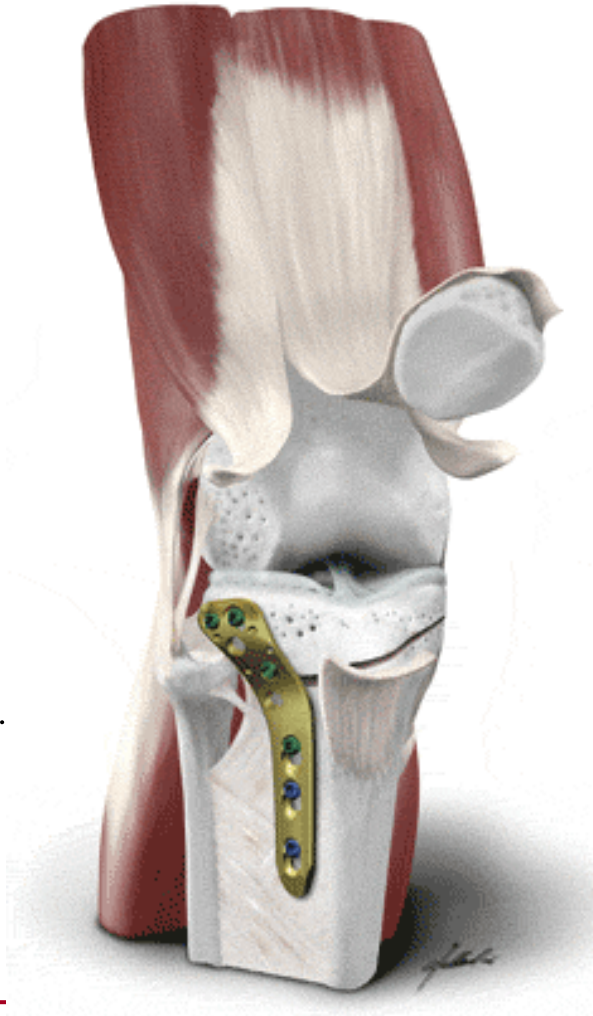
eCM XV

Cartilage & Disc: Repair and Regeneration

June 16 - 18, 2014
Congress Center, Davos Platz, Switzerland



eCM WELCOMES YOU TO DAVOS

Dear colleagues

Although bone has a remarkable propensity to repair, there are a number of situations where the repair process fails. This leads to many complications both social and economic. To meet these challenges, a complete understanding of bone biology, at the molecular, cellular and mechanical level is required. Furthermore the knowledge gained must be implemented into clinically relevant applications. This conference aims to address precisely these topics while exploring issues such as biomaterials and clinical approaches. The outcome should be an increased understanding of the topic and a fostering of the interaction between scientists and clinicians. Since the inaugural meeting in 1999 eCM meetings have been the place where scientists and clinicians meet to move clinical problems to the scientists and to initiate projects to translate the scientific solutions back to the clinics. The afternoons are free for excursions into the mountains or networking opportunities.

This international forum continues the eCM congress series held in Davos. The limited number of participants (150) ensures clinicians, biologists, engineers and material scientists will have ample opportunities for knowledge sharing in basic, translational and clinical research in addition to developments in the field of bone fixation, repair and regeneration. As the conference does not have parallel sessions it permits in-depth multidisciplinary discussions about how to advance this research area.

Yours sincerely

Local Conference Organizers

Dr, Martin J Stoddart
Principal Investigator
Musculoskeletal Regeneration
AO Research Institute Davos

Dr Sybille Grad
Principal Investigator
Musculoskeletal Regeneration
AO Research Institute Davos

Dr. David Eglin
Principal Investigator
Musculoskeletal Regeneration
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Registration & Conference dinner tickets eCM XV

Monday, June 16th Registration and pick up of conference bags at the Convention Center from 7.30 am on

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All **invited talks** are 20 minutes (Max) (20-30 slides)
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eCM provides a forum for publication of preclinical research in the musculoskeletal field (Trauma, Maxillofacial (including dental), Spine and Orthopaedics) and the cells & materials used in the replacement, repair or regeneration of these tissues.

Detailed Scope

• Assessment of materials for biomedical use in the musculoskeletal field & interaction with tissues/ prokaryotic / eukaryotic cells.

Manuscripts must have an important biological dimension reporting effects at the cellular, tissue or organismic levels. Papers focussing purely on material properties will not be entertained. In vitro cytocompatibilty studies should use primary cells or more than one cell line and provide more than just simple descriptions of cell viability, spreading, proliferation, mineralisation etc. Major improvements in in vitro or in vivo models are considered.

• Tissue Engineering and Regenerative Medicine.

Manuscripts concerning aspects of the repair or regeneration of connective and mineralized tissues within the musculoskeletal field will be considered.

• Structure, function, biology of connective tissues.

Manuscripts concerning the structure of bone, teeth, cartilage, intervertebral discs, skeletal muscle, tendons and ligaments within the musculoskeletal field will be considered.

• Stem and Progenitor Cells.

All manuscripts concerning stem cell characterization and mechanisms of differentiation as they relate to the connective and mineralized tissues of the musculoskeletal field will be considered. Note: The use of pooled donor cells should be avoided. When unavoidable, their use needs to be justified convincingly within the manuscript (and within the cover letter).

Ten good reasons for publishing a paper in eCM

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Scientific Program eCM XV 2014

Monday, June 16th

Welcome

08:40 – 08:50 **Martin J. Stoddart (CH)**
AO Research Institute Davos

08:50 – 09:00 **Sibylle Grad (CH)**
Welcome to Davos

Session 1 Opening Session

Chair: Brian Johnstone

09:00 – 09:45 **Peter Roughley (CA)**
The life of aggrecan

09:45 – 10:30 **Charlie Archer (UK)**
The regenerative potential of articular cartilage

10:30 – 11:00 Coffee Break

Session 2 Structure and Characterisation

Chair: Ilyas Khan

11:00 – 11:30 **Andrew A. Pitsillides (UK)**
Transcriptional profiling in osteoarthritis

11:30 – 12:00 **Laura B. Creemers (NL)**
Cytokine profiles and pathways in trauma and degeneration

12:00 – 12:15 Identification of disc degeneration markers in the human AF by microarray
Z Kazezian, R Gawri, L Haglund, J Ouellet, F Mwale, A Pandit, M Alini, S Grad

12:15 – 12:30 Interleukin 16 in control of proliferation of mesenchymal stem cells (MSC), in MSC chondrogenesis and in early osteoarthritis
B Ranera, C O'Flatharta, G McKenna, T Jungwirth, G Shaw, L Howard, WW Cruikshank, M Tuzova, M Leijs, GJVM van Osch, F Barry, JM Murphy

Free afternoon

Conference Social Walk (optional)

Session 3 **Pathology**

Chair: Mauro Alini

17:00 – 17:30 **Di Chen (US)**
The role of β-catenin signaling in cartilage

17:30 – 18:00 **Christopher B. Little (AU)**
Pre-clinical models of inter-vertebral disc disease (IVDD): can we learn from the new paradigms of organ-wide pathology and disease phenotypes in osteoarthritis?

18:00 – 18:15 Human cartilaginous endplate degeneration is regulated by the extracellular calcium-sensing receptor
MP Grant, LM Epure, J Antoniou, F Mwale

18:15 – 18:30 Establishment and characterisation of a non-invasive model of post-traumatic knee osteoarthritis
SJ Gilbert, C Bonnet, M Davies, C Elford, LB Meakin, VC Duance, DJ Mason, EJ Blain

18:30 – 18:45 Human adipose mesenchymal stromal cells transplantation promotes intervertebral disc regeneration in biglycan-deficient murine model of chronic and progressive disc degeneration
G Marfia, SE Navone, C Di Vito, L Giammattei, M Egidi, M Zavanone,P Rampini, L Riboni, R Campanella

19:00 – 20:30 Poster session (Drinks & Snacks)

Tuesday, June 17th

Session 4 **Mechanobiology**

Chair: Robert Mauck

08:30 – 09:00 **Clark T. Hung (US)**
Chondrocyte mechanobiology: applications to cartilage tissue engineering

09:00 – 09:30 **Niamh C. Nowlan (UK)**
Developmental biomechanics of the prenatal skeleton

09:30 – 09:45 Mechanics and biology of the intervertebral disc: long-term studies in a loaded disc culture system
TH Smit, K Emanuel, PP Vergroessen, KP Paul, AJ van der Veen, I Kingma

09:45 – 10:00 Articulation reduces IL-1β effect on cartilage biosynthesis and matrix degradation
RL Trevino, CA Pacione, S Chubinskaya, AM Malfait, MA Wimmer

10:00 – 10:15 Chondrocyte de-differentiation is associated with primary cilia elongation and a reduced capacity for hedgehog signaling
AKT Wann, CL Thompson, EM Tan, MM Knight

10:15 – 10:30 Low-frequency high-magnitude mechanical strain of articular chondrocytes activates p38 MAPK and induces phenotypic changes associated with osteoarthritis and pain
DH Rosenzweig, TM Quinn, L Haglund

10:30 – 11:00 **Coffee Break**

Session 5 **Scaffolds and Matrices**

Chair: David Eglin

11:00 – 11:30 **Samuel I. Stupp (US)**
Biomimetic and bioactive scaffolds for regeneration

11:30 – 11:45 Fibrin-genipin annulus fibrosus sealant as a delivery system for anti-TNFα drug
M Likhitpanichkul, Y Kim, Z Kazezian, A Pandit, AC Hecht, JC Iatridis

11:45 – 12:00 A therapeutic effect of hyaluronic acid based-hydrogels in *in vitro* inflammation model of nucleus pulposus
IL Mohd-Isa, D Tiernan, A Srivastava, P Rooney, A Pandit

12:00 – 12:15 Osteochondral defect repair using a biomimetic hydrogel
F Babaei, J Parry, P Kok, S Kakar, YW Lam, MJ Yaszemski, MDadsetan

Free afternoon

18:00 Conference Dinner

Wednesday, June 18th

Session 6 **Tissue Engineering and Regenerative Medicine**

Chair: Chris Evans

08:30 – 09:00 **Sue J. Kimber (UK)**
Pluripotent stem cells for cartilage repair

09:00 – 09:30 **Farshid Guilak (US)**
Engineering new biological therapies for for osteoarthritis

09:30 – 09:45 Manipulation of chondrogenic progenitor cells from late stages of osteoarthritis for cartilage repair
B Schminke, H Muhammad, G Cingöz, C Bode, S von der Heyde, N Miosge

09:45 – 10:00 Intermittent but not continuous parathyroid hormone-related protein exposure augments mesenchymal stromal cell chondrogenesis and reduces hypertrophy via cAMP/PKA signaling
J Fischer, A Aulmann, V Dexheimer, T Grossner, W Richter

10:00 – 10:15 Evaluation of bone marrow mesenchymal stem cell (MSCs)-based intervertebral disc regeneration using quantitative T2 mapping: a study in a rabbit model
F Cai, XT Wu, XH Xie

10:15 – 10:30 A mouse model of joint surface injury: contribution of functional mesenchymal stem cells to cartilage repair
AR Armiento, F Dell'Accio, C De Bari

10:30 – 11:00 Coffee Break

Session 7 Inflammation Effects

Chair: Sibylle Grad

11:00 – 11:30 Gerjo van Osch (NL)
Inflammation and cartilage regeneration

11:30 – 12:00 Makarand V. Risbud (US)
Emerging role of PHDs in intervertebral disc degeneration and iflammation

12:00 – 12:15 Epigallocatechin 3-gallate suppresses interleukin-1β-induced inflammatory responses in intervertebral disc cells *in vitro* and reduces radiculopathic pain *in vivo*
O Krupkova, M Sekiguchi, J Klasen, S Konno, SJ Ferguson, K Wuertz-Kozak

12:15 – 12:30 Immuno-regulation by articular chondrocytes and synovial stem cells in inflammatory environment: prime effect of platelet lysate in joint repair
RC Pereira, A Poggi, R Benelli, D Martinelli, R Cancedda, C Gentili

12:30 – 12:45 M2-macrophages instruct mesenchymal stem/progenitor cell to better chondro-differentiate
SB Sesia, R Duhr, C Medeiros da Cunha, E Padovan, I Martin, A Barbero

12:45 – 14:15 Lunch Break (no lunch provided)

Session 8 Clinical Approaches (sponsored by AOTRAUMA)

Chair: Henning Madry

14:15 – 14:45 Mats Brittberg (SE)
Clinical approaches to cartilage repair

14:45 – 15:15 Joji Mochida (JP)
Clinical approaches to intervertebral disc repair with activated nucleus pulposus cell transplantation. A twenty-year journey from basic and translational studies to a novel clinical study.

15:15 – 15:30 Norepinephrine: chondroprotective effects in human OA-chondrocytes
J Lorenz, RH Straub, A Pasoldt, J Schaumburger, J Grifka, S Grässel

15:30 – 15:45 Evaluation of articular cartilage progenitor cells for the repair of articular defects in an equine model
D Frisbie; HE McCarthy; CW Archer; MF Barrett; CW McIlwraith

15:45 – 16:00 Agili-C™ induced cartilage regeneration: insights in to the mode of action, *in vitro* and *in vivo* data
S Chubinskaya, AS Levy, D Robinson, E Kon, AA Hakimiyan, L Rappoport, A Margulis, K Zaslav, P Davidson, N Altschuler

16:00 – 16:15 Cartilage tissue engineering from nose to knee: early results of a Phase 1 Clinical Trial
M Mumme, A Barbero, S Miot, A Wixmerten, DJ Schaefer, U Studler, A Hirschmann, T Schwamborn, I Martin, M Jakob

16:15 – 16:30 Robert Mathys Student prize announcements, F1000 Poster prize

16:30 – 16:45 Mauro Alini (CH)
Summary & Conference closing

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F1000Posters F1000Research AN OPEN SCIENCE JOURNAL FOR THE LIFE SCIENCES

Conference End

Posters

1. The effect of fluid-dependent dissipation on chondrogenesis
   P Abdel-Sayed, S Rissone, DP Pioletti
2. Link N suppresses Interleukin-1β induced human osteoarthritic cartilage degradation through down-regulation of NF-κB signaling
   M Alaqeel, MP Grant, LM Epure, O Salem, OL Huk, J Antoniou, F Mwale
3. Cartilage growth on Insulin and collagen-coated PLGA/PCL scaffolds
   A Basiri, G Amoo-Abedini, M Soleymani, B Khorvash, M Vasei
4. *In vitro* cell mobility: a mesenchymal stem cells marker for multipotency?
   A Bertolo, A Gämperli, M Gruber, B Ritter-Gantenbein, T Pötzel, M Baur, JV Stoyanov
5. Characterization of hydrogels under physiological loading conditions for cartilage tissue engineering
   R Bohm, S Biechler, S Williams, R Fei
6. A collagen 2 reporter for online identification of chondrogenic microenvironments
   AK Born, E Despot Slade, M Zenobi-Wong
7. Injectable hydrogels for cartilage repair
   SK Both, R Wang, PJ Dijkstra, M Karperien
8. Degenerated human intervertebral discs contain autoantibodies against extracellular matrix proteins
   S Capossela, P Schläfli, A Bertolo, T Janner, BM Stadler, T Pötzel, M Baur, JV Stoyanov
9. Stem cell effect on mechanically-loaded nucleotomised intervertebral discs
   S Caprez, M Peroglio, M Janki, M De Wild, LM Benneker, M Alini, S Grad
10. Intervertebral disc cell response to torsion as a function of duration and magnitude
    SCW Chan, J Walser, S Ferguson, B Gantenbein-Ritter
11. Expression of HSP72 and HSF1 in nucleus pulposus in response to compressive loading
    WH Chooi, SCW Chan, B Gantenbein-Ritter, BP Chan
12. Generating nucleus pulposus-like cells from human adipose stromal cells: a first step towards the regeneration of intervertebral disc
    P Colombier, M Ruel, J Lesoeur, A Moreau, C Robiou-Dupont, O Hamel, L Lescaudron, J Clouet, J Guicheux
13. Behaviour of human disc cells in collagen and fibrin-based matrix
    A Colombini , S Lopa, C Ceriani, AB Lovati, A Di Giancamillo, G Banfi, M Moretti
14. Retinoid acid receptor signaling during mesenchymal stromal cell chondrogenesis: differential sensitivity of chondral versus endochondral pathways
    S Diederichs, K Zachert, W Richter
15. Biomechanics of the degenerating human intervertebral disc: a 10-day axial loading study
    KS Emanuel, PPA Vergroesen, I Kingma, TH Smit
16. Articular cartilage contains a nestin positive stem cell population
    CR Fellows, IM Khan, CW Archer
17. Comparing the secretomes of unstimulated and mechanically loaded MSCs
    OFW Gardner, CW Archer, M Alini, MJ Stoddart

18. Chitosan-based flock scaffolds for potential application in tissue engineering of articular cartilage
E Goßla, A Bernhardt, D Aibibu, RD Hund, A Walther, M Gelinsky

19. Development of a whole bovine long-term organ culture system that retains vertebral bone for intervertebral disc repair and biomechanical studies
MP Grant, LM Epure, O Salem, O Ciobanu, M Alaqeel, J Antoniou, F Mwale

20. 3D printing from biodegradable polyurethane for tissue engineering applications
KC Hung, CS Tseng, Sh Hsu

21. Microencapsulation of bone marrow derived stem cells using electrohydrodynamic spraying for minimally invasive tissue repair of the IVD
LJ Kelly, SM Naqvi, CT Buckley

22. Studies on electrical hysteresis in bovine cartilages
DS Khanam, MS Syed, RJ Kumar, A Ahmad

23. Transplantation of bone marrow derived mesenchymal stem cells embedded in poly(trimethylene carbonate) scaffold: mechanical and biological repair of ruptured annulus fibrosus
Z Li, T Pirvu, SBG Blanquer, DW Grijpma, LM Benneker, M Alini, D Eglin, S Grad

24. Hypoxia mimicking glasses for osteochondral tissue engineering
E Littmann, AK Solanki, H Autefage, M Alini, M Peroglio, MM Stevens

25. Failure strength characterization of composite repair for annulus fibrosus injury
RG Long, KP Cole, M Likhitpanitchkul, AC Hecht, JC Iatridis

26. Biophysical characterisation of articular cartilage-derived stem cells using atomic force microscopy
B Morgan, LW Francis, RS Conlan, IM Khan

27. The LINK to regeneration of human intervertebral discs
F Mwale, R Gawri, J Ouellet, P Önnerfjord, B Alkhatib, T Steffen, D Heinegård, PJ Roughley, J Antoniou, L Haglund

28. Degradation study of mechanically-controlled drug delivery system for knee cartilage
M Nassajian Moghadam, DP Pioletti

29. Acute mechanical injury of human intervertebral discs initiates events associated with degeneration and pain
DH Rosenzweig, B Alkhatib, E Krock, L Beckman, T Steffen, MH Weber, JA Ouellet, L Haglund

30. Comparison of swelling properties of bovine nucleus pulposus and poly-ethylene-glycol hydrogel
A Schmocker, A Khoushabi, PE Bourban, C Schizas, C Moser, DP Pioletti

31. Evidence of balloon cells in lumbar spine disc herniation. Preliminary morphological results of patients versus SPARC null mice
I Sitte, M Klosterhuber, V Kuhn, RA Lindtner, I Vietor, A Kathrein

32. Tailoring silk based nano fibres for human intervertebral disc repair
T Studer, G Fortunato, N Gadhari, D Frauchiger, R Rossi, B Gantenbein-Ritter

33. Autonomous formation of cartilage tissue by expanded chondrocytes is dependent on a functional TGFβ-signalling
A Tekari, R Luginbuehl, W Hofstetter, R Egli

34. Histochemical evaluation of second-look osteochondral biopsies: symptomatic vs asymptomatic MACI-treated patients
F Tessarolo, M Fedel, R Tatti, E Bonomi, I Caola, M Molinari, P Dorigotti, G Nollo

35. Lithium protects cartilage from interleukin-1β induced degradation and preserves the mechanical integrity of articular cartilage
CL Thompson, A Wiles, H Yasmin, A Varone, CA Poole, MM Knight

36. Lithium chloride triggers primary cilia elongation and inhibits hedgehog signalling in articular chondrocytes
CL Thompson, A Wiles, CA Poole, MM Knight

37. An explanatory model for intervertebral disc degeneration: a degenerative cycle
PPA Vergroesen, I Kingma, KS Emanuel, BJ van Royen, JH van Dieen, TH Smit

38. Microspheres of BCP produced by Snowballing technique for multipurpose application
KB Violin, TS Goia, K Ishikawa, JC Bressiani, AHA Bressiani

39. The chondrocyte primary cilium modulates NFκB signaling, defining the response to interleukin-1β
AKT Wann, JP Chapple, MM Knight

40. Platelet-rich plasma (PRP) induces articular cartilage maturation - A possible mechanism for repair
YD Zhang, B Morgan, IM Khan

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June 20 – 23, 2016, Congress Center, Davos Switzerland (tentative)

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eCM XV

Cartilage & Disc: Repair and Regeneration

June 16 – 18, 2014
Convention Center
Davos Platz, Switzerland

Abstracts Oral Presentations

(in order of presentation)

The life of aggrecan

PJ Roughley

Shriners Hospital for Children, Montreal, Canada

The name aggrecan was officially adopted in 1990, when it replaced the cumbersome name of the large aggregating chondroitin sulphate/keratan sulphate proteoglycan of cartilage. However, the origins of aggrecan date back to the 1860's when the presence of chondroitin sulphate (CS) was recognised in cartilage, though it took another 90 years before the presence of keratan sulphate (KS) was confirmed. At this time it was still believed that the CS and KS chains existed in isolation, but this changed in 1958 when the attachment of CS to serine was recognized and the proteoglycan era was born. However, it was not until the development of dissociative extraction in 1969 and the avoidance of proteolysis, that the large size of aggrecan became apparent. This was followed in 1972 by the recognition of its ability to specifically interact with hyaluronan (HA) to form aggregates and the stabilization of the aggregates by link protein, and in 1975 electron microscopy revealed the structural beauty of aggrecan.

By the early 1980's it was appreciated that the CS and KS substitution of aggrecan varied considerably during juvenile development, with the abundance and chain length of CS decreasing with age while those of KS increased. It was not until 1987 when the aggrecan gene was first cloned that the structural features of its core protein and the sites of CS and KS substitution became apparent.

Since that time many investigators have devoted their careers to understanding the changes in aggrecan structure that occur during its lifetime in the extracellular matrix. Foremost amongst these changes has been the role of proteolysis in fragmenting the aggrecan core protein. While such fragmentation takes place in both articular cartilage and intervertebral disc, mainly due to the action of matrix metalloproteinases (MMPs) and aggrecanases, fragment accumulation differs between the two tissues. Proteolytic cleavage of aggrecan results in two distinct fragments, an amino terminal fragment that retains the ability to interact with HA and a carboxy terminal one that does not. In cartilage these latter non-aggregating fragments are rapidly lost by diffusion, whereas in the disc they are retained.

Aggrecanase-mediated cleavage of aggrecan can be stimulated by a variety of inflammatory cytokines, whereas excessive mechanical load has been associated with MMP action, and both mechanisms contribute to aggrecan fragmentation during life. In addition to proteolysis of the aggrecan core protein, the aggregate structure can be destroyed by cleavage of the HA, by either hyaluronidases or free radicals, or by non-enzymic glycation of the core protein. In the disc this latter process contributes to the large abundance of non-aggregating fragments of aggrecan present in the tissue. The half-life of the aggrecan fragments that are retained within either cartilage or disc is about 20 years, and their abundance increases with age.

Any change that decreases the content, size, charge or aggregation of aggrecan can be viewed as being detrimental to tissue function, and such changes have been implicated in the development of cartilage and disc degeneration. This would include all the mechanisms described above, but in the human there is also an additional mechanism. The human aggrecan core protein exhibits size variation in its CS-attachment region, due to a unique VNTR polymorphism in the region of the gene encoding its CS1 domain. It has been postulated that individuals with the shortest CS1 core protein lengths, and therefore bearing the lowest number of CS chains, may be more susceptible to tissue degeneration. If true, then care must be taken when selecting donor cartilage for tissue engineering purposes.

In recent years aggrecan has received considerable attention by those who wish to repair lesions in cartilage and disc, though often its presence in such processes is evaluated by message expression or CS analysis. These techniques are convenient because they are simple to perform, but they tell nothing about the size and structure of aggrecan, which are essential for retention and function in the tissue.

During the 40 years that I have been involved in aggrecan research much has been learned, but many questions remain unanswered and the life of aggrecan is not yet an open book.

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The Regenerative Potential of Articular Cartilage

CW Archer

College of Medicine, Swansea University

INTRODUCTION:

It is well established that cartilage as a whole and particularly articular cartilage has a poor reparative potential following injury. This presentation aims to review the literature relating to this reparative potential from various model systems. These range from a variety of differing animals in addition to the potential effects of the aging process. Within the presentation, the role/potential role of innate stem cells will be discussed, in addition to the use of harvested/expanded stem cells has in the reparative process. Whilst the presentation will focus on articular cartilage, attention will also be given to cartilages at differing locations.

Transcriptional profiling in osteoarthritis

Andrew A. Pitsillides
Comparative Biomedical Sciences, The Royal Veterinary College, London, UK

INTRODUCTION:

One powerful epidemiological risk factor for osteoarthritis (OA) is chronological age. How ageing and OA interact at the molecular level to produce this OA risk to the integrity of the articular cartilage (AC) is however unclear. This was recently been addressed in several studies using global transcriptome profiling. We utilised in vivo time-course analysis and inter-strain variability for natural murine OA susceptibility to address this key question. By profiling AC transcriptomes of non-prone CBA and OA-prone Str/ort mice, which bare significant histological resemblance to human OA pathology, we aim to discover molecular signatures linked with both OA vulnerability and progression. Joint mechanics is another major OA risk and we therefore also report upon studies exploring the transcriptional profile in destabilised mouse joints and in immobilised developing skeletal elements.

Array profiling revealed that Str/ort mouse OA exhibits a molecular phenotype resembling human OA, pin-pointing a central role for NFκB signalling and an emerging immune-related signature in OA cartilage over time. Strikingly, young healthy AC was found to possess a highly expressed skeletal muscle isoform gene expression program, which is switched off during maturation, but is intriguingly retained during OA in Str/ort mice. Profiling of destabilised mouse joints affirms close links with both NFκB and the skeletal muscle isoform program, while developmental immobilisation provides new mechanistic insights.

Failure of AC chondrocytes to down-regulate a high-abundance gene-expression program, shared with skeletal muscle, is associated with inappropriate expression of NFκB signalling pathways at OA induction and with an emerging immune-related signature during progression of OA.

This work was supported by the Osteoarthritis Research Society International Training Rotation scholarship, the University of London Central Research Fund, Biotechnology and Biological Sciences Research Council UK and by Arthritis Research UK.

Acknowledge: Blandine Poulet, Andrea Pollard, Frank Beier and James A. Timmons

Cytokine profiles and pathways in trauma and degeneration

LB Creemers[1]

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INTRODUCTION:

Profiling of tissues under pathological conditions can serve multiple purposes. Underlying pathways of disease may be elucidated and profiling may be used as diagnostic or prognostic tool. In cartilage and intervertebral disc disease, cell-based markers, extracellular matrix markers and secretory factors have been investigated. Among the latter, inflammatory cytokines have been of special interest, as they are thought to have an important role in cartilage and disc pathology. Cytokine profiling may be a promising approach towards the identification of targets for treatment. However, their role in the complex joint environments may not be as straightforward as they seem, while in vitro studies often are based on oversimplified models. Moreover, the multitude of factors present and the inherent biological variation in human subjects may require a more systematic approach towards unravelling their role in disease and treatment than monofactorial approaches.

Identification of disc degeneration markers in the human AF by microarray

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INTRODUCTION: The pathophysiological complications preceding degeneration of the disc incorporate a combination of environmental and genetic factors. Disc degeneration is accompanied by oxidative stress and inflammation that are the major factors leading to cell toxicity and apoptosis.[1] However, the biological and molecular factors that lead to disc degeneration are still ill identified. Identification of molecular markers associated with impaired cell function and apoptosis will lead to better understanding of the degenerative process. Therefore, we studied a set of microarray data from human healthy (grade I-II) and degenerative (grade III-V) disc, with particular emphasis on annulus fibrosus (AF) cells, for the purpose of identifying the molecular markers associated with degenerative pathways.

METHODS: Analysis of the microarray data. Healthy (n=8) and degenerative (n=16) AF cell microarray data (Affymetrix® Human Genome) was analysed by using IPA (Ingenuity® Systems, www.ingenuity.com). Genes were selected according to p<0.05 with log2 fold change >+/-1.5.

Gene expression analysis. Genes with significant differences between healthy and degenerative AF in microarray comparison were further analysed by real-time RT-PCR. RNA from AF cells of n=8 healthy and n=10 degenerative discs was subjected to RT and custom designed Gene Expression Arrays (Applied Biosystems).

RESULTS: Microarray data analysis revealed dysregulation of several pathways, including cellular growth and proliferation (Fig. 1).

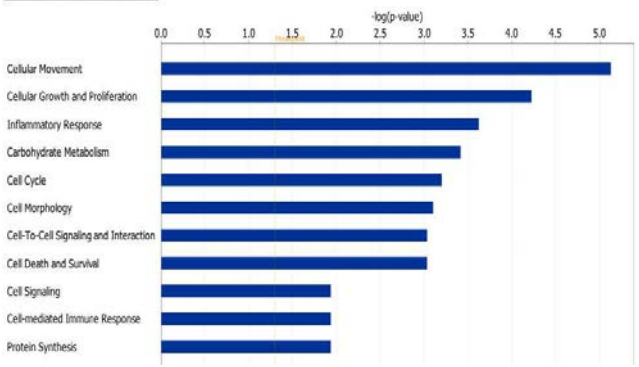

Fig. 1. *Dysregulated pathways in degenerative AF*Furthermore, various genes expressed differently (log2 fold >1.5) in human degenerative compared to healthy AF disc tissue were identified (Table 1).

Table 1. *Microarray Gene Expression comparison. P<0.05, fold +/- 1.5 (degenerative vs. healthy AF)*

Symbol	Description GenBank	p-value	log2 fold Change
IBSP	integrin-binding sialoprotein	0.002	-3.46
BANK1	B-cell scaffold protein with ankyrin repeats 1	0.006	-2.09
LYVE1	lymphatic vessel endothelial hyaluronan receptor 1	0.011	-1.85
LOXL4	lysyl oxidase-like 4	0.032	-1.83
CHAD	chondroadherin	0.02	-1.8
SOD3	superoxide dismutase 3, extracellular	0.002	-1.64
GPR64	G protein-coupled receptor 64	0.022	-1.59
C5orf62	chromosome 5 open reading frame 62	0.026	1.53
GBP1	guanylate binding protein 1, interferon-inducible	0.006	1.86
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	0.032	1.89
IFIT1	interferon-induced protein with tetratricopeptide repeats 1	0.013	1.96
TFPI	tissue factor pathway inhibitor (lipoprotein-associated coagulation inhibitor)	0.002	2.01
MGST1	microsomal glutathione S-transferase 1	0.001	2.02
IFIT3	interferon-induced protein with tetratricopeptide repeats 3	0.004	2.03
GDF15	growth differentiation factor 15	0.003	2.29
MMP1	matrix metallopeptidase 1 (interstitial collagenase)	0.053	2.36
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	0.01	2.49
GREM1	gremlin 1	0.002	2.54
IGFBP3	insulin-like growth factor binding protein 3	0.003	2.85

Gene expression analysis by qRT-PCR partially supported the microarray data, showing significant increases in IGFBP3, IFIT3 and TFPI expression in degenerative AF (Fig. 2).

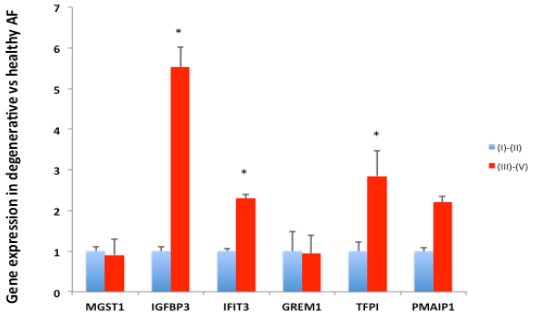

Fig. 2. *Expression of genes up-regulated in degenerative (III-V) discs. *p<0.05 vs (I-II).*

DISCUSSION & CONCLUSIONS: Analysis of the dysregulated gene expression by the IPA system indicates that genes induced by interferon (IFN) pathways are up-regulated in degenerative discs. This may lead to AF cell growth arrest through (pro-apoptotic) IFIT and IGFBP3 induction. Further studies will investigate the role of IFN signalling in disc degeneration.

REFERENCES: [1] Kepler *et al* (2013) Spine J. 13(3):318-30.

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Interleukin 16 in control of proliferation of mesenchymal stem cells (MSC), in MSC chondrogenesis and in early osteoarthritis

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INTRODUCTION: Interleukin 16 (IL16) was identified as a hypomethylated gene in MSC chondrogenesis with increased gene expression. IL16 isoforms (pro and neuronal) are cleaved by caspase 3 to generate the C-terminal mature cytokine and the N-terminal protein (N-proIL16). Cytoplasmic localisation of proIL16 has been associated with T-cell proliferation whereas binding of N-proIL16 to the Skp2 complex inhibits cell cycle progression. We hypothesised that epigenetic modification of the IL16 gene initiates a cascade of events for regulation of proliferation in early chondrogenesis and that this pathway may be upregulated in early OA.

METHODS: IL16 gene and protein expression were assessed by quantitative PCR (qPCR) and western blot (WB) with secreted cytokine levels determined by ELISA. Intracellular distribution of proIL16 was detected by immunostaining using antibodies specific to N-proIL16 and the C-terminal portion. Lentiviral-mediated siRNA knockdown (KD) of IL16 was confirmed by qPCR and cell cycle progression analysed by expression of p27 and skp2. Detection of IL16 in normal, early and late OA cartilage was achieved by IHC and WB. Finally, OA chondrocytes were exposed to 10ng/ml IL1β for 48 h to assess the effect of the OA environment on expression of IL16 isoforms.

RESULTS: IL16 knockdown in MSCs was associated with the inability of MSCs to proliferate. A temporal increase in full-length proIL16 protein was observed in early MSC chondrogenesis. Detection at day 2 preceded secretion of the mature cytokine, peaking between days 3-6. Additionally, IFF/IHC confirmed early cytoplasmic localization of IL16 and translocation of N-proIL16 into the nucleus on day 3, correlating with proliferation in early chondrogenesis [1]. Expression of proIL16 transcript was similar but preceded p27 up to day 4 of chondrogenesis; conversely, skp2 showed a diametrically opposed pattern to p27 (Fig. 1). Normal, aged chondrocytes did not stain for IL16; however, cytoplasmic IL16 was detected in proliferating chondrocytes in early OA cartilage. IHC staining correlated with significantly increased levels of proIL16 by WB and increased proIL16 protein levels correlated with disease progression. WB analysis of IL16 protein in OA chondrocytes indicated a similar pattern of expression to MSCs. Treatment with IL1β resulted in the upregulation of proIL16, similarly to early OA cartilage.

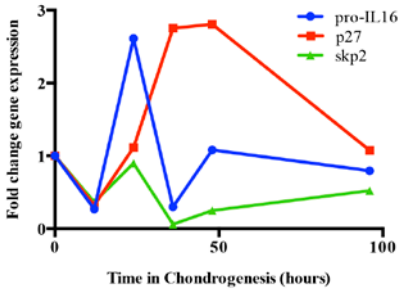

Fig. 1: Fold change of gene expression with respect to time 0 for pro-IL16, p27 and skp2.

DISCUSSION & CONCLUSIONS: Expression of IL16 seems to be essential for MSC proliferation; siRNA KD of the gene in myeloma cells also resulted in decreased growth [2]. Our results suggest that IL16 is associated with regulation of the required proliferative step in early chondrogenesis but is also crucial for commitment to differentiation with caspase-3 generated N-proIL16 trafficking to the nucleus to inhibit proliferation. The pro-inflammatory environment in early OA may lead to epigenetic changes and expression of cytoplasmic proIL16 to promote proliferation of resident chondrocytes and initiation of the degenerative process associated with the disease. These results highlight IL16 as a target for therapeutic modulation in OA.

REFERENCES: [1] V. Dexheimer et al (2012) *Stem Cells Dev*, 21(**12**): 2160-9. [2] D. Atanackovic, et al (2012) *J Natl Cancer Inst* 104(**13)**:1005-20.

