within the synovial membrane for at least five days indicating that they could prolong the action of intraarticularly applied medication be used for treating acute or chronic joint diseases.

REFERENCES: [1]In collaboration with ANTIA SA, University of Geneva (E. Doelker, O.Jordan) and Hospital Universitaire Genève (D. Rüfenacht)
[2]In collaboration with Inselspital Bern (M. Reinert, A. Bregy)
[3]In collaboration with MSRU, Equine Hospital, Vetsuisse Faculty, University Zurich, (B. von Rechenberg et al.)

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Use of cell therapy to enhance the bone tendon healing, validation of a small animal model and results with the use of chondrocytes in rat.

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Introduction:
Current clinical data reports almost 50% of non healing rate after cuff repair in shoulder surgery. These poor healing rates are reported independently of the surgical procedure (2); and non healing is statistically related to poorer clinical outcomes (2,3,6). One of the reasons of this failure is related of aging of tissues involved in the repair and many studies have demonstrate the poor cell contain of local tissue (7). The first aim of this study was to validate a small animal model close to human data, with 50% of failed repaired cuff tear, and the second was to evaluate the effect of chondrocytes injections during the initial repair.

Material and Methods
a) Validation of the Model
In order to validate the model, after IRB approval, 10 male wistar rats (300 g) were scarified and Achilles' tendon and calcaneum were harvested together. Histological data were obtained to demonstrate the presence of an enthesis at the bone tendon junction. Ten other rats were scarified and histological confirmed the efficiency of the destruction of the enthesis with a burr. 30 male wistar rats had surgery (group 1: G1). Under general anesthesia, Achilles' tendon of the left leg was exposed and released from the calcaneum. The tendon was cut upon the insertion on the bone, and the enthesis was destroyed with a burr. Using a sterile needle, 2 tunnels were performed through the calcaneum and a 4/0 non absorbable suture was used to fix the tendon back to the bone. Skin was closed by staples. They were sacrificed at 15, 30 and 45 days in order to explore biomechanical data and compare it to cell therapy group. Failure of the healing was defined by: migration of the tendon proximally with a local gap, breakage of the suture, distance between the bone and the tendon superior to 1 cm (spontaneous healing after failure). The global healing rate was 47%, validating our model with a rate comparable with the one reported in human shoulder cuff surgery.
b) Cell therapy Study
Cell isolation*:* 16 wistar rats (4 days old) were sacrificed after IRB approval. Hip and knee cartilage were harvested according to our laboratory protocol (8). Cartilage fragments were digested in 2 baths of collagenase. Solution was filtered in 60 µm strainers, and cells were washed 3 time in PBS with 1% antibiotic solution before use. 1 million cells were used to perform immunostaining and demonstrate collagen 2 contain. Chondrocytes were used fresh, the day after harvesting, in order to prevent dedifferentiation.
Cell injection: cells were suspended in a 40 mg concentration bovine fibrinogen solution and mixed with equal volume in a 20 UI bovine thrombin solution as reported in literature (9). Final concentration was 4 millions cells in 100 µl solution as recommended in other studies(1,9). The solution was mixed during the surgery, and it was injected at the site of repair. (image 2). 30 rats had surgery (Group 2 G2) with the same technical procedure than in first group, but with injection of cells.
c) Measurement
Measurement were performed at 15, 30 and 45 days after surgery, by sacrifice of 10 animals each time.
Biomechanical testing: n=8 each time. The failure to load of the neo-enthesis during a tensile test was the main element we looked at using a custom made device. The global stiffness of the bone-enthesis-tendon sample and the local displacements at bone-tendon junction was also analyzed. Global failure rate was identified using previous described criteria.
Histological data: n=2 each time. Cell proliferation was explored using H&E staining by pathologists used to work in animal model histology. Local production of a neo–enthesis was performed by type II collagen immuno-staining of samples.

Results
Biomechanical data: n=8 for each group
At 45 days failure to load was 82 N in G2 versus 62 N in G1, with a statistically significant difference ($p<0,05$). (table 1) No difference was seen in failure to load in both groups at 15 days. The global stiffness increased from 40 N/mm in G1 to 45 N/m in G2. Maximum constrains in G1 were 0,7 MPa vs 0,75 in G2. The global healing rate increased from 47% (G1) to 70% (G2) ($p=0,11$).
Histological data: n=2 for each group
For each group, local identification of chondrocytes was carried out by 3 independent readers. Typical aspect of chondrocytes in chain was looked for, as soon as 15 days the typical aspect was seen in G2 but never in G1. Immunostaining using anti-collagen I and anti-collagen II antibody demonstrates local production of type II collagen at the bone tendon junction only in G2.

Discussion.
The current study, to our knowledge, is the first study exploring the efficiency of cell therapy regarding failure to load in one hand and global healing rate in the other hand. Studies published about efficiency of cell therapy in tendon healing or bone tendon healing are mostly focusing one the cinematic of the healing more than on the global healing rate (1,10). Because this rate is low in clinical practice, we consider that having a good, non expensive, animal model is a crucial step in bone tendon healing research. The surgery is easy to do, and does not require any training like should rat shoulder surgery. Animal models that are recognized today are mostly large animal model, like goat or sheep, and for them, no global healing rate have been reported (4,5).
Use of chondrocytes, by local production of type II collagen, seems to be an efficient therapy to stimulate bone tendon healing with a statistically significant difference at 45 days. Most studies published are reporting a difference, but rarely statistically significant (1,10). Use of fibrin glue (9) demonstrate one more time its interest, with no difference at 15 days in failure to load, confirming the lack of biomechanical properties of this scaffold. In the future we will work with different kind of cells, to become more clinically relevant (plated chondrocytes, MSC).