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The Role of β-Catenin Signaling in Cartilage

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INTRODUCTION: β-catenin is a central molecule in the canonical Wnt signaling pathway and plays a critical role in chondrocyte formation and maturation. Genetic studies have provided evidence regarding the role of β-catenin in skeletal development. However, this information is limited due to the embryonic or early postnatal lethality of β-catenin gene deletion. Global knockout (KO) of the β-catenin results in lethality at E7.5, before mesenchymal cell condensation. Conditional deletion of β-catenin in early mesenchymal progenitor cells (targeted by Prx1-Cre or Dermo1-Cre) results in enhanced chondrogenesis, suggesting that β-catenin inhibits early mesenchymal cell differentiation into chondrocytes. Mice with conditional deletion of β-catenin in chondrocytes (targeted by Col2a1-Cre) die at birth from respiratory distress caused by a cleft secondary palate and a small rib cage. Conditional activation of β-catenin in chondrocytes (targeted by Col2a1-Cre) in which exon 3 of the β-catenin gene was deleted results in embryonic lethality around E18.5 with markedly diminished cartilage formation. Chondrocyte maturation is accelerated in these mice but occurs in the setting of severe chondrodysplasia. Exon 3 of the β-catenin gene encodes for the serine and threonine residues that are phosphorylated by GSK-3β and targeted by protein degradation mechanism. Overall, although conditional gene deletion and activation approaches have provided insight regarding the role of β-catenin in early cartilage development, roles of β-catenin in articular cartilage, in intervertebral disc (IVD) and in temporomandibular joint (TMJ) at postnatal and adult stages remain unknown. In order to determine if β-catenin signaling is activated during OA development and disc degeneration, we have examined changes in β-catenin protein levels in articular cartilage derived from patients with OA and from disc samples derived from patients with disc degeneration by IHC. We found that β-catenin expression was significantly upregulated in articular cartilage tissues of OA patients and disc tissues from patients with disc degeneration.

To determine the mechanism of β-catenin signaling in these tissues, we created β-catenin conditional activation mice. In order to specifically target β-catenin signaling in chondrocytes we first developed Col2-CreER transgenic mice. We bred Col2-CreER mice with Rosa-Tomato (mT/mG) and Rosa-lacZ (Rosa26) reporter mice to determine the tissue specificity and Cre recombination efficiency of these mice. We found that Col2-CreER mice can efficiently target growth plate and articular chondrocytes in long bone, inner annulus fibrosus cells and growth plate cartilage cells in IVD and chondrocytes in TMJ. We then bred these mice with β-catenin(ex3)flox/flox mice. Removing exon3 of the β-catenin gene will create a truncated β-catenin protein which is resistant to GSK-3β-induced phosphorylation and subsequent proteasome degradation of β-catenin protein

β-catenin(ex3)Col2ER conditional activation mice display a progressive OA-like phenotype in knee joints, in IVD and in TMJ tissues. Expression of several chondrocyte differentiation marker genes, including Runx2, Mmp13 and Adamts5 and Col10a1, were up-regulated, suggesting that chondrocyte maturation process is accelerated in β-catenin(ex3)Col2ER mice. Through a series of in vitro studies, we demonstrated that β-catenin activates Mmp13 and Adamts5 expression through a Runx2-dependent mechanism. We then generated (β-catenin(ex3)/Mmp13)Col2ER and β-catenin(ex3)Col2ER/Adamts5-/- double mutant mice to determine if deletion of the Mmp13 or Adamts5 gene will rescue cartilage defects observed in β-catenin(ex3)Col2ER mice. We found that deletion of Mmp13 or Adamts5 can significantly and partially reverses cartilage defects observed in IVD and TMJ tissues in β-catenin(ex3)Col2ER mice, suggesting that Mmp13 and Adamts5 may serve as critical downstream target genes of β-catenin signaling during the development of degenerative defect of these tissues. Our studies provide novel insights into the role of β-catenin signaling in degenerative cartilage diseases.

Pre-clinical models of inter-vertebral disc disease (IVDD): can we learn from the new paradigms of organ-wide pathology and disease phenotypes in osteoarthritis?

C Little

Director, Raymond Purves Bone and Joint Research Laboratories

Kolling Institute of Medical Research

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INTRODUCTION: The underlying principle of biomedical research is that it is only through a better (complete) understanding of the pathophysiological mechanisms of a given disease that rational and effective therapies will be developed. How robust is this if the disease in question is really just an end-stage diagnosis based on a collection of pathological findings? There may be many initiating causes and many pathways to reach this final point of tissue failure. The challenge is to identify clear patterns in this heterogeneity that will allow us to better sub-categorize the disease in question, and then define the associated pathophysiology of these different phenotypes. The emerging paradigm in osteoarthritis (OA) research is that rather than being a single entity, OA is actually a collection of disease sub-types with similar end-stage pathology. These phenotypes may be defined clinically e.g. post-traumatic OA versus age-associated OA, or OA with and without associated obesity and metabolic syndrome. Importantly these clinical divisions recognize that these disease subtypes have distinct underlying molecular pathophysiology. In addition to clinically defined phenotypes, it is increasingly recognized that OA is a disease of the entire joint organ, with varying pathology in all tissues. Thus some OA subtypes may be categorized by the relative involvement or disease in the different tissues – e.g. degree of synovitis, or OA with bone erosion versus bone formation. Pre-clinical research in OA, and particularly that using in vivo models, is increasingly embracing the study of the joint as an organ and interpreting data in the context of the different OA phenotype that is being modeled. While yet to be proven, it is hoped this approach will improve the presently poor translation of pre-clinical research to clinical trails and better-targeted treatment strategies for OA. To date there has been limited sub-typing of IVDD into different phenotypes either clinically or in pre-clinical research. This talk will discuss whether it is time to explore such an approach for IVDD, and what we might learn from the growing knowledge base in OA.

Human cartilaginous endplate degeneration is regulated by the extracellular calcium-sensing receptor

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INTRODUCTION: Intervertebral discs (IVDs) are the largest avascular structures in the body, mostly relying on the diffusion of nutrients and metabolites from blood vessels that partially penetrate the subchondral plate, and terminate at the cartilaginous endplate (CEP) during development of the spine[1]. Progressive degeneration of the CEP ultimately leads to IVD degeneration, as the integrity of the CEP is vital for disc function. Degeneration of the CEP involves decreased proteoglycan and type I and II collagen (Col I and II) content, thinning, and calcification[1]. Although biological repair strategies of the degenerative disc involving supplementation of growth factors are being pursued, disc nutrition is an important factor in considering their success.

The extracellular calcium-sensing receptor (CaSR) is a G protein-coupled receptor, and can sense both local and systemic calcium fluctuations[2]. The mechanism(s) of CEP degeneration are currently unknown. The purpose of the present study was to determine how activation of the CaSR influences disc degeneration and if this effect can be suppressed.

METHODS: Human lumbar spines were obtained through the organ donation program of Héma-Québec. Immunohistochemistry was performed on human CEP from donor IVDs of Thompson grade 2, 3 and 4 to compare the expression of the CaSR. Ionic calcium levels were measured from the CEP tissue using a calcium detection kit (Abcam). CEP cells isolated from Thompson grade 2, 3 and 4 IVDs (G2-4) were cultured and measured for the expression of the CaSR. Grade 2 (G2) CEP cells were cultured in various concentrations of Ca^{2+} (0.5-5 mM Ca^{2+}), CaSR allosteric agonist (cincalcet, 1 uM), or 2.5 mM Ca^{2+} in the presence of a CaSR antagonist for 7 days, and monitored for changes in markers of degeneration by Western blotting.

RESULTS: Ionic calcium content in CEP tissue increased significantly with IVD degeneration (Fig. 1A). Immunohistochemistry on CEP tissue also revealed an increase in the expression of CaSR that paralleled the degree of IVD degeneration. Western blotting on lysates from isolated CEP cells demonstrated an upregulation of the CaSR (Fig. 1 B) with Thompson grade.

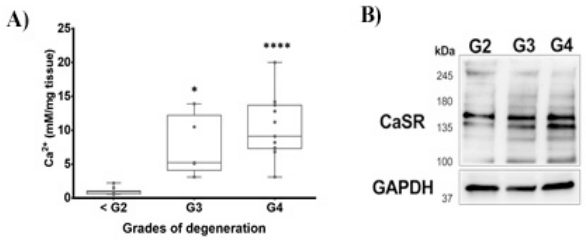

Fig. 1: ***Ca^{2+} content and CaSR expression in CEP from G2-4 IVDs.*** *A) CEP tissue from G2-4 IVDs was measured for Ca^{2+} content. B) Expression of CaSR by Western blotting in isolated CEP cells from G2-4 IVDs.*

Agg, Col I and II expression decreased dose-dependently in G2 CEP cells treated with Ca^{2+}, or cinacalcet. The effects of Ca^{2+} on matrix protein synthesis were abrogated in the presence of a CaSR antagonist. Upregulation of matrix metalloproteinase (MMP)-13 was increased following activation of the CaSR.

DISCUSSION & CONCLUSIONS: It has been suggested that CEP calcification is involved in the progression of disc degeneration by decreasing nutrient availability to the disc. In this study, we show that activation of the CaSR either by its agonist, calcium or cinacalcet, resulted in decreased expression of Agg, Col I and II, and upregulated metalloproteinase expression. In principle, therefore, suppression of the CaSR could be an option for treating disc degeneration.

REFERENCES: [1] Lotz, J.C., Fields, A.J. and Liebenberg, E.C. (2012) *Global Spine J.* **3**:153-164. [2] Brown, E.M. (2013) *Best Practice & Research Clin. Endo. Metab.* 27:333-343

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Establishment and characterisation of a non-invasive model of post-traumatic knee osteoarthritis.

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INTRODUCTION: Post-traumatic osteoarthritis (PTOA) of the knee commonly occurs following joint injuries such as anterior cruciate ligament (ACL) rupture. The use of animal models to study PTOA is crucial, since the disease process can be studied in a controlled environment over a shortened time line [1-2]. However, most models of PTOA are invasive and/or non-physiological often involving surgical transection of the ACL or induction of bilateral ACL injury. The current study aims to establish and characterise a non-invasive model of PTOA of the knee that mimics clinically-relevant injury conditions.

METHODS: Loads (9N, 40 cycles [3], 360N/s; ElectroForce® 3200, BOSE, USA) were applied to the right knees of anaesthetised 12-week-old C57Bl6 mice (n=6) on 2 occasions, 2 days apart using custom built cups to hold the knee in flexion; mice moved freely between loading sessions. Ligament rupture occurred during the second episode of loading, revealed by a continued increase in displacement following release of the applied compressive force during a loading cycle. Left knees served as unloaded controls. All procedures were in compliance with the Animals (Scientific Procedures) Act 1986. Knees were assessed 72 hours after the last loading episode for joint swelling (callipers), joint degeneration and synovial inflammation (histology and OARSI [4] score). Data were analysed by Minitab.

RESULTS: Loaded knees exhibited significantly more swelling than unloaded knees (Student's T-test: $P<0.0001$; n=6). Mice with the ACL injury also showed signs of lameness on their right leg. Extensive joint damage was observed in the knee following ACL rupture (figure 1). There was evidence of cartilage erosion, synovial inflammation, and proteoglycan loss in both lateral compartments of the knee. Significantly higher OARSI scores were observed in injured knees compared to uninjured knees (GLM ANOVA: $P<0.0001$; n=4-6 mice with scores representing an average score from 2 sections per leg). Cartilage and subchondral bone damage and proteoglycan loss all contributed to the higher scores. The total inflammatory score in injured knees was significantly higher than uninjured knees with

significantly higher exudate scores (Kruskal Wallis $p<0.05$; n=5-6 mice, 1 section scored per leg).

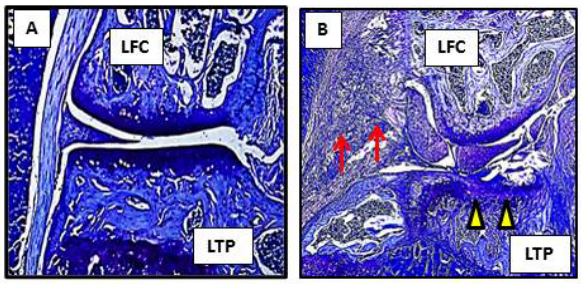

Fig1. Toluidine blue stained sections showing the lateral femoral condyle (LFC) and lateral tibial plateau (LTP) from unloaded (A) and loaded (B) knees. Synovial inflammation (red arrows) and cartilage erosion (yellow arrow heads) are shown.

DISCUSSION & CONCLUSIONS: This pilot study describes a potential non-invasive mouse model of post-traumatic knee joint degeneration due to ligament rupture. An acute inflammatory response and cartilage and bone damage occurred rapidly following the injury providing a model of non-invasive joint injury that can be defined over a short time frame. Longitudinal characterisation of this model will thus aid in the understanding of the changes that occur in the musculoskeletal tissues and cell signalling pathways immediately following joint injury and assist in testing therapeutic interventions.

REFERENCES: [1]T.S. Onur et al (2014) *J Orthop Res* **32**:318-323; [2]K.A. Lockwood et al (2014) *J Orthop Res* **32**:79-88; [3]B. Poulet et al (2011) *Arthritis Rheum* **63**:137-47; [4]S. Glasson et al. (2010) *Osteoarthritis Cartilage* **18** (Suppl 3): S17-23.

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Human adipose mesenchymal stromal cells transplantation promotes intervertebral disc regeneration in Biglycan-deficient murine model of chronic and progressive disc degeneration

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INTRODUCTION: Intervertebral disc (IVD) degeneration is a major cause of back pain and represents an endemic problem for which treatment is costly and relatively ineffective. The purpose of the present study is to assess disc regeneration by adipose-derived stromal cells (ADSCs)[1] transplanted in Biglycan (BGN) deficient mice (Bgn−/0). BGN is an important proteoglycan of the extracellular matrix and its decrease has been correlated with intervertebral disc aging and degeneration. Bgn−/0 mice lack this protein and undergo spontaneous IVD degeneration during aging[2], thus representing a valuable *in vivo* model to study therapies that may delay IVD degeneration.

METHODS: To evaluate ADSCs efficacy, mice were injected with $8x10^4$ cells intradiscally (L1-L2) at 16 months old, an age in which mice showed severe IVD degeneration, confirmed by both 7T Magnetic Resonance Imaging (MRI)[3] and histology. Placebo and ADSCs treated Bgn−/0 mice were assessed by 7TMRI analysis until 12 weeks post-transplantation. Mice were then sacrificed and investigated by histology and by immunohistochemistry for the presence of human cells, using anti-human nuclei (HuNu), and for the expression of BGN and aggrecan in the IVD treated area.

RESULTS: After treatment, *in vivo* 7TMRI showed a visible increase in signal intensity over time within the disc of mice that received ADSCs, while the placebos did not show such any variation. Moreover, in mice with ADSC-injected discs, ultrastructural analyses demonstrated the presence of human (anti-HuNu positive) cells even at 12 weeks after transplant, and these cells were found positive for the expression of the BGN proteoglycan. Furthermore this treatment determined an increase of aggrecan tissue levels. These results demonstrate that the injection of ADSCs into Bgn−/0 mouse model of spontaneous IVD degeneration promotes the new expression of human BGN and increases aggrecan levels, as demonstrated by MRI, histological and immunofluorescence findings.

DISCUSSION & CONCLUSIONS: In conclusion, we used a Bgn−/0 model to explore the effect of ADSCs in a progressive and spontaneous degenerative disc disease. Bgn−/0 mouse represents a unique model to study *in vivo* the time progression of IVD degeneration as a chronic and linear degenerative pattern, similar to the human IVD pathophysiology. Our data show, for the first time, that ADSCs transplantation is beneficial in arresting IVD degeneration in a mouse model, and suggest that this approach might be an effective treatment to delay degenerative disc disease.

REFERENCES:

[1] S.E. Navone, G. Marfia, L. Canzi, et al (2012) Expression of neural and neurotrophic markers in nucleus pulposus cells isolated from degenerated intervertebral disc. *J Orthop Res* **30**:1470-7. **[2]** M. Sato, T. Asazuma, M. Ishihara, et al (2003) An experimental study of the regeneration of the intervertebral disc with an allograft of cultured annulus fibrosus cells using a tissue-engineering method. *Spine* **28**:548-53. [3] X.D. Chen, S. Shi, T. Xu, et al (2002) Age-related osteoporosis in biglycan-deficient mice is related to defects in bone marrow stromal cells. J *Bone Miner Res* **17**:331-40.

Chondrocyte mechanobiology: applications to cartilage tissue engineering

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INTRODUCTION: Our laboratory has exploited the chondrocyte's ability to respond to mechanical stimuli for purposes of enhancing its biosynthetic activity and matrix elaboration for cartilage tissue engineering. Deformational loading (Study A) and osmotic loading (Study B) are two components of the chondrocyte milieu in situ (Fig. 1).

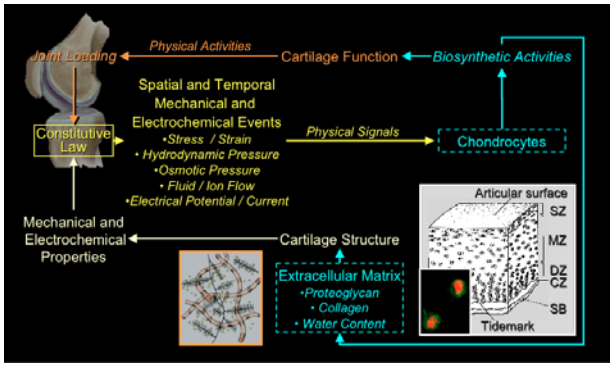

Fig. 1: Feedback loop coupling chondrocyte biosynthetic activities with joint loading stimuli.

We first discuss the application of dynamic deformational loading that provides both a mechanical stimulus to chondrocytes, as well as means for convective transport of nutrients. We will then focus on the osmotic environment of chondrocytes. Theoretical analyses have indicated that the magnitude of osmotic perturbations induced by joint loading-induced tissue deformation are negligible when compared to the baseline osmolarity created by the highly negatively charged glycosaminoglycans (GAGs) that make up the tissue. As such, chondrocytes exist in a static hyperosmotic environment. These approaches can become intertwined by controlled GAG removal via enzymatic digestion, which has been found to increase collagen content. **METHODS:** Juvenile chondrocytes were passaged and seeded in agarose at 30 million cells/mL. Constructs were maintained in serum-free chondrogenic media (CM, 330 mOsm) with TGF-β3 supplementation for the initial 2 weeks. In Study A, constructs were subjected to digestion with chondroitinase ABC (cABC), 0.15 U/mL for 48 h) on day 14 of culture and subjected to applied deformational loading (10% deformation at 1 Hz for 3 h daily) or free-swelling. Study B) Constructs were culture in CM at 300, 330 and 400 mOsm. Hyperosmotic media was produced by adding Na^+/K^+. Constructs were assessed for material and biochemical properties.

RESULTS: Study A results show that applied DL increased tissue mechanical properties, modulated spatial distribution of depth-dependent properties, and increased GAG and collagen content (not shown) (Fig. 2). In Study B, hyperosmotic loading increases tissue properties (Fig. 3) versus isotonic (330 mOsm) or hypo-osmotic (300 mOsm).

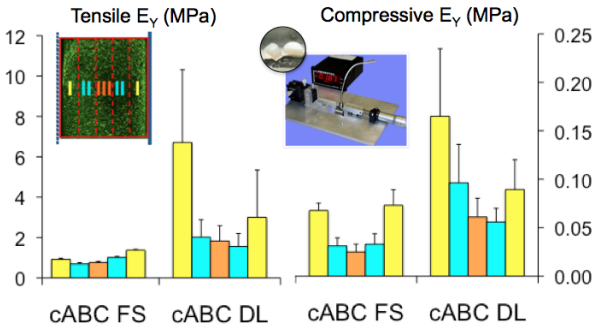

Fig. 2: Effects of loading (DL) on functional ECM development through the depth of day 56 cartilage constructs. The central region of the construct is coded zone III in orange. The axial faces of the construct are coded zone I in yellow.

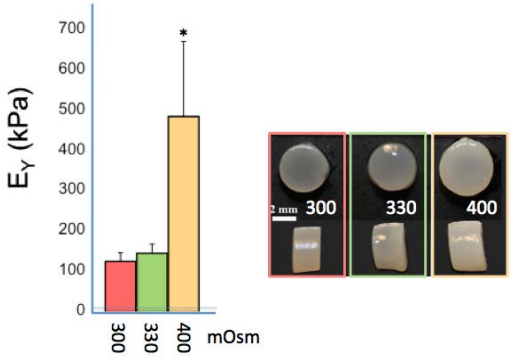

Fig. 3: Culture osmolarity effects on ECM functional development in day 56 chondrocyte-seeded agarose constructs. Adapted from[1].

DISCUSSION & CONCLUSIONS: *Applied physical and osmotic forces can be used to modulate development of engineered cartilage.*

REFERENCES: *[1] Sampat SR et al. (2013)* J Biomech ***46****:2674-81.*

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Developmental biomechanics of the prenatal skeleton

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Fetal movements have long been of interest to the medical and scientific communities as a possible measure of fetal health. However, the importance of fetal movements for skeletal development has only recently been explored in depth. Of particular interest in our lab is joint shape morphogenesis, the process in which joints obtain their distinctive shapes. Several medical conditions involving abnormal joint shape or orientation are related to abnormal prenatal movements, the most common of which is developmental dysplasia of the hip (DDH).

We employ a range of approaches in investigating the role of mechanical forces in prenatal skeletal development, including animal embryonic model systems of abnormal prenatal movement (both genetic and pharmacological), *in vitro* culture of limb explants, and computational simulation of growth and development. 3D imaging and image registration techniques of joint shapes are of key importance to our work.

We recently proposed the immobilised chick embryo as the first model system for prenatal-onset hip dysplasia [1]. Using this system, we showed that prenatal movements are not necessary for joint shape development prior to joint cavitation, but that immobilisation during and after cavitation leads to dramatic effects on the hip joint. These effects were similar to those seen in infants with severe, early-onset hip dysplasia that occurs when a neuromuscular disorders is present.

A number of avenues remain relatively unexplored in the field of developmental biomechanics. Despite knowing that mechanical forces are important for prenatal skeletal development, we have no idea what the biomechanical environment of the fetus is as it moves or grows. Computational modelling will be the only feasible means of quantifying the forces and stresses induced by fetal movements. It is unclear what the relative contributions of mechanical forces due to muscle contraction, forces due to the baby's interaction with the uterine wall, and even external mechanical forces [2] are during prenatal skeletal development are. Finally, for the field's research to be truly translational, we need a much greater understanding of human fetal movements, in particular what constitutes a normal frequency and intensity of fetal movement is over the course of a pregnancy and how these quantities vary between individuals. In order to investigate these measures, novel wearable sensor technologies will be necessary, which is another focus of our ongoing work.

REFERENCES: [1] Nowlan NC, Chandaria V, Sharpe J (2014), *Journal of Orthopaedic Research* **32**: 777-785. [2] NC Nowlan, G Dumas, S Tajbakhsh et al (2012) , PJ Prendergast PJ, Murphy P (2012) *Biomech Model Mechanobiol* **11**: 207-219.

ACKNOWLEDGEMENTS: Funding from the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013) / ERC Grant Agreement no. 336306-MechJointMorph is acknowledged.

Mechanics and biology of the intervertebral disc: long-term studies in a Loaded Disc Culture System

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INTRODUCTION: Degeneration of the intervertebral disc is assumed to be an important causal factor of low back pain. Intervertebral discs are continuously loaded mechanically and both positive and negative effects have been attributed to different loading conditions. In order to study mechanical loading effects, degeneration-associated processes and/or potential regenerative therapies in intervertebral discs, it is imperative to maintain the disc's structural integrity. While *in vivo* models provide comprehensive insight in intervertebral disc biology, an accompanying organ culture model can focus on a single factor, such as loading or osmotic pressure, and may serve as a prescreening model to reduce life animal testing. The loaded disc culture system also can be used for characterizing the time-dependent, non-linear properties of the intervertebral disc.

METHODS & RESULTS: In one study, we investigated the effect of prolonged dynamic loading on intradiscal pressure, disc height and compressive stiffness in healthy goat intervertebral discs. We found a strong linear relationship between axial compression and intradiscal pressure and they were also highly correlated with disc height: all decreased during high loading, and increased during low loading regimes. In contrast, compressive stiffness increased during high loading, and was inversely related to intradiscal pressure and disc height.

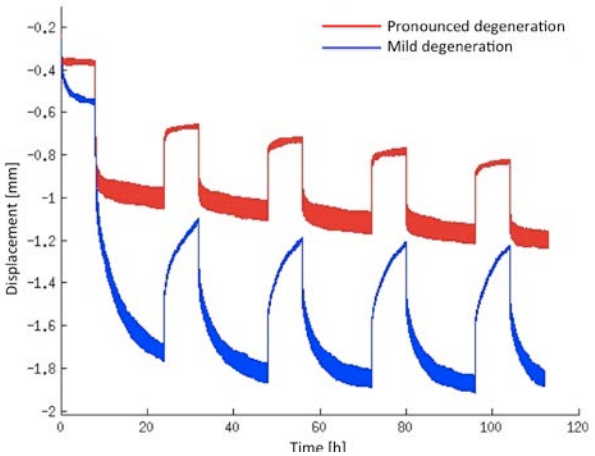

Figure 1: Changes in disc height in pronounced (red) and mildly (blue) degenerated human IVDs over a period of five days.

In a second study we investigated the dynamic behavior of healthy and degenerated human discs subjected to a five-day period of night-day rhythm. The healthy discs showed more variability in disc height and a much larger viscous component in the deformation curve (see Figure 1). Both indicate a larger potential for fluid flow and recovery of disc height. Degenerated discs had a tendency to show continued disc height loss over time, indicating plastic deformation or mechanical failure of (parts of) the specimen, presumably the vertebral bone. Using caprine lumbar discs as a healthy model for the intervertebral disc, we further investigated the effect of loading on cell behavior in a long-term (21d) culture period. It was found that moderate physiological loading was required to keep cells alive, but that overloading caused cell death in both, the nucleus pulposus and the annulus fibrosis. Further, the expression of catabolic and inflammation-related genes was up regulated from the onset of mechanical loading. Loss of water and glycosaminoglycans, on the other hand, were changed significantly after 21 days.

DISCUSSION & CONCLUSIONS: Bioreactors allow quantitative studies on the mechanics and biology of intervertebral discs. Characterization of dynamic disc behavior may provide a way to non-destructively quantify disc degeneration *in vitro*. It appears from our studies that several days are required to reach a state of homeostasis in response to a mechanical day- and night rhythm. The described cascades in the prolonged loading studies provide leads for the development of new pharmacological and rehabilitative therapies to halt the progression of disc degeneration.

REFERENCES: [1] Paul CP et al (2013): Dynamic and static overloading induce early degenerative processes in caprine lumbar intervertebral discs. PLoS One. 2013 Apr 30;8(4): e62411

ACKNOWLEDGEMENTS: The development of the culture system was supported by the ZonMw Program "Alternatives for life animal testing" (grant #11400090) and the BioMedical Materials program BMM (grant #P2.01 IDiDAS).

Articulation reduces IL-1β effect on cartilage biosynthesis and matrix degradation

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INTRODUCTION: Compressive loading of cartilage explants has shown to be effective in mitigating IL-1β induced matrix damage [1-2]. Our laboratory utilizes a novel *in vitro* joint motion simulator that better replicates *in vivo* motion through dual-rotating articulation in the presence of compressive loading. We hypothesized that articulating motion would decrease the negative effects of IL-1β as observed on cartilage biosynthesis and on matrix degradation.

METHODS: Oval cartilage explants were removed from the femoral trochlear groove of immature bovine joints. Samples were randomized into articulation or no articulation and tested either in the presence or absence of IL-1β (n≥3 each). Groups exposed to IL-1β (100 ng/ mL) experienced pretreatment for one day before testing with continued exposure throughout the test. After a five-day pre-culture, testing was conducted in a joint motion simulator under physiologic conditions (Fig.1), utilizing a moving contact point to preserve biphasic lubrication [3].

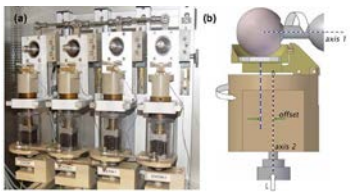

Fig. 1: (a) Joint motion simulator with (b) model of contact between the ball and explant.

Explants were loaded to 40N (~2 MPa) while articulated against a ceramic ball for three hours per day for three days. Explants were examined for cell viability and for metabolic response (^{35}S-incorporation) immediately after day three testing. The collected media from test and rest periods was examined for total GAG content (DMMB assay). Two-way ANOVA and student t-tests were used with $p < 0.05$ being significant.

RESULTS: IL-1β exposure did not impact cell viability of explants, although increased superficial zone cell death was observed due to articulation against the ceramic ball. As expected, articulation increased ^{35}S-incorporation in both the absence ($p=0.055$, dashed line) and presence of IL-1β (Fig. 2, top). The presence of IL-1β significantly decreased the positive effect of articulation on metabolic activity. While there was a significant increase in cumulative GAG release due to the presence of IL-1β ($p<0.05$) and a trend towards a decrease in GAG release due to articulation ($p=0.087$), there was no interaction effect detected. From the day three test period, though, IL-1β caused a significant increase in GAG release in both the absence and presence of articulation (Fig.3, bottom). Likewise, articulation caused a significant decrease in GAG release in both the presence and absence of IL-1β.

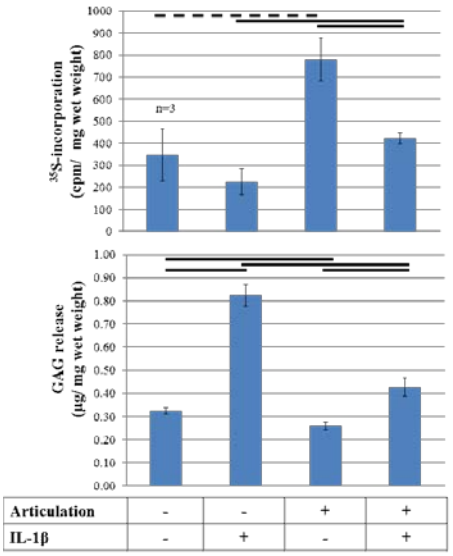

Fig. 2: (top) Metabolic response. (bottom) GAG release during day three test. n=4, except where noted. Mean ± SEM. Solid line denotes p<0.05.

DISCUSSION & CONCLUSIONS: In line with previous research [1-2], this study found a reduction in IL-1β induced GAG release due to articulation using a novel joint motion simulator. Interestingly, articulation is able to recover some metabolic activity lost due to IL-1β exposure, but not to levels observed with articulation only.

REFERENCES: [1] PA Torzilli, et al (2010) *Osteoarthritis and Cartilage* **18**:97-105. [2] PA Torzilli, et al (2011) *Cartilage* **2**:364-73. [3] MA Wimmer, et al (2004) Tissue Eng **10**:1436-45.

ACKNOWLEDGEMENTS: Support through Rush Arthritis and Orthopaedics Institute.

Chondrocyte de-differentiation is associated with primary cilia elongation and a reduced capacity for hedgehog signalling

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INTRODUCTION: The expansion of chondrocytes for clinical implantation therapies is associated with loss of the differentiated phenotype with monolayer passage[1]. The hedgehog signalling pathway, modulated within the primary cilia compartment, plays a critical role in chondrogenic differentiation[2] and the development of osteoarthritis[3]. Activation of the hedgehog pathway has been demonstrated to be capable of rescuing chondrocyte phenotype in passaged, dedifferentiated cells[4]. Primary cilia signalling, including hedgehog signalling, is modulated by cilia length[5, 6] itself altered by physicochemical changes to the cellular microenvironment[7, 8]. This study quantified hedgehog signalling responsiveness and changes in primary cilia length during chondrocyte dedifferentiation associated with serial passage in monolayer.

METHODS: Isolated primary bovine articular chondrocytes were expanded to passage five. Hedgehog signalling pathway activation in response to addition of indian hedgehog was quantified by expression of Gli1 and Patched 1 (PTCH1).Primary cilia prevalence and length were assessed by confocal immunofluorescence of acetylated alpha-tubulin.

RESULTS: Following 24hrs r-Ihh treatment, P0 chondrocytes exhibited a 5.4 and 4.9 fold increase in the expression of Gli1 and PTCH1 respectively. P5 chondrocytes exhibited a diminished response such that a 3.7 fold increase in Gli1 expression was observed while PTCH1 expression was not significantly altered. Both these responses in P5 cells were statistically significantly smaller than those observed in P0 cells (Fig. 1A).

A statistically significant increase in ki67 staining, an indicator of cell proliferation, was observed with passage ($p<0.001$). Despite this increase in the proliferative status of chondrocytes cilia prevalence also increased with passage from 29% (P0) to 49% at P5 ($p<0.001$). In freshly isolated bovine articular chondrocytes primary cilia varied in length from 0.3 µm to 2.3 µm with a median length of 1.21 µm. Length positively correlated to passage number ($p=0.0035$) such that a steady increase in cilia length was observed with passage (Fig. 1B).

A **B**

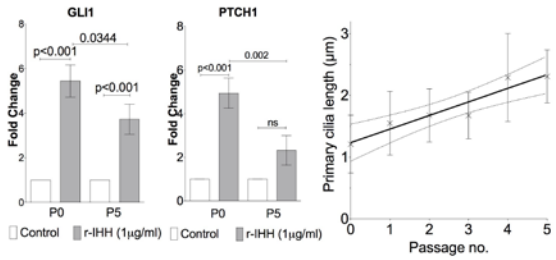

*Fig. 1: Passage of chondrocytes in monolayer was associated with reduced capacity to activate hedgehog signalling, quantified by Gli1 and PTCH1 gene expression (**A**), and with elongation of the primary cilia (**B**)*

DISCUSSION & CONCLUSIONS: These data indicate chondrocyte dedifferentiation is associated with a reduced responsiveness of the hedgehog pathway and a gradual increase in primary cilia length. Given recently published findings of Lin *et al. 2014* [8], who showed activation of the hedgehog pathway aids chondrocyte re-differentiation following monolayer expansion, we propose chondrocyte dedifferentiation may be driven, in part, by changes to cilia structure and function. The primary cilium structure may be both a useful biomarker and but also a target to exploit for the manipulation of chondrocyte phenotype.

REFERENCES: [1]Schiltz, Mayne and Holtzer., (1973) Dedifferentiation 1(2) p97-108. [2] Haycraft & Serra (2008) Curr Top Dev Biol 85 p303-32. [3]Lin et al. (2009) Nat Med 15(12) p1421-5. [4] Lin et al., (2014) PlosOne 9(2). [5]Cruz et al. (2010) Development 137(24) p4271-82. [6]Thompson, Chapple and Knight (2014) OA Cartilage. [7]Pitaval et al., (2010) J Cell Biol 191(2) p303-12. [8]McGlashan et al., (2008) Dev Dyn 237(8) p2013-18.

ACKNOWLEDGEMENTS: Humphrey's and Sons for supply of bovine forefeet.

Low-frequency high-magnitude mechanical strain of articular chondrocytes activates p38 MAPK and induces phenotypic changes associated with osteoarthritis and pain.

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INTRODUCTION: Osteoarthritis (OA) is often accompanied by inflammation and pain ENREF_1, whereby cytokines associated with chronic OA upregulate expression of neurotrophic factors such as nerve growth factor (NGF). Studies have shown a role for cytokines and NGF in OA pain [1], however effects of changing mechanical properties in OA tissue on chondrocyte metabolism remain unclear. We recently showed that high mechanical strain (HMS) to human disc cells promotes NGF and inflammatory factor secretion associated with disc degeneration and pain using our novel culture technique [2]. Here, we apply the same culture technique using high-extension silicone rubber membranes to examine if HMS to articular chondrocytes increases inflammatory gene expression and release of NGF.

METHODS: Primary bovine articular chondrocytes were isolated from femoropatellar groove of skeletally mature steer as previously described [3]. $2x10^5$ cells were seeded on flexible silicone dishes or static silicone dishes that were salinized and collagen coated. 20% magnitude cylical mechanical strain at 0.0001Hz was applied to HMS cultures for 8 hours/day over 2 days as described [2]. Conditioned media was collected and cells were divided for protein and RNA isolation. Quantitative RT-PCR was performed to assess TLR, NGF, TNF, MMP and ADMATS gene expression. Western blot was performed to assess intracellular MAPK activity. Conditioned media was applied to PC12 cells, and neurite outgrowth was assessed.

RESULTS: HMS cultured chondrocytes displayed up-regulated NGF, TNFα and ADAMTS4 gene expression while decreasing TLR2 expression, as compared to static-cultured controls. Changes in gene expression correlated with increased p38 MAPK activity. Conditioned medium from HMS cultures induced significant neurite sprouting and increased cell death in PC12 cells, as compared to conditioned media from static-cultured controls.