References: 1 Awad HA.Tissue Eng. 1999. 2 Burkhart SS. J Am Acad Orthop Surg. 2006. 3 Boileau P. J Bone Joint Surg Am. 2005. 4 Coleman SH. J Bone Joint Surg Am. 2003. 5 Gerber C. J Bone Joint Surg Am. 1999. 6 Gill TJ. *Instructional Course Lectures*. 1999. 7 Goutallier D. Rev Chir Orthop Reparatrice Appar Mot. 2005, 8 Salvat C. Osteoarthritis Cartilage. 2005. 9 Silverman RP. Plastic & Reconstructive Surgery. 1999. 10 Wong MW. J Biomed Mater Res B Appl Biomater. 2004

Microvascular Bone Reconstruction with Induction of Ectopic Bone Formation by Multipotent Human Adipose Stem Cells - Case Report

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INTRODUCTION: Currently, the most reliable method of reconstructing large bony defects involves painful harvest of bone. This causes donor site morbidity and a higher risk of infection. The use of other biological materials such as autograft bone carries the risk of virus transmission. Microvascular reconstruction is the state of the art in many fields of defect surgery today. Stem cell based custom made implants have been thought to become a new treatment modality in the near future. Here we describe the first case report of microvascular custom made ectopic bone flap employing Good Manufacturing Practice (GMP)-level adipose stem cells (ASCs).

METHODS: Our patient had a hemimaxillectomy due to a large keratocyst. After a follow-up of 36 months, with no signs of recurrency, the defect was reconstructed with a custom made microvascular flap by inducing ectopic bone formation with the patient's own ASCs combined with beta-TCP biomaterial and BMP-2 growth factor. Adipose tissue was subjected to cell extraction and cell expansion techniques[1] modified according to GMP guidelines without animal derived materials in clean room laboratories. ASCs were characterized by flow cytometry. ASCs multipotency e.g. osteogenic, chondrogenic and adipogenic differentiation potential and biocompatibility of biomaterial were evaluated in vitro. ASCs were cultured on beta-TCP granules and combined with BMP-2 prior bone tissue ossification in the patient's rectus abdominis muscle.

RESULTS: Our cell surface marker expression data is in accordance with the results reported earlier with ASC and other mesenchymal stem cells[2]. Furthermore, our in vitro data shows that ASCs are multipotent stem cells owning the capacity to undergo osteogenic, chondrogenic and Adipogenic differentiation.

After a follow up of 8 months, the flap was raised and transferred to the defect area of the maxilla. At the time of flap surgery, small biopsy was taken. The bone was clinically vital and vasculature had developed within the newly regenerated bone. Histological sample showed mature bone in the custom made implant. Postoperative healing has been uneventful, radiologically the area resembles bone and further rehabilitation with dental implants can be planned.

DISCUSSION & CONCLUSION: To our knowledge, this is the first successful reported clinical case where ectopic bone has been formed from the patient's own multipotent ASCs combined with microvascular reconstruction and it will pave the way for new clinical trials in this field.

REFERENCES: [1]Zuk PA, Zhu M, Mizuno H, et al. (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 7: 211-228. [2]Strem BM, Hicok KC, Zhu M, et al. (2005) Multipotential differentiation of adipose tissue-derived stem cells. *Keio J Med* 54: 132-141

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Integrin α_2: Deficiency Causes Impaired Bone Stability *in vivo* and Reduced Mineralization of the Extracellular Matrix *in vitro*

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INTRODUCTION: Integrins, heterodimeric, transmembrane proteins, act as receptors for extracellular matrix molecules and are involved in important cellular processes. The subunit α_2 which is widely expressed in different cells like platelets, endothelial cells, fibroblasts, and epithelial cells was shown to bind collagen I with high affinity. Integrin α_2 was investigated in recent studies with regard to platelet-collagen-I-interaction. It was shown that integrin $\alpha_2\beta_1$ contributed to synthesis and activation of several matrix metalloproteinases in tissue remodelling. The influence of Integrin α_2 (ITGA) on bone development, bone structure and fracture healing remains unknown.
Since collagen I is the most abundant protein in bone, we hypothesize an important function of this cell surface protein for bone metabolism and fracture healing. In this study the structural and biomechanical features of bone of integrin α_2 deficient mice were compared to wild type mice.

METHODS: Twenty right femurs of 4 and 12 month old female wild type and integrin α_2 knockout mice were dissected. Biomechanical testings were performed and maximum torque, angle at max. torque were determined and torsional stiffness was calculated. For cell culture experiments calvaria of newborn wild type and knockout mice were prepared for isolation of osteoblast progenitor cells. Osteoblast differentiation was induced by adding β-glycerophosphate, L-ascorbate and dexametasone. Mineralization was determined up to 25 days by von Kossa staining, and quantified after alizarin red staining by an image analysing system.

RESULTS Biomechanical stability with regard to maximum torque increased with age in both groups (highly significant in the ITGA $^{-/-}$ group). Torsional stiffness of the femurs of 1 year old ITGA knockout mice, was significantly higher compared to the 4 month old integrin α_2 deficient animals and tends also to higher values compared to both wild type groups.
Osteoblastic cells derived from osteoprogenitor cells isolated from integrin α_2 deficient mice revealed no differences cell morphology compared to wild type cells. With regard to mineralization of the extracellular matrix as visualized by von Kossa staining of the cell layer up to 25 days after induction of differentiation, considerably differences appeared (Fig 1).