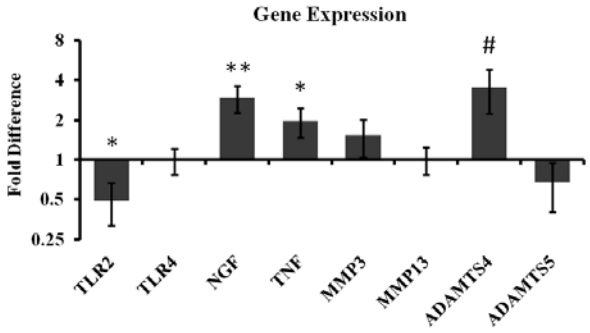

*Fig. 1: Quantitative real-time PCR analysis immediately following HMS culture. All samples compared to static controls. Error bars: ± SEM, n = 6. Student's t-test. * indicates p < 0.05; ** indicates p < 0.01. # indicates p = 0.06*

DISCUSSION & CONCLUSIONS: Low-frequency high-magnitude mechanical strain of primary articular chondrocytes *in vitro* drives factor secretion associated with degenerative joint disease and joint pain. This study provides evidence for a direct link between cellular strain, secretory factors, neo-innervation, and pain in OA pathology.

REFERENCES: [1] L Aloe, MA Tuveri, U Carcassi, et al. 1992. Nerve Growth-Factor in the Synovial-Fluid of Patients with Chronic Arthritis. Arthritis Rheum-Us 35:351-355. [2] R Gawri, DH Rosenzweig, E Krock, et al. 2014. High mechanical strain of primary intervertebral disc cells promotes secretion of inflammatory factors associated with disc degeneration and pain. Arthritis Res Ther 16:R21. [3] DH Rosenzweig, M Matmati, G Khayat, et al. 2012. Culture of primary bovine chondrocytes on a continuously expanding surface inhibits dedifferentiation. Tissue Eng Part A 18:2466-2476.

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Biomimetic and Bioactive Scaffolds for Regeneration

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Departments of Chemistry, Materials Science and Engineering, Medicine, and Biomedical Engineering

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INTRODUCTION: The interactions of cells with molecularly engineered structures is of critical importance in emerging areas such as regenerative medicine, precision targeting of therapies, and control of the immune response. Mammalian cells are naturally surrounded by one-dimensional filamentous nanostructures that provide them with mechanical support and most importantly host the signaling molecules that determine cell survival, fate, proliferation, and other aspects of cell behavior.

This lecture describes strategies that utilize supramolecular self-assembly to create bioactive and biomimetic nanostructures that emulate components of the extracellular matrix which are specifically designed to signal cells. These biodegradable and biocompatible systems can display varying densities of biological signals and trigger regenerative processes. The lecture will discuss their use in spinal cord injury, regeneration of cartilage and bone, and on-demand angiogenesis in cardiovascular therapies.

Fibrin-genipin annulus fibrosus sealant as a delivery system for anti-TNFα drug

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[1]*Leni & Peter W. May Department of Orthopaedics, Icahn School of Medicine at Mount Sinai, NY, USA*

[2]*Collaborative Research Partner Annulus Fibrosus Rupture Program of AO foundation, Davos, Switzerland*

[3]*Network of Excellence for Functional Biomaterials, National University of Ireland, Galway, Ireland*

INTRODUCTION: Regenerative repair of intervertebral discs (IVDs) with ruptured annulus fibrosus (AF) is a clinical challenge due to the large spinal load that must be applied immediately after repair and the associated inflammation that can prevent tissue regeneration. Fibrin-genipin (Fib-Gen) adhesive hydrogel is an effective sealant for AF defects and can restore IVD biomechanical behaviors [1], and these properties make it an attractive carrier for drug delivery. TNFα is expressed in painful IVDs and is a key initiator of pro-inflammatory cytokines and matrix degrading enzymes; therefore blocking TNFα may inhibit cell catabolism and promote tissue regeneration. This study functionalized Fib-Gen for sustained release of an anti-TNFα drug, infliximab, using direct incorporation of the drug within Fib-Gen and by embedding drug-loaded collagen hollow spheres (CHS) [2] into the Fib-Gen gel.

METHODS: Four drug delivery systems were prepared: 1) CHS alone (1µm, 400µg) loaded with 320 µg infliximab, 2) Fib-Gen alone (250 µl) directly mixed with 2.5 mg infliximab, 3) Fib-Gen+CHS loaded with both doses above, and 4) Fibrin alone (250 µl) directly mixed with 394 µg infliximab. Infliximab doses were selected from pilot studies maximizing drug release in that system. TEM compared microstructures of each system. To obtain drug release kinetics, 300 µl of DMEM was added to each sample, all systems were incubated in 37°C on a shaker, and media was collected and freshly replaced every 3-4 days for 35 days. Drug released to the media was measured with an infliximab ELISA [3]. To test bioactivity of the released drug, collected media was applied on TNFα-treated (10 ng/ml) human AF cells (10^5 cells/ml, 37°C, 5% O_2, 5% CO_2). Pro-inflammatory cytokines (TNFα, IL1-β, IL-6, IL-8) in AF cell supernatant were measured using a multiplex ELISA and averaged.

RESULTS: CHS was successfully loaded into Fib-Gen (Fig. 1). Drug release kinetics (Fig. 2) showed higher drug concentration released from CHS but no differences were observed between Fib-Gen and Fib-Gen+CHS. Fibrin released large amounts of drug but degraded completely within 6 days and is not amenable to sustained release. Pro-inflammatory cytokines produced by AF cells was significantly reduced (Fig. 3) confirming infliximab remained bioactive following release.

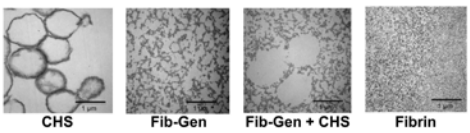


Fig. 1: TEM of drug delivery systems.

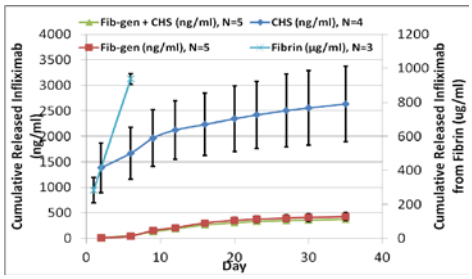
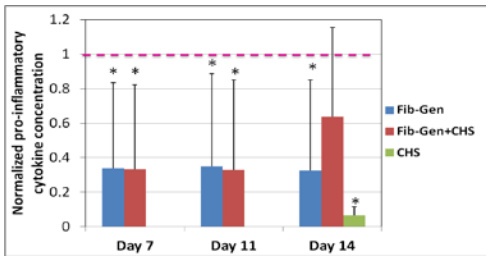


Fig. 2: Mean±SD release of infliximab.



*p<0.05

Fig. 3: Effects of released drug on AF cells. Mean±SD of average pro-inflammatory cytokine concentration normalized by untreated control.

DISCUSSION & CONCLUSIONS: Fib-Gen was an effective carrier for sustained delivery of infliximab with no benefit of adding CHS. CHS was more effective at delivering sustained high doses of infliximab yet requires a carrier for retention in the IVD space. Fib-Gen was an attractive carrier for drug delivery since it is capable of sealing AF defects and lasts longer than fibrin. Future *in vivo* assessment is warranted.

REFERENCES: [1]Likhitpanichkul+ (2013) Trans ORS; [2]Browne +(2012) Mol Phar **9** 3099-3106, [3]Ternant +(2006) Ther Drug Monit **28** 169-174.

ACKNOWLEDGEMENTS: Funded by AO foundation, NIH NIAMS (R01AR057397) & Mount Sinai Inst of Tech (4D/NCATS programs).

A Therapeutic Effect of Hyaluronic Acid Based-Hydrogels in *In Vitro* Inflammation Model of Nucleus Pulposus

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INTRODUCTION: Inflammatory process plays a crucial role in intervertebral disc (IVD) degeneration[1] and is associated with production of neurotrophins in sensitizing innervation into the disc[2]. A biomaterial-based approach of using high molecular weight (HMw) Hyaluronic Acid (HA) hydrogels become a potential therapy in IVD degeneration as it exhibits anti-inflammatory properties and mimics the native extracellular matrix. The hypothesis of the study was that HA hydrogels attenuate inflammatory receptor and neurotrophins expression in IL-1β induced inflammation model of NP cell culture. Specifically, to test this hypothesis a HA hydrogel system was developed and the therapeutic effect in inflamed NP cell culture was investigated, activation of receptors IL-1R1, MyD88, CD44 by immunofluorescent and neurotrophin expression of Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF), by Elisa and qPCR

METHODS: HA hydrogels were synthesized by mixing 0.75% (w/v) sodium HA Mw 1.2 x 10^6 Da (Lifecore Biomedical, USA), 0.15g 4-arm PEG amine Mw 2000Da, 0.15g N-hydroxysuccinimide (NHS) and 0.0877g 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) (100µL) in 1mL distilled water. The hydrogels were obtained through pipetting a channel volume of 5µL onto a hydrophobic modified glass slide and incubated at 37°C. The *in vitro* degradation of HA hydrogels with hyaluronidase (HAase) (5U/mL) and PBS was assessed at 1, 3, 7, 14, 21 and 28 days. The potential cytotoxicity of hydrogels was assessed against ADSCs by alamarBlue®. NP cell viability and distribution in 2D culture was examined for day 1, 3 and 7 using Live/Dead® staining kit. Isolated NP cells were cultured for 10 days (density 5 x 10^3) stimulated with IL-1β (10ng/ml) and treated with 100µL (5µL × 20) hydrogels for day 1, 3 and 7. IL-1R1, MyD88 and CD44 receptors were determined by immunofluorescent imaging. Neurotrophins expression of NGF and BDNF were examined by ELISA and qPCR.

RESULTS: The gelation time of spherical-shaped hydrogels formation was 5.5 minutes. The hydrogels showed stability in PBS and over 70% degradability in HAase. Cytotoxicity data showed 94.75 ± 1.18%, 97.95 ± 4.69% and over 73% metabolic activity of ADSCs in 100µL and 200µL hydrogels after day 1 and 3 in culture (Fig. 1.A). NP cells showed 100% and over 90% viability in presence of hydrogels (Fig. 1.B). IL-1R1 and MyD88 were down-regulated after hydrogel treatment in inflamed NP cells (Fig. 1.C). Up-regulation of NGF (fold change 3.2 and 8.1) from qPCR results were seen in inflamed model.

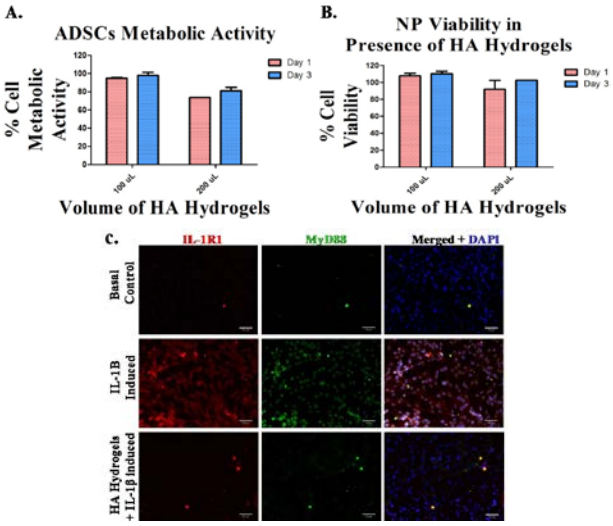

Fig. 1. A. Percentage ADSCs metabolic activity alamarBlue® test shows over 90% and 73% viability in presence of 100µL and 200µL hydrogels respectively. **B.** NP cells viability in presence of HA hydrogels shows 100% and over 90% viability for 100µL and 200µL hydrogels. **C.** Inflammatory receptors of IL-1R1 and MyD88 showed decrease immunoreactivity after treating with 100µL HA hydrogels compared to IL-1β control group.

DISCUSSION & CONCLUSIONS: HA hydrogels show therapeutic effect by reducing inflammatory receptors and neurotrophins expression in inflamed NP cell cultures.

REFERENCES: [1]Wuertz *et. al*, ECM Vol.23 (2012) [2]Freemont *et. al*, J. Pathol. 197 (2002)

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Osteochondral defect repair using a biomimetic hydrogel

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[2]*Department of Biology and Chemistry, City University of Hong Kong, Hong Kong SAR, China*

INTRODUCTION: The treatment of cartilage injuries remains one of the most difficult challenges in Orthopaedic Surgery because damaged cartilage has a limited capacity to regenerate. The past several decades have seen the evolution of biodegradable macromolecular scaffolds with optimized internal architecture, chemical properties, and mechanical properties to function as temporary supports for the engineering of living constructs in cartilage tissue regeneration applications.

In this study, we utilize a novel biodegradable synthetic hydrogel, oligo (polyethylene glycol) fumarate (OPF), conjugated to a negatively charged methacrylate group, as a scaffold for chondrogenic cells. We have previously shown that this hydrogel is highly biocompatible, and that it mimics the function of chondrocyte extracellular matrix [1]. Our goal in this study was to investigate the effect of scaffold chemical composition, TGF-β1 and xenograft human adipose derived stem cells (hADSCs) on cartilage regeneration in a rabbit osteochondral knee defect.

METHODS: OPF was synthesized using a previously described method [2] and copolymerized with sodium methacrylate (SMA) to introduce negative charge into the OPF hydrogel. Sodium chloride was added to the mixture to create porosity in the hydrogel. Scaffolds were implanted bilaterally into 4×6 mm osteochondral defects in New Zealand rabbit knees. There were four experimental groups: hydrogels with and without TGF- β1 (Groups 1 & 2), hydrogel with hADSCs and TGF- β1 (Group 3), and hydrogel with hADSCs (Group 4). After 10 weeks, the rabbits were sacrificed and specimens were harvested for micro-CT analysis, mechanical testing and histology.

RESULTS: Gross images of the defects showed a greater amount of new cartilage in the treatment groups that had TGF-β1 (Groups 1 and 3). Safranin-O/Fast Green staining revealed new cartilage and new subchondral bone in the defects after 10 weeks. A full thickness cartilage repair was observed in the defects treated with hydrogel+TGF-β1 (Group 1). A continuous tidemark was observed in this group using Von Kossa/Safranin-O staining. Cartilage repair was graded using the O'Driscoll histological scoring system and compared to normal cartilage. The results showed O'Driscoll scores of 88% for hydrogel+TGF-β1, 58% for hydrogel+ADSCs with and without TGF-β1, and 36% for hydrogel alone. Functional testing of the regenerated cartilage and subchondral bone was carried out by push-out testing. All groups with either/both hADSCs and TGF-β1 (Groups 1, 3, and 4) showed greater push-out strength than the group with only hydrogel.

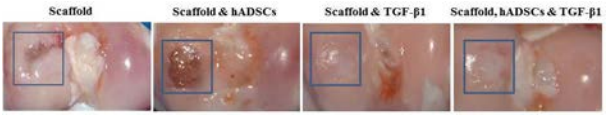

Fig. 1: Gross morphology of cartilage repair

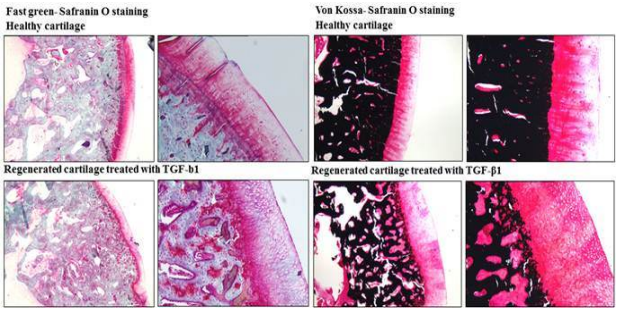

Fig. 2: Histology of healthy and regenerated cartilage tissue

DISCUSSION & CONCLUSIONS: These data demonstrate that TGF- β1 in conjunction with the negatively charged hydrogel has a significant effect on development of a new cartilage in the rabbit osteochondral defects. Integration of reparative and host cartilage was observed at their interface. These findings suggest that negatively charged OPF hydrogel has a potential to be used as both a scaffold and a delivery vehicle for cartilage repair in osteochondral defects.

REFERENCES: [1] M. Dadsetan, M. Pumberger, M.E. Casper, et al (2011) *Acta Biomater.* ***7****:2080-90.* [2] S. Jo, H. Shin, AG. Mikos (2001) *Biomacromolecules* ***2****:255–261.*

ACKNOWLEDGEMENTS: Mayo Center for Regenerative Medicine for funding. F.B. was supported by a PhD student grant from the University Grant Council, Hong Kong.

Pluripotent Stem Cells for Cartilage repair

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INTRODUCTION: Both human embryonic (hESC) and induced pluripotent (iPSC) stem cells have tremendous promise for regenerative medicine because they can self renew indefinitely and are able to form cell types from all three germ layers. However their plasticity is a problem as well as a benefit and one barrier to their use is the difficulty in targeting their differentiation only to a particular desired cell type for clinical therapy. We have generated a protocol to efficiently target differentiation of hESCs towards chondrocytes (Oldershaw et al 2010 Nature Biotech 28, 1187-1193) with very high efficiency in 5/6 lines tested. This 3-stage protocol induces both hESCs and iPSCs to differentiate through developmental intermediate stages using substrates of known matrix proteins and chemically-defined medium to give 94-97% SOX 9 positive cells expressing COL2A1 in Safranin O positive aggregates. We have now shown that these cells are also able to repair an osteochondral defect in immunocompromised nude rats and that the cells give good quality matrix in which human cells can still be detected at 12 weeks. However, although our extensive gene characterisation reveals reproducible progression of hESC through mesendoderm, and mesoderm like stages, before differentiating into chondrogenic aggregates, our cells do not produce the equivalent amount and organisation of matrix in 2D culture that can be found in hyaline cartilage. We have therefore refined the protocol and investigated the role of a number of differentiation modulation steps to identify when and though which pathways we can enhance the chondrogenic phenotype. We found that during hESC-chondrogenesis SOX9 was transcriptionally paused until day 5 in the protocol at which time transcription is released. Low Oxygen conditions have limited effect in improving chondrogenic differentiation and although ER stress increased as the cells differentiated, their proteasome function was maintained suggesting this is not detrimental to extracellular matrix protein synthesis. Through interrogation of RNAseq data obtained from two different cell lines during the protocol we are starting to identifying micro RNAs and matrix synthesis and assembly pathways for modulation. Our protocol is highly efficient, scalable and reproducible, enabling further understanding of the mechanisms involved in targeted differentiation and with our ongoing refinements opening up the potential for translation to disease models and clinical therapies.

Engineering new biologic therapies for osteoarthritis

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INTRODUCTION: Osteoarthritis is a painful and debilitating disease of the synovial joints that is characterized by progressive degeneration of the articular cartilage that lines the joint surfaces. The etiology of osteoarthritis is poorly understood, although it is now well accepted that biomechanical factors play an important role in the onset and progression of this disease.

The primary goal of these studies has been to develop new bioengineering approaches for the treatment of osteoarthritis based on regenerative medicine or a fundamental understanding of the mechanisms of mechanical signal transduction occurring in normal or osteoarthritic cartilage.

METHODS AND RESULTS: Using a hierarchical approach to span different systems, ranging from clinical studies and *in vivo* animal models to studies at the tissue, cellular, and subcellular scale, we have identified specific mechanical signaling pathways that are critically involved in cartilage physiology, pathology, and mechanically-induced regeneration[1]. These pathways may provide novel pharmacologic targets for the modification of inflammation or cartilage degeneration in osteoarthritis.

Additionally, our studies have focused on tissue engineering approaches for repairing cartilage damage with osteoarthritis. Using textile processes that allow weaving of biomaterial fibers in three dimensions, we have created cell-instructive bioactive scaffolds that can recreate many of the complex biomechanical properties and anatomic features of articular cartilage[2,3]. In combination with multipotent adult stem cells[4] or induced pluripotent stem cells[5], our work is focusing on developing a tissue-engineering approach for repair of cartilage defects or complete resurfacing of osteoarthritic joint surfaces.

DISCUSSION & CONCLUSIONS: These studies emphasize the important role of biomechanics and mechanobiology in the health, disease, and regeneration of the joint.

REFERENCES: [1]O'Conor et al. *Proc Natl Acad Sci USA* 111:1316-1321, 2014. [2]Moutos et al. *Nat Mater* 6:162-167, 2007. [3]Brunger et al. *Proc Natl Acad Sci USA* 111:E798-806, 2014. [4]Estes et al. *Nat Protoc* 5:1294-1311, 2010. [5]Diekman et al. *Proc Natl Acad Sci USA* 109:19172-19177, 2012.

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Manipulation of chondrogenic progenitor cells from late stages of osteoarthritis for cartilage repair

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INTRODUCTION: Regeneration of diseased hyaline cartilage continues to be a great challenge, mainly due to degeneration, caused either by major injury or by age-related processes. This usually overextends the tissue's self-renewal capacity.

METHODS: Up to date, more than 600 samples of adult osteoarthritic cartilage, without signs of rheumatoid involvement, were obtained from the knee joints of patients (ages: 65 – 75 years) suffering from late-stage osteoarthritis (OA) after total knee replacement. Recently, we also included meniscus specimens from late OA (n = 150), as well as samples from rheumatoid arthritis (n = 40) in our investigation of progenitor cells *in situ*. Histology, immunohistochemistry and staging was carried out as described (Bock et al. 2001). Standard explant cultures were performed (Koelling et al., 2009). A transcriptome analyses and proteome analysis further characterized the progenitor cell populations. We performed dilution cloning and lentiviral transfection of the cells with hTERT. Quantitative real-time RT-PCR, as well as Western blotting was applied after overexpression and RNA silencing experiments for various chondrogenic mediators, for example, sox9, smad2 or HMGB2. Cell stimulation experiments involved TGFß3, PDGF or BMP6.

RESULTS: Recently, we showed, that repair tissue from late stages of OA in humans harbors a unique progenitor cell population, termed chondrogenic progenitor cells (CPCs). These exhibit stem cell characteristics such as clonogenicity, multipotency, and migratory activity. Down-regulation of the osteogenic transcription factor runx-2 enhanced the expression of the chondrogenic transcription factor sox-9. This, in turn, increased the matrix synthesis potential of the CPCs (Koelling et al., 2009). Recently, we extended our investigation to include also cartilage specimen from rheumatoid arthritis as well as meniscus from late stage OA patients. We discovered a unique population of meniscus progenitor cells (MPCs), as well as one of RA-CPCs from rheumatoid arthritis. The two were characterized in terms of their differentiation potential, their stem cell marker profiles and their migratory potential. All proved to be different from the known CPCs, even though belonging to the family of osteochondro progenitor cells. In an elaborate combined proteome analysis, pull-down and immune precipitation approach, we identified first candidate genes to enhance the chondrogenic potential of these cells.

DISCUSSION & CONCLUSIONS: Our results offer novel insights into the biology of progenitor cells in the context of diseased cartilage tissue and strengthen the concept of progenitor cells *in situ*. The CPCs and MPCs are under the control of a balanced interaction of sox9 and runx2. In MPCs, the TGFß pathway with its mediators smad2/3 and 4 play an essential role in the regulation of the expression levels of sox9. While the RA-CPC seem to be mainly influenced via IL17, with Secukinumab® ameliorating its anti-chondrogenic influence. In the future, our work will be relevant in the development of novel therapeutics for the later stages of osteoarthritis, diseased meniscus and rheumatoid arthritis.

Muhammad H, Schminke B, Miosge N (2013) Current concepts in stem cell therapy for articular cartilage repair. Expert Opin Biol Ther, 13:541-8.

Koelling S, Kruegel J et al. (2009) Migratory chondrogenic progenitor cells from repair tissue during the later stages of human osteoarthritis.
Cell Stem Cell, 4:324-35.

Bock HC et al. (2001) The small proteoglycans decorin and biglycan in human articular cartilage of late-stage osteoarthritis. Osteoarthritis Cartilage, 9:654-63.

Intermittent but not continuous parathyroid hormone-related protein exposure augments mesenchymal stromal cell chondrogenesis and reduces hypertrophy via cAMP/PKA signalling

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**both authors contributed equally*

INTRODUCTION: The use of human mesenchymal stromal cells (MSC) for cartilage regeneration is prevented by phenotype instability and pre-mature hypertrophy[1]. Aim of this study was to investigate whether intermittent application of parathyroid hormone-related protein (PTHrP) can, in contrast to constant treatment, positively influence MSC chondrogenesis and to explore the molecular mechanisms behind differential responses to both treatment modes.

METHODS: Human MSC were subjected of chondrogenic induction in high-density pellet culture for 6 weeks. Cells received either chondrogenic medium alone (control) or supplemented with PTHrP(1-34), forskolin, dbcAMP or PTHrP(7-34) either constantly or via 6 hour pulses (3-times weekly). Proteoglycan and DNA content, collagen type II and -X deposition, gene expression of chondrogenic and hypertrophic markers and alkaline phosphatase (ALP) activity were quantitatively assessed at different time points during chondrogenic induction.

RESULTS: Constant application of PTHrP(1-34) suppressed chondrogenesis of MSC (Fig. 1) in line with previous results[2], whereas pulsed application significantly increased collagen type II gene (COL2A1) expression and collagen type II deposition, as well as proteoglycan and DNA content of pellets after 6 weeks. Collagen type X gene and protein expression levels were not altered, but indian hedgehog (IHH) gene expression and ALP activity were significantly reduced by pulsed PTHrP. Stimulation of cAMP/PKA signaling by forskolin reproduced major effects of both treatment modes, whereas application of the N-terminally truncated PTHrP(7-34), capable to activate PKC- but not cAMP/PKA signalling, was ineffective.

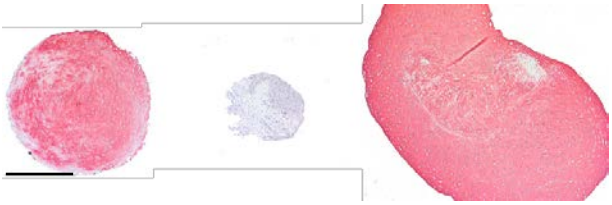

Figure 1: Effect of constant versus intermittent application of 2.5 nM PTHrP(1-34) on MSC chondrogenesis. Paraffin sections of day 42 pellets were stained for collagen type II deposition by immunohistochemistry. Representative results from one of four donors are shown. Bars represent 500 µm.

DISCUSSION & CONCLUSIONS: Intermittent PTHrP application stimulated chondrogenesis and simultaneously reduced undesired endochondral differentiation of MSC, and represents thus a novel and simple means to regulate chondrogenic matrix deposition independently from hypertrophic marker expression. cAMP/PKA was the major signalling pathway triggering the opposing effects of both treatment modes, indicating that signal timing might be the decisive variable. Intermittent application of PTHrP represents an important novel means to improve chondrogenesis of MSC and may be considered as a supporting clinical treatment mode for MSC-based cartilage defect regeneration.

REFERENCES: [1] Pelttari K, A Winter, E Steck et al (2006) *Arthritis Rheum* 54:3254-66. [2]Weiss S, T Hennig, R Bock et al (2010) *J Cell Physiol* 223:84-93.

ACKNOWLEDGEMENTS: This work was supported by funds of the German Research Foundation (DFG Ri707/7-1) and the Orthopaedic University Hospital Heidelberg.

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Evaluation of bone marrow mesenchymal stem cell (MSCs)-based intervertebral disc regeneration using quantitative T2 mapping: a study in a rabbit model

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INTRODUCTION: Studies have shown that MSCs-based therapy was effective on treatment of intervertebral disc degeneration (IVDD) by histological grading, immunohistochemistry, and Western blotting. Though the data yielded by these methods are valid and reliable, they cannot be used for clinical evaluation because of their invasion in nature. It is important for us to translate the laboratory findings or methods to the clinic application according to the acquirement of translational medicine[2]. So it is necessary to find a non-invasive and effective way to measure the biological effects of IVDD treatment and facilitate future clinical application. This study is to explore the curative effects of MSCs transplantation on intervertebral disc regeneration and to investigate the feasibility of quantitative T2 mapping method on evaluating the repair of nucleus pulposus after implantation of MSCs.

METHODS: Forty-eight New Zealand white rabbits were used to built up the lumber disc degenerative model by stabbing the annulus fibtosus and randomly divided into four groups. Two weeks after that, MSCs or PBS (Phosphate Buffered Saline) were transplanted into degenerative discs(MSCs group and PBS group). The rabbits with operation but without implantation of MSCs or PBS worked as sham group and the rabbits without operation worked as normal control group. At week 2, 6 and 10 after operation, the T2 values and disc height indices (DHI) were calculated by MRI (3.0T) and the gene expression of type II collagen (*COL2*) and aggrecan (*ACAN*) in degenerative discs were evaluated by real-time RT-PCR. Correlations between T2 values and gene expression of *ACAN* and *COL2* were sought.

RESULTS: Cell clusters, disorganized fibers, interlamellar GAG matrix and vascularisation were observed in lumber degenerative discs,. MSCs could be found to survive in intervertebral discs and differentiate into nucleus pulposus-like cells expressing *COL2* and *ACAN*. The gene expression of *COL2* and *ACAN* increased during 10 weeks after transplantation as well as the T2 signal intensity and T2 value. The DHI in MSCs group decreased slower than that in PBS and sham group. The T2 value correlated significantly with the gene expression of *ACAN* and *COL2* in nucleus pulposus.

Correlations for ACAN and COL2 expression with T2 relaxation time

Group	ACAN expression Vs. T2 relaxation time			COL2 expression Vs. T2 relaxation time		
	n	R^2	p	n	R^2	p
MSCs group	16	0.7855	< 0.0001*	16	0.5448	0.0011*
PBS group	16	0.7127	< 0.0001*	16	0.5293	0.0014*
Sham group	16	0.7045	< 0.0001*	16	0.5475	0.0010*
Normal control group	16	0.5454	0.0011*	16	0.2819	0.0343*
Total	48	0.9278	< 0.0001*	48	0.7211	< 0.0001*

*Significant correlations have P less than or equal to 0.05.

Fig. 1: Correlations for ACAN and COL2 expression with T2 relaxation time.

DISCUSSION & CONCLUSIONS: Increased matrix synthesis is indicative of disc regeneration after MSCs transplantation[3]. The regeneration process was reflected by T2 values, which increased as *COL2* and *ACAN* levels rose in the nucleus pulposus. Thus, T2 mapping may allow the regeneration of the nucleus pulposus to be sensitively and non-invasively evaluated. It may also be used to define regeneration stages quantitatively. In conclusion, T2 mapping of IVD matrix and water contents may be useful for diagnosing degenerative disc diseases and evaluating treatment effectiveness in clinical settings.

This study is the first time to evaluate disc degeneration and regeneration process using a sensitive and non-invasive detection means, T2 mapping, developed on an experimental rabbit model. It is a great advantage over other methods for detecting pathology. What is more, T2 values can been used to define regeneration stages quantitatively.

REFERENCES: [1] D. Sakai, J. Mochida, T. Iwashina, et al (2005) *Spine (Phila Pa 1976)* 30:2379-87. [2] L. Qin (2013) *JOT* 1: 3-5. [3] D. Sakai, J. Mochida, Y. Yamamoto, et al (2003) *Biomaterials* 24:3531-41.

A mouse model of joint surface injury: Contribution of functional mesenchymal stem cells to cartilage repair.

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INTRODUCTION: We have previously validated a murine model of articular cartilage injury and regeneration[1]. In the present study we investigated the contribution of mesenchymal stem cells (MSCs) to articular cartilage repair and the activation of cell signalling pathways involved in skeletogenesis.

METHODS: Joint surface injury and an *in vivo* double nucleoside analogue-labelling scheme[2] were used in DBA/1 mice, which display healing ability after cartilage injury, to identify functional MSC. Briefly, mice received the first nucleoside analogue (iododeoxyuridine - IdU) for 20 days followed by 28 days of wash-out, and then were subjected to joint surface injury. Animal received the second analogue (chlorodeoxyuridine - CldU) for the final 4 days prior to sacrifice, starting at different time points following injury.

Immunofluorescence was performed to firstly detect double nucleoside analogue-positive cells in knee joint tissues and, subsequently, to characterize the phenotype of these cells using putative MSC markers. Double immunofluorescence was also adopted to study activation of signalling pathways, such as TGFβ, bone morphogenetic proteins (BMPs), Wnt and Notch, in response to articular cartilage injury.

RESULTS: Populations of slow-cycling cells were identified in synovium and bone marrow. Following articular cartilage injury, these cells proliferated, as indicated by the presence of double nucleoside analogue-positive cells, with a marked accumulation at 4 days post injury (dpi). Slow-cycling cells were also identified in the residual articular cartilage but no proliferation was observed following injury.

At the site of injury, double-positive cells were observed as early as 8 dpi, with a complete filling of the defect at 12 dpi. The heterogeneous population of cells contributing to articular cartilage repair included slow-cycling cells.

Immunofluorescence analysis using putative MSC markers such as CD146, CD105, CD44, PGFRα and LNGFR, revealed a phenotype compatible with MSCs.

In synovium and bone marrow, BMP, TGFβ and Notch pathways were activated in slow-cycling cells between 2 and 8 dpi, as revealed by nuclear co-localization of pSMAD1-5-8, pSMAD2 and Notch intracellular domain (NICD). β-Catenin had nuclear localization in both the uninjured control and injured sample until 8 dpi; at 12 dpi it showed cytosolic distribution in IdU-positive cells suggesting inactivation of canonical Wnt signalling.

DISCUSSION & CONCLUSIONS: Our data demonstrated that within the knee joint there are resident MSCs able to respond to joint surface injury by proliferating and thus directly contribute to cartilage repair tissue. The analysis of pathways involved in skeletogenesis during development, revealed activation of BMP and Notch signalling during the repair process. These signalling pathways might be involved in the proliferative response during the early stage of the healing process when β-Catenin, a repressor of chondrogenic differentiation in MSCs during development[3], is inactive. Later in the healing process, activation of TGFβ and inactivation of canonical Wnt pathway would drive chondrogenic differentiation of MSCs.

REFERENCES: [1]Eltawil NM *et al*. *Osteoarthritis and Cartilage* 2009; 17(6):695-704. [2]Kurth TB *et al*. *Arthritis Rheum* 2011; 63(5):1289-300. [3]Guo X *et al*. *Genes Dev* 2004; 18(19):2404-17.

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Inflammation and cartilage regeneration

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INTRODUCTION: It is well known and accepted that inflammation leads to cartilage *de*generation in (osteo)arthritis. However the role of inflammation in cartilage *re*generation is less clear. We investigate the effect of joint inflammation factors on cartilage regeneration. In particular we study the effects of factors secreted by tissues of diseased joints on chondrogenesis of Mesenchymal Stem Cells (MSC) and hypothesize a role for macrophages in this process.

RESULTS: In osteoarthritic synovium of patients that obtained total knee replacement surgery, clear signs of inflammatory cells (macrophages and T-cells) were present. The synovial fluid of affected joints contained many inflammatory factors, albeit the level of pro-inflammatory factors in patients with established osteoarthritis was not very high. The synovial fluid composition reflects factors secreted by synovium, cartilage and other tissues, such as fat pads.

To investigate the effect of factors secreted by osteoarthritic knee synovium or osteoarthritic Hoffa's fat pad on MSC chondrogenesis we have collected conditioned medium of these tissues. This medium inhibited the chondrogenic differentiation of human bone marrow derived MSCs.

Synovium and adipose tissues both contain a relatively large number of macrophages. We have separated the macrophages and the fibroblast-like cells from synovium. When conditioned medium of these cell fractions was used on MSCs it appeared that the inhibition of chondrogenesis of MSCs is mainly due to the macrophage-like cells.

Synovium and fat pad tissue contain both classically (M1) and alternatively (M2) activated macrophages. To further investigate the role of different macrophage subtypes on the inhibition of chondrogenesis we have isolated CD14+ monocytes from human peripheral blood and stimulated them with LPS/IFN to an inflammatory phenotype (M1) or with IL4 to an anti-inflammatory phenotype (M2). The factors secreted by M1 inhibited chondrogenesis of MSCs whereas the factors secreted by M2 had no effect.

Specific inhibition of inflammatory pathways with tofacitinib (JAK-inhibitor) and/or oxozeaenol (TAK1 inhibitor) during MSC chondrogenesis could partly prevent the inhibition of chondrogenesis by factors secreted by synovium. However, this only worked when the first stages of chondrogenic differentiation had taken place; Inhibition of these pathways during early chondrogenesis prevented chondrogenic differentiation indicating that inflammatory pathways are required to induce repair processes but can inhibit regeneration in later phases.