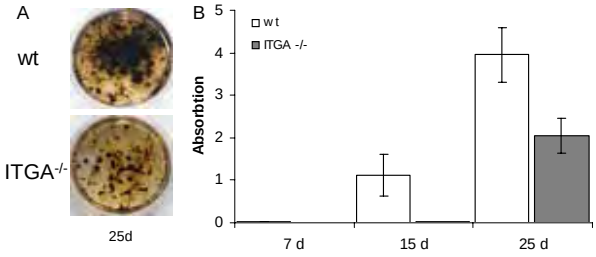

Fig. 1: Mineralization of extracellular matrix in vitro in ITGA$^{-/-}$ cells compared to wild type (A) B Quantification of calcification using alizarin red staining

Calcification of the extracellular matrix was delayed and reduced about 50% in cells deficient for integrin α_2 compared to wild type.

DISCUSSION: In conclusion, biomechanical stability of the femurs of the ITGA knockout mice was lower in young animals and higher with increasing age compared to the wild type group. Interestingly this contradicts to our *in vitro* data that point to an osteoblast defect with regard to mineralization of the extracellular matrix. Knowing that integrin α_2 is a receptor of collagen I, the dominant fibrous protein in hard tissues, these results point to an important function of integrin α_2 for the interaction of osteoblasts and collagen I, directly.
To our opinion integrin α_2 may be an interesting new target in bone development and remodeling.

Generation of human tendon- and ligament-derived cell lines, via immortalization with hTERT, for tissue engineering applications

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INTRODUCTION: In comparison to bone, cartilage and muscle, the cellular and molecular bases of tendon/ligament (T/L) physiology have not been fully understood due to the lack of appropriate cell lines. Establishment of such cell lines could enable the long-term molecular analyses of tenocytes, as well as, contribute to the development of novel strategies for T/L tissue engineering. The purpose of this study was to generate, by overexpressing hTERT, and characterize human T- and L-derived cell lines in vitro.

METHODS: Fibroblasts were isolated by collagenase digestion from human Achilles T (HTD5 cells) and periodontal L (PDL cells). The experimental protocol was approved by the Ethical Commission of the LMU (Project: 166-08). The cells were infected with hTERT-lentivirus using the ViraPower leniviral expression system (Invitrogen, Germany) as described [1]. HTD5-hTERT and PDL-hTERT cells were characterized by growth curve analysis (calculation of population doubling), immunohistology with anti-hTERT antibody (CalBiochem, Germany) and PCR for a set of T/L-related genes (Scleraxis, tenomodulin, Tenascin C and etc.). Furthermore, PDL-hTERT cells were used to analyse the biocompatibility of two different titanium scaffolds, machined (MA) and sandblasted/acid-etched (SLA). The scaffolds (15 mm diameter and 1 mm thickness) were generously provided by Straumann (Germany). The cells were cultured on the scaffolds and their cell spreading (membrane and actin staining), growth (nucleus counting) and survival (live-death assay) were investigated.

RESULTS: HTD5 and PDL cells were successfully infected and after 7 days of blasticidin selection homogeneous populations of hTERT-overexpressing cells were obtained. Both cell types demonstrated a strong overexpression of the hTERT transgene which was detected at RNA and protein levels. Importantly, HTD5-hTERT and PDL-hTERT cells kept proliferating for more then 250 days in culture, while HTD5 and PDL underwent senescence after approx. 90 days. Next, we tested whether these cells still retain T/L-like characteristics. PCR-based analysis demonstrated clear expression of several T/L-related genes in the cell lines. Finally, we used the PDL-hTERT cells in a dental tissue engineering model wherein we cultured and analysed their behaviour on MA and SLA-titanium scaffolds. Interestingly, PDL-hTERT cells were spread better on the MA scaffolds; they had a spindle-like shape and their F-actin was organized in parallel structures. In contrast, the cells on SLA scaffolds displayed a polygonal shape and mesh-like actin. PDL-hTERT cells favoured the MA scaffolds also in terms of cell growth and survival. They proliferated faster and the number of dead cells was 4 fold less than on the SLA scaffolds.

DISCUSSION & CONCLUSIONS: Our data clearly demonstrated the successful establishment of human T- and L-derived cell lines. Furthermore, we used PDL-hTERT cells to compare the biocompatibility of dental implants. PDL is important for the anchorage of the tooth root to the bone socket and it is known to be very difficult to reconstruct in diseased patients. Thus, PDL-hTERT, as well as, HTD5-hTERT will provide a good basis for further molecular analysis and the development of tissue engineering models for T/L therapy. Nevertheless, additional testing will be required to prove whether these cell lines can indeed regenerate T/L tissue in vivo.

REFERENCES: [1] W. Bocker, Y, Zhanhai, I Drosse, et al (2008). J Cell Mol Med. Epub ahead of print 2008 Mar 4.

ACKNOWLEDGEMENTS: We acknowledge the support of the AO Research Fund of the AO Foundation (Project No. S-07-18D) and the FöFoLe Research Program of the Medical Faculty of the LMU (Project No. 37/2006).

Mechanism of Decreased Expression of Type X Collagen in Human Mesenchymal Stem Cells from Osteoarthritis Patients Cultured on Nitrogen-Rich Plasma Polymers: Implication of Cyclooxygenase-1

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Recent evidence indicates that a major drawback of current cartilage and intervertebral disc (IVD) tissue engineering is that human mesenchymal stem cells (MSCs) from osteoarthritic patients rapidly express type X collagen, a marker of late-stage chondrocyte hypertrophy (associated with endochondral ossification). We recently discovered that a novel thin film substrate, named "nitrogen-rich plasma-polymerized ethylene" (PPE:N), is able to inhibit type X collagen expression in committed MSCs. Here we sought to elucidate mechanisms responsible for the selective inhibition of this collagen by PPE:N surfaces. Specific inhibitors of cyclooxygenases, protein tyrosine kinases, EGFR kinases, protein kinase C, and protein kinase A were used to assess their effect on the expression of type X collagen. We found that cyclooxygenase-1 (COX-1) and 5-lipoxygenase (5-LOX) may be implicated in the inhibitory effect of PPE:N. However, since PPE:N reduced COX-1 expression, whereas 5-LOX genes were not expressed in human MSCs, the results suggested that PPE:N inhibited type X expression through COX-1. These findings points to the mechanisms by which type X collagen expression is suppressed by PPE:N and may be important for tissue engineering applications.

EARLY VASCULARIZATION AND NEW BONE FORMATION IN A CRITICAL BONE DEFECT IS IMPROVED BY CO-CULTURE OF ENDOTHELIAL PROGENITOR CELLS (EPC) AND MESENCHYMAL STEM CELLS (MSC) IN RATS

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1

Department of Traumatology, University of Frankfurt, Germany

Introduction: Early vascularization of bone defects is a prerequisite for ingrowth of osteogenic reparative cells to regenerate bone *in vivo*. The size of the bone defect may limit the ingrowth of bone forming cells, since lack of vessels does not ensure a sufficient nutritional support of the bone graft. Mobilized EPC may contribute to neovascularization (1). This investigation tests after one week the ability of human endothelial progenitor cells (EPC) in coculture with mesenchymal stem cells (MSC) seeded on β-tricalcium phosphate scaffold (β-TCP) to improve the vascularization and therefore accelerate the healing process of large bone defects *in vivo*.

Material and Methods: For this *in vivo* study, EPC were isolated from buffy coat and MSC were isolated from bone marrow aspirate by density gradient centrifugation (2). Cultivated EPC and MSC were loaded onto ß-TCP in vitro. 35 critical-sized segmental bone defects (5mm) were created surgically in the femoral diaphysis of adult athymic rat and stabilized with an external fixateur. 7 empty defects served as control. The remaining defects were filled with β-TCP granules (n=7), human EPC seeded on β-TCP (n=7), MSC seeded on β-TCP (n=7) or coculture of EPC and MSC seeded on β-TCP (n=7). Athymic rats were used to avoid graft-versus-host reactions. After 1 week the rats were sacrificed. Histology and Immunohistology for qualitative determination of ingrowth-behaviour in decalcified serial sections (staining of HE, vWF and PECAM) as well as quantitative analysis of vascularization and new bone formation in an image-analysis-system were performed.

Results: Formation of a primitive vascular plexus could also be demonstrated in the TCP and MSC group, but on a significant higher level in the EPC group. Moreover, in the coculture group (EPC and MSC) we found the highest values for early vascularization (p= 0.01). One week after implantation, gross morphology revealed an instable critical bone defect. Histological inspections showed newly formed bone structures (osteoid) in all specimens. Compared to the TCP group there was significant more new bone formation in the EPC group and in the MSC group observed. But no difference between EPC and MSC group was detectable. Also in the analysis for new bone formation the coculture of EPC and MSC showed highest values, which were significant compared to MSC group (p=0.03), but non-significant compared to EPC group.

Discussion: After one week early vascularization and new bone formation in the bone defect seems to be improved by implantation of coculture of EPC and MSC onto ß-TCP scaffold. This investigation displays a promising approach in developing capillary networks inside of bone constructs in vivo. Moreover, beside MSC also EPC showed enhanced new bone formation. It seems that the coculture of EPC and MSC has a synergistic effect. Cell-seeded β-TCP seems to be a potential osteogenic construct for in vivo application.

Project no. 05-S23 was supported by the AO Research Found of the AO Foundation, Switzerland.

Literature:

1. Lee DY, Cho TJ, Kim JA, Lee HR, Yoo WJ, Chung CY, Choi IH.Mobilization of endothelial progenitor cells in fracture healing and distraction osteogenesis Bone. 2008;42(5):932-41
2. Crosby JR, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. Circ Res. 2000 ; 87(9):728-730.

EXTRACORPORAL BONE GENERATION

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Introduction

A need for bone tissue supplementation exists in a wide range of clinical conditions involving surgical reconstruction following trauma or other pathological conditions in limbs and spine.

The amount and purpose of bone supplementation determines the origin of the used tissue, i.e. for bone inductive purposes a supplementation of fresh autologeous cancellous bone that contains cellular, mineral and humeral components, is required and usually is taken from non involved body site, and a material for bone conductive-support purposes might require either autogrtafts or allografts.

Both solutions for bone supplementation, either autografts or allografts, bear potential serious side effects and complications. Availability of autografts is usually limited and their harvesting anatomical site and causes a considerable surgical morbidity. The success of use of allografts is limited because of a high risk of "docking site" nonunion (or rejection of whole graft) and infection.

Therefore a possibility for ex vivo generation of sufficient amount of autologeous bone for inductive and conductive purposes should resolve these difficulties and complications.