DISCUSSION & CONCLUSIONS: The regeneration capacity of cartilage in mammals is limited. This could be due to the inflammatory environment in damaged joints. An acute inflammatory phase, which is typical for classical wound healing processes, is lacking. Our data indicate that early inflammatory processes are required to induce tissue repair. On the other hand osteoarthritic joints are expressing a chronic inflammatory phenotype. Our research suggests that macrophages present in various osteoarthritic joint tissues might play several roles in cartilage repair, depending on stage of the disease. A therapy, specifically targeting macrophages might provide a tool to improve the results of cartilage repair procedures such as microfracture.

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Emerging Role of PHDs in Intervertebral Disc Degeneration and Inflammation

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INTRODUCTION: Recent studies from our laboratory have shown a differential role of prolyl hydroxylases (PHD) in controlling hypoxia-inducible factor (HIF)-α degradation and activity in hypoxic nucleus pulposus (NP) cells. Moreover, our studies suggest that under inflammatory conditions, PHD expression is dysregulated and may play an important role in controlling overall inflammatory response. Results suggest that in NP cells, PHDs control actions of TNF-α through modulation of NF-κB signaling pathway. Loss-of-function studies clearly indicate that PHDs serves as co-activator of NF-κB signaling activity in NP cells; PHDs interacts with, and co-localizes with, p65. Moreover, suppression of PHDs result in significant decrease in TNF-α-induced expression of catabolic markers, and at the same time, restore aggrecan and collagen 2 expression. It is noteworthy that hydroxylase function of PHDs is not required for mediating cytokine-dependent gene expression changes. These findings suggest that by enhancing the activity of inflammatory cytokines, PHDs may serve a critical role in degenerative disc disease.

Epigallocatechin 3-gallate suppresses interleukin-1β-induced inflammatory responses in intervertebral disc cells in vitro and reduces radiculopathic pain in vivo

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INTRODUCTION: Intervertebral disc (IVD) degeneration is characterized by are-related catabolic changes in the adult disc. These changes lead to mechanical dysfunction, hence increasing the risk for load-induced structural failure in the disc. Pain sensation can be provoked by leakage of nucleus pulopsus material through the annulus fibrosus and consequent irritation of spinal nerves and/or nerve infiltration into the compromised disc. However, even without a disc prolapse, the degenerated IVD can be painful, especially if high levels of pro-inflammatory mediators are secreted[1,2]. The purpose of this study was to analyse the potential of biologically active polyphenol from green tea, Epigallocatechin 3-gallate (EGCG), for the treatment of painful IVD degeneration by identifying and explaining its anti-inflammatory and anti-catabolic activity.

METHODS: Human IVD cells were isolated from patients undergoing surgery due to IVD degeneration (n=25). Cells cultured in 2D or 3D were pre-stimulated with IL-1β for 2 hours before treatment with EGCG and the expression of target genes was measured by qPCR. Protein activity was determined by Western blotting, Immunofluorescence and Transcription factor assay. The small molecule inhibitor SB203580 was used to examine the involvement of the p38 pathway in the observed effects. The effect of EGCG on radiculopathic pain was studied by von Frey filament test in Sprague-Dawley rat model of radiculopathy induced by conditions similar to disc herniation (n=60).

RESULTS: EGCG inhibits IRAK-1 - NF-κB/p38/JNK signaling pathways, which can lead to its ability to inhibit the expression of pro-inflammatory mediators (IL-6/-8, TLR2, NGF, iNOS, COX-2) and matrix metalloproteinases (MMP1/3/13) *in vitro* (Fig. 1). EGCG reduces radiculopathic pain *in vivo* and this effect might be mediated by the inhibition of p38-dependent expression of iNOS and COX-2.

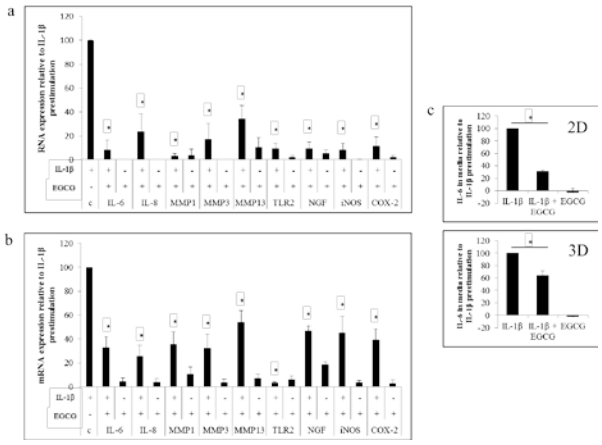

Fig. 1: After 18 hours, EGCG significantly reduced mRNA expression of inflammatory mediators and matrix metalloproteinases, both in adherent cell culture (a) and in alginate beads (b). IL-6 protein secretion in cell culture media was significantly decreased as well (c) ($p < 0.05$, Student´s T-test).

DISCUSSION & CONCLUSIONS: *The effects of the bioactive polyphenol EGCG on IVD-related pathologies were examined* in vitro *and* in vivo. *EGCG exhibits anti-inflammatory and anti-catabolic as well as analgesic activity, which may be mediated by its inhibition of IRAK-1 - NF-κB/p38/JNK signaling pathways. Although the precise mechanism of EGCG action in IVD cells still need to be elucidated, we demonstrated promising therapeutic potential of EGCG in the treatment of painful degenerative disc disease.*

REFERENCES: *[1] C.L. Le Maitre, A.J. Freemont, J.A. Hoyland (2005)* Arthritis Res Ther ***7****: R732-745.* *[2] K. Wuertz, N. VO, D. Kletsas, N. Boos (2012)* Eur Cell Mater ***23****: 103-119; discussion 119-120*

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Immuno-regulation by articular chondrocytes and synovial stem cells in inflammatory environment: prime effect of platelet lysate in joint repair

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INTRODUCTION: In articular cartilage disorders, cartilage breakdown and synovial inflammation are linked to joint swelling and pain. In this environment, articular chondrocytes (HAC) and synoviocytes play a role in the immuno-regulation of T lymphocytes. Blood-derived products, such as Platelet Lysate (PL), are key sources of factors and active molecules. Used as a supplement to either cell-free or cell-loaded scaffolds, we recently reported that PL is applied to repair cartilage lesions [1].

The aim of our work was to analyze whether PL can affect the immuno-regulatory effects of HAC and synoviocytes on T lymphocytes. The balance of anabolic/catabolic cellular and molecular signals was also considered.

METHODS: Primary human articular chondrocytes (HAC) and synoviocytes from bioptic specimens were cultured under both physiological and inflammatory conditions (mimicked with 100U/ml of TNFα) with or without PL (Crt; TNFα; PL and TNFα+PL). PL was prepared according to Zaky et al [2]. Peripheral blood mononucleated cells (PBMC) were separated from blood of healthy donors [3]. HAC and synoviocytes, treated as indicated above, were co-cultured with PBMC at different ratios (1:10; 1:5, 1:2) in medium or with either PHA (phytoemoagglutinin A) or anti-CD3 mAb or staphyloenterotoxin E (SEB). Proliferation was evaluated as decrement of green fluorescence of PBMC labeled with carboxy-fluorescein succiminidyl ester (CFSE). The surface expression of major histocompatibility complex (MHC) class-I and -II, ICAM1, and VCAM1 and the gene expression and protein secretion of IL6, IL8, CXCL1 and COX2, was analyzed.

RESULTS: PL triggered a strong short-term down-regulation of MHC classes I and II in HAC and synoviocytes under physiologic and inflammatory conditions with rescue of ICAM1 and VCAM1 expression at 96h (fig1A). Furthermore, HAC and synoviocytes primed with PL sharply increased their inhibiting effect on T lymphocyte proliferation (e.g. SEB stimulus, fig.1B). Moreover, the analysis of cytokines shown that the proinflammatory effect of the PL was a transient phenomenon, after an initial upregulation, we observed a reduction of NF-kB activity and repression of the inflammatory COX-2. The medium of HAC cultured in presence of PL and inflammatory stimuli showed a significant increase of the chemoattractant activity versus untreated chondrocyte and synoviocytes cell.

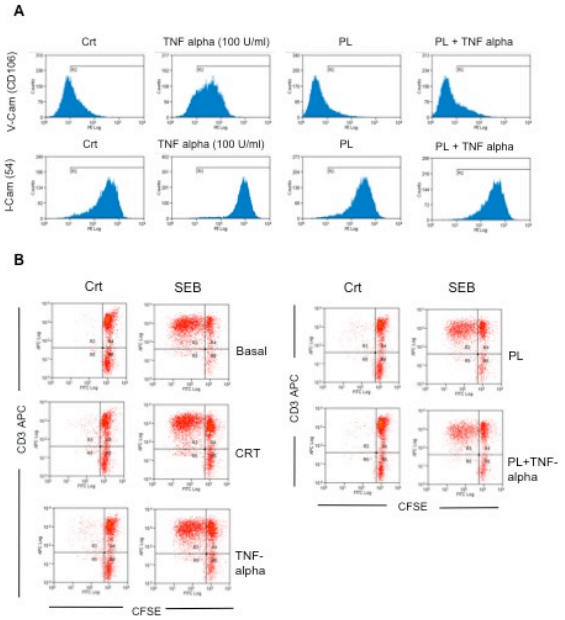

Fig.1 Flowcytometric analysis of VCAM and ICAM expression after TNF-α and PL treated and untreated conditions (A). Proliferation was assessed as decrement of CFSE cell staining in $CD3^+$ T cells in direct contact with HAC (B)

DISCUSSION & CONCLUSIONS: Our results indicate that, in joint disorders, PL can act as a prime agent in the immunoregulation of T lymphocytes response both to antigen specific and unspecific mitogenic stimuli with simultaneous reduction of inflammatory cell/molecular signals. In this perspective, our findings claim a key function role of PL in cartilage active mechanisms of immune evasion in tissue repair perspectives.

REFERENCES; [1]Siclari A. Clin Orthop Relat Res. 2012 470(3): 910-9. [2]Zaky S. J.Tissue Eng. and Reg. Med. 2008, 2(8). [3]Poggi A, Haematologica, 2011, 96.

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M2-macrophages instruct mesenchymal stem/progenitor cell to better chondro-differentiate

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INTRODUCTION: Monocytes play a pivotal role in controlling tissue inflammation and repair. They infiltrate the tissues and develop into two main subsets of macrophages in response to local signals: inflammatory (M1-) and tissue-repair (M2-) macrophages (Mφ). Here we investigated whether M1 and M2φ differentially modulate chondrogenesis of bone marrow derived mesenchymal stem/stromal cells (BM-MSC).

METHODS: Monocytes isolated from buffy-coats were cultured for 5 days with GM-CSF or MCSF to induce respectively M1- or M2φ-polarization. BM-MSC were isolated from the bone marrow of a total of 9 patients and expanded for two passages. BM-MSC and polarized macrophages were cultured in collagen scaffolds up to 3 weeks alone or after being mixed (at the ratio 1:1) in medium containing TGF-β3. For specific experiments BM-MSC were labeled with carboxyfluorescein succinimidyl ester (CFSE) before being cocultured in the collagen scaffolds. Constructs were analysed histologically and bochemically to assess collagen and glycosaminoglycans (GAG) contents or enzymatically digested. BM-MSC (isolated from monoculture constructs) and *Instructed* BM-MSC (isolated from coculture constructs) were analysed by FACS to estimate their numbers or further cultured at single cell levels to assess CFU-f or cultured at high density with TGF-β3 to assess their chondrogenic capacity. To investigate the role of soluble factors in modulating BM-MSC differentiation, conditioned media harvested from macrophages were supplemented during the chondrogenic culture of BM-MSC.

RESULTS: Coculture of BM-MSC/M2φ in scaffolds resulted in statistically significant 1.9-fold higher GAG content than what would be expected (defined as *chondro-induction*). Chondro-induction was lower (1.3±0.4) and less reproducible when coculture was performed with M1φ. GAG contents of constructs generated by solely macrophages were undetectable. Histological analyses of constructs confirmed the biochemical results. As compared to monocultures, in coculture BM-MSC decreased less markedly in number (Fig.1). *Instructed* MSC were more clonogenic (35% higher CFU-f), generated tissues with better cartilaginous features (Fig.2) and accumulated higher contents of GAG (44.5% more GAG/wet weight). Chondrogenic capacities of BM-MSC were not modulated by macrophages conditioned media.

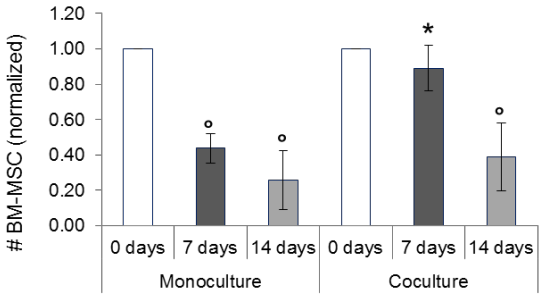

*Fig. 1: Number of BM-MSC estimated after the digestion of monoculture and coculture constructs (N=4). °=p<0.05 from 0 days (same culture group), *=p<0.05 from monoculture (same time)*

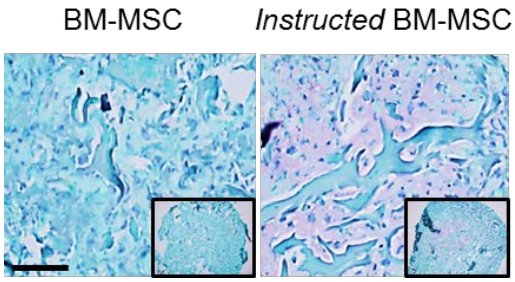

Fig. 2: Representative Safranin O pictures of cartilaginous tissues generated by BM-MSC and Instructed MSC. Bar = 100μm

DISCUSSION & CONCLUSIONS: Our results demonstrated that coculture of BM-MSC/Mφ2 results in synergistic cartilage tissue formation. Such effect is not mediated by soluble factors alone and seems to result from the survival of a BM-MSC subpopulation with high chondrogenic capacity. In vivo studies are necessary to assess the clinical relevance of our findings in the context of cartilage repair.

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Clinical Approaches to Cartilage Repair

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INTRODUCTION: The end stage of cartilage loss is osteoarthritis, disability producer in many millions of people all over the world. To find reliable methods to early repair cartilage injuries seems of uttermost importance. Articular cartilage lesions that do not penetrate to the subchondral bone fail to heal spontaneously while lesions that penetrate to the subchondral bone elicit an intrinsic repair response that yields a fibro cartilaginous repair tissue with less biomechanical quality compared to hyaline articular cartilage. Many cartilage repair strategies employed utilise this intrinsic repair response to induce the production of a repair tissue within the cartilaginous lesion. The task is to produce a repair tissue that has a near as possible the functional and mechanical properties of hyaline articular cartilage. Besides surgically making holes through the subchondral plate like the Pridie drilling there was no other treatment option available that repairs cartilage until 1994 when the first cell therapeutic approach for cartilage repair, Autologous Chondrocyte Implantation (ACI), found its way into the clinic. Cell based transplantation methods currently involve the transplantation of expanded autologous chondrocytes to the defects to form a repair tissue. Current research is exploring the potential use of mesenchymal stem cells as source for cartilage repair, as well as the combination of cells within biodegradable scaffolds. Although current repair strategies improve joint function in a joint with focal lesions, further research is required to see if the repair also can halt or slow down the progression of degenerative lesions; prevent development of post traumatic osteoarthritis.

The principal goal of biological cartilage repair strategies is to provide pain relief and improved joint function. Most of the available repair strategies meet this aim more or less but still many of the techniques have poor long-term durability. The repair tissue is often of a fibrous to fibro cartilaginous nature. Subsequently, the repair tissue often fail to withstand the mechanical demands of articular cartilage and as often repaired with a lack of successful integration to adjacent cartilage, a successful long-term outcome is at risk. In the future research may need to focus on a combination of biodegradable scaffolds and autologous or allograft chondrogeneic cells to produce a mechanically functional repair tissue. Due to costs and new regulations related to manipulations of cells outside the body, many companies now focus on one-stage procedures. Of special interest are then the fourth generation ACI: s recently appearing. One is the isolation of chondrons mixed with stem cells while the other is the use of morselized cartilage for the repair of the cartilaginous defects. Old and new techniques for cartilage repair will be presented and discussed.

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Clinical approaches to intervertebral disc repair with activated nucleus pulposus cell transplantation. A twenty-year journey from basic and translational studies to a novel clinical study

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INTRODUCTION:
Degeneration of the lumbar intervertebral discs is believed to be a significant cause of low back pain, but it is an irreversible occurrence with no treatment currently available. Building upon experimental studies that demonstrated the importance of the nucleus pulposus (NP) in preserving overall disc structure, the authors' group demonstrated that cultured NP cells activated annulus fibrosus cells, and that reinsertion of NP cells slowed further disc degeneration. We also showed that direct cell-to-cell contact in a NP cell and mesenchymal stem cell (MSC) coculture system significantly upregulated the viability of NP cells in animal models. In an in vivo animal study using chondrodystrophic beagles, we reported that the reinsertion of activated NP cells attenuated further disc degeneration. Based on the promising data acquired with animal models, we initiated an in vitro clinical study to determine whether autologous MSC-mediated NP cell activation is applicable to human cells, followed by an in vivo clinical study to treat degenerated lumbar discs.

METHODS and RESULTS: 1. In vitro study: Human NP tissue and autologous MSCs were obtained from 10 surgical patients and cultured in three conditions:. NP cells cultured alone (Group A), NP cells conventionally cocultured with MSCs (Group B), and NP cells cocultured with MSCs having direct cell-to-cell contact (Group C). Cell proliferation, DNA synthesis, and PG synthesis were significantly upregulated in Group C, confirming the positive effects of the direct contact coculture system in human cells. No chromosomal abnormality or tumorigenesis was observed in the activated NP cells. 2. Clinical approach: Candidates for the clinical study were patients aged 20 to 29 who had Pfirrman's Grade III intervertebral disc degeneration at the level adjacent to the level for which they were scheduled to have posterior lumbar intervertebral fusion. At the first fusion surgery, intervertebral disc tissue was collected. NP cells were extracted and cocultured in direct contact with autologous bone marrow derived mesenchymal stem cells at a certified cell processing center. One million activated human NP cells were transplanted into the degenerated disc adjacent to the fused level at 7 days after the first fusion surgery.

Ten patients were enrolled in this clinical study that was strictly evaluated and approved by the Japanese Ministry of Health, Labour and Welfare. There were no adverse effects observed during the perioperative period or during the 3-year follow-up period. MRI scans did not show any detrimental effects to the transplanted discs and revealed improvement of signal intensity in 3 cases. No cases have reported any low back pain during the follow-up period.

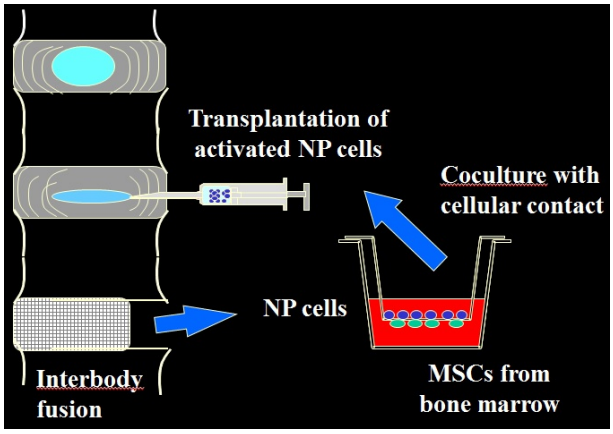

Fig1. Design of the clinical study. One million activated human NP cells were transplanted at 7 days after the first fusion surgery

CONCLUSION: Our clinical study confirmed the safety of activated NP-cell transplantation, and provided promising findings that suggest the efficacy of activated NP-cell transplantation for repair of degenerated human intervertebral discs.

REFERENCES:
[1]Y Yamamoto, J Mochida, D Sakai, et al (2004) *Spine* 29; 1508-1514. [2]J Mochida. (2005) *J Orthop Sci* 10; 112-118. [3]T Watanabe T, D Sakai, Y Yamamoto, et al (2010) *J Orthop Res* 28; 623-630

Norepinephrine: chondroprotective effects in human OA-chondrocytes

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INTRODUCTION: Norepinephrine (NE) belongs to the catecholamine family of tyrosine-derived neurotransmitters of the sympathetic nervous system. Tyrosine-hydroxylase (TH) positive sympathetic nerve fibers have been identified in synovia, bone marrow, subchondral bone, the periosteum and in bone-adherent ligaments (1) indicating that growth and metabolic activity of bone and joint tissues is modulated by sympathetic neurotransmitter (2). NE can regulate cell proliferation or apoptosis in chondrocytes (3), osteoblasts and osteoclasts (4). Catecholaminergic effects are mediated by the adrenergic receptor family comprising a1, a2- and ß2-adrenergic receptors. Here, we aim to understand the role of the catecholaminergic neurotransmitter norepinephrine (NE) in human osteoarthritic chondrocytes in the context of inflammation and its impact on metabolic activity.

METHODS: All chondrocytes were isolated from post-surgery discarded human osteoarthritic articular cartilage. Expression of adrenergic receptors/tyrosine-hydroxylase (TH) in articular cartilage chondrocytes was tested with standard PCR and immunohistochemical methods. Employing 3D cell cultures embedded in fibringel, effects of NE on interleukin-1β (IL) induced expression of pro-inflammatory cytokines and matrix metalloproteinases (MMP) and on extracellular matrix components were analyzed with real-time PCR, ELISA and DMMB-Assay. The impact of NE on cell metabolism was determined in 3D-cultured chondrocytes with PCNA/TUNEL immunohistochemistry. To specify adrenergic receptors, specific adrenergic receptor antagonists were included in BrdU/caspase3/7-ELISAs. Cell cycle analyses with FACS were performed to determine impact of NE on cell population distribution.

RESULTS: Chondrocytes cultured in monolayer and in 3D under non- and inflammatory conditions expressed TH, α1D-, α2A/B/C- and β2-adrenergic receptors. TH, α1D- and ß2-adrenergic receptors were detected on protein level in osteoarthritic cartilage explants as well. Stimulation with NE significantly reduced IL1ß induced expression of IL8 and MMP-13 in human osteoarthritic chondrocytes cultured in fibringel. NE also mitigated IL1ß induced decrease in extracellular glycosaminoglycan and collagen II concentrations. Additional, NE (10^{-8} M) increased number of TUNEL-positive and decreased (10^{-6} M) number of PCNA-positive chondrocytes cultured in 3D. Apoptosis induction was reversed after addition of α1-adrenergic receptor antagonist doxazosin. Proliferation decrease was reversed with β1-3-adrenergic receptor antagonist nadolol. Cell cycle analyses revealed an increase in G0/1-phase cell population and a decrease in S-phase population after treatment with NE (10^{-6} M) compared to untreated controls.

DISCUSSION & CONCLUSIONS: Neurotransmitters of the sympathetic nervous system like norepinephrine presumably mediate an anti-inflammatory / chondroprotective effect in human osteoarthritic chondrocytes via opposing IL1β induced increase of IL8 and MMP-13 and decrease of glycosaminoglycan and collagen II concentrations. Furthermore, norepinephrine is able to modulate metabolic activity of chondrocytes by a cell cycle slow down via β2-adrenergic receptor signaling and by induction of apoptosis via α1D-adrenergic receptor signaling. We therefore assume a yet not reported function of catecholaminergic neurotransmitters in adult articular cartilage that might have an impact on osteoarthritis pathology.

REFERENCES:

1. Imai S, Matsusue Y. MicroscResTech. 2002;58(2):61-9. 2. Amling M, Takeda S, Karsenty G. Bioessays. 2000;22(11):970-5.
3. Opolka A, Straub RH, Pasoldt A, Grifka J, Grassel S. Arthritis Rheum. 2012;64(3):729-39.
4. Elefteriou F, Ahn JD, Takeda S, Starbuck M, Yang X, Liu X, et al. Nature. 2005;434(7032):514-20.

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Evaluation of articular cartilage progenitor cells for the repair of articular defects in an equine model.

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INTRODUCTION: A chondrocyte progenitor population has demonstrated functional equivalence in their differentiation capacity when compared with MSCs but have the advantage of retaining the highly desirable stable chondrocyte phenotype[1]. These cells exhibit delayed senescence and retain their chondrogenic potency following extensive *in vitro* expansion[2]. Here, autologous and allogenic articular cartilage derived progenitor cells are evaluated for the repair of critical size cartilage defects in an equine model.

METHODS Cartilage defects of 15mm diameter were created bilaterally on the medial trochlear ridge of the stifle. Three experimental groups were compared with empty defect controls: fibrin only, autologous cells plus fibrin (Auto), and allogenic cells plus fibrin (Allo); n = 4 per treatment. Horses underwent treadmill exercise throughout the 12-month study and evaluations included lameness (pain), arthroscopic, radiographic, gross and histological analyses.

RESULTS: Arthroscopic evaluation demonstrated significantly better repair in Auto treated defects compared to empty or fibrin only ($p<0.02$). This was represented by attachment to surrounding cartilage and bone as well as firmness of repair tissue compared to normal cartilage. Radiographic appearance of Allo compared to Auto treated defects were more pathologic but not significantly different than other treatments. Auto treated defects had significantly improved microscopic repair tissue (modified O'Driscoll score) when compared with fibrin or empty defects (Figure 1, $p<0.05$, $p<0.01$). Safranin-O staining was significantly improved in the Auto repair tissue compared to the other treatment groups ($p<0.02$). A greater proportion of repair tissue labelled positive for collagen type II in both the Auto and Allo repair tissue compared with the control and Fibrin groups (Figure 2). Allogenic cells added little benefit to fibrin alone with the exception of type II collagen.

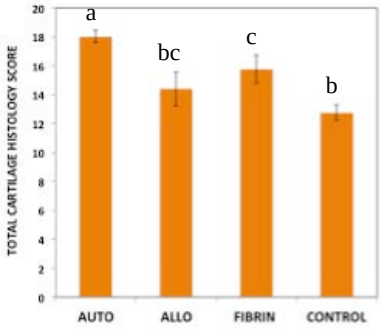

Figure 1: Total histology score plotted by treatment group (maximum score of 25). Like letters indicate no significant differences between groups.

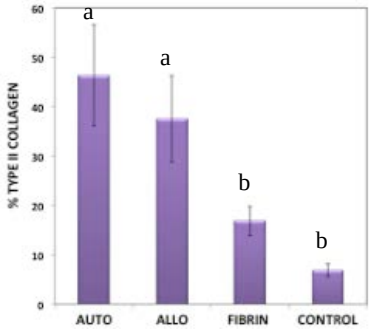

Figure 2: Percentage of repair tissue labelling positive for type II collagen plotted by treatment group Like letters indicate no significant differences between groups.

DISCUSSION & CONCLUSIONS: Autologous chondroprogenitor cells provided significant benefit compared to debridement alone (control) as well as fibrin alone for many outcome parameters. However, allogenic chondroprogenitor cells did not show any significant benefit in improving repair tissue. Disparate results between these two groups were unexpected and suggest immune system compatibility issues that need to be further investigated.

REFERENCES: [1]McCarthy et al (2012). Vet. J. 192 345-351 [2] McCarthy & Archer (2013) Eur Cell Mater 2013; 26(Suppl 3):48.

Agili-C™ Induced Cartilage Regeneration: insights in to the mode of action, in vitro and in vivo data

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INTRODUCTION: Regeneration of hyaline cartilage is the ultimate goal of orthopedics. To date four major approaches for treating focal cartilage defects dominate the clinical arena: 1) Osteochondral grafting; 2) Bone marrow stimulation; 3) Acellular scaffolds, and 4) Cell-based technologies. Each of them presents several drawbacks. Acellular scaffold, capable of attracting stem cells, that guides a regenerative process culminating in the formation of a hyaline-like cartilage could be an ideal solution. The Agili-C™ is a first-in-class acellular implant that has been designed to allow regeneration of hyaline cartilage and subchondral bone. The current work describes the scientific evidence.

METHODS: *In-vitro experimentation*: Response of normal human adult articular chondrocytes to the Agili-C™ implant was studied in a model, in which the chondral phase of the Agili-C™ implant was placed inside cartilage plugs and cultured for up to 120 days. Chondrocyte survival, migration, proteoglycan (PG) synthesis, gene expression of collagen type II and aggrecan, histology were performed to characterize cellular responses. *In-vivo experimentation*: A long-term study was conducted on 24, mature, female, 55-60 kg, Saanen goats. A critical size focal osteochondral defect (6mmx10mm) was created in the medial femoral condyle. In 16 of the goats Agili-C™ implants were inserted into the defect. Eight goats served as empty defect control. The goats were followed for 6 and 12 mo. Following sacrifice macroscopic evaluation by independent blinded experts (according to Fortier et al. and ICRS), micro CT, synovial fluid analysis, 7 Tesla MRI utilizing T2 mapping and blinded histology and immunohistochemistry with modified ICRS II and O'Driscoll scores (by NAMSA).

RESULTS: *In-vitro data*: Chondrocytes migrated into the implant either when cultured as monolayers in the presence of Agili-C™ construct or as tissue explants implanted with Agili-C™ construct. The migrating cells remained viable within and around the construct for the entire duration of the experiment. Signs of the extracellular matrix formation were evidenced inside 3D structure of the scaffold. PG synthesis and gene expression of collagen type II and aggrecan were elevated by more than 2.5 fold in cartilage with the scaffold vs corresponding controls. *In-vivo data*: Hyaline cartilage and bone regeneration were found in the Agili-C™ implanted goats but not in the controls. Results of Gross Appearance Macroscopic Evaluation (Max=15) at 6 mo after implantation (n=9) were 12±2 vs. 5±2 in the control group (n=5, $p<0.05$) and at 12 mo (n=7) 13±1 vs. 9±1 in the control group (n=3, $p<0.01$). Agili-C™ histological specimens at 6 mo were associated with formation of articular hyaline cartilage and subchondral bone, resulting in a total score of 31 (max=38) vs 16 in the control. Agili-C™ histological result scored 33/38 at 12 mo, while control group was 18. New MOCART score average of the Agili-C™ treated animals was 32±1 (max= 34) vs. 17±1 in the untreated control group.

DISCUSSION & CONCLUSIONS: The Agili-C™ implant is an example of smart nanomaterial, capable of inducing articular cartilage regeneration in both in vitro and in vivo settings. The regenerated tissue resembles hyaline cartilage evidenced by the expression of specific markers, lack of collagen type I expression, PG aggregation with typical zonation phenomenon at the 12 month follow-up time point, as well as subchondral bone plate regeneration, lack of intra-cartilaginous osteophyte formation and self-reconstructing of the articular contour. It appears that the Agili-C™ implant, in contrast to empty defects, prevents interleukin-1 expression and deterioration of surrounding articular cartilage. **In summary**, the Agili-C™ is a first-of-a-kind acellular scaffold capable of inducing regeneration of cartilage and subchondral bone. It appears to be useful in the treatment of focal articular cartilage defects and might also prevent deterioration of the surrounding articular surface acting to prevent progression to osteoarthritis.

Cartilage tissue engineering from nose to knee: early results of a Phase 1 Clinical Trial

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INTRODUCTION: When injured, cartilage has a limited intrinsic repair capacity leading to progressive joint damage. The implantation of autologous articular chondrocytes does not reproducibly support durable regeneration in the long-term. As an alternative, an engineered cartilage tissue graft with biochemical and mechanical properties mimicking those of native cartilage, could result in a more durable repair in the long-term. To reduce the variability in the quality of the engineered tissue grafts inherent to the use of articular chondrocytes, a cell source with more reproducible cartilage forming capacity such as nasal chondrocytes could be used.

The purpose of this phase-1 study is to demonstrate safety and feasibility of the procedure. We here demonstrate early results after 6 months.

METHODS: Seven patients below 55years with symptomatic post-traumatic full-thickness cartilage lesion (2-8cm2) on the femoral condyle and/or trochlea have been treated so far. The patients underwent a nasal septum cartilage biopsy in an outpatient procedure. Nasal chondrocytes were isolated and expanded using autologous serum. Cells were seeded and cultured in a collagen membrane (Chondrogide®, Geistlich) in the context of a quality management system and Good Manufacturing Practice facility. Four weeks later, the tissue engineered nasal cartilage autograft was implanted into the cartilage defect and secured with sutures and fibrin glue via mini-arthrotomy.

Patients were followed up using MRI according to MOCART score, delayed Gadolinium-enhanced MRI of cartilage (dGEMRIC) for the evaluation of glycosaminoglycan content of repair tissue and clinically through IKDC and KOOS scores.

RESULTS: No complication occurred during nasal cartilage biopsy or the implantation of the engineered cartilaginous tissues. All the operated patients could follow the established rehabilitation program.

Five patients with 7 cartilage defects reached 6 months follow up so far. At this time, MRI revealed the presence of the nasal cartilage graft in situ. The mean MOCART score was 57.5 (45-60). The dGEMRIC revealed a relative ΔR1 of 1.63 (0.89-2.28).

The IKDC pre-surgery and 6 months post-surgery was 61.8 and 68.1 respectively. The KOOS pre-surgery and 6 months post-surgery was 85.7 and 79.3 (Symptoms), 90.6 and 88.9 (Pain), 91.2 and 96.2 (ADL), 56.0 and 71.0 (Sport), 51.3 and 53.8 (QoL).

DISCUSSION & CONCLUSIONS: The early results show that engineered constructs based on autologous nasal chondrocytes were stable, could integrate with the surrounding tissues and participate to the repair of articular cartilage defects in the knee. These data so far indicate safety and feasibility of the procedure.

The mean relative ΔR1 of 1.63 (1.0 for native cartilage) of the first treated patients indicates, that a hyaline repair tissue can be achieved and, if compared to MACT[1] (relative ΔR1 2.18) or ACT (2.40, Trattnig et al 2007), might even result in improved repair tissue quality. Two year follow up of all included patients is necessary to confirm these early observations.

This study opens the way for a new approach in biological cartilage regeneration with engineered tissue and nasal chondrocytes as autologous cell source.

REFERENCES: [1] S. Trattnig, et al (2008) *Eur Radiol* Jun;18(6):1251-9. [2] S. Trattnig, et al (2007) *J Magn Reson Imaging* Oct;26(4):974-82.

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eCM XV

Cartilage & Disc: Repair and Regeneration

June 16 – 18, 2014
Convention Center
Davos Platz, Switzerland

Abstracts Poster Presentations

(in alphabetical order)

The effect of fluid-dependent dissipation on chondrogenesis

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INTRODUCTION: Mechanical stimulation has been shown to induce chondrogenesis in cell-seeded scaffolds. However, the effects of mechanical stimuli on engineered cartilage may vary substantially between different scaffolds. This advocates for the need to identify an overarching mechanobiological variable. Energy dissipation of scaffolds subjected to dynamic loading has been proposed to be such a variable[1]. The energy dissipation would encompass all the transient physical phenomena related to poroelasticity and viscoelasticity that would induce a biological response. The aim of the present study is to assess the effect of fluid-dependent dissipation related to poroelasticity on the expression of chondrogenic genes.

METHODS: Different HEMA-based scaffolds were produced as described elsewhere[1], having different levels of dissipation when subject to the same loading in terms of frequency and amplitude of deformation. Scaffolds were seeded with chondro-progenitor cells[2] at 3 mio cells/scaffold. Cell-scaffold constructs of the different groups were subject to dynamic compression of 1 Hz and 10% strain for 2 h/day during 4 consecutive days. After the 4th day of testing, immediately after mechanical stimulation, gene expressions of TGF-beta, Sox9, Col2a and Aggrecan were analyzed for the loading group and the free-swelling group used as control. The dissipation was calculated from the load-displacement graphs as the integral of the area enclosed by the hysteresis curve. To determine the contribution of fluid-dependent dissipation, level of saturation with fluid was altered in scaffolds.

RESULTS: Scaffolds of 8% EGDMA had a higher dissipation with a contribution of the fluid-phase that was also higher compared to two other groups of scaffolds (Fig.1). 8%-scaffolds had higher mRNA level for chondrogenic markers (Fig.2).

DISCUSSION & CONCLUSIONS: Effect of dissipation in mechanobiology has already been observed for bone, where cellular response of mouse bones was maximal when the dissipation was maximal[3]. For cartilage, it has been shown that dissipation is sufficient to raise temperature in the tissue, which in turn influence cell metabolism[4,5]. The results of this study suggest that the fluid-flow related to energy dissipation may also play a role in cartilage mechanobiology.