Methods

For the purpose of supplementation of autologeous bone graft that will suffice the requirements of an individual patient we developed a method for ex vivo bone generation. By this method bone matrix generating cells, osteoblasts, were seeded on an inorganic supporting matrix (porous calcium triphosphate) in a specially designed bioreactor allowing exposure of cells to osteogenic medium and their stimulation by biomechanical activation by mechanical vibration in the infrasonic range of frequencies (1). The source for osteoblasts was from a mesenchymal precursor cells originated from disposable human cancellous bone samples, which were collected during elective knee arthroplasties. The osteoblasts were initially grown as explant primary cultures, in special bone inductive medium (2) and presented osteoblastic characteristics, e.g. osteopontin and osteocalcin expression, cellular alkaline phosphatase activity and positive Von Cossa staining.

Results

Three days after treatment of cells in the bioreactor there was a histological evidence (H&E staining) of bone matrix formation (Figure 1) which became abundant after two weeks (Figure 2) and has been kept intact in the bioreactor conditions for 6 months (Figure 3). There was also an evidence of collagen deposition by the osteoblasts after one week of treatment in the bioreactor (trichrome staining – Figure 4).

Figure 1: Low power micrograph, HE staining

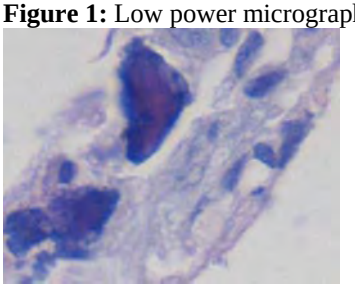


Figure 2: Low power micrograph, HE staining

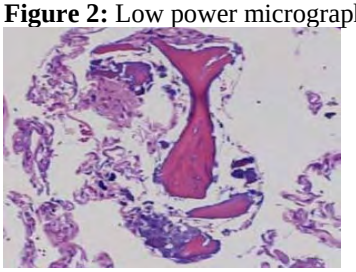


Figure 3: Low power micrograph, HE staining

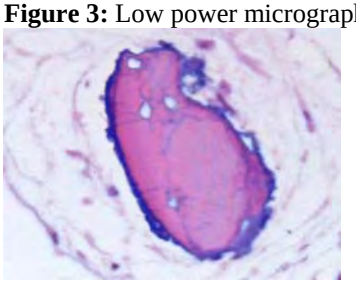
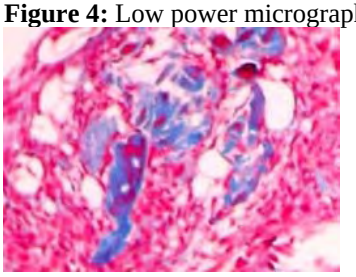


Figure 4: Low power micrograph, trichrome staining



Conclusion

We show that the interaction of the cellular, inorganic and mechanical components in the described ex vivo bioreactor rapidly generated bone tissue that may be kept viable ex vivo for a significant time period. Further in vivo studies will be done in order to determine the ability of generated tissue to incorporate into bone defects for its use as a bone graft.

References

1.N. Rosenberg(2003) *The role of the cytoskeleton in mechanotransduction in human osteoblast-like cells.* Human Exp Toxicology 22:271-274

2. R. Gundle, K. Stewart, J. Screen, JN. Beresford (1998) *Isolation and culture of human bone-derived cells.* In: Beresford N, Owen ME, editors. Marrow stromal cell culture. Cambridge: Cambridge University press; pp 43-66.

TIME-DEPENDENT VEGF EXPRESSION MODULATES ECTOPIC BONE FORMATION MEDIATED BY MUSCLE-DERIVED STEM CELLS

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INTRODUCTION:
Previous work by our group has demonstrated synergy between angiogenic and osteogenic factors in bone formation, indicating that the former is very important for endochondral ossification.[1] We also noticed that excessive amounts of VEGF in relation to BMP4 impair bone healing. Therefore, tight regulation of growth factor expression may be necessary for successful bone tissue engineering. The effects of timely induced angiogenesis on bone regeneration have not been determined. Here we hypothesized that time-dependent VEGF up-regulation could affect bone formation mediated by muscle-derived stem cells (MDSCs) expressing bone morphogenetic protein 4 (BMP4).

METHODS:
We constructed a self-inactivating tet-on retroviral vector expressing VEGF under the control of a tetracycline-inducible promoter.[1,2] MDSCs were isolated and transduced as described previously.[3] The amount of protein secreted by BMP4-transduced cells (B4) was detected using BMP bioassay.[4] ELISA was used to confirm VEGF expression by MDSCs transduced with conventional (VE) or inducible retrovirus (TVE). TVE cells were exposed to different doses of doxycycline (Dox; 0, 1, and 2 μg/mL), and the amount of VEGF in conditioned medium was measured over 2, 4, and 6 days. Based on in vitro results, a normalized number of B4 and TVE cells (B4+TVE) was seeded onto Gelfoam scaffolds and implanted into the right thigh muscles of normal mice. Control mice received scaffolds containing B4 and VE cells (B4+VE). Left thigh muscles of all mice received scaffolds containing B4 cells only (B4). The 25 animals then were divided into 5 groups depending on whether or not they received Dox-supplemented drinking water and when Dox was administered after surgery. Mice in group 1 (control) and group 2 received no Dox. Dox administration began on day 0 after surgery in group 3, on day 3 in group 4, and on day 7 in group 5, and continued until scheduled euthanization. Ectopic bone formation at the implantation sites was monitored radiographically and histologically at 3 and 6 weeks post-surgery. Northern Eclipse imaging software was used to quantify ectopic bone area and bone photodensity. Cryosections of the specimens harvested at 3 weeks were stained for Von Kossa to detect mineralized bone deposition. At the end of the experiment at 6 weeks we isolated bony nodules from the muscles of remaining mice and measured their dry weight.