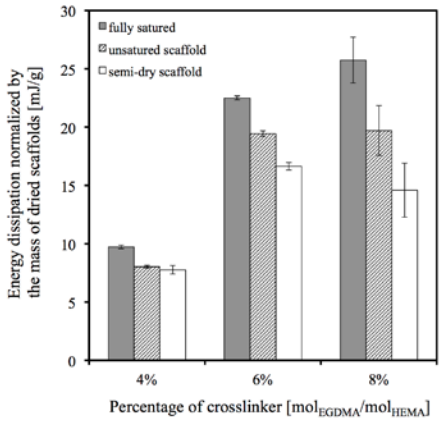

Fig. 1: Energy dissipation of poly(HEMA-co-EGDMA) scaffolds.

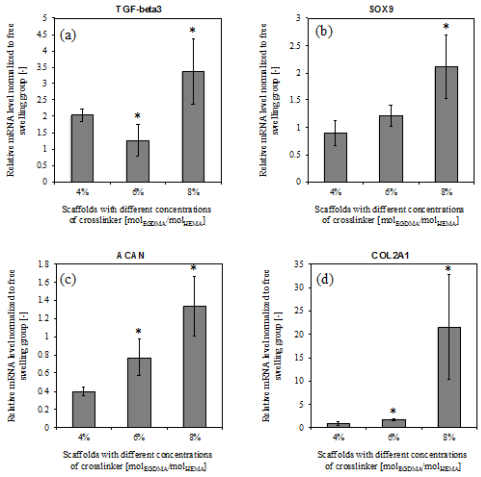

Fig. 2: Gene expression graphs for (a) TGF-beta3, (b) Sox9, (c) Col2 and (d) Acan.

REFERENCES: *[1]Abdel-Sayed et al*, Biomaterials. *35, 6, 1890-7, 2014. [2]Darwiche et al*, Cell Medicine. *4, 23-32, 2012. [3]Kunnel et al*, Calcif Tissue Int, *71, 6, 499-507, 2002. [4]Abdel-Sayed et al*, J Mech Behav Biomed Mater. *30, 123-30, 2014. [5]Abdel-Sayed et al*, eCM, *26, 171-8, 2013.*

ACKNOWLEDGEMENTS: *This project was supported by the Inter-Institutional Center for Translational Biomechanics EPFL-CHUV-DAL.*

Link N suppresses Interleukin-1β induced human osteoarthritic cartilage degradation through down-regulation of NF-κB signalling

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INTRODUCTION: Osteoarthritis (OA) is a chronic degenerative joint disorder that affects millions of people. It is characterized by the destruction of articular cartilage due to an imbalance in the anabolic and catabolic activities of chondrocytes. Matrix degradation is mediated by matrix metalloproteinases (MMPs) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs)-4 and ADAMTS-5, induced by Interleukin-1 (IL-1β), the major cytokine implicated in OA. IL-1 receptor antagonist (IL-1Ra) therapy has proven clinically beneficial in the treatment of rheumatoid arthritis; however, in the treatment of OA, the results have been disappointing. There are currently no therapies that reverse or repair cartilage degradation in OA patients.

Link N (DHLSDNYTLDHDRAIH) is a naturally occurring peptide generated *in vivo* by the N-terminal proteolytic fragmentation of the link protein during tissue turnover. We have previously shown that Link N is able to stimulate the synthesis of aggrecan (Agg) and collagen (Col II) while down-regulating metalloproteinase expression in degenerate human intervertebral discs. The purpose of this study was to determine whether Link N can stimulate matrix production in human OA cartilage under inflammatory conditions (presence of IL-1β) and the possible cellular mechanisms involved.

METHODS: OA cartilage was obtained from four donors undergoing total knee arthroplasty (range 50-65 years) with informed consent. OA cartilage-bone explants and OA chondrocytes were prepared from each donor and stabilized for 7 days under standard culture conditions. Normal chondrocytes (PromoCell, Heidelberg, Germany) were expanded under the same conditions and used as control. After stabilization, the cells and cartilage explants were exposed to IL-1β (5ng/ml), Link N (1µg/ml) or co-incubated with IL-1β+Link N for 7 and 21 days, respectively. The expression of Col II, Agg, Col X and MMP-13 was evaluated by Western blotting. The total glycosaminoglycan (GAG) content in the tissue was quantified using the dimethylmethylene blue (DMMB) assay.

RESULTS: OA cartilage explants showed a statistically significant increase in proteoglycan synthesis in response to Link N that was retained in the matrix. Link N significantly induced proteoglycan production in the presence of IL-1β, in OA explants and chondrocytes. Similar results were obtained for Col II. Link N also suppressed MMP-13 activation and Col X expression. Of special interest was the finding that in OA and normal chondrocytes, IL-1β-induced activation of NF-κB was dose-dependently suppressed by Link N (Fig. 1).

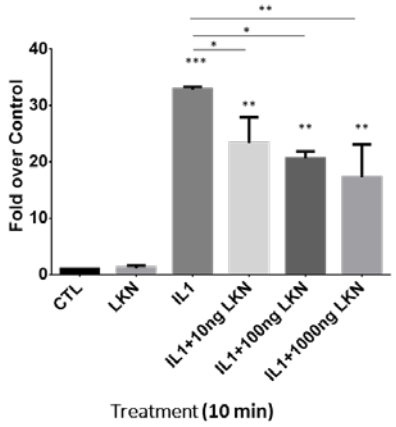

Fig. 1: ***NF-κB activation in OA chondrocytes.*** *OA chondrocytes were incubated with IL-1β alone, or co-incubated with increasing concentrations of Link N [10-1000ng/ml]. Western blotting was performed on cell lysates for active NF-κB (P-p65) and normalized to p65 by densitometry.*

DISCUSSION & CONCLUSIONS: Link N stimulated proteoglycan and collagen synthesis in an inflammatory milieu. One mechanism for Link N in preserving matrix protein synthesis may, in part, be due to its ability in suppressing IL-1β-induced activation of NF-κB. Link N has therapeutic potential in the regeneration of cartilage in OA.

ACKNOWLEDGEMENTS: We thank the Canadian Institute of Health Research (CIHR).

Cartilage growth on Insulin and collagen-coated PLGA/PCL scafolds

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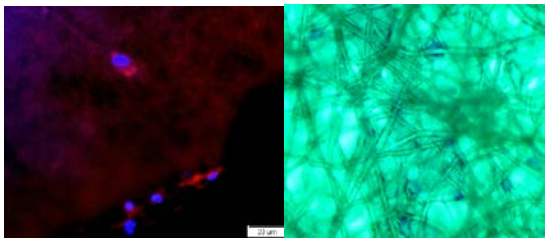
INTRODUCTION: Poly lactic-co-glycolic acid (PLGA) and polycaprolactone (PCL) are commonly used biodegradable and biocompatible polymers as cartilage scaffolds[l]. Insulin is an additive in chondrogenic growth and differentiation [2]. In this study insulin was loaded on PLGA with and without collagen coating to assess these nanofiber-based-scaffolds in growth and differentiation of human chondrocytes.

METHODS: PCL/PLGA, PCL/PLGA-insulin, PCL/PLGA-collagen, and PCL/PLGA-insulin-collagen nanofiber were prepared. Collagen coating was done by immobilization (on surface of scaffolds) and approved by FTIR test. Insulin was dissolved in PLGA and PCL/PLGA hybrid nanofiber was prepared. Insulin release was measured in triplicates every other day from day 2 to 22. Chondrocyte from human's nasal septum were seeded on the 4 scaffolds within differentiating media. Scanning electron microscopy was used for evaluation of cell morphology and distribution. Viability and proliferation were assessed by MTT and mRNA of aggrecan, collagen I, and collagen II was measured by RT-PCR on days 7, 14, and 21 and protein expression was assessed by immunofluorescent study.

RESULTS: The seeded cells were evenly distributed in all area of scaffolds in DAPI and H&E stainings. SEM and alcian blue staining showed that the attached cells had round chondrocyte-like appearance and penetrated the pores of the insulin-loaded scaffold. Insulin release in media had zero-order kinetic model and did not show burst release pattern. MTT results showed better survival rates of chondrocytes in collagen coated and insulin loaded scaffolds. Collagen II and aggrecan productions were significantly higher in insulin loaded scaffolds than the others.



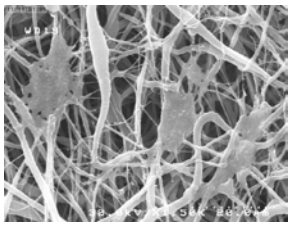


Fig 1: a- Collagen II was revealed by immunofluorescent staining (Phycoerythrin). b- Alcian blue stains the chondrocytes containing glycosaminoglycan. c- Scanning electron microscopy reveals insertion and adhesion of cells within the nanofibers.

DISCUSSION & CONCLUSIONS:

PCL/PLGA scaffold with controlled release of insulin can be introduced as an effective three-dimensional scaffold for enhancing proliferation and differentiation of chondrocytes.

REFERENCES: [1] Jeong JY, Ha NK, Eun HB,et al (2011). Culture of Human Chondrocytes in a Macroporous PLGA Scaffold for up to Sixteen Weeks. *Tissue Engineering and Regenerative Medicine* 8: 300-305. [2] Kristin A, Rolf Z, Maja K, et al (2011). Biodegradable insulin-loaded PLGA microspheres fabricated by three different emulsification techniques: Investigation for cartilage tissue engineering. *Acta Biomaterialia* 7: 1485-1495.

In vitro cell mobility: a mesenchymal stem cells marker for multipotency?

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INTRODUCTION: Mesenchymal stem cells (MSCs) are fundamental players in future cell-based therapies. These cells possess high proliferative ability, multilineage potential and immunomodulatory properties, all requisites essential for cell therapies.

Nevertheless, intra-donor variability of MSCs is not fully characterized yet, leading to uncertain outcomes in the use of these cells.

Here we propose a life imaging tool to characterize population of human MSCs derived from different donors, based on the *in vitro* mobility of the cells and to correlate results to the respective differentiation potential.

METHODS: Bone marrow human MSCs from 20 donors and at various *in vitro* passages (from P3 to P13) were evaluated. Track length of 70 cells per population were measured over a period of 24 hours, and distances were compared with the respective adipogenic, chondrogenic and osteogenic differentiation potential.

RESULTS: We found that senescent populations were enriched with slow moving cells, compared with younger ones (Fig. 1). Cells with larger cell body were moving less compared to smaller ones, while spindle shaped cells had an average speed.

Our preliminary data showed that both fast cells and slow cells were characterized by low differentiation potential, while average moving cells were more effective in undergoing all three lineage differentiation.

Heterogeneity in single cell motility within a population correlated with faster moving cells, while populations rich in slow moving cells tended to homogeneity (only senescent cells).

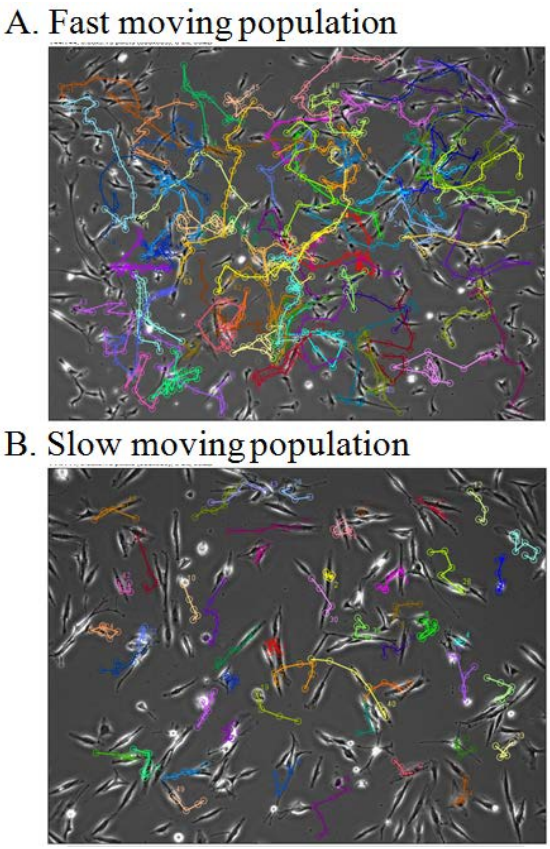

Fig. 1: Representative microphotographs of fast (A) and slow (B) moving MSC population. The coloured lines depict single cell paths over a period of 24 hours.

DISCUSSION & CONCLUSIONS: In conclusion, *in vitro* cell mobility might be an useful tool to characterize MSC population and recognize prior to use the differentiation potential of MSCs. Based on this set of results, we propose a fast method which may give a reliable quality control of MSCs, instead of depending only on mechanistic passage number.

ACKNOWLEDGEMENTS: This work was supported by the Swiss Paraplegic Foundation and Swiss National Foundation Grant CR3I3_140717/1.

Characterization of hydrogels under physiological loading conditions for cartilage tissue engineering

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INTRODUCTION: Joint cartilage degeneration results in pain and reduced quality of life. Because adult cartilage has limited tendency to self-heal, degeneration is addressed with mosaicplasty and chondrocyte implantation. Unfortunately, donor site morbidity and limited availability of autologous osteochondral plugs hinder these treatment possibilities, so researchers have focused efforts to replace damaged cartilage with tissue-engineered cartilage constructs. Hydrogels are often used in these efforts because they not only provide a boundary for retention of cells, but also act as a substrate to which anchorage-dependent chondrocytes can adhere.

Mechanical stimulation is known to direct cell differentiation and proliferation during three-dimensional cell culture. However, these dynamic loading waveforms are often not representative of the physiological loading pattern that cells experience in vivo, and further, the scaffold materials are not characterized in physiologically-relevant conditions prior to selection. In this study, the mechanical response of polyethylene glycol (PEG) hydrogels was observed was observed with a waveform to mimic dynamic compressive strain on knee cartilage during a walking gait cycle.

METHODS: Specimens were punched from PEG hydrogel sheets (12 mm in diameter and 6 mm in height) and soaked in 37C saline for two hours prior to placement on the mechanical loading frame. A preloading force of 0.1 N was applied initially to ensure that the entire scaffold surface was in contact with the compression platens prior to application of the walking gait waveform (Fig. 1). In addition to individual sample loading, simultaneous loading of 24 hydrogel samples was performed in a multi-specimen compression bioreactor to assess the loading accuracy when stimulating multiple samples with the same actuator. A waveform model (Fig. 2) of strain vs. gait cycle based on simulation of human walking was used in this study[1]. The extracted waveform was constructed based on a cycle of 1.1s, and the strain was scaled according to specimen thickness.

Fig. 1: A single hydrogel specimen was loaded between compression platens for comparison to load data taken on 24 samples simultaneously.

RESULTS: Similar test results of three specimens were achieved, and reliable repeatability of the testing method was demonstrated. Compared to the original extracted waveform, the majority of the waveform definition was retained, including micron-level changes in strain. Similarly, when compressing 24 samples simultaneously, the sample stage was found to synchronously and evenly distribute the load to all 24 specimens.

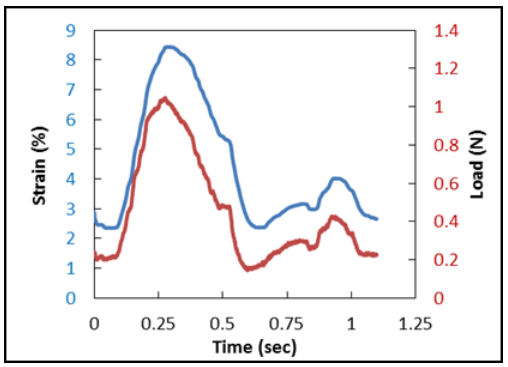

Fig. 2: Representative walking gait waveform and load response on a single hydrogel sample.

DISCUSSION & CONCLUSIONS: In this study, a walking gait waveform was used to dynamically compress hydrogels for the application of cartilage tissue engineering. The waveform was determined to be applicable to future cell culture studies in which cell-seeded constructs will be loaded over an extended period of time.

REFERENCES: [1]KS Halonen, et al. (2013) J. Biomech. 46(6): 1184-92.

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

A collagen 2 reporter for online identification of chondrogenic microenvironments

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INTRODUCTION: Cell-based tissue engineering is a promising method for repair of injured cartilage, however, the outcome depends highly on the continued expression of cartilage markers by the transplanted cells. Collagen 2 is a main structural component of cartilage and hence a method to monitor its expression under changing experimental microenvironments would be of great value. In this study, the human collagen II promoter was cloned into a lentiviral vector and used to drive the expression of green fluorescent protein (GFP) in human chondroprogenitor cells. The resulting promoter reporter gene construct provides the opportunity for non-invasive and repeated imaging of chondrocyte differentiation.

METHODS: A vector containing the 6100 bp human collagen 2 promoter [1] (gift of KSE Cheah, University of Hong Kong) and a 3100 bp fragment was sub-cloned into a lentiviral vector to control the expression of green fluorescent protein (GFP). Lentiviral particles were produced using 293T HEK cells and human epiphyseal chondroprogenitors (gift of Lee-Ann Applegate, EPFL) were transduced in the presence of 6 μg/ml DEAE-dextran 500 followed by puromycin selection of positively transduced cells for 5 days. To induce chondrogenic re-differentiation in 2D, chondroprogenitors were cultured in the presence of 1 nM staurosporine and the MEK inhibitor PD98059 (10 μM) which was previously shown to induce re-expression of the chondrogenic phenotype [2]. Relative expression of Col 2 was analysed by RT-PCR and synthesis of glycosaminoglycans was evaluated by alcian blue staining. Expression of GFP was evaluated microscopically.

RESULTS: After 7 days of 2D culture in the presence of staurosporine or a combination of staurosporine and PD98059, transduced chondroprogenitor cells started to produce glycosaminoglycans as shown by positive alcian blue staining. This staining correlated with a rounding of the cell morphology, indicative of a switch to a cortical actin-like cytoskeleton. RT-PCR further showed that the combination of staurosporine and PD98059 increased the expression of Col 2 to a higher extent than staurosporine alone. While no GFP expression was observed at early days of culture, GFP expression of transduced cells was observed in the majority of chondroprogenitors cultured for 7 days under both chondrogenic medium conditions.

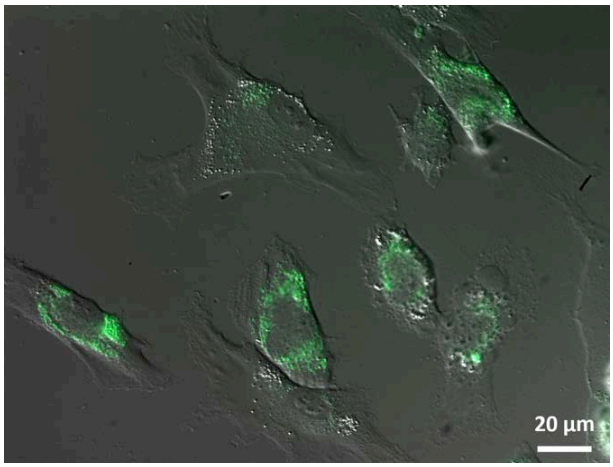

Fig. 1: Transduced chondroprogenitors were cultured in 2D with 1nM staurosporine and 10 μM PD98059 for 7 days. Collagen 2 promoter activity is indicated by intracellular expression of green fluorescent protein.

DISCUSSION & CONCLUSIONS: The responsiveness of our differentiation-specific reporter was validated in 2D culture of human chondroprogenitors. RT-PCR further confirmed the progression into the chondro-specific lineage of transduced chondroprogenitors. Together our data demonstrate the functionality of the lentiviral reporter gene as a versatile tool for online monitoring of chondrogenesis. Other possible applications of this tool include high-throughput screening of inhibitor libraries, selection for chondrogenic cells from a mixed population and systematic design and selection for chondroinductive biomaterials and niches.

REFERENCES: [1]Leung, K.K., et al. *J Cell Biol*, 1998 141(6): p. 1291-300. [2] Rottmar, M. et al. *Exp Cell Res*, 2014 320(2):175-87.

ACKNOWLEDGEMENTS: Center for Applied Biotechnology and Molecular Medicine (CABMM) start-up grant and SNF.

Injectable hydrogels for cartilage repair

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INTRODUCTION: Osteoarthritis is a prevalent degenerative cartilage disease. If left untreated it will have a severe impact on the quality of life. However, treatment options are limited and are still suboptimal. Our aim is to create an injectable hydrogel that can be used to plaster eroded cartilage surfaces and / or to fill up focal cartilage defects in a minimally invasive arthroscopic procedure. The plaster should protect the damaged cartilage surface against further cartilage erosion and possess an optimized environment for cartilage regeneration.

METHODS: The dextran-tyramine conjugate (Dex-TA) and Hyaluronic acid-TA (HA-TA) conjugates were synthesized using slightly modified procedures as described previously [1,2]. This procedure renders an injectable hydrogel which gelates in a peroxidase mediated reaction initiated by non-toxic concentrations of H_2O_2. The adhesive properties of the injectable plaster were compared to clinically used fibrin glue in a customized set up whereby rheological analysis and a tensile test were performed. Finally pilot studies were performed in fresh horse cadavers to test whether the plaster could be applied in focal cartilage defects in an arthroscopic procedure. The repaired joint was manually flexed to study the plaster's resistance to force.

RESULTS: The hydrogel solution was injected in between two bovine cartilage specimens (gap size of 3 mm) using a double syringe (fig. 1). The gel was formed almost instantaneously and after 5 min a tensile test was performed at a distraction rate of 100 µm/s. An average ultimate stress of approximately 17 kPa and ultimate strain of 8 % were found for the DEX-TA/HA-TA gel.

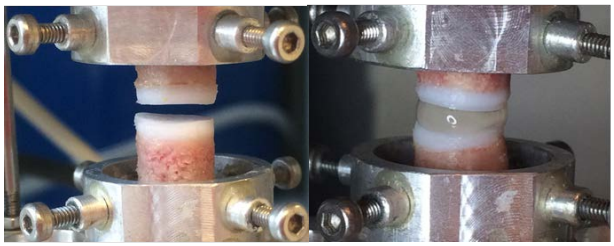

Fig. 1: Schematic representation of the tensile tests performed. The gap in between the osteochondral plugs was first filled with hydrogel before distraction.

These values were 10-fold higher compared to commercially applied fibrin glue.

Next we tested our Dex-TA/HA-TA injectable plaster in a minimally invasive surgical procedure. Thus the gel was injected via arthroscopic procedure under CO_2 insufflation in a full thickness defect in the joint of a horse. Subsequently, the knee of the horse was flexed manually to test the injectable plaster which remained in its place (Figure 2).

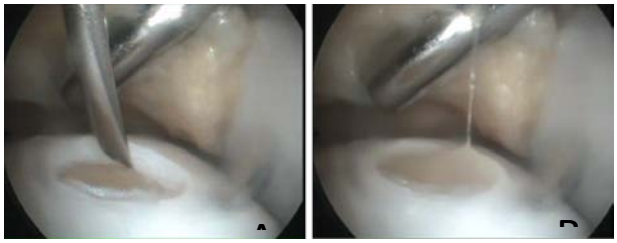

Fig. 2: Applying the injectable plasters in a full thickness chondral defect during an arthroscopic procedure. The joint was insufflated by CO_2 Before (A) and after (B) the injection of the plaster. The initial fluidity ensures complete filling of the defect. The plaster remained fixed at the defect site after manual flexion of the joint

DISCUSSION & CONCLUSIONS: We have developed a method to integrate enzymatically crosslinked hydrogels to cartilage tissue and subchondral bone and measured their adhesive properties. The rheological properties of a 10% Dex-TA/HA-TA hydrogel as well as bonding to damaged cartilage is in the order of 1 to 2 logs better than the properties of clinically applied fibrin glue. Moreover we demonstrated that this hydrogel can be applied in a minimally invasive arthroscopic procedure in a horse knee in an surgically easy, straightforward procedure. We are now moving forward to test our concept for treatment of chondral defects in live horses.

REFERENCES: [1] R Jin, C. Biomaterials 2007, 28, 2791-2800. [2] JWH Wennink, *Macromolecular Symposia* **2011**, *309-310*, 213-221.

ACKNOWLEDGEMENTS: This work is supported by a long term program grant of the Dutch Arthritis Association.

Degenerated human intervertebral discs contain autoantibodies against extracellular matrix proteins

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INTRODUCTION: Degeneration of intervertebral discs (IVDs) is associated with back pain and elevated levels of inflammatory cells. It has been hypothesised that discogenic pain is a direct result of vascular and neural ingrowth along annulus fissures, which may expose the avascular nucleus pulposus (NP) to the systemic circulation and induce an autoimmune reaction. In this study, we confirmed our previous observation of antibodies in human degenerated and posttraumatic IVDs cultured in vitro. We hypothesised that the presence of antibodies was due to an autoimmune reaction against specific proteins of the disc. Furthermore we identified antigens which possibly trigger an autoimmune response in degenerative disc diseases.

METHODS: Human degenerated and post-traumatic IVDs (AF and NP separately) were obtained from patients (n = 20), undergoing surgical intervention. Samples were washed 3-4 times in phosphate-buffered saline (PBS) to eliminate residual blood, and cultured in vitro. After three days, the presence of autoantibodies in culture medium was analysed by immunoblotting and immunohistochemistry.

RESULTS: We demonstrated that degenerated and post-traumatic IVDs contain IgG antibodies against typical extracellular proteins of the disc, particularly proteins of the NP. We identified IgGs against collagen type II and aggrecan, confirming an autoimmune reaction against the normally immune privileged NP. We also found specific IgGs against collagens types I and V, but not against collagen type III. This probably means that the autoimmune reaction in the disc is not generic to the whole collagen family.

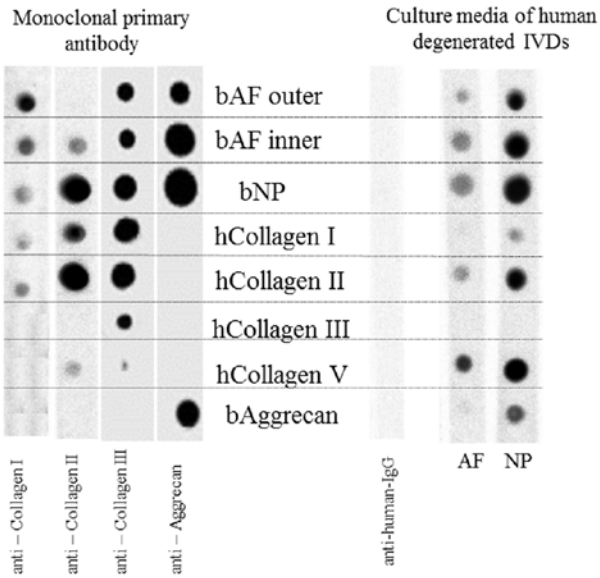

Fig. 1: IgGs present in human degenerated IVDs target native ECM proteins. Dot blot on healthy bovine disc protein extracts (bovine AF outer, AF inner and NP) and purified ECM proteins (human Collagen type I, type II, type III, type V and bovine aggrecan) shows the presence of specific IgGs for all detected proteins except Collagen type III, in culture media collected at day 3 from human degenerated IVDs. Monoclonal primary antibodies (anti-Collagen type I, type II, type III and anti-aggrecan) were used as positive controls, while antihuman-IgG antibody was used as a negative control. Rows represent spotted proteins, while columns represent monoclonal antibodies and disc culture media used as primary antibodies.

DISCUSSION & CONCLUSIONS: In conclusion, this study confirmed the association between disc degeneration and autoimmunity, and may open the avenue for future studies on developing prognostic, diagnostic and therapy-monitoring markers for degenerative disc diseases.

ACKNOWLEDGEMENTS: This work was supported by the Swiss Paraplegic Foundation and Swiss National Foundation Grant CR3I3_140717/1.

Stem cell effect on mechanically-loaded nucleotomised intervertebral discs

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[1]AO Research Institute Davos, Davos, CH. [2] School of Life Sciences, University of Applied Sciences Northwestern Switzerland, CH. [3]Department of Orthopaedic Surgery, Inselspital, University of Bern, Bern, CH.

INTRODUCTION: Mesenchymal stem cell (MSC) therapy has been described for the treatment of intervertebral disc (IVD) degeneration[1,2]. Two mechanisms have been suggested for the MSC regenerative effect: MSCs can differentiate into disc cells or release trophic factors that will in turn influence the endogenous disc cells. The goal of this study was to investigate the effect of MSCs on the gene expression of IVD cells.

METHODS: Bovine caudal discs were loaded for 7 days in either physiological (3 hours/day at 0.5 ± 0.3 MPa at 0.1 Hz, culture in high glucose medium) or degenerative (3 hours/day at 0.5 ± 0.3 MPa at 10 Hz, culture in low glucose medium) conditions. One disc per group was kept for gene expression analyses and the other discs were nucleotomised and filled with: (1) fibrin gel (final fibrinogen concentration=60 mg/mL), (2) human MSCs in fibrin gel ($6x10^6$ cells/mL), (3) human MSCs in saline solution, (4) saline. Discs were loaded for additional 7 days and analysed for gene expression.

RESULTS: Following one week of physiological loading, the expression of type I collagen (COL1) and aggrecan (ACAN) was maintained both in nucleus pulposus (NP) and annulus fibrosus (AF) tissues. Instead, one week of degenerative loading induced significant COL1 down-regulation (in NP and AF tissue) and ACAN down-regulation (in NP tissue). Type II collagen (COL2) was down-regulated under both loading regimes and for all tissues.

In the second week all discs were nucleotomised, assigned to subgroups and loaded physiologically. It was found that MSCs induced an up-regulation of COL2 expression (Figure 1) in physiologically-loaded discs. A similar trend was also observed for COL1. Interestingly, in degenerative-loaded discs MSCs induced an up-regulation of COL1 and COL2 only in the outer AF.

No difference in terms of endogenous response was observed when MSCs were supplied with a fibrin carrier or a saline solution. The presence of MSCs had no effect on the expression of ACAN, MMP3 or ADAMTS4 in disc tissue.

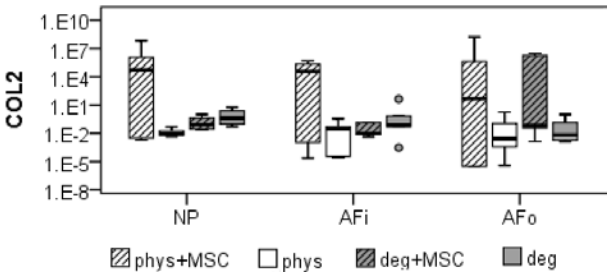

Fig. 1: Application of stem cells induced an up-regulation of type II collagen in physiologically-loaded discs (data are expressed as 2^{-ddCt} relative to discs collected on day 0, n=4).

DISCUSSION & CONCLUSIONS: This is the first study to show the effect of MSCs on intervertebral discs cultured ex-vivo under dynamic load. Our results are in agreement with *in vivo* studies reporting regenerative effects in IVDs in the presence of MSCs[1] and *in vitro* studies proving MSC/disc cell cross-talk[2].

A high variability in the response of MSC-treated discs was observed, probably due to the high inter-donor variation of MSCs. The fact that MSCs did not influence the response of NP and inner AF tissue in degenerative-loaded discs could be due to a higher damage of these tissues during the first week of loading.

In conclusion, physiologically-loaded discs showed a stronger gene expression response to the application of MSCs than degenerative-loaded discs. In the latter case, only the outer annulus fibrosus responded to the treatment. The main effect of MSCs was up-regulated of COL1 and COL2, while minor changes were observed in catabolic gene expression.

REFERENCES: [1]D. Sakai and J. Mochida (2014) Use of stem cells for regeneration of the intervertebral disc in *The Intervertebral disc* (eds I.M. Shapiro and M. Risbud), Springer, pp.373-83. [2]G. Vadalà, R.K. Studer, G. Sowa (2008) *Spine* **33**:870-6.

ACKNOWLEDGEMENTS: Fibrin components were provided by Baxter Biosurgery, Vienna. This study was supported by AOSpine and a NASS Research Grant.

Intervertebral disc cell response to torsion as a function of duration and magnitude

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INTRODUCTION: It has been suggested that a low degree of axial rotation of the spine at low frequency might increase the spinal length, and therefore, enhance the nutrition and waste exchange,[1] whereas a high torsion angle would cause damage to the disc.[2] In this study, we aimed to investigate the relationship between the magnitude and duration of torsion applied to the IVD and the level of NP cell viability. In this study, the biological response of the intervertebral disc (IVD) to different magnitudes and duration of dynamic torsion were tested in a bovine organ culture model.

METHODS: Bovine caudal discs were harvested and cultured in a custom-built dynamic loading chamber.[3] First, torsion of increasing magnitudes (±0°, ±2°, ±5°, ±10°) with 0.2 MPa static loading at 0.1 Hz was applied to the explants for 1 hour/day for five days. Second, the beneficial torsion magnitude determined from the first study was used for the second study, where torsion of ± 2° was applied to the samples at a frequency of 0.2 Hz for durations of 0, 1, 4 and 8 hours per day, repeated over 7 days. After the last day of loading, disc tissue was dissected for analysis of cell viability and gene expression.

RESULTS: Disc explant cell viability (CV) maintained between 70- 80% when torsion was applied to the explants for 1 hour/ day on top of the 24 hour static loading. (Table 1) CV was the highest in both NP and AF when ± 2° torsion was applied to the explant.

In the second part of the study, disc explants were cultured in unloading condition except the duration of torsion application. In general, disc CV maintained above 80% when static loading was not applied. Disc NP CV remained above 85 % after torsional loading for 0, 1, or 4 hours per day. CV was statistical significantly reduced to below 70 % when torsion was applied for 8 h per day ($p = 0.03$). (Table 2) The daily duration of torsional loading did not affect the AF cell viability (> 80% for all loading durations).

*Table 1. Cell viability of the disc after different magnitudes of torsion. Mean ± SD. N= 6, * $p < 0.05$.*

Magnitude of Torsion	NP	AF
0	71.91 ± 15.90	71.65 ± 14.43
2	79.6 ± 13.73	78.61 ± 12.34
5	74.46 ± 18.34	71.56 ± 14.51
10	79.45 ± 5.12	70.83 ± 22.39

*Table 2. Cell viability of the disc after different durations of torsion. Mean ± SD. N= 6, * $p < 0.05$.*

Time of Torsion	NP	AF
0	87.17 ± 10.89	89.87 ± 7.34
1	91.24 ± 6.31*	92.05 ± 12.27
4	89.31 ± 12.35	81.38 ± 13.89
8	67.19 ± 16.07 *	84.45 ± 13.96

DISCUSSION & CONCLUSIONS: Results showed that short-term physiological low magnitude (2°) torsion improved cell viability in the inner annulus of the disc in organ culture as compared to static loading alone, however, an extended duration of low magnitude torsion could inhibit the survival of NP cells within the IVD in organ culture.

REFERENCES: [1] van Deursen et al, Applied Ergonomics 31: 95-98, 2000.
[2] Farfan HF et al. J Bone Joint Surg Am 52: 468-497, 1980. [3] Chan et al. PLOS One 2013, 8(8), e72489.

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Expression of HSP72 and HSF1 in nucleus pulposus in response to compressive loading

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INTRODUCTION: Many studies have demonstrated that expression of extracellular matrix proteins in nucleus pulposus cells (NPC) can change under mechanical loading.[1] However, few have studied whether the NPC respond to the mechanical stress by expression of heat shock proteins (HSP). It has been demonstrated that expression of HSPs can be confirmed in cell line chondrocytes under hydrostatic pressure. On the other hand, studies have also shown that intervertebral disc (IVD) cells secrete HSP70 in response to oxidative stress. This study aims to understand the stress response in the IVD in response to compressive loading and how it relates to the disc cells adaption to the loading.

METHODS: Adult bovine caudal discs were harvested and cultured with dynamic compressive loading applied at physiological range magnitude, 0.1-0.6 MPa. The mechanical loading parameters were 2 h of dynamic loading, followed by 22 h of resting for two days. Samples were retrieved at different time points: right after loading (Dyna) and right after resting (Dyna+rest). Positive control discs were put under static loading (0.35 MPa, static) and heat shock (43 °C, HS) exposed for 2 h per day during two days. Nucleus pulposi (NP) were retrieved for cell activity assay and gene expression. The cellular stress response genes Heat Shock Protein-72 (HSP72) and Heat Shock Factor-1 (HSF1) were monitored for relative gene expression. The expression was normalized to free swelling control (Free).

RESULTS: Cell activity of different groups did not differ from the free swelling group, showing the cells were alive after the loading protocols. This agrees with the results from earlier studies.[2,3] Both positive controls expressed high level of HSP72, confirming their expression in the NP tissues and their response to heat stress. For the experimental groups, expression of both HSP72 and HSF1 were up-regulated after loading and decreased after resting (Fig. 1). The expression of both genes was up-regulated again after the second round of loading and decreased after resting on Day 2. The relative expression of the two genes was after two rounds of loading was higher than after a single round of loading (Fig. 1). This may indicate transcriptional up-regulation is increased after repeated cycles of loading.

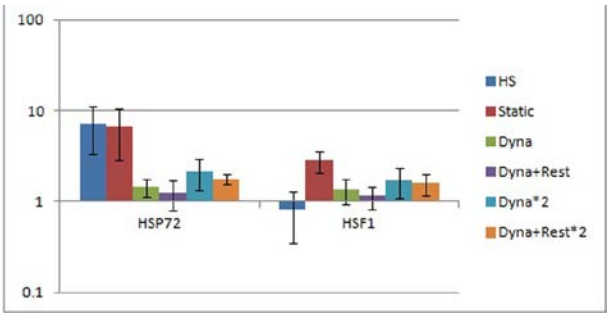

Fig. 1: Stress response protein expression in the NP cells normlized to free swelling control.

DISCUSSION & CONCLUSIONS: This study demonstrated that NP cells up-regulate stress response proteins in response to loading induced stress. The NPC express HSP72 and HSF1 in response to the stress. The increase in HSP72 and HSF1 expression after two rounds of loading may indicate that the cells take longer than two days to adapt to the stress induced by mechanical loading.

REFERENCES: [1] SC Chan et al. *European Spine Journal* **20.11** (2011):1796-812. [2] B Gantenbein et al *Spine* **31.23** (2006): 2665-2673. [3] C Paul et al *PloS one* **7.3** (2012): e33147.

ACKNOWLEDGEMENTS: This project was funded by AO Spine (AOSPN) (grant number: SRN_2011_14), the Swiss National Science Foundation (SNF project 310030_153411) and a fellowship exchange award by AO Spine Scientific Research Network (SRN).

Generating *Nucleus pulposus*-like cells from human Adipose Stromal cells: a first step towards the regeneration of intervertebral disc

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INTRODUCTION: Intervertebral disc (IVD) degeneration is one of the major causes of low back pain. It is now well-established that the *Nucleus pulposus* (NP) is prematurely affected by degenerative events, mainly starting with the apoptotic death of the NP resident mature cells namely the nucleopulpocytes. In this context, repopulating a damaged NP with functional regenerative cells seems to be a promising approach for long term regeneration of IVD.
Since Growth Differentiation Factor 5 (GDF5) plays a crucial role in IVD anabolism and biological activity of nucleopulpocytes [1], we questioned whether GDF5 may be an efficient tool to drive the nucleopulpogenic differentiation of human Adipose Stromal Cells (hASC). In this context, the aim of the present work was to investigate the effects of supplementing a chondrogenic medium with GDF5 on the *in vitro* production of functional nucleopulpocytes from hASC.

METHODS: Human ASC have been cultivated in pellets (500.000 cells/pellet) for 28 days in the presence of low oxygen tension (5%) with a chondrogenic medium enriched or not with GDF5 (100ng/ml). The nucleopulpogenic differentiation of hASC has been evaluated by determining the expression levels of ACAN, COL2A1, PAX1, OVOS2 and CD24 transcripts by RT-qPCR. The levels of the corresponding proteins in hASC pellets have been assessed by immunohistochemistry analyses. The specificity of the hASC commitment has been determined by analysing the expression of osteogenic-, adipogenic- and chondrogenic-related markers by Taqman Low Density Array. Furthermore, hASC were cultivated in the presence of the GDF5-enriched chondrogenic medium with or without SB505124 (5µM) or dorsomorphin (5µM) to decipher the involvement of Smad proteins.

RESULTS: Our RT-qPCR and immunohistochemistry data showed that hASC cultivated in the presence of a GDF5-enriched chondrogenic medium expressed nucleopulpocyte markers (ACAN, COL2A1, PAX1, OVOS2 and CD24) at the transcript and protein levels. A time course experiment demonstrated that hASC express nucleopulpocyte markers as early as 21 days of culture. Furthermore, the osteogenic-, adipogenic- and chondrogenic-related markers remain at a barely detectable level of expression. Concerning the role of Smad pathways during the nucleopulpogenic differentiation of hASC, our western blot data demonstrated that SB505124 and dorsomorphin respectively inhibit the phosphorylation of Smad2/3 and Smad1/5/8 and thus respectively block the TGF-β and GDF5 canonical pathways. Whereas SB505124 was found to prevent the nucleopulpogenic differentiation of hASC, dorsomorphin did not significantly affect this differentiation process.

DISCUSSION & CONCLUSIONS: Our data demonstrate that human ASC cultivated in the presence of a GDF5-enriched chondrogenic medium can be committed towards the nucleopulpocyte lineage. This commitment seems to be reproducible, robust and specific. Our results also suggest that the canonical TGF-β pathway (Smad2/3) plays a pivotal role for the nucleopulpogenic commitment of hASC. Whether the *in vitro* generation of functional nucleopulpocytes may help us design regenerative medicine strategies remains to be further evaluated in suitable animal models of disc degeneration.

REFERENCES: 1.X. Li (2004) *Collagen and proteoglycan abnormalities in the GDF-5-deficient mice and molecular changes when treating disk cells with recombinant growth factor*. Spine, 2004. **29**(20): p. 2229-34.

Behaviour of human disc cells in collagen and fibrin-based matrix

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INTRODUCTION: The intervertebral disc (IVD) presents a very limited self-repair ability and cell-based therapy has been suggested as a challenging preventive and/or conservative strategy to treat this damaged tissue[1]. Fibrin-based scaffolds could be promising candidates as cell carriers, able to adapt to the lesion shape and to support/retain the injected cells in the desired location[2], with potential application in IVD tissue engineering[3,4]. We evaluated the behavior of nucleus pulposus (NP) and annulus fibrosus (AF) cells in a clinically employed hemostatic collagen-enriched fibrin matrix that has never been tested before for orthopedic applications.

METHODS: Human NP and AF cells were isolated and expanded, dispersed in fibrin gel with or without collagen granules (Fibrin/Collagen) and cultured *in vitro* for 24 hours (T1). The cell-loaded constructs were implanted subcutaneously in athymic nude mice for 28 days (T2). At T1 and T2, quantitative gene expression of chondrogenic markers, glycosaminoglycans (GAGs) quantification, Safranin-O staining and immunohistochemistry for collagen I and II were performed.

RESULTS: A short period of *in vitro* culture within the collagen-enriched fibrin gels was not sufficient to promote the synthetic activity of disc cells. After *in vivo* implantation in mice subcutaneous tissue, human IVD cells were still present in the explants and produced chondrogenic matrix, showing a significantly higher GAGs content and collagen I and II expression in comparison with cells cultured *in vitro* for 24 hours (Figure 1, T1 vs T2). Both fibrin gel, with or without collagen granules, seemed to be suitable materials for AF cells, since a homogeneous synthesis of collagen type I, similar in composition to the native fibro-cartilaginous tissue, was observed. On the contrary, fibrin alone seemed a suitable matrix for the culture of NP cells, since a homogeneous deposition of both glycosaminoglycans and collagen II was observed in the explants.

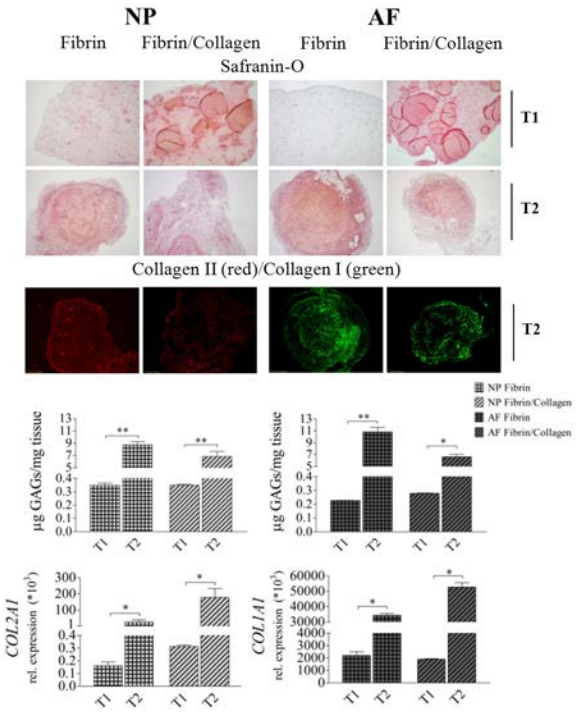

Fig. 1: Safranin-O staining and immunohistochemistry for collagen II and I, GAGs production, COL2A1 and COL1A1 expression.

DISCUSSION & CONCLUSIONS: These results suggest to combine AF cells with fibrin enriched or not with collagen granules and NP cells with fibrin alone to promote the maintenance of the typical features of this different kind of cells in three-dimensional culture, indicating that these clinically-employed materials could represent a viable option for the treatment of IVD lesions.

REFERENCES: [1] R. Kandel, S. Roberts, and J.P. Urban (2008) *Eur Spine J* **17**:480-91. [2] P.B. Malafaya, G.A. Silva, and R.L. Reis (2007) *Adv Drug Deliv Rev* **59**:207-33. [3] H.E. Gruber, K. Leslie, J. Ingram, et al (2004) *Spine J* **4**:44-55. [4] S.H. Yang, C.C. Wu, T.T. Shih, et al (2008) *Artif Organs* **32**:70-3.

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Retinoid acid receptor signaling during mesenchymal stromal cell chondrogenesis: differential sensitivity of chondral versus endochondral pathways

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INTRODUCTION: During growth plate chondrogenesis, retinoid signaling inhibits maturation of prechondrocytes and enhances endochondral ossification. Antagonists of the retinoid acid receptors (RAR) might therefore be attractive factors to improve chondrogenic differentiation of mesenchymal stromal cells (MSCs). Aim of this study was to investigate whether the synthetic RARβ antagonist LE135 is able to drive in vitro chondrogenesis of human MSCs or improve differentiation by suppressing hypertrophic chondrocyte development.

METHODS: Chondrogenesis of human MSCs was induced in micromass pellet culture for 6 weeks. Effects of LE135 alone and in combinatorial treatment with transforming growth factor (TGF)-β on deposition of cartilaginous matrix including collagen type II and glycosamino-glycans, on deposition of non-hyaline cartilage collagens type I and X, and on hypertrophy markers including alkaline phosphatase (ALP), indian hedghehog (IHH) and matrix metallo-proteinase (MMP)-13 were assessed.

RESULTS: LE135 failed to stimulate deposition of collagen type II and glycosaminoglycans. Moreover, addition of LE135 to TGF-β-treated pellets strongly inhibited cartilaginous matrix deposition and gene expression of COL2A1 even at a low concentration. In contrast, even a high LE135 concentration was not sufficient to completely block COL1A1 and COL10A1 gene expression, indicating that these non-hyaline cartilage collagens were less sensitive to LE135. Interestingly, cell proliferation was active under treatment with a high LE135 concentration. Moreover, LE135 had only little influence on terminal and osteogenic differentiation markers (IHH, MMP13, ALP).

DISCUSSION & CONCLUSIONS: These data show that LE135 treatment is inadvisable during standard protocols of MSC chondrogenesis in vitro, because it strongly blocks phenotypic differentiation of the cells and deposition of a cartilaginous matrix.

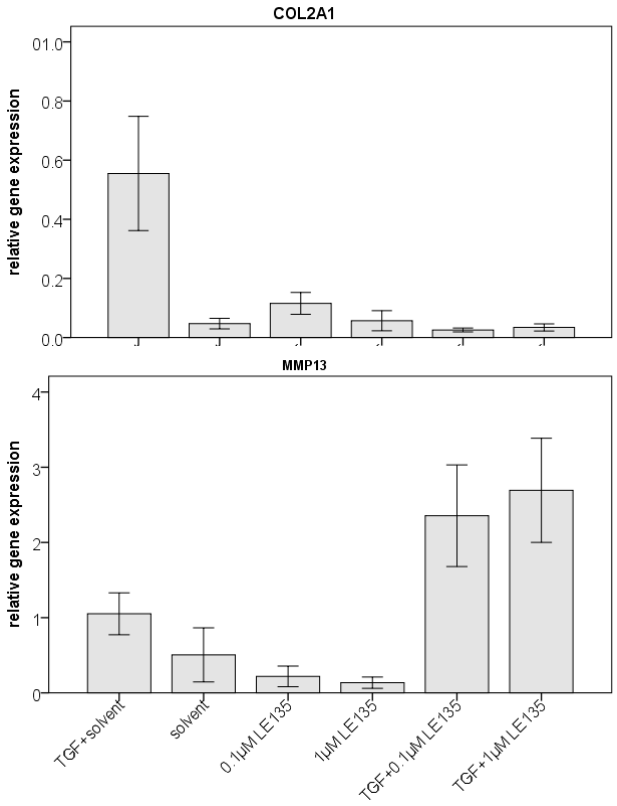

Fig. 1: Micromass pellet cultures consisting of 500,000 MSCs were treated for 6 weeks with LE135 (0.1 or 1 µM) and TGF-β. Mean relative gene expression +/- SEM for COL2A1 (top) and MMP13 (bottom) is given for n=7 MSC donors.

Notably, LE135 affected matrix deposition and endochondral differentiation with deviating efficiency, suggesting that only selected TGF-β effects are sensitive to RARβ while others are not. These results are highly interesting, because uncoupling the induction of the desired deposition of cartilaginous matrix from the concomitant induction of undesired collagen types and hypertrophic differentiation is a yet to overcome challenge for MSC chondrogenesis in vitro.

ACKNOWLEDGEMENTS: This study was supported by the German Research Foundation (Deutsche Forschungsgemeinschaft DFG, RI707/71).

Biomechanics of the degenerating human intervertebral disc: a 10-day axial loading study.

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INTRODUCTION: Although the changing biomechanical properties of intervertebral discs (IVDs) are believed to be a major cause of low-back pain[1], it is still not abundantly explored what biomechanical parameters change in several stages of IVD degeneration. The objective of this study was to provide insight in the biomechanical changes during IVD degeneration by applying a simulated physiological axial loading for 10 days in human spine segments at a range of degeneration levels.

METHODS: after obtaining MRI T2-weighted images, lumbar IVDs were harvested from 12 fresh-frozen human cadaveric spines. With parallel cuts through the vertebral bodies, the IVDs were isolated with intact endplates. After removing the posterior elements, the IVDs were implemented in a Loaded Disc Culture System and axially loaded for 10 days. A diurnal regime with 16 hours of high dynamic pressures of 370 +/- 130 kPa followed by 8 hours of low dynamic pressure of 73 +/- 10 kPa were applied on the endplate. Disc height was monitored to give information about the elastic and viscous properties of the IVD.

RESULTS*:*

As can be seen in the typical examples shown in Figure 1, clear differences between discs are seen in the biomechanical data. The more severely degenerated discs show a large collapse at the start of the experiment. After day one it can be seen that the instantaneous, elastic properties of the disc are similar to the mildly degenerated disc. However, the slow, viscous properties greatly differ, both within days and over days. After 4 days, the mildly degenerated disc comes to a steady state. The severely degenerated disc continues to subside for 10 days.

DISCUSSION & CONCLUSIONS:

The results show that IVD degeneration mostly effects the viscous properties of the IVD, rather than the elastic properties. Although MRI grading methods by Griffith[2] and Pfirrmann[3] are predictive for some of the biomechanical parameters, great variety within similar-graded IVDs was observed, which may partly explain the lack of direct relation between MRI degeneration grading and low-back pain.

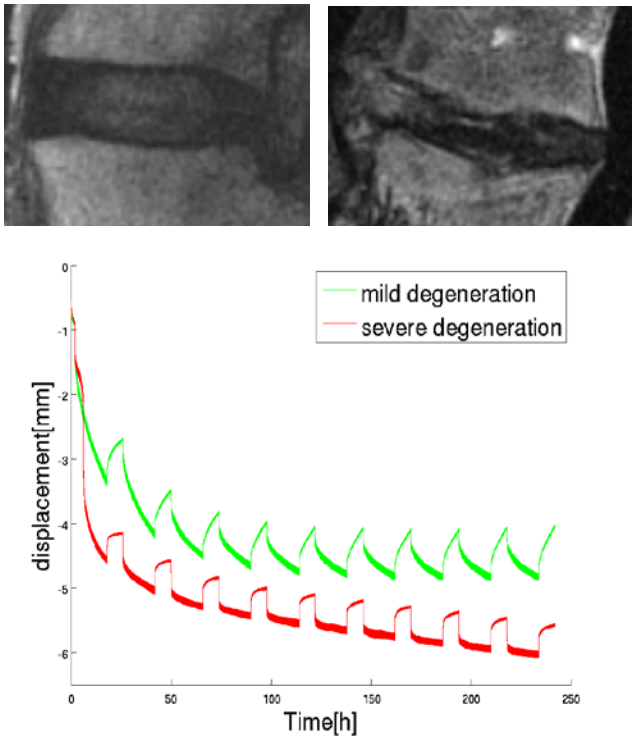

Figure 1. Typical example of disc height changes over 10 days of loading, showing differential behaviour within days and over days between a mildly degenerated and a severely degenerated disc.

REFERENCES: [1]M. Adams (2004) Biomechanics of back pain, *Acupunct Med* **22**:178-188 [2]J. Griffith, YX. Wang, G. Antonio et al. (2007), *Spine* **24:**708-712 3 C. Pfirrmann, A. Metzdorf, M. Zanetti et al. (2001) *Spine* **26:1873-8**

Articular cartilage contains a nestin positive stem cell population

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INTRODUCTION: Stem cells co-ordinate tissue homeostasis and, if required, facilitate a reparative response. The discovery of tissue-specific stem cells within articular cartilage has important implications for understanding tissue maintenance, degeneration and endogenous repair[1]. The challenge we face is to examine the quantity, location and function of stem cells in healthy and diseased tissue with respect to replicative and regenerative capacities.

Nestin, an intermediate filament protein, is widely utilised as a marker of proliferating and migrating adult stem cells and in previous studies across multiple tissue types is up-regulated in response to injury or pathology[2, 3]. The aim of this study was to determine if tissue specific adult stem cells derived from human articular cartilage express nestin, and if so, whether its expression in these cells is affected by osteoarthritis.

METHODS: Stem cell numbers in normal and osteoarthritic cartilage were quantified using colony forming assays. Cellular growth kinetics were assessed through population doublings and BrdU labelling. Stem cell phenotype was confirmed through qPCR and immunocytochemical detection of nestin.

RESULTS: There was a ~2-fold increase in percentage of colony forming cells derived from OA cartilage when compared to normal cartilage. Colony forming cells were positive for both gene and protein expression of the adult stem cell marker nestin. Normal and OA stem cell lines exhibit a 7.8 fold greater level of *NESTIN* gene expression than chondrocytes. Growth kinetics showed both OA and normal stem cell lines have capacity for extended culture, greater than that of differentiated chondrocytes; however OA-derived stem cell lines diverge into two sub-populations, early senescing and later-senescing that could be distinguished on the basis of proliferative kinetics. The duration of proliferative culture significantly correlates to *NESTIN* gene expression ($P=4.8x10^{-5}$)

DISCUSSION & CONCLUSIONS: Our data show that OA cartilage significant numbers of adult stem cells and that these cells are composed of early and late senescing sub-populations. These

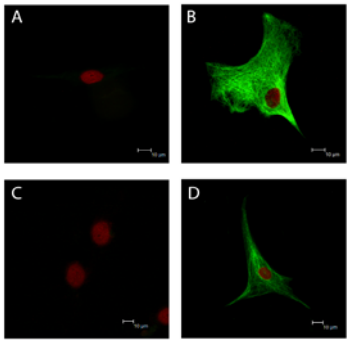

Figure 1 *– Immunocytochemistry for* NESTIN *protein expression. Cytoskeletal labelling in normal and OA cartilage stem cells* ***(B, D)****. No labelling full depth chondrocytes for either normal or OA cartilage* ***(A, C)****.*

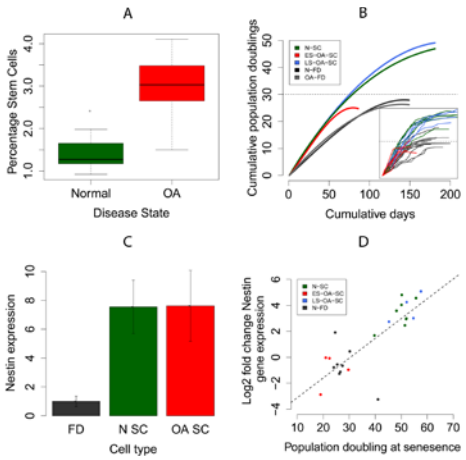

Figure 2 *– Percentage stem cells in normal and OA cartilage* ***(A)****. Growth kinetics for normal and OA stem cell (N-SC, OA-SC) and full depth chondrocyte cell lines (N-FD, OA-FD); OA contains two subpopulations, early senescing (ES-OA-SC) and late senescing (LS-OA-SC)* ***(B).*** *NESTIN gene expression in full depth chondrocytes and stem cell lines* ***(C)****, NESTIN gene expression correlates to proliferative longevity* ***(D).***

stem cells express the widely established stem cell intermediate filament nestin, with gene expression levels correlating to the proliferative potential of the cell line. From the perspective of endogenous repair capacity of OA cartilage, it is encouraging to note that a viable adult stem cell population is present in significant quantity. For autologous therapies it is hopeful that cell line proliferative potency can be screened using nestin expression improving therapeutic efficacy.

REFERENCES: [1] R.Williams et al (2010), *PLoS ONE* **5**(10):e13246. [2] J.E. Eriksson, et al (1992) (0955-0674). [3] K.Michalczyk, M. Ziman (2005) (0213-3911).

Comparing the secretomes of unstimulated and mechanically loaded MSCs

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INTRODUCTION: The chondrogenic differentiation of human mesenchymal stem cells (hMSCs) was first described in a high density pellet culture model in which the MSCs were exposed to 10ng/ml of transforming growth factor beta type 1 (TGF-β1) [1]. Based on this work the chondrogenic induction of MSCs has been performed in a number of 3D models, but always in the presence of an isoform (1, 2 or 3) of TGF-β. Work by our group has shown that the application of multiaxial mechanical load can lead to the induction of chondrogenesis in the absence of exogenous growth factors, as a result of the exogenous production of TGF-β1 by the stimulated MSCs. In order to investigate the role of other factors in this chondrogenic induction secretome analysis was performed on media collected from both loaded and non-loaded control samples. Analysis then focused on the relative levels of different factors between the media of loaded and control samples.

METHODS: Human bone marrow MSCs were isolated from marrow aspirates with full ethical approval. Following isolation MSCs were expanded and seeded into fibrin-polyurethane scaffolds as previously described [2].

Following seeding, scaffolds were cultured in a 'chondropermissive media' without any exogenous growth factors: serum-free DMEM High Glucose (4.5g/l), 1% penicillin-streptomycin, Ascorbic Acid (50μg/ml), Dexamethasone ($1x10^{-7}$M), 1% ITS+1, 1% NEAA and ε-aminocarproic acid (5μM). Mechanical load (10-20% compression and of rotation of ±25°, both at a frequency of 1Hz) was applied for one hour a day, five days a week over four consecutive weeks, a total of 20 loading sessions per scaffold, controls were kept in free swelling culture. Culture media was changed and collected three times a week. Secretome analysis was performed using the RayBio® Human Cytokine Antibody Array G-Series 2000 (Cat# AA-CYT-G2000-8) on media collected from a loaded scaffold after two weeks of loading and again after four weeks of loading, analysis was also performed on a control sample after four weeks of culture.

RESULTS: After four weeks of culture more factors were expressed at a higher level in loaded samples than in non-loaded controls (Table.1). In contrast a similar number of factors were upregulated after both two and four weeks of load (83 and 89 respectively). Of the factors that peaked at week 2 a greater number showed a ≥2-fold difference compared to week four than factors that peaked at week 4.

	Number of factors
Higher in Control at W4	44
≥2.0 Fold difference Control/Load W4	21
Higher in Load at W4	126
≥2.0 Fold difference Load/Control W4	51
No Change:	4

	Number of factors
Higher in Load at W2	83
≥2.0 fold difference W2 Load/W4 Load	33
Higher in Load W4	89
≥2.0 fold difference W4 Load/W2 Load	12
No Change:	2

Table 1. A comparison of the upregulation of factors in different groups.

DISCUSSION & CONCLUSIONS: These results show that the secretomes of loaded and non-loaded scaffolds differ over time in culture. Further work will allow for greater understanding of the role of the difference in secretome in MSC chondroinduction.

REFERENCES: [1]Johnstone B, Hering TM, Caplan AI, Goldberg VM, Yoo JU. In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp Cell Res 1998 Jan 10;238(1):265-72.[2]Li Z, Kupcsik L, Yao SJ, Alini M, Stoddart MJ. Chondrogenesis of human bone marrow mesenchymal stem cells in fibrin-polyurethane composites. Tissue Eng Part A 2009 Jul;15(7):1729-37.

ACKNOWLEDGEMENTS: The authors would like to thank David Eglin and Marcus Glarner for producing the poly-urethane sponges. The fibrin used in these experiments was generously provided by Baxter Biosurgery (Vienna, Austria).

Chitosan-based flock scaffolds for potential application in tissue engineering of articular cartilage

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INTRODUCTION: Electrostatic flocking is a method for the generation of scaffolds with high compressive strength and porosity [1]. Short fibers are applied vertically on a membrane-like substrate, covered with an adhesive layer, forming a highly anisotropic and porous structure. Those properties are beneficial for the application in cartilage engineering [2]. One critical point in this process is the surface modification of fibers to enable a suitable electric conductivity which is prerequisite for the alignment in the electrostatic field during flocking. This was done successfully with non resorbable polyamide fibers first [1]. In the current work, we have developed a process for flocking of fully resorbable chitosan fibers and present first *in vitro* experiments.

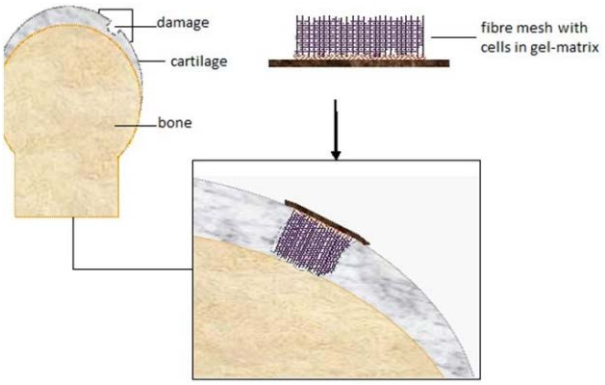

Fig. 1: Concept of articular cartilage repair by flock-scaffold-based bioconstructs, manufactured from chitosan. The flocking of wet-spun chitosan-fibers leads to scaffolds with interconnecting pores of defined and scalable size. Cells in a gel matrix are embedded in these pores [2].

METHODS: Chitosan fibers were produced by a wet-spinning process. A chemical treatment enabled the electrostatic charge. After cutting, fibers were flocked on a chitosan membrane coated with a viscous chitosan solution as adhesive.

Human mesenchymal stem cells (hMSC) from bone marrow were cultivated directly and indirectly with the chitosan scaffolds. Activity of cytosolic lactate dehydrogenase (LDH) was determined to evaluate the cytocompatibility. Examination of cell adhesion and morphology was performed by confocal laser scanning microscopy after DAPI/phalloidin-staining of the cells.

RESULTS: The chemically treated chitosan fibers were successfully utilized in the flocking process. Chitosan solubilized in hydrochloric acid turned out to be a good adhesive to bond the fibers to the chitosan membrane. The resulting highly anisotropic structure and the parallel aligned fibres led to high mechanical strength despite the high porosity of the scaffolds. The indirect and direct cultivation of hMSC did not reveal any cytotoxic effects of both the chitosan material and the chemicals used for the treatment in this process.

Microscopic evaluation revealed the attachment of hMSC to the chitosan fibers.

DISCUSSION & CONCLUSIONS: This is the first demonstration of a fully resorbable scaffold manufactured by electrostatic flocking. Substrate, fibers and adhesive are composed of chitosan, which has been widely used for tissue engineering applications due to its biodegradability and biocompatibility [3].

Further experiments will involve the infiltration of the flocked scaffolds with different hydrogels and simultaneous embedding of mesenchymal stem cells or chondrocytes to obtain functional cell-matrix constructs for the treatment of articular cartilage defects.

REFERENCES: [1] A. Walther, B. Hoyer, A. Springer, et al. (2012) *Materials* **5** 540-557. [2] E. Steck, H. Bertram, A. Walther, et al. (2010) *Tissue Eng Part A* **16** 3697-3707. [3] T. Kean and M. Thanou (2010) *Adv Drug Deliv Rev* **62** 3-11.

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Development of a whole bovine long-term organ culture system that retains vertebral bone for intervertebral disc repair and biomechanical studies

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INTRODUCTION: Degenerative intervertebral disc disease is a common cause of low back pain. Testing potential therapeutics in the regeneration of the disc requires the use of model systems. Although several animal models have been developed to test intervertebral disc (IVD) regeneration, application becomes costly when used as a screening method. The bovine IVD organ culture system offers an inexpensive alternative, however, in the current paradigm, the bony vertebrae is removed to allow for nutrient diffusion to disc cells. This provides limitations on the conditions and strategies one can employ in investigating IVD regeneration and mechanisms in degenerative disc disease (i.e. combination of axial and torsional loading). Although one method has been attempted to extend the survival of bovine vertebrae containing IVDs (vIVD) cell viability declined after two weeks in culture. Our goal was to develop and validate a long-term organ culture model with vertebral bone, which could be used subsequently for studying biological repair of disc degeneration and biomechanics.

METHODS:

Preparation of vIVDs: Tails of 22- to 28-month-old steers were obtained from the local abattoir within 4 hrs of slaughter. The largest 4 IVDs were prepared for organ culture by parallel cuts through the adjacent vertebral bodies at 1cm from the endplates using a IsoMet®1000 precision sectioning saw (Buehler, Germany). Eight vIVDs were incubated for one hour in Disc Isolation Solution (DIS – under patent) and eight were incubated in DMEM. All discs (referred to as vIVDs w and w/o DIS) were cultured up to 5 months in sterile vented 60ml Leakbuster™ Specimen Containers (Starplex) in 3mL/g tissue of medium containing high glucose DMEM, L-glutamine (2mM), penicillin-streptomycin (1x), ascorbic acid (50µg/ml) and heat inactivated FBS (10%) and with no external load applied.

Live/Dead Assay: vIVDs cultured for 1 or 5 months were dissected to separate the NP and inner AF (iAF) and outer AF (oAF) regions. A 4mm biopsy punch was used to prepare specimens for cell viability using a live/dead fluorescence assay (Live/Dead®, Invitrogen) and visualized by confocal microscopy.

Glucose Diffusion: vIVDs that were prepared with or without DIS were cultured for 1 month. Discs were incubated for 72hrs in diffusion medium containing PBS (1x), $CaCl_2$ (1mM), $MgCl_2$ (0.5mM), KCl_2 (5mM), 0.1% BSA and 150µM 2-[N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NDBG), a D-glucose fluorescent analogue. Discs were dissected and regions (NP, iAF, oAF) were incubated in extraction buffer (4M guanidinium chloride, 0.1M Tris HCl, pH 7.5). Extracts were measured for fluorescence using a FLUOstar OPTIMA (BMG Labtech) spectro-photometer.

RESULTS: After 5 months of organ culture, vIVDs prepared with DIS demonstrated approximately 95% cell viability in all regions of the disc. However, dramatic reductions in vIVD viability were measured in discs w/o DIS (~5%) after 1 month. Interestingly, vIVD viability was related to the amount of 2-NDBG incorporated into the disc tissue.

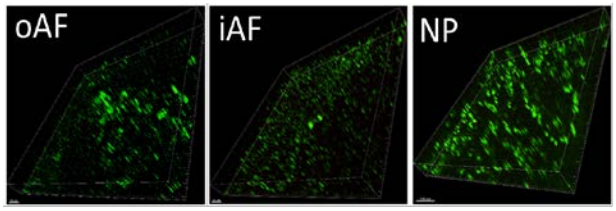

Fig 1: ***Cell viability in vIVDs treated w DIS after 5 months in culture*** *measured using a live/dead fluorescence assay and visualized by confocal microscopy (live - green and dead – red).*

DISCUSSION & CONCLUSIONS: We have developed a novel method for isolating IVDs with vertebral bone capable of long-term viability. This method may not only help in the discovery of novel therapeutics in disc regeneration, but could also advance our understanding of complex loading paradigms in disc degeneration and repair.

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3D printing from biodegradable polyurethane for tissue engineering applications

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INTRODUCTION: Three-dimensional (3D) printing is a rapid prototyping and fabrication technology where 3D solid objects are made by layer-by-layer deposition through computer-aided design (CAD). Tissue engineering scaffolds can be custom-made from 3D printing. During the manufacturing process, the use of a toxic photo-initiator, heat, or organic solvent is often inevitable.[1] Biodegradable polymers such as polylactide and polyglycolide are commonly used to fabricate scaffolds. Nevertheless, these materials are in lack of the proper elasticity that matches to that of living tissue.[2] In this study, a novel biodegradable waterborne polyurethane (PU) was synthesized in the form of nanoparticles (NPs). We then employed a 3D printing strategy to fabricate elastic scaffolds from the water dispersion of PU. We attempted to establish a green and sustainable 3D printing platform to derive non-toxic and highly elastic scaffolds for customized tissue engineering applications.

METHODS: The biodegradable PU elastomers were synthesized from a water-based process. The soft segment consisted of two oligodiols, i.e. poly(ε-polycaprolactone) diol and polyethylene butylene adipate diol. The hard segment was isophorone diisocyanate, 2,2-bis(hydroxymethyl) propionic acid and ethylenediamine. 3D scaffolds were printed from a feed containing PU NP dispersion and polyethylene oxide (PEO). The rheological properties of PU/PEO solutions were determined by a rheometer. The mechanical properties and degradation rate of the scaffolds were analyzed. Chondrocytes were seeded in the scaffolds to evaluate the potential in cartilage tissue engineering.

RESULTS: Scaffolds were successfully fabricated by the 3D printing process from the dispersion of PU NPs (Fig. 1). The elastic recovery of PU scaffolds was significantly better than that of polylactic-co-glycolic acid (PLGA) scaffolds made from the solution in organic solvent. The compressive modulus was close to that of native cartilage. The seeding efficiency, proliferation, and matrix production of chondrocytes in PU scaffolds after 7 and 14 days (Fig. 2) were superior to those in PLGA scaffolds.

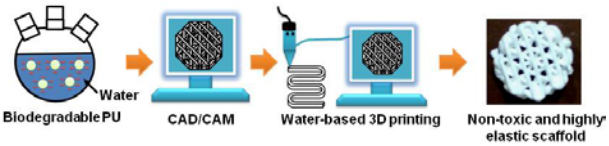

Fig. 1: Flow chart for the fabrication of non-toxic and elastic PU scaffolds by the water-based 3D printing.

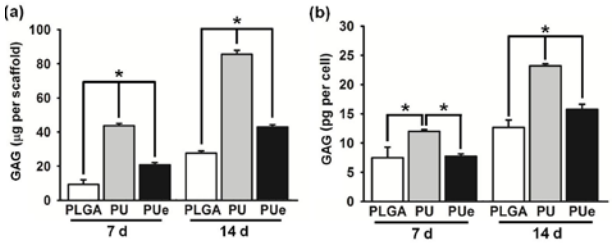

*Fig. 2: The matrix production of chondrocytes in 3D-printed scaffolds. a) Total glycosaminoglycan (GAG) contents, and b) the GAG contents normalized to cell number in various scaffolds after 7 and 14 d of culture. *p < 0.05 among the indicated groups.*

DISCUSSION & CONCLUSIONS: Elastic biodegradable scaffolds were fabricated by 3D printing. Not any toxic organic solvent, crosslinker, or initiator was used. The environment-friendly process generated a highly elastic scaffold with good affinity to cells. The green and sustainable 3D printing platform offers a useful way to fabricate biodegradable/elastic scaffolds for tissue engineering applications.

REFERENCES: [1] W.-Y. Yeong, C.-K. Chua, K.-F. Leong, et al. (2004) *Trends Biotechnol* **22**:643-52. [2] Q. Chen, S. Liang, G.A. Thouas (2013) *Prog Polym Sci* **38**: 584-671.