RESULTS:
VEGF secretion in vitro: ELISA confirmed that in the absence of Dox TVE cells secreted almost undetectable or very low levels of VEGF over the period of 6 days. Stimulated with 2 μg/mL of Dox for 2, 4, or 6 days TVE cells secreted nearly constant amount of VEGF (7 to 8 ng/ml per million cells/24h) which was 4 times less than the amount of VEGF secreted by VE cells (34 ng/ml per million cells/24h)(data not shown). *Radiographic and histological examination of heterotopic bone:* No difference in bone formation at any time was noticed between groups treated with B4 cells alone (Fig.1). Control mice demonstrated exorbitant ossification at B4+VE cell implantation sites. There was no apparent difference in bone formation between B4 cell and B4+TVE cell implantation sites without Dox treatment. Larger ectopic bone was evident in Dox-treated animals at B4+TVE implantation sites compared to B4 implantation sites any time VEGF expression was induced by Dox administration (Fig.1). Radiomorphometry showed larger bone area at B4+TVE implantation sites in Dox-treated mice than in nontreated mice. There was a significant increase in bone area and density after 6 weeks when Dox was administered at times other than immediately after surgery (Fig.1). *Quantification of ectopic bone by weight:* Bone harvested from B4+TVE implantation sites of Dox-treated animals weighted more than bone from contralateral B4 implantation sites, but the increase was significant only when VEGF expression was induced on day 3 after surgery. VEGF induction time had no significant effect on the ectopic bone weight in Dox-treated mice (data not shown).

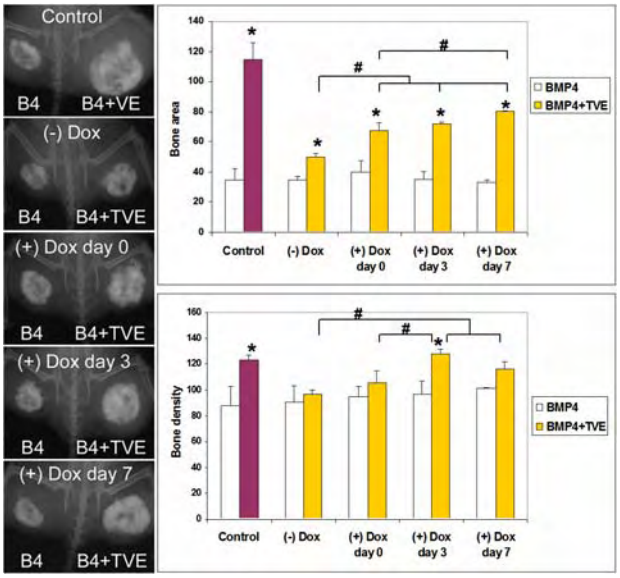

Figure 1. Radiographic (left panel) and radiomorphometric evaluation of ectopic bone formation 6 weeks after implantation (upper right panel: ectopic bone area; lower right panel: photodensity of ectopic bone; N=3; *p<0.001; #p<0.05)

DISCUSSION:
Our study shows that controlled VEGF expression is achievable using an inducible tet-on retroviral vector. This vector enables tight regulation of growth factor secretion by transduced MDSCs. Dox-induced TVE cells secreted constant amounts of VEGF in vitro and enhanced ectopic bone formation mediated by BMP4-expressing MDSCs in vivo. We were able to modulate bone formation by altering the induction time of VEGF expression. Radiomorphometry and measurement of bone weight performed 6 weeks after implantation of cell-seeded scaffolds revealed the presence of larger, denser and heavier bony masses when neovascularization was triggered not immediately after the surgery, but 3 or 7 days later. This finding indicates that proper timing of VEGF expression is crucial for bone regeneration. Our study has important implications for the development of novel bone tissue engineering strategies based on angiogenic gene regulation.

ACKNOWLEDGEMENTS:
We wish to thank Jim Cummins for his editorial assistance. This work was supported by a grant to Dr. Johnny Huard from the National Institutes of Health (NIH R01-DE 13420).

REFERENCES:
1. Peng H, et al. J Clin Invest 2002; 110:751-9.
2. Peng H, et al. Mol Ther 2004; 9:885-894.
3. Lee JY, et al. J Cell Biol 2000;150:1085-1100.
4. Peng H, et al. Mol Ther 2001; 4:95-104.

HUMAN OSTEOBLASTS INDUCE PROLIFERATION AND NEO-VESSEL FORMATION OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS IN A LONG-TERM 3D CO-CULTURE ON POLYURETHANE SCAFFOLDS

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INTRODUCTION: The improvement of vascularization by implementation of endothelial cells or angiogenic growth factors may represent a key solution for engineering bone constructs of large size. In this study, we focused on the process of cell organization and neo-vessel formation in endothelial cell cultures on polyurethane scaffolds. In particular, we addressed the question of how the parameters of endothelial cell proliferation and differentiation would be influenced by differentiated human osteoblasts in a three-dimensional long-term culture that is relevant for bone tissue engineering.

METHODS: Static mono- and co-cultures of primary human osteoblasts and human umbilical vein endothelial cells (HUVEC) were established employing resorbable polyurethane scaffolds, platelet rich plasma, and FCS-free media. Cell proliferation and differentiation was analyzed using histological, immunohistological, and confocal laser microscopic methods. Differential gene expression of osteoblastic (collagen Type I, osteonectin, and osteopontin) and endothelial (CD31, von Willebrand Factor, and collagen Type IV) markers was examined using quantitative RT-PCR.