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Microencapsulation of bone marrow derived stem cells using electrohydrodynamic spraying for minimally invasive tissue repair of the IVD

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INTRODUCTION: Biomaterial cell-based microencapsulation may be a promising approach to repair damaged tissue in a minimally invasive manner [1]. One such method to microencapsulate cells is through the use of electrohydrodynamic (EHD) spraying. This technique generates micron sized spheres by spraying and subsequent crosslinking of a polymer/cell solution through a needle connected to a high voltage generator [2]. Bone marrow-derived stem cells (BMSCs) are actively being explored as an alternative cell source for IVD regeneration. In this study BMSCs were encapsulated in alginate microspheres using EHD spraying and assessed in isolation at different seeding densities (***Study 1***) and in co-culture with nucleus pulposus (NP) cells (***Study 2***).

METHODS: ***Study 1:*** BMSCs were combined with 1% alginate at varying seeding densities $4x10^6$, $8x10^6$ and $12x10^6$cells/ml. The biomaterial/cell suspension was electrosprayed and ionically crosslinked with 102mM calcium chloride producing microspheres of diameter 135.7±53.37µm. Microspheres were evaluated in terms of cell viability after 24 hrs, 21 and 35 days of culture in hypoxia. ***Study 2:*** NP cells ($4x10^6$ cells/ml) were co-cultured with BMSCs encapsulated either in microspheres or in a mixed co-culture at a 1:1 ratio in cylindrical alginate constructs (3Hx5Ø). All cultures were maintained in low oxygen (5%) and low glucose conditions.

RESULTS: ***Study 1:*** Live/Dead staining revealed that BMSCs encapsulated in alginate microspheres remained viable after 21 days for all seeding densities investigated (Fig 1A). Furthermore with increased culture time (day 35) aggregation of microspheres was observed whilst viability was also maintained (Fig 1B). ***Study 2:*** BMSCs co-cultured in microsphere form with NP cells maintained higher viability compared to mixed cultures (Fig 2A, B) with higher DNA observed by day 21 (Fig 2C). No significant differences were observed for sGAG or collagen biochemical content (Fig 2C).

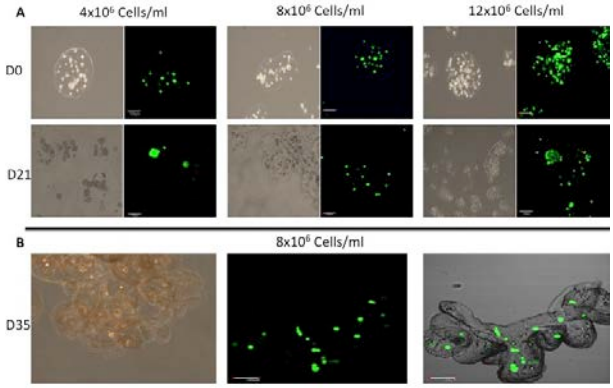

Fig. 1- Study 1: Microscopic images and Live/Dead staining A) Day 0 and 21 at seeding densities $4x10^6$, $8x10^6$ and $12x10^6$ cells/ml B) Aggregation of microspheres was observed by Day 3.

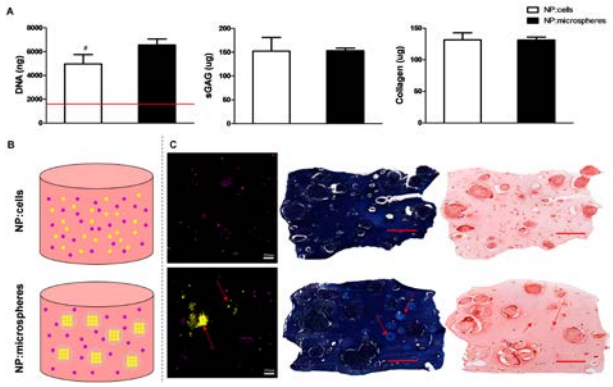

Fig. 2- Study 2: A) Schematic representation of two configurations; magenta = NP cells, yellow = BMSCs B) Confocal microscopy of labeled cells and histology at day 21; Red arrows = microspheres; Scale bar = 1mm C) Biochemical content of NP: Cells and NP: Microspheres at day 21; # indicates $p<0.05$; Red line = Day 0.

DISCUSSION & CONCLUSIONS: BMSCs remained viable after encapsulation using EHD and in a co-culture system; higher BMSC viability was observed when cultured in microsphere form. Taken together, these findings illustrate the potential of EHD as a versatile and effective method to microencapsulate cells for minimally invasive tissue repair.

REFERENCES: [1] Wang L. et al. Cells Tissues Organs, 197; 333, 2013 [2] Gasperini L. et al. Journal of Bioactive and Compatible Polymers, 28;413, 2013.
ACKNOWLEDGEMENTS: Supported by the Graduate Research Engineering Programme, PRTLI Cycle 5 and MSc Bioengineering Programme, TCD.

studies on electrical hysteresis in bovine cartilages

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INTRODUCTION: The aim of this paper is to investigate the electrical behaviour of bovine cartilage samples. In mammals cartilage is gristly, flexible tissue usually found attached to, or associated with the joint surfaces of bones or forming attachments between certain bones. It is the substance from which the majority of the bones of the skeleton develop [1]. The voltage-current characteristic of soft and hard tissues has not been studied in detail by the researchers [2], so the present investigation is focused on voltage-current characteristics, of bovine cartilage in different physiological conditions in order to understand mechanisms involved in the electrical conduction processes in different cartilages and to develop new low cost cartilage repair techniques.

METHODS: In the present investigation we studied 20 bovine cartilage samples in different physiological conditions (fresh and oven dried). We measure the current (NADC) as a function of voltage in the interval of the 10 volts to 300 volts, both in increasing and decreasing order[3].

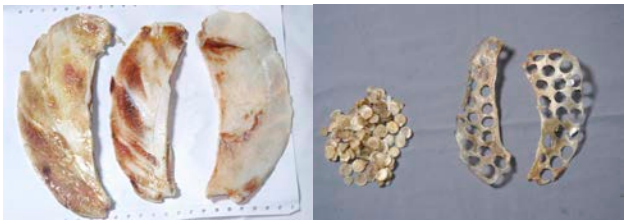
RESULTS: The characteristics voltage-current curve shows a hysteresis loop. The area within the loop represents electrical energy stored in the material per unit volume per cycle. Voltage measurements should be considered perturbative with respect to electrical response of the samples.

Table 1 The energy stored in bovine cartilages in different physiological conditions.

	Fresh bovine cartilages	Oven dried bovine cartilage
Energy stored	1.93±1.4 to 5.9± 2.1 μ J $cm^{-3}cycle^{-1}$	0.291± 0.17 to 1.917±0.76 μ J $cm^{-3}cycle^{-1}$

DISCUSSION & CONCLUSIONS: From the voltage-current characteristics of cartilage it is obvious that fresh cartilage is non-ohmic, while oven dried cartilage exhibit ohmic behaviour. The energy stored in fresh bovine cartilages calculated by electrical hysteresis loops (V-I characteristics) is in the range 1.93±1.4 to 5.9± 2.1 μ J $cm^{-3}cycle^{-1}$ and 0.291± 0.17 to 1.917±0.76 μ J $cm^{-3}cycle^{-1}$ for oven dried bovine cartilages. Significant variation was observed. This large variation may be due to water content. Water plays a key role in the physics of cartilage. Since cartilage is gristly, flexible tissue of chondrocytes embedded in a dense mucoprotien matrix, usually found attached to, associated with, the joint surfaces of bones or forming attachments between certain bones, its electrical properties are complex. This paper constitutes a step towards the application of electrical hysteresis for in vivo cartilage diagnosis.

Fig. 1: Images of bovine cartilages in fresh and oven dried condition and samples were cut in the shape of pellets.



REFERENCES: [1] Bourne, G.H. (1972) *The Biochemistry and Physiology of Bone*, New York: Academic.[2]Aschereo.G, Romano.S.M, Gizdulich.P (1995) *Evidence of electrical hysteresis in bones*, Electro and magneto biology, 14(3), pp 199-215. [3]A Ph.D thesis: Dr. Syed Mohammed Shoaib, (2008) *Studies on ferroelectric properties of animal bone*.

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Transplantation of bone marrow derived mesenchymal stem cells embedded in poly(trimethylene carbonate) scaffold: mechanical and biological repair of ruptured annulus fibrosus

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INTRODUCTION: Recurrent intervertebral disc herniation and degenerative disc disease have been identified to be the most important factors contributing to increased pain and disability after surgical discectomy. An annulus fibrosus (AF) closure device which provides immediate closure of the AF rupture and restores the mechanical properties of discs is an unmet clinical need. Furthermore, cellular and biochemical components should be preserved to prevent further disc degeneration and enhance self-repair capacities. In this study, designed poly(trimethylene carbonate) (PTMC)-based scaffolds seeded with bone marrow derived mesenchymal stem cells (MSCs) were assessed in an organ culture model for AF rupture repair.

METHODS: PTMC scaffolds with AF mimicking pore architecture were prepared by stereolithography in the shape of a truncated cone (diameter 2 and 3 mm, length 4 mm, pore channel size 450 μm). Bovine caudal discs were dissected from 15 animals (6-9 months old). A defect was created through the AF (diameter 2 mm, depth 7 mm), and then refilled with PTMC scaffolds. Scaffolds were infused with either fibrin gel only or fibrin gel containing 0.2×10^6 human bone marrow derived MSCs. The scaffolds were covered with poly(ester-urethane) (PU) membrane which was sutured to the adjacent AF tissue. After 1 week of pre-culture, sinusoidal dynamic load was applied on discs (3 hours/day at 0-0.1 MPa, 0.1 Hz) for another week. Non-loaded discs served as control. Analyses include histology, disc height measurement, and gene expression measurement of implanted MSCs and host disc cells.

RESULTS: Safranin O/Fast Green stained sections of discs revealed that PTMC scaffolds and PU membrane were able to withstand the dynamic loading applied on the discs. PTMC scaffolds remained stable within the AF defect and no protrusion of nucleus pulposus (NP) was observed. Disc heights of discs treated with PTMC scaffolds were increased compared with defect only negative control, and comparable with intact disc positive control both after dynamic loading and after free swelling recovery.

Type V collagen (potential AF marker) gene expression of MSCs increased 2 fold in unloaded discs and 2.7 fold in loaded discs compared with cells before seeding into discs.

Compared with unloaded non MSC implanted discs, implantation of MSCs, dynamic load, and combination of both all showed a trend of up-regulation on COL1, COL2 and ACAN gene expression in the AF tissue next to the PTMC scaffold. The combination of MSC implantation and dynamic load significantly down-regulated MMP13 gene expression ($p<0.001$). In AF tissue opposite the PTMC scaffold, dynamic load up-regulated ACAN gene expression ($p<0.05$), and down-regulated MMP13 gene expression ($p<0.05$).

DISCUSSION & CONCLUSIONS: Designed PTMC implants combined with sutured PU membrane restored part of the mechanical properties of annulotomized discs, and prevented protrusion of NP, hence may be suitable as an AF rupture repair device. Implanted MSCs obtained partially the phenotype of AF cells after 2 weeks of culture in discs. In addition, MSCs may prevent further degeneration of native disc tissue by up-regulating anabolic gene expression and down-regulating catabolic gene expression in host disc cells.

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Hypoxia mimicking glasses for osteochondral tissue engineering

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INTRODUCTION: Replacement of damaged cartilage with osteochondral scaffolds may provide a promising treatment approach for osteoarthritis, a degenerative joint disease affecting both cartilage and the underlying subchondral bone. Bioactive glasses (BGs) are smart, tailorable materials that have been shown to promote bone formation *in vitro* and to release bioactive ions[1]. Hence BGs are attractive candidates for the bone substrate of osteochondral scaffolds. Cobalt is known to mimic hypoxia by activating the HIF-1 pathway and thus may be suitable for promoting cartilage formation[2]. Hereby, the influence of ions released from cobalt containing BGs on human mesenchymal stem cells (hMSCs) in the context of osteochondral tissue engineering is evaluated.

METHODS: To determine the influence of cobalt on hMSCs, cobalt was incorporated in the glass composition $3SiO*0.07P_2O_5*(1.4-X)CaO*1.6Na_2O*X$ (X=CoO) by substitution with calcium (0%, 1%, 1.5% and 2mol% substitution)[3]. Bioactive glass conditioned medium was obtained by incubating 1.5mg/ml of glass powder in DMEM medium for 4h. Ion release from the glasses into the medium was quantified by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Passage 3 bone marrow derived hMSCs were cultured either as pellets or on electrospun polycaprolactone (PCL) membranes in bioactive glass conditioned or control medium (DMEM) with chondrogenic supplements for up to 21 days. For DNA and GAG quantification, samples were digested in Proteinase K. The DNA content was measured by Hoechst and the amount of GAG was quantified by 1,9-Dimethyl-Methylene Blue zinc chloride double salt (DMMB) respectively. Cell viability was measured by Alamar Blue.

RESULTS: ICP-OES analysis of bioactive glass conditioned media (0% CoBG, 1% CoBG, 1.5% CoBG and 2% CoBG) showed similar values for the silicon, calcium and phosphorous ion contents. The amount of cobalt increased corresponding to its molar content in the glasses and was similar to values in the therapeutic range. A cytotoxicity assay indicated that cell viability of hMSCs was not affected by the bioactive glass conditioned media after 24h. To evaluate if the behaviour of hMSCs seeded on PCL membranes was comparable to the well-established pellet culture, experiments using both techniques were carried out in parallel. Scaffold and pellet cultures showed similar trends in terms of GAG and DNA.

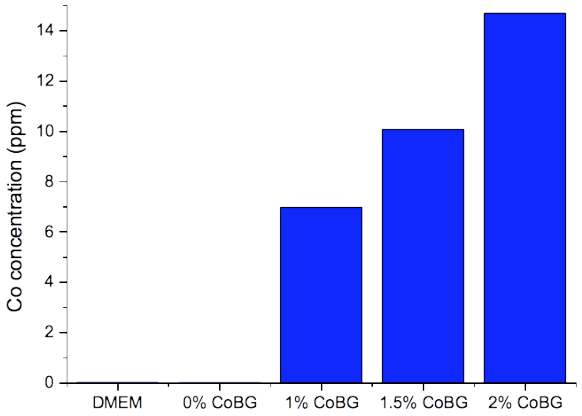

Figure 1: Cobalt ion release into DMEM increases corresponding to its molar content in the glasses.

DISCUSSION & CONCLUSIONS: Here, we evaluate the influence of ions released from cobalt containing glasses on hMSCs in the context of cartilage tissue engineering. Our data suggest that hMSCs cultured in bioactive glass conditioned medium showed a similar GAG/DNA profile on PCL membranes and in pellet culture. Further characterisation of key chondrogenic gene expression markers and *in vitro* tissue formation will provide more insight into the effect of cobalt bioactive glass conditioned media on chondrogenesis.

REFERENCES: [1]E Gentleman et al. (2010) Biomaterials 31(14):3949-56, [2]Liu et al. (2010) BBRC 401:509–515, [3]MM Azevedo (2010) J. Mater. Chem., 20:8854–8864

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Failure strength characterization of composite repair for annulus fibrosus injury

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INTRODUCTION: Lumbar microdiscectomy is an effective treatment for lower back pain related to intervertebral disc (IVD) herniation but the benefits of surgery are less clear after 10 years likely due to progressive IVD degeneration. A successful annulus fibrosus (AF) repair strategy could augment discectomy procedures by restoring biomechanical properties and preventing accelerated degeneration of the IVD.

METHODS: We evaluated a composite AF repair strategy consisting of a fibrin-genipin adhesive hydrogel (FibGen), a poly(ester urethane) protective membrane (8mm diameter, 0.12±0.03mm thick to prevent herniation) and two sizes of poly(trimethylene carbonate) (PTMC) scaffold (Small cylindrical: ∅5mm, 7mm length; Large conical: ∅4to6mm, 10mm length). Bovine IVDs were randomly distributed to 5 experimental groups: Intact (no injury), Injury (∅5mm punch), Small scaffold, Large scaffold and FibGen (no scaffold). Each specimen was subjected to 16 rounds of cyclic torsion increasing from ±0.5°to±19°. Torsional stiffness and torque range were calculated, and herniation was diagnosed with photographs.

RESULTS: FibGen herniated at significantly larger rotation angles than the Large and Small groups. The nominal axial compressive stress at herniation was not significantly different between repair groups and was in the physiological range.

Table 1. Herniation characterization: Maximum rotation, torque, and nominal axial stress at herniation. Maximum rotation angle of the FibGen group was significantly different than the Small and Large groups.

Group	n	Max Rotation Angle ± SD (°)	Max Torque ± SD (N*m)	Nominal Axial Stress ± SD (kPa)
Small	5	6.3± 2.2 *	3.1±0.8	-345.5±100.5
Large	6	5.2 ± 2.0 *	3.0±1.3	-320.14±122.6
FibGen	4	14.5 ± 6.2 *	8.1±6.8	-441.3±184.0

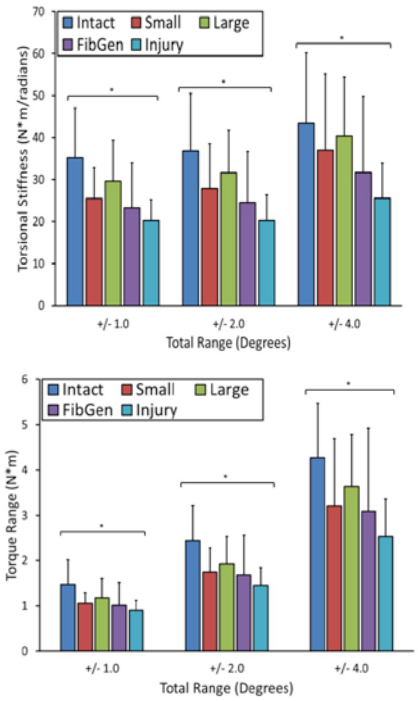

Fig. 1: (A) Torsional Stiffness & (B) Torque Range prior to herniation. Values for the Intact group were significantly larger than for the Injury group.

Torque range and torsional stiffness were greatest for Intact and lowest for Injury groups with repaired groups having values in between. The repair with the large scaffold had values that most closely approached Intact levels.

DISCUSSION & CONCLUSIONS: The composite repair strategies partially restored torsional stiffness and torque range to values approaching intact spinal segments but introduced a risk of herniation at high rotation angles. The FibGen repair reduced the risk of herniation at high ranges of motion but can be improved to better enhance the mechanical function of this repair strategy.

ACKNOWLEDGEMENTS: Funded by AO Foundation & NIH/NIAMS (R01AR057397). We thank Drs. Grijpma, Blanquer & Eglin of the AF Rupture Program for providing materials & helpful discussions.

Biophysical characterisation of articular cartilage-derived stem cells using atomic force microscopy

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INTRODUCTION: Osteoarthritis is the loss of articular cartilage in joints [1]. It is the largest single cause of physical disability in the world [2]. Treatments to reduce symptoms are limited in their success due to a lack of suitable cell sources to repair damaged tissue [3]. It is known that articular cartilage contains a population of stem cells capable of repairing damaged tissue [4], however, there are no biomarkers to allow their efficient isolation [5]. This study will therefore combine molecular biology and biophysics to characterise stem cell populations and identify biomarkers that define chondrogenic potential. Our previous work has shown stem cells can be activated by growth factors FGF2 and TGFβ1, and we used this information as the basis to understand, at single cell resolution, the biophysical changes that occur during growth and development.

METHODS: Colony-forming clonal stem cell populations were isolated from mature and immature bovine metacarophalangeal joint cartilage and expanded in presence and absence of FGF2 and TGFβ1. Atomic force microscopy (AFM) using the JPK (NanoWizard II) and Bruker (BioScope Catalyst) systems were used to obtain force values for individual cells and tissues.

RESULTS: Following biophysical characterisation of adult stem cells by AFM, we found decreased membrane stiffness from 0.49kPa to 0.24kPa in mature stem cells after FGF2 and TGFβ1 exposure, and this correlated with increased proliferation. Interestingly, we did not see this change in stem cells from immature tissue.

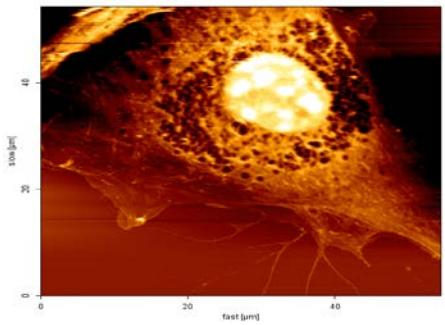

Figure 1. AFM scan image showing AFM cantilever positioned above a fixed bovine articular cartilage stem cell.

DISCUSSION & CONCLUSIONS: These data show that stem cells react to external stimuli in a developmentally encoded fashion, a phenomenon that has not been appreciated previously for this cell type. We will attempt to correlate biophysical changes following growth factor stimulation with changes in cytoskeletal architecture. Furthermore, the effect of growth factor stimulation on chondrocyte differentiation will be characterized through pellet culture of expanded cells. AFM has been useful in understanding stem cell phenotype in the absence of defined biological markers, it is hoped that this work will result in more effective cartilage repair strategies.

REFERENCES:

[1] G. Peat, R. McCarney, and P. Croft, “Knee pain and osteoarthritis in older adults: a review of community burden and current use of primary health care.,” *Ann. Rheum. Dis.*, vol. 60, no. 2, pp. 91–7, Feb. 2001.

[2] S. N. Redman, S. F. Oldfield, and C. W. Archer, “Current strategies for articular cartilage repair.,” *Eur. Cell. Mater.*, vol. 9, no. 0, pp. 23–32; discussion 23–32, Jan. 2005.

[3] G. P. Dowthwaite, J. C. Bishop, S. N. Redman, I. M. Khan, P. Rooney, D. J. R. Evans, L. Haughton, Z. Bayram, S. Boyer, B. Thomson, M. S. Wolfe, and C. W. Archer, “The surface of articular cartilage contains a progenitor cell population.,” *J. Cell Sci.*, vol. 117, no. Pt 6, pp. 889–97, Feb. 2004.

[4] A. J. Hayes, D. Tudor, M. A. Nowell, B. Caterson, and C. E. Hughes, “Chondroitin sulfate sulfation motifs as putative biomarkers for isolation of articular cartilage progenitor cells.,” *J. Histochem. Cytochem.*, vol. 56, no. 2, pp. 125–38, Feb. 2008.

ACKNOWLEDGEMENTS: This work was supported by the European Union Regional Development Fund (ERDF) and Progenteq.

The LINK to regeneration of human intervertebral discs

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INTRODUCTION: Back pain affects a large portion of the population across all ages and intervertebral disc (IVD) degeneration is its most common cause. Presently, there are no established treatments to prevent, stop or even retard disc degeneration, and surgery is often the offered option. Previous studies have shown that Link N can act as a growth factor and stimulate the synthesis of proteoglycans and collagens in bovine IVD *in vitro* and in intact human IVDs *ex vivo*, as well as increase disc height in a rabbit model of disc degeneration. However, the sequences in Link N involved in modulating cellular activity are not well understood. The aims of this study are to determine (1) the sequence specificity of Link N, (2) whether Link N is processed by disc cells and the resulting fragments retain biological activity, and (3) the identity of any proteinase involved.

METHODS: Human lumbar spines were retrieved through organ donation program of Transplant Quebec. Six spinal segments (mean age 30) were retrieved and IVD cells were isolated from nucleus pulposus (NP) and inner annulus fibrosus (iAF) regions. Isolated IVD cells embedded in 1.2% alginate were exposed to native, scrambled or reverse sequences of Link N and $^{35}SO_4$ incorporation was used to assess sequence specificity in promoting proteoglycan synthesis. To determine if disc cells can proteolytically process Link N, human disc cells in monolayer were exposed to native Link N over a 48 h period (Fig. 1) and fragmentation analyzed by mass spectrometry. The proteinase capable of fragmenting Link N was identified by incubating it with a variety of proteinases reported to be active in the degenerate IVD.

RESULTS: Mass spectrometric analysis revealed that a peptide spanning residues 1 to 8 was generated in the presence of AF cells but not NP cells. Analysis of proteases associated with disc degeneration showed that only cathepsin K was able to cleave this site. Link N 1-8 significantly induced proteoglycan production in bovine and human NP and AF cells, but Link N 9-16 did not, confirming that the biological activity is maintained within the first 8 amino acids of the peptide. A reversed or scrambled Link N peptidehad no biological effect.

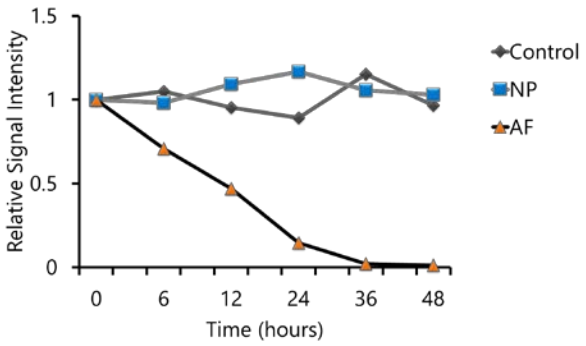

Fig. 1: **The proteolytic processing of Link N by human NP and AF cells.** AF cells have the ability to process the Link N peptide after 48 h of incubation.

DISCUSSION & CONCLUSIONS: Link N 1-8 peptide is a promising therapeutic alternative for treating early disc degeneration before major collagen degradation has occurred. One advantage in using this shorter 8 amino acid peptide rather than the original 16 amino acid Link N in therapy is the production cost. More importantly, this small size should be amenable to medicinal chemists allowing them to optimize the structure for maximal biological activity and stability.

ACKNOWLEDGEMENTS: This work would not have been possible without funding from AO Spine, the North American Spine Society (NASS), the Canadian Institutes of Health Research (CIHR), the Crafoord Foundation, and the Inga-Britt & Arne Lundberg Foundation.

Degradation study of mechanically-controlled drug delivery system for knee cartilage

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INTRODUCTION: It has been proposed that drug delivery (especially growth factors) may enhance the healing process of cartilage defects. Nevertheless, it is how these growth factors are delivered that holds the key for tissue regeneration. It has been shown that some growth factors' receptors on cells are load-sensitive. So a mechanically controlled drug delivery system is of great interest [1, 2].
Biodegradable hydrogels are proposed to be good drug carriers to keep and release drug long term. The main drawback of biodegradable hydrogels is their mechanical weakness, which limits their application at load bearing tissues like cartilage. Here we developed biodegradable and biocompatible HEMA hydrogels by copolymerizing with Dimethacryloyl hydroxylamine (DMHA), a hydrolyzable cross-linker. The obtained hydrogels present high mechanical strength and their hydrophobic property prevents high passive release, making them mechano-sensitive. Their degradation under mechanical loading is evaluated.

METHODS: DMHA was prepared by the reaction of hydroxylamine hydrochloride with methacryloyl chloride in pyridine[3]. PHEMA hydrogel cross-linked with 2% mol DMHA as cross linker and 62.5% aqueous phase or 10% mol DMHA and 40% aqueous phase were polymerized under UV for 15 min. We studied the degradation by incubating them in 5 ml PBS (pH=7.4) at 34°C. Thirty min cyclic compression load was applied on hydrogels once a week (15% deformation amplitude at 1 Hz). The elastic modulus and weight lose of hydrogels where measured every 4 weeks.

RESULTS: Figure 1 shows that the elastic modulus of hydrogels drops by about 60% after 4 months. For the non-mechanically stimulated hydrogels, the modulus drop was less marked (max 48%).

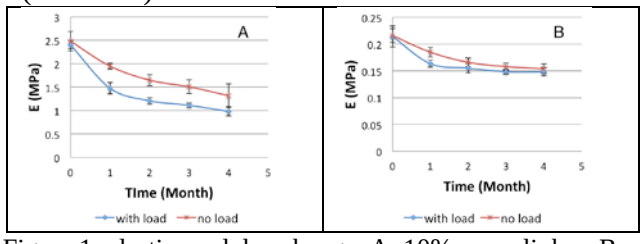

Figure 1: elastic modulus change, A: 10% cross-linker, B: 2% cross-linker (blue: with load; red: without load).

Figure 2 shows that the weight lost of hydrogels after 4 month was about 17% for more dense hydrogels and 10% for softer hydrogels.

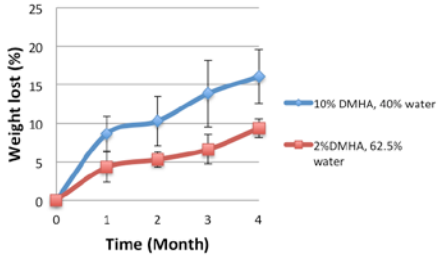

Figure 2: weight lost of hydrogels due to degradation

DISCUSSION AND CONCLUSION: In this study, we quantified the degradation properties of HEMA-DMHA hydrogels under mechanical load. This kind of study has not being reported previously in the literature. Short length of DMHA can provide high mechanical strength comparing to natural biodegradable molecules (from several Kpa to Mpa: Figure 1).
The degradation results after 4 months showed that at the early stage of degradation, the degradation more shows itself at losing the mechanical stiffness of hydrogels. This can be due to the hydrolyzation of the DMHA cross-linker and increasing the porosity inside the gel. But since the back-bone is still interconnected, the weight lost was less significant during first 4 months and it seems that gels need more time. This is an advantage, because it let the gel keeps its integrity for months and supports the regeneration of the new tissue especially for tissues like cartilage, that the tissue repair is very slow. The faster degradation of hydrogels, which were subjected to mechanical load comparing to without applying load can be due to the effect of load at removing degradation products and expediting chain breakage.

REFERENCES

[1] P. Blount, *Neuron* **2003**, 37, 731.
[2] K. Y. Lee, M. C. Peters, D. J. Mooney, *Advanced Materials* **2001**, 13, 837.
[3] A. B. South, L. A. Lyon, *Chemistry of Materials* **2010**, 22, 3300.

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Acute mechanical injury of human intervertebral discs initiates events associated with degeneration and pain.

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INTRODUCTION: Intervertebral disc degeneration is a major cause of low back pain, a chronic disorder among individuals world-wide. Mechanical overload has been proposed as a potential initiating mechanism of degeneration, and previous studies have demonstrated that high mechanical loading of lumbar motion segments disrupts disc cell metabolism and increases matrix-degrading enzyme activity [1]. This study investigated changes in cellular metabolism, matrix composition, inflammatory responses and pain markers in human intervertebral discs following single compression, acute mechanical injury.

METHODS: Healthy human lumbar discs acquired from consenting organ donors were isolated as described [2]. Discs were subjected to a single-ramp compression of either 5% or 30% strain at 30% per second using an MTS machine, and then cultured up to two weeks. Proteoglycan content was assessed by tissue histology and quantified by DMMB assays. Cytokine arrays and ELISAs were performed to analyze conditioned media.Western blot probed tissue extracts for cleaved aggrecan. Conditioned media was applied to PC12 cells and neurite outgrowth was assessed.

RESULTS: Disc injury caused approximately 50% and 60% cell death in the nucleus pulposus and annulus fibrosus respectively. Uninjured discs maintained approximately 85% viability. Proteoglycan content was diminished in injured disc tissue correlating with significant GAG content released to medium. Safranin-O staining confirmed GAG loss in injured discs. Conditioned media from injured discs caused significant neurite outgrowth in PC12 cells compared to uninjured control media. ELISA analysis showed significant increase in NGF in injured disc-conditioned media compared to controls. Cytokine arrays revealed significantly elevated levels of IL-5, IL-6, IL-7, IL-8, MCP-2, GROα, and MIG released from injured discs. Western blot analysis of injured disc tissue revealed increased aggrecan cleavage by MMP and ADAMTS enzymes compared to controls.

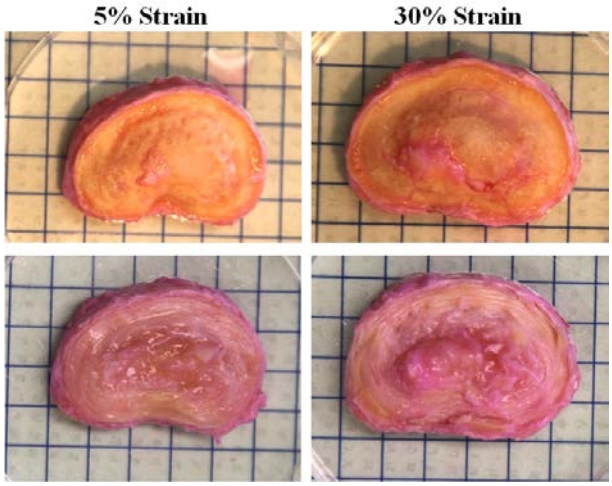

Fig. 1: Images of uninjured (5%) and injured (30%) discs with (top) and without (bottom) cartilage endplate. Grids represent 1cm x 1cm.

DISCUSSION & CONCLUSIONS: These data demonstrate that a single traumatic load event can initiate matrix disruption, inflammation, and release NGF which may be directly related to disc degeneration, nerve in-growth and chronic pain. Further investigation may yield insights to novel diagnostics and therapies for painful degenerative disc disease.

REFERENCES: [1]BA Walter, CL Korecki, D Purmessur, et al. 2011. Complex loading affects intervertebral disc mechanics and biology. Osteoarthritis Cartilage 19:1011-1018. [2] Gawri R, Mwale F, Ouellet J, et al. 2011. Development of an organ culture system for long-term survival of the intact human intervertebral disc. Spine (Phila Pa 1976) 36:1835-1842.

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comparison of swelling properties of bovine nucleus pulposus and poly-ethylene-glycol hydrogel

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INTRODUCTION: Despite its invasiveness, spinal-fusion remains the main treatment for painful degenerative disc disease (DDD)[1]: other currently available implant- and treatment-options do not seem to be more efective.[2,3] However, an attractive and less invasive alternative would be to replace the core of the intervertebral disc, the nucleus pulposus (NP), with a synthetic material.[4] Such a material should on one hand be stiff in order to avoid extrusion of the implant out of the disc and on the other hand be soft enough to avoid implant subsidence. Only poro-elastic materials are able to fulfil such requirements due to their unique ability to regulate their mechanical properties via swelling or deswelling. In order to design the implant, the swelling characteristics of the NP have to be investigated together with other properties like its adhesion or rupture strength.

METHODS: Swelling, fatigue and adherence properties of bovine NP tissue and highly poro-elastic poly-ethylene-glycol dimetacrylate (6 kD) hydrogel samples were tested. A semi-confined compression setup was developed which monitors compressed tissue samples' volumes and applied pressure in real time. It allowed applying a constant pressure to a sample under large strains and performing creep measurements while controlling tissue hydration. Short- and long-term behaviours under constant or cyclic loading of NP and hydrogel were evaluated. Adherence of hydrogel/tissue interfaces was tested in shear and traction.

RESULTS: Under constant pressure in the physiological range[5] (0.1 - 0.5 MPa) the equilibrium swelling ratio of NP tissue rapidly decreases from 10.5 to 6 [-]. When compared to the hydrogel (equilibrium swelling ratio decreases from 9 to 6 [-]), the NP tissue relaxed and adapted faster (Fig. 1). However, when testing the equilibrium swelling pressure the hydrogel was able to withstand higher loads (0.2 MPa) than the NP tissue (NP: ~ 0.1 MPa). Under cyclic compression the NP tissue was able to take-up and release water faster than the hydrogel. In both cases the samples reached an equilibrium state faster under cyclic compression compared to uniform loading conditions. Interestingly, creep tests over several hours showed that an NP under physiological loading conditions is compressed within ~5 hours and expands within ~8 hours, the latter being close to a typical diurnal cycle.

DISCUSSION & CONCLUSIONS: In general our results indicate that the NP can adapt faster to an applied load compared with the hydrogel. One option to improve the hydrogel system would be to increase its porosity (e.g. by changing the polymer's chain length).

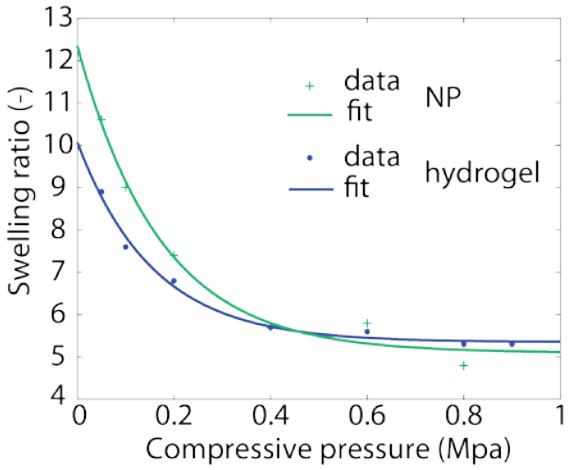

Fig. 1: Swelling ratio after two hours of constantly applied compressive load

REFERENCES: [1] K. Zarghooni, J. Siewe, and P. Eysel (2011) *Der Orthopäde* **40(2)**:141–7. [2] C. Schizas, G. Kulik, and V. Kosmopoulos (2010) *European cells & materials* **20:**306–15 [3] R.W. Hu, S. Jaglal, T. Axcell, et al (1997) *Spine* **22(19)**: 2265–2270. [4] M.H. Pelletier, W.R. Walsh, and N. South (2011) *Nucleus Replacement*, Elsevier Ltd., pp171–190. [5] H.J. Wilke, P. Neef, M. Caimi, et al (1999) *Spine* **24**:755–62.