RESULTS: Histological evaluation of hOB-monocultures after a long-term culture period revealed that these cells were able to form mono and multilayers, which were associated with the predetermined scaffold surface (Fig. 1a). A significant number of HUVECs did not survive the long-term culture period of six weeks in a monoculture (Fig. 1b) and could only be detected as an amorphous layer containing residues of cell nuclei. Co-cultures of hOB/HUVEC were characterized by impressive formation of multiple sprouts and tube-like structures (Fig. 1c). These lumens were branched, interconnected, formed predominantly of a single elongated cell layer, and strongly positive for CD31 and vWF. According to the histological results, which revealed tube-like formation only in co-cultures, expression of collagen type IV was significantly induced in this group

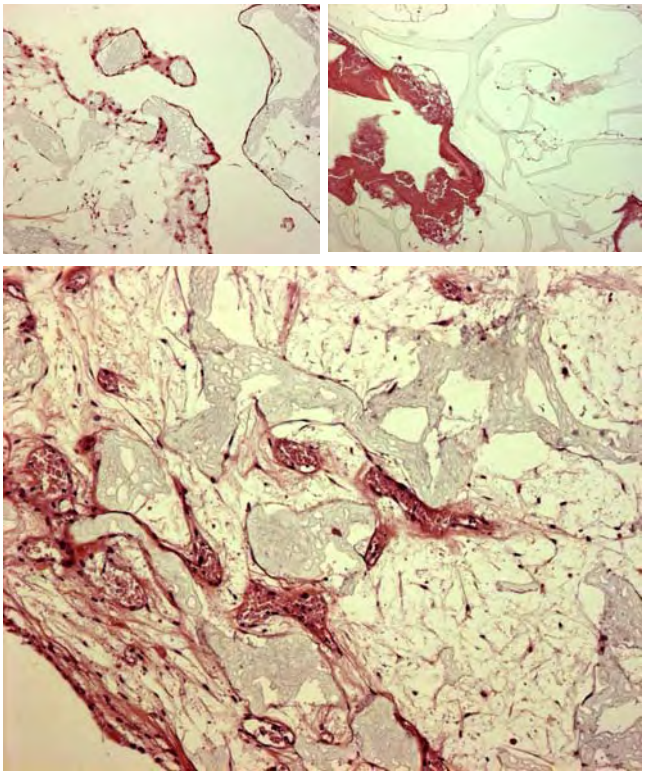

Fig. 1: Effect of hOB on neo-vessel formation by HUVEC. A: hOB monoculture, B: HUVEC monoculture, C: hOB/HUVEC co-culture. HE-staining.

DISCUSSION & CONCLUSIONS: We could clearly show that hOB support cell proliferation and spontaneous formation of multiple tube-like structures by HUVEC. Immunohistochemical and qPCR analyses of gene expression revealed that cell differentiation of hOB and HUVEC was stable in the long-term co-culture. The three dimensional, FCS-free co-culture system could provide a new basis for the development of complex tissue engineered constructs with a high regeneration and vascularization capacity.

ACKNOWLEDGEMENTS: This work was supported by AO-Foundation grant 05-H63. The full text manuscript has been accepted for publication in Biomaterials.

Controlled release of antibiotics from Bone Cement.

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Introduction. It is a common practice in orthopedic surgery to blend antibiotics into beads of bone cement to prevent infection. Bone cement (poly (methyl methacrylate) (PMMA)) is used for this purpose because it has established biocompatibility in these surgical settings. However, the material is nondegradable and nonporous so that less than 5% of the encapsulated drug is released. The objective of this study was to use pharmaceutically acceptable, water-soluble additives to increase the porosity of the cement so that more drug (Vancomycin or Fusidic acid) might be released over the timecourse of implantation.

Methods: Model beads and films were made by blending the drug (2.5% or 5% w/w) with the powdered PMMA (Pelacos® bone cement, Zimmer, Wehrheim, Germany) and the additives (Polyethylene glycol, (PEG), sodium chloride or dextran T70). Polymerization was initiated with methyl methacrylate, followed by mixing and centrifuging 500mg of the mixture into 2 ml plastic eppendorf tubes. For thin films, 500 mg aliquots of the mixed paste were squeezed between Teflon coated glass slides and then cut into approx. 15x4x1mm sections. Beads and films were then weighed and placed in 5 ml of phosphate buffered saline (PBS pH 7.4) and the amount of drug released each day was quantitated using HPLC. Sodium chloride or dextran release were measured by conductivity or refractive index changes, respectively. The effect of the additives on the mechanical strength (Young's modulus) of the implants was measured using a flexure adaptor on a TMA.

Results: Sodium chloride released from the cement over a two month period and resulted in a concentration dependent increase in the release rates of both Vancomycin and Fusidic acid from the cement with no effect on Young's modulus. Similar results were found for the addition of dextran at 18% loading. The addition of PEG resulted in smaller increases in the burst phase of drug release only and caused a large decrease in the mechanical strength of the cement.

Conclusion: The addition of water soluble additives like sodium chloride and dextran (but not PEG) greatly increase the release rate of Vancomycin and Fusidic acid from bone cement and offer a simple method of modifying a clinically relevant procedure for an improved antibacterial action.