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Evidence of balloon cells in lumbar spine disc herniation. Preliminary morphological results of patients versus SPARC null mice.

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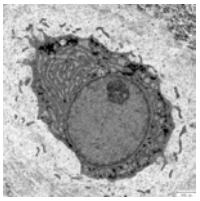
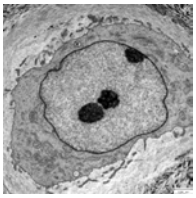
Introduction: SPARC (secreted protein, acidic rich in cysteine) is a multifunctional glycoprotein that belongs to the matricellular group of proteins. Recent publications reported disc degeneration with herniations in SPARC null mice (KO - targeted deletion of the SPARC gene). [1] M. Battie showed in her twin studies up to 74% genetic influence on disc degeneration in human patients. Ultrastructural studies in the cervical spine with the pathology of disc herniations showed besides disc cell death, a new disc cell morphology, so called balloon cell.[2] These cells presented themselves viable, with homogenous nuclei, composed mainly of euchromatin and with the ability to proliferate into clusters. All patients with a degeneration grade (DG) II and III presented balloon cells mainly (mean 32%) in the inner annulus fibrosus in contrast to higher DGs were nearly no balloon cells were found. The incidence of balloon cells identified by ultrastructural analysis in SPARC-null mice suggested a possibility of similar processes leading to the changes in the cell morphology and corroborating the genetic influence on this specific disease in humans.

Materials & Methods: Three human disc herniations (54 yrs/DGIII; 65 yrs/DGIII; 67 yrs/DGIV) from the lumbar spine (LS) and 6 mouse lumbar spines (LS: 5 discs each; 1, 6, 12 month) (KO LS n=15, wild type LS n=15) were investigated with MRI/micro-CT scans for gross morphology and ultrastucture to qualify different disc morphologies (disc cell death, healthy cells, balloon cells) in tissue samples and cell culture. Cells were separated from matrix and cultured in DMEM/F12 for 15 days (3 human herniation's and 3 discs of KO mice).

Results: MRI showed progressive sclerosis of the endplates with more severe DG and mass prolapse in 2 patients. Similar findings of progressive sclerosis of endplates were seen in CT scans of SPARC KO mice with age/DG, while the wild type (WT) presented minor changes. Disc cell death was highest in tissues of human discs herniations. Balloon cells were evident in one human (Fig.a: 54 yrs/DGIII) and in 80% of SPARC null mice (1 and 6 m) discs (Fig.b: 1 m KO), mainly in L2-L4 segments. There were no balloon cells and less disc cell death found in samples from WT mice. There were higher numbers of balloon cells found in all cell culture samples derived from patients and discs from SPARC KO mice.

Fig. a  Fig. b 

Discussion & Conclusion: Our data presented here suggest a similar process in human cervical / lumbar disc herniation and in SPARC null mice. Endplate sclerosis diminishes the nutrient supply to discs and might be responsible for the absence of balloon cells in the investigated tissues with higher DGs. Moreover, in herniations the supply is reduced. At the moment it is impossible to say if SPARC or suppressors of SPARC enable changes found in disc morphologies in the human samples. A genetic mutation leading to balloon cells needs to be identified. However, this study might give an impact to new ways of repair strategies in future.

Reference: (1) Millecamps M, Tajerian M, Naso L, Sage EH, Stone LS. 2012. Pain. 153(6):1167-79.
(2) Sitte I, Kathrein A, Pedross F, Freund MC, Pfaller K, Archer C. 2012. Eur Spine J 21(7):1396-1409.

Tailoring silk based nano fibres for human intervertebral disc repair

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INTRODUCTION: Low back pain linked to intervertebral disc (IVD) degeneration is a highly abundant problem in the aging modern society. Until today there is no biological solution available based on the patient's autologous cells to restore or repair the IVD. We hypothesized that electrospun silk scaffolds can mimic the extracellular matrix of the IVD cells: i) a random orientation of the fibres would be ideal for nucleus pulposus cells (NPC) and ii) an alignment of the fibres would be favourable for annulus fibrosus cells (AFC).

METHODS: *Silk liquefaction:* Silk fibres from *Bombyx mori* (Swiss Silk) were cut in small pieces and boiled in 0.2M Na_2CO_3 for 30 min to remove the sericine. Then, the silk fibres were rinsed three times in ultrapure water (UPW) and dried overnight. The dry silk was then dissolved in 9.3 M LiBr solution and dialysed against UPW for 48 h and purified by high speed centrifugation.[1] *Electrospinning:* Silk was mixed with 5% (wt/vol) 900kDa-PEO to generate a solution of 6.4% silk and 1 % (wt/vol) PEO. This solution was electrospun on a flat collector for randomly oriented fibres and on a rotating mandrel for aligned fibres. Of each electrospun mat N=40 samples of 6mm diameter were punched out. N=20 of the randomly aligned samples were ultra-sonicated for 1 min at 80 Watts to increase their porosity.[2] *Cyto-compatibility:* 40k human derived NPC and AFC (ethically approved) were seeded per carrier and grown for 7 days. On day 1 and 7 cell spreading (LIVE/DEAD® and confocal laser scanning microscopy) and cell activity (Alamar Blue®) and DNA content (PicoGreen®) was monitored.

RESULTS: The electro-spinning process revealed two completely different scaffold and micro environments for cells as confirmed by SEM (Fig. 1, A,B) Live/dead stain of IVD cells confirmed their alignment in the direction of the parallel-oriented fibres (Fig. 1, C, D). On day 7 cell activity decreased for AFC to the contrary for NPC were it increased per cell (Fig. 2) Generally, it was noted that cells adhered and proliferated better on ultra-sonicated non-oriented scaffolds than on aligned scaffolds (data not shown).

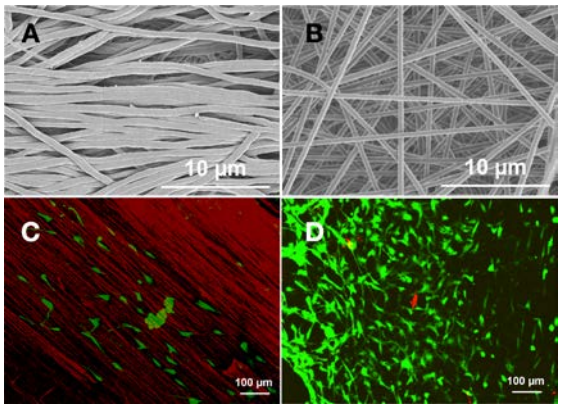

Fig 1: A-B) Scanning electron microscopy images of scaffolds A) Aligned silk fibres (mandrel speed=30Hz) B) Randomly distributed silk fibres C-D) Human IVD cells on scaffolds C) AFC aligned in the direction of silk fibres (red) D) NPC randomly oriented in ramdomly aligned silk nano fibres.

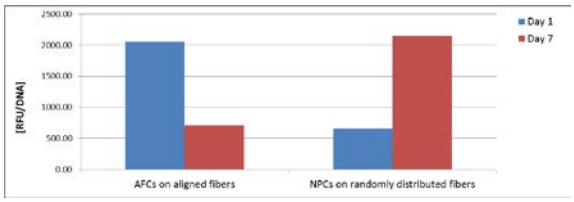

Fig. 2: Cell activity of human NPC and AFC measured with Alamar Blue Assay on day 1 and 7.

DISCUSSION & CONCLUSIONS: By modification of the silk composition and the electro-spinning parameters the 3D environment of a scaffold can be controlled. This is crucial for cell adhesion and proliferation of primary cells. The general direction of cell growth can be controlled by the arrangement of the silk nano fibres. Future research will focus on the control of porosity and integration of adhesion molecules and/or cytokines to tailor the IVD cell specific niche.

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REFERENCES: [1]D N Rockwood (2011) *Nat. Protoc* **6**:1612-31. [2]J B Lee (2011) *Tissue Engineering* **17**:2695-2702.

Autonomous formation of cartilage tissue by expanded chondrocytes is dependent on a functional TGFβ-signalling

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INTRODUCTION: Articular chondrocytes are exposed to a microenvironment which is substantially changed when they are isolated from the tissue and expanded in monolayer cultures as required for cell-based cartilage repair strategies. Upon expansion, the cells loose the competence to autonomously form a cartilage-like tissue in an environment supporting chondrogenesis, an observation which has not been explained conclusively. Since formation of cartilage by these cells can be rescued by the addition of chondrogenesis-inducing factors, such as transforming growth factor (TGF)β1, we hypothesized that changes in the TGFβ signalling pathway occurring early during expansion are governing the loss of the potential for autonomous cartilage formation. The aim of the present study was therefore to assess the expression of components of TGFβ signalling during expansion and their contribution in the formation of cartilage-like tissues by expanded articular chondrocytes.

METHODS: Articular chondrocytes were isolated from cartilage of bovine shoulder joints (2-4 year old animals) and expanded in monolayer cultures. Confluent cells were released with trypsin and re-plated until 20 population doublings (PD) were reached. At each passage, cells were analysed by real-time PCR for the levels of transcripts coding for TGFβ1, TGFβ2, TGFβ3, TGFβ receptor 1 (TGFβR1) and TGFβR2. The potential of the expanded cells to form a cartilage-like tissue was assessed in high density 3D pellet cultures in a serum-free medium with or without exogenous TGFβ1.

RESULTS: Autonomous formation of a cartilaginous tissue in pellet cultures was observed only with cells from passage 1 (P1) and P2, a process which was blocked by the inhibition of the TGFβR1 kinase with SB-505124 (Fig. 1).

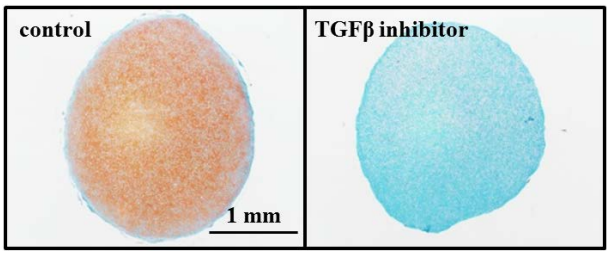

Fig. 1: Cells of P1 were subjected to pellet cultures without TGFβ1. Addition of the TGFβR1 kinase inhibitor SB-505124 abrogated the potential for autonomous cartilage formation. (Safranin O/ Fast green staining).

Cells from P1 – P6 developed cartilage-like tissue in the presence of exogenous TGFβ1, albeit with a reduced efficiency in later passages as evidenced by decreased staining intensity for glycosaminoglycans. At P3 (8.3±2.2 PD) and later passages of the monolayer culture, levels of transcripts encoding TGFβR1 and TGFβR2 were reduced by >50% and >70%, respectively, as compared to freshly isolated chondrocytes. Transcripts coding for TGFβ1 and TGFβ2 were expressed at similar levels but 10-fold higher than TGFβ3 in freshly isolated chondrocytes. Levels of transcripts coding for TGFβ1 and TGFβ3 remained unchanged throughout expansion, while levels of TGFβ2 were reduced by >80% at P3 and later passages.

DISCUSSION & CONCLUSIONS: The autonomous formation of a cartilage like tissue *in vitro* by expanded chondrocytes relies on TGFβ signalling. While the reduced expression of TGFβ2 may result in lower total concentration of TGFβs in the self-established microenvironment within pellet cultures and thus presenting with a lower chondroinductive potential, the reduced receptor expression may render the cells insensitive to the endogenously produced TGFβs. Initially, this can be compensated by the addition of exogenous TGFβ1, which, however, becomes inefficient in later passages. This suggests that changes in the expression of additional factors can be made accountable for the loss of autonomous formation of cartilage tissue, which cannot be compensated for by TGFβ1.

Histochemical evaluation of second-look osteochondral biopsies: symptomatic vs asymptomatic MACI-treated patients

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INTRODUCTION: In the last years, autologous chondrocyte implantation after in vitro expansion has been further improved by MACI (Matrix-induced autologous chondrocyte implantation) [1]. To assess the efficacy of long-term treatments, the histological evaluation of osteochondral tissue plays a key role in the distinction between hyaline and fibrous cartilage. This study aimed at highlighting different histochemical features of osteochondral tissues treated with MACI in order to distinguish between successful and unsuccessful treatments.

METHODS: Two osteochondral biopsies were collected with a Jamshidi needle during second-look arthroscopy from a symptomatic (M 51 years, lateral femoral condyle) and an asymptomatic patient (M 50 years, medial femoral condyle), 1 and 5 years after MACI, respectively (NOVOCART® 3D, Tetec AG, Germany). Tissues were fixed in 10% buffered formalin for 24 h, decalcified in EDTA for 24 h, embedded in paraffin, and cut in 5 µm slices. Sections were subjected to one of the following protocols: Hematoxylin-Eosin Y (HE), Safranin-O (SO), Alcian Blue pH 2.5 (AB), Toluidine Blue (TB), Astra Blue pH 2.5 (AsB), Azan (Az) and Picrosirius Red (PR). Polarized light was used for PR-stained sections. Tissue biopsies were evaluated for proteoglycan content, cell distribution and collagen fibre orientation. Surface regularity was not considered because of artefacts induced during sampling procedure.

RESULTS: Newly formed tissue in the biopsy from symptomatic patient presented hyaline-like cartilage in the deep zone and intact tidemark, but fibrous cartilage in the middle zone (Fig. 1a), with proteoglycan depletion (Fig. 1c), and loss of normal collagen fibres orientation (Fig. 1e). A tear compromising tissue integrity was found between the two zones, showing an unsuccessful tissue regeneration (arrows in Fig. 1). Differently, full thickness hyaline cartilage was found in the asymptomatic patient (Fig. 1b, d, f).

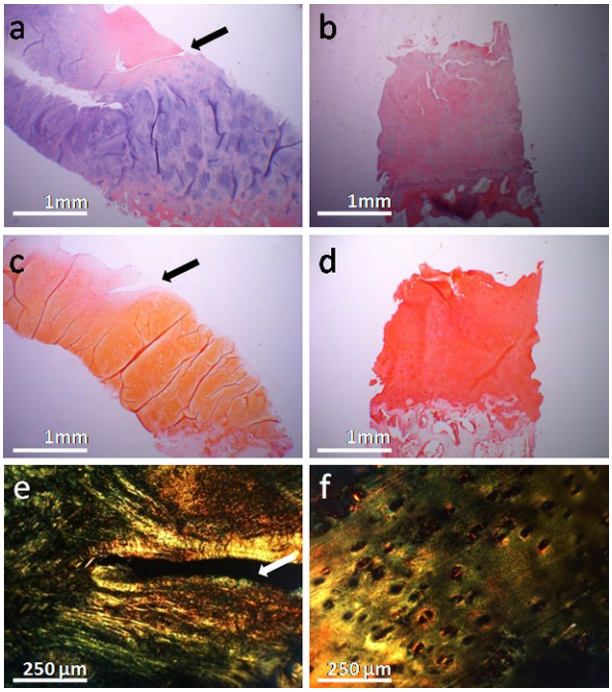

Fig. 1: Histochemical characterization of tissue unsuccessfully (left) and successfully (right) treated with MACI. HE (a, b), SO (c, d), PR (e, f).

DISCUSSION & CONCLUSIONS: HE successfully characterized morphology and spatial organization of the chondrocytes. SO (Fig. 1c), AB, AsB and TB showed proteoglycan depletion in fibrocartilaginous areas, but AB and AsB staining were sensitive to pH and temperature. Both Az and PR revealed fibres alignment in hyaline areas and disordered microfibrillar aspect in fibrous areas (Fig. 1e, f). The histochemical analysis provided a comprehensive description of second-look osteochondral biopsies after MACI. Structural and compositional differences in the newly-formed tissue were reported between symptomatic and asymptomatic patients.

REFERENCES: [1] S. Marlovits, G. Striessnig, F. Kutscha-Lissberg, et al. (2005) *Knee Surg Sports Traumatol Arthrosc.* **13**: 451-7.

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Lithium protects cartilage from interleukin-1β induced degradation and preserves the mechanical integrity of articular cartilage

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INTRODUCTION: Lithium chloride (LiCl) is commonly used for the treatment of bipolar disorder. It is a potent inhibitor of inflammatory signalling and prevents cartilage degradation in response to inflammatory cytokines *in vitro* [1]. Recent studies have shown that intra-articular injection of LiCl ameliorates cartilage destruction in surgical models of osteoarthritis (OA) highlighting its potential as an OA therapeutic [2]. However, LiCl can affect many cellular processes that can negatively influence cartilage function including chondrocyte viability, differentiation and matrix synthesis. This study aims to investigate the effect of LiCl on the biomechanical properties of healthy and inflamed cartilage using an *in vitro* model and examines the consequences of long term exposure to LiCl on cartilage health *in vivo*.

METHODS: *In vitro* studies used isolated bovine cartilage explants treated with 0-50mM LiCl for up to 12 days in the presence or absence of 5ng/ml interleukin 1β (IL-1β). The upregulation of inducible nitric oxide synthase (iNOS) expression was assessed in response to IL-1β by western blotting, and nitric oxide (NO) release measured using the Greiss assay. Matrix catabolism was monitored using the dimethyl-methylene blue assay to monitor sulphated glycosaminoglycan (sGAG) release, and the effects on mechanical integrity determined using unconfined compression testing. For *in vivo* studies, male wistar rats were fed a high lithium diet (60 mmol lithium/kg food) for up to 9 months to achieve clinically relevant serum levels of Li (approx. 1mmol/L). The consequences for joint health were monitored by histological staining and assessed according to the Mankin Grade for OA.

RESULTS: *In vitro*, LiCl treatment did not significantly affect chondrocyte viability, matrix catabolism or the biomechanical properties of the tissue relative to control. Consistent with these findings, histology revealed that LiCl did not adversely affect cartilage health in rats fed a high lithium diet. However, in the face of an inflammatory stimulus, LiCl blocked the upregulation of iNOS and the associated downstream NO release and prevented matrix catabolism *in vitro* such that sGAG release was completely abolished by 50mM LiCl (Fig1A). Consequently the loss of mechanical integrity observed with IL-1β treatment alone was inhibited (Fig1B).

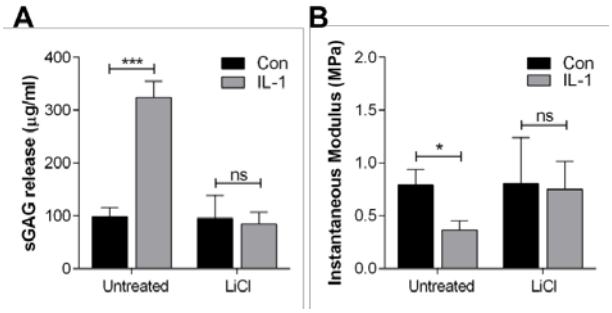

Fig. 1.LiCl (50mM) treatment for 12 days (A) blocks IL-1β induced sGAG release and (B) prevents the IL-1β induced loss of mechanical integrity (cartilage instantaneous modulus).

DISCUSSION & CONCLUSIONS: Our results indicate that LiCl does not induce matrix catabolism nor significantly affect the biomechanical properties of articular cartilage. Moreover, long term exposure does not induce osteoarthritis *in vivo*. *In vitro*, LiCl prevented matrix degradation and the associated loss of mechanical integrity that occurs in response to an inflammatory stimulus providing further support for LiCl as a novel compound for the treatment of arthritis.

REFERENCES:

[1] Hui W et al. *Lithium protects cartilage from cytokine-mediated degradation by reducing collagen-degrading MMP production via inhibition of the P38 mitogen-activated protein kinase pathway.* Rheumatology, 2010. **49**(11): p. 2043-2053. [2] Minashima T et al., *Lithium protects against cartilage degradation in osteoarthritis.* Arthritis Rheumatol, 2014.

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Lithium chloride triggers primary cilia elongation and inhibits hedgehog signalling in articular chondrocytes

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INTRODUCTION: Hedgehog signalling is activated in osteoarthritis (OA) and promotes chondrocyte hypertrophy and the expression of catabolic enzymes such as ADAMTS-5 and MMP-13 [1, 2]. Inhibition of this signalling pathway has been shown to reduce cartilage degradation in surgical models of OA [1]. The primary cilium is a microtubule-based organelle and is present on the majority of chondrocytes. Components of the hedgehog pathway are trafficked through the ciliary compartment making it essential for pathway regulation. Recent studies indicate that lithium chloride (LiCl) is chondroprotective due to its anti-inflammatory properties [3, 4]. LiCl modulates cilia structure in numerous cell types [5]. We hypothesise that LiCl may also affect cilia-mediated signalling pathways in chondrocytes, like hedgehog, through the regulation of ciliary structure.

METHODS: For *in vitro* studies bovine primary chondrocytes (BPC) were treated with 0-50mM LiCl for up to 24hrs. To assess the effects of LiCl on cilia *in vivo*, male wistar rats were fed a high lithium diet (60 mmol lithium/kg food) for up to 9 months to achieve clinically relevant serum levels of Li (approx. 1mmol/L). Confocal imaging and immunocytochemistry for acetylated alpha tubulin and Arl13b were used to measure primary cilia length and prevalence. Activation of the hedgehog signalling pathway in response to recombinant Indian hedgehog (r-Ihh) was quantified using real-time PCR for GLI1 and PTCH1.

RESULTS: In BPC, LiCl triggered the dose dependent elongation of primary cilia such that the mean length of cilia was maximally increased by 95% at 50mM (Fig1A). Cilia elongation was rapid, with the majority of growth occurring within the first hour of treatment. Histological analyses revealed that cilia length was also increased *in vivo* in a region specific manner.

Analysis of the hedgehog response in BPC revealed that in untreated cells r-Ihh increased the expression of GLI1 and PTCH1 by 5.22 and 4.23-fold respectively (Fig1B). In LiCl treated cells, dose dependent inhibition of this response was observed such that 50mM LiCl completely abolished the changes in GLI1 and PTCH1 expression (Fig1B).

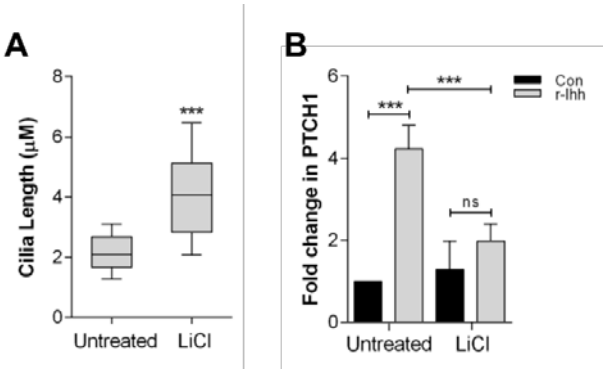

Fig.1. LiCl treatment (50mM) for 24hrs (A) increases primary cilia length and (B) inhibits r-Ihh induced hedgehog signalling, as measured by changes in PTCH1 gene expression.

DISCUSSION & CONCLUSIONS: These data show that LiCl stimulates rapid, dose dependent cilia elongation in primary articular chondrocytes and inhibits hedgehog signalling *in vitro*. The mechanism by which this inhibition is achieved is unclear, however recent studies show that the modulation of ciliary structure by mechanics can effect this pathway [6]. We therefore propose that this inhibition may be linked to the effects of LiCl on cilia structure, future studies will investigate this by examining the effects of LiCl on the ciliary trafficking of hedgehog pathway components. This study highlights the potential for targeting the ciliary structure as a novel therapeutic approach to modulate hedgehog signalling and matrix catabolism in OA.

REFERENCES: [1] Wei, F. et al. Osteoarthritis and Cartilage, 2012. 20(7): p. 755-63. [2] Lin, A.C. et al. Nature Medicine, 2009. 15(12): p. 1421-5. [3] Minashima, T., et al. Arthritis Rheumatol, 2014. [4] Hui, W., et al., Rheumatology, 2010. 49(11): p. 2043-2053. [5] Ou, Y., et al. Exp Cell Res, 2009. 315(16): p. 2802-17. [6] Thompson et al. Osteoarthritis and Cartilage, 2014. 22(3): p. 490-8.

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An explanatory model for intervertebral disc degeneration: a degenerative cycle

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INTRODUCTION: Intervertebral disc degeneration (IVDD) is viewed as the main cause of low back pain; a major health problem which affects many patients and has a large macro-economic impact. Unfortunately, the exact pathophysiology of IVDD has not been elucidated, nor is there any therapeutic intervention available to decelerate or cure the disease. However, there are numerous changes described in IVDD: the main pathological changes occur in the cells and the extracellular matrix (ECM), which leads to changes in the biomechanical behaviour[1,2]. Unfortunately, researchers and clinicians focus on either of these three degenerative properties, where an integrated approach could be more successful. We propose a model which entails a positive feedback loop over these three domains of IVDD.

THE DEGENERATIVE CYCLE:

Cells: The loss of notochordal cells and increase of chondrocyte-like cells in the human IVD respectively lead to decreased anabolic potential, and increased matrix remodelling. Additionally, with progression of age and degeneration, there is increased production of catabolic agents such as MMPs and ADAM-TSs by the IVD cells.

ECM: The main ECM proteins in the IVD are: Aggrecan, Collagen II (Nucleus) and Collagen I (Annulus). The chondrocytes that maintain the ECM at an adult age increasingly produce Collagen I in the nucleus, while the increased catabolic agents slowly disintegrate Aggrecan, which results in reduced negative charge, and thus a reduction of hydrostatic pressure.

Biomechanics: The loss of hydrostatic pressure leads to a loss of disc height; an increase in compressive stiffness; and an increase of the neutral zone in bending. Altered biomechanics can induce catabolic activity of cells in both the nucleus and the annulus[3]. This results in the deduction that there is a positive feedback loop in IVDD which contains: Catabolic cellular behaviour – loss of proteoglycans – altered biomechanics (Fig. 1).

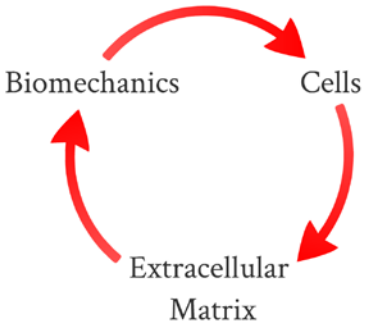

Fig. 1: The degenerative cycle of intervertebral disc degeneration. Red arrows show the positive feedback loop.

PATHWAYS INTO THE DEGENERATIVE CYCLE: Both in animal models and human epidemiology there is evidence that one can enter this degenerative cycle at any point as IVDD occurs in e.g.: tail suspension models; chemonucleolysis models; annulus puncture models; chondrodystrophic dogs; diabetics; astronauts; and elite gymnasts. Because disturbances in any domain lead to similar IVDD, all must be considered equally important.

DISCUSSION & CONCLUSIONS: Previously, there has been a dichotomy in IVDD literature where researchers weighed either biology or biomechanics as more important than the other. However, recent mechanobiology research links both as biomechanical forces influence biology[4]. With the degenerative cycle we intend to provide a model in which all parts are equally important and interdependent. The pathways into the degenerative cycle have important implications for both researchers and clinicians: they should aim for results in all domains, rather than one. As such, this model can aid in addressing the issues and challenges surrounding IVDD and low back pain. Possibly, a comparable feedback loop is present in osteoarthritic joints.

REFERENCES: [1] Adams, M.A., Dolan, P. and McNally, D.S. (2009) *Matrix Biol.* **28,** 384–9. [2] Colombini, A. et al. (2008) *Int. J. Biochem. Cell Biol.* **40,** 837–42. [3] Paul, C. P. L. et al. (2013) *PLoS One* **8,** e62411. [4] Hsieh, A.H. and Twomey, J.D. *J. Biomech.* **43,** 137–45 (2010).

Microspheres of BCP produced by Snowballing technique for multipurpose application

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INTRODUCTION: Biphasic calcium phosphates (BCP), a combination of hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP), are widely used in dental, orthopedics and drug delivery systems (DDS) nowadays. In an effort to broaden the range of application, architecture and microstructures are major aspects of concern. Considering manufacturing granules of spherical shape, micro-scale size of less than 1mm and loose arrangement still present a challenge, despite the existence of many process to obtain such structures involving several steps [1]. Microspheres are of great interest because they possess a high packing capability combined with the possibility of being injected through less invasive procedures, as bone fillers [2]. The microarchitecture represented by microporosity gives an adsorption potential to microspheres. Snowballing technique is a new process shown to be a suitable method for obtaining ceramic spheres without additives or binders [3]. This work presents the obtaining of microspheres through Snowballing technique.

METHODS: Ceramic powders of HAp and β-TCP, HAP-200 and β-TCP-100, respectively, from Taihei Chemical Industrial Co. Ltd., Osaka, Japan, were mixed in a 1:1 by weight composition to form the BCP. The amount of 25g of BCP powder mixture was added to a plastic bottle of ~1000mL (9cm x 17cm) and put in a small ball mill device for transverse mixing at 85 rpm speed for 6 hours. The BCP material were sintered at 1150ºC/1 hour and separated by sieve of 1mm and 500µm to form two groups. Microspheres were characterized by scanning electron microscopy (SEM) S-3400N, Hitachi and X-ray diffraction (XRD) D8 Advance, Bruker.

RESULTS: The XRD analysis showed that no α-TCP phase transformation occurred during the sintering process with the BCP. The resulting microspheres were separated into two groups: microspheres ranging between 1mm and 500µm and microspheres with less than 500µm, both groups differing only in microspheres' diameter size. The SEM analysis shows the morphology of the microspheres with round shape and smooth surface, while under higher magnification the topography of microspheres shows an intricate rough microstructure pattern with microporosity, Fig.1 and Fig. 2. Both groups of samples presented good sintering parameters with neck formation between particles and good mechanical strength allowing the handling of microspheres.

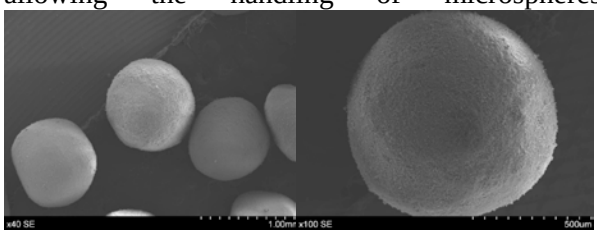

Fig. 1: BCP microspheres ranging between 1mm and 500µm – SEM (1mm bar / 500µm bar)

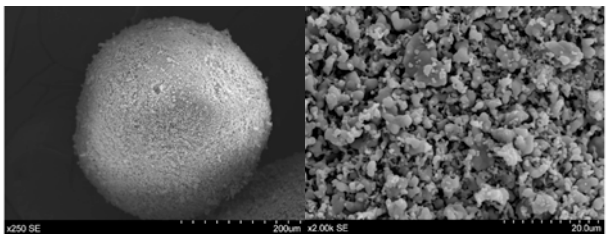

Fig. 2: BCP microspheres with less than 500µm – SEM (200µm bar / 20µm bar)

DISCUSSION & CONCLUSIONS: *The Snowballing technique allowed to obtain microspheres with less than 1 mm and less than 500µm in diameter without additives or binders. The samples surface presented overt microporosity, which is a desirable feature for protein adsorption, cell attachment and DDS. The round shape gives to the material fluidity, a potential feature for use as an injectable material in less invasive procedures, as vertebral disk filling with material for vertebral fusion (arthrodesis).*

REFERENCES: *[1] M. Bohner, S. Tadier, N. Garderen, et al (2013)* Biomatter ***3****:2 e25103-1-15.* *[2] D.H. Kim, H.H. Chun, J.D. Lee, et al (2014)* Ceramics International ***40****:4 5145-55.* *[3] K.B Violin, T.S. Goia, K. Ishikawa, et al (2014)* Advances in Science and Technology ***(CIMTEC 2014 - in press)***.

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The chondrocyte primary cilium modulates NFκB signalling, defining the response to interleukin-1β.

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INTRODUCTION: Most forms of arthritis exhibit inflammatory signalling triggered, in part, by cytokines such as interleukin-1β. Previously, we have shown the primary cilium, an organelle now established to be a master regulator of cytosolic signalling, is required for inflammatory responses in chondrocytes[1]. NFκB signalling, is triggered when IL-1 activates a cascade of kinases, including inhibitor of kappa kinase (IKK). IKK phosphorylates inhibitor of kappa B (IκB) triggering its polyubiquitination and destruction. This releases a burst of transcription factors, such as p65, into the nucleus activating a plethora of targets including many components of cartilage pathology. A complex array of molecules regulate pathway activation, including chaperones such as heat shock proteins. The unique ciliary compartment traffics signalling components and its architecture is subsequently adapted during inflammatory insult[2]. We hypothesised that fundamental components of the inflammatory signalling cascade must be trafficked to the ciliary compartment enabling the cilium to tune signalling.

METHODS: To investigate the mechanism of ciliary influence over IL-1 signalling, experiments used chondrocytes isolated from wild type (WT) and heterozygous *ORPK* mice with a hypomorphic mutation in *Tg737* encoding IFT88/Polaris. Following exposure to IL-1β, western blotting (WB), a DNA-binding ELISA and immunofluorescence (I.F) were used to assess NFκB targets and signalling.

RESULTS: *ORPK* cells exhibited low primary cilia prevalence compared with WT (Fig1A). WB indicated IL-1β-induced COX2 and iNOS protein expression was absent in *ORPK*. I.F staining revealed, in *ORPK*, p65 translocation was delayed. WB indicated, at 20 min, *ORPK* nuclear fractions contained less p65 than WT and NFκB DNA transcript binding, assessed by ELISA, was also reduced in *ORPK* cells. Upstream, IL-1β-induced IKK phosphorylation was not significantly different in WT and *ORPK*. However, subsequent IKK mediated phosphorylation of IκBα was drastically muted in *ORPK*, as was later progressive destruction of IκBα (Fig 1C). After 30 mins IκBα protein was still present in *ORPK* (immeasurable in WT). Activated IKK (Fig 1B) and the molecular chaperone hsp27, previously shown to influence IKK activity, were found concentrated to the basal body and primary cilium respectively.

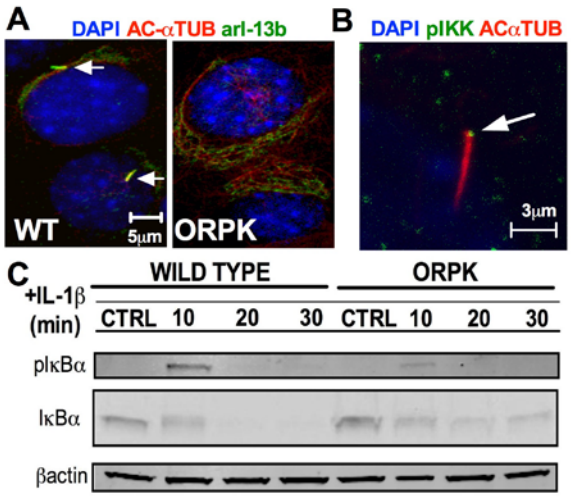

Fig. 1: (A) I.F indicates cilia (arrows) in WT but not ORPK chondrocytes and (B) activated IKK at the base of the cilium. (C) WB showing IKK activity inhibited in ORPK.

DISCUSSION & CONCLUSIONS: These data imply trafficking within the chondrocyte primary cilium is hugely influential to the temporal dynamics of NFκB signalling at the level of IκK activity. This reveals spatial regulation of chondrocyte inflammatory signalling and unveils the unique cilia machinery as a potential target in the resolution of cartilage inflammatory disease.

REFERENCES: [1] AKT Wann & MM Knight (2012) *Cellular and molecular life sciences* **69** (17):2967-77 [2] AKT Wann, CL Thompson, JP Chapple et al (2013) *Cilia* ***2*****:** *17.*

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Platelet-rich plasma (PRP) induces articular cartilage maturation - A possible mechanism for repair

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INTRODUCTION: Autologous PRP has been used as a biologic solution in the treatment of a wide range of musculoskeletal disorders [1, 2] as it has been shown to releases a number of cytokines and growth factors that stimulate the healing of bone and soft tissue. However, ongoing controversy remains regarding its mechanism of action and clinical efficacy *in vivo*, especially in regard to cartilage repair.

We hypothesised that one mechanism by which PRP may exert its reparative effects is through stimulation of articular cartilage maturation[3]. We tested this hypothesis by investigating whether PRP could functionally substitute for growth factors FGF2 and TGFβ1 in an *in vitro* model of articular cartilage maturation.

METHODS: Explants were prepared from the metacarpophalangeal (MCP) joints of immature male bovine calves, and