To All Guests

2nd International Symposium on Biotechnology in Musculoskeletal Repair

October 2 - 4, 2008 / Beaulieu Convention Centre, Lausanne, Switzerland

Dear Madam, dear Sir,

Before the 2nd Symposium on Biotechnology starts next week in Lausanne, we like to inform you in detail about the venue, the social events and the possibilities of transport:

1. Conference Venue

The Convention Centre Beaulieu is situated in the upper region of Lausanne. The access plan is shown below. We will provide a bus from the Hotel Mövenpick to the Convention Centre (departure time on Friday and Saturday at about 7:45 in the morning). This bus will bring you back to the hotel on Friday afternoon. On Saturday no further bus will be provided in the afternoon as many of you may leave at different times. Public transport is available from the hotel to the conference venue by using bus **Line 2 :** Ouchy – Saint-François – Beaulieu.

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Parking Beaulieu Lausanne.**:**

The Beaulieu car park is open to the public 24 hours a day. Its capacity of 580 parking spaces in the city centre is a plus for your convenience. We have ordered parking tickets for you, please let us know if you need one.

Further Information: Beaulieu Lausanne, Av. des Bergières 10, CH-1000 Lausanne 22 ,

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2. Hotel Mövenpick

Most of you are booked in the Hotel Mövenpick. It is situated directly at the lake side in the part of Lausanne called OUCHY.

The Hotel Beau Rivage, which will host all guest for our Welcome Apéro is situated in walking distance (5 minutes by foot) and the harbour, which is the starting point for the dinner on the boat is situated directly opposite to the hotel also in walking distance.

Information: Mövenpick Hotel Lausanne, 4, Avenue de Rhodanie, 1006 Lausanne
Phone:+41 21 612 7 612, Fax:+41 21 612 7 611, E-Mail: hotel.lausanne@moevenpick.com, http://www.moevenpick-hotels.com

3. Hotel AuLac

The Hotel AuLac is situated 2 minutes by foot from the Hotel Mövenpick and welcomes also some guests of this symposium.

Information: HOTEL AULAC **-** Place de la Navigation 4 - CH - 1000 Lausanne 6 - TEL 0041 (0) 21 / 613 15 00 - FAX 0041 (0) 21 / 613 15 15 , aulac@cdmgroup.ch, http://www.aulac.ch

4. Hotel Beau Rivage – Welcome Apéro

The Welcome Apéro offered by the AO Foundation within the scope of the 50 years Anniversary will be held in the Library of the Hotel Beau Rivage Lausanne Ouchy on October 2nd 2008. It will start at about 6 p.m. and end about 8:30 p.m. The hotel is in walking distance to the hotels Mövenpick and AuLac.

Please turn to the left when you come out of your hotel. Guests from Hotel Mövenpick have to cross two streets, those of Hotel AuLac have to cross one street. The entrance is on the opposite side of the lake.

Information: Hotel Beau-Rivage Palace, Place du Port 17-19 - CH 1000 Lausanne 6 - Switzerland - Tel: (+41) 21/613 33 33 - Fax: (+41) 21/613 33 34 - eMail : reservation@brp.ch; http://www.brp.ch/

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5. Dinner on the Boat

The dinner on the boat will be held on October 3rd, 2008. The steam boat is called RHONE and has been built in 1927.

Time Table:

19:15 Embarkment Lausanne Ouchy

19:30 Start of the Dinner Cruise on the Lake Geneva

22:30 Return to Lausanne Ouchy

Please be at the embarkment early enough.

Information: COMPAGNIE GENERALE DE NAVIGATION SUR LE LAC LEMAN, Avenue de Rhodanie 17, 1000 Lausanne 6; **INFOLINE +41 (0) 848 811 848**

As you may see by this sketch, the embarkment and the disembarkment is at the outer end of a little peninsula, you can walk over there within 5 minutes from your hotel.

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6. Transport

a. from Geneva or Zurich airport to Lausanne

The easiest way is to take the train as the train station is situated in the basement of each of the airports and the trains are as fast as the car. You can get the ticket at the ticket office or by a ticket machine; in case you pay by card or cash (Swiss Franks in some cases also EUR).

The train will leave half-hourly and takes about 2h 30 from Zurich to Lausanne (departure time always xx:13 or xx:43) and 42 minutes from Geneva if you take the trains which leave at xx:01 or xx:36.

b. from Lausanne train station to your hotel

You can either take the bus called MB which takes 10 minutes from the train station to the hotel or you take a taxi.

In all cases please use the webpage www.sbb.ch. You can enter your point of departure and the point of arrival (Lausanne Ouchy) and you will find all information on trains and buses you need.

7. Other Information

The Lausanne Tourist Office will help you with all necessary information, It is situated directly beside the Hotel Mövenpick (http://www.lausanne-tourisme.ch/UploadedAsp/26958/2/F/HPLT.asp?Check=True&Language=E)

You can download city maps from the following WebPage:
http://www.lausanne-tourisme.ch/view.asp?domId=63222&language=E

Lausanne Tourisme
Pl. de la Gare 9 + pl. de la Navigation 4 (Ouchy), 1000 Lausanne
Phone +41 21 613 73 73, Fax +41 21 616 86 47 Gare: 7/7 09h00-19h00
The office is open in Ouchy: 7/7 09h00-18h00 Gare+Ouchy: ligne 2 et Metrobus (MB)

We hope we could deliver all necessary information and wish you a safe trip to Lausanne and an interesting Symposium. We are looking forward to welcoming you at the lake of Geneva. Should you have further questions, please do not hesitate to contact us.

With kind regards,

Margarethe Hofmann and BAB Members
Chairperson BAB

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Chairperson Dr.-Ing Margarethe Hofmann
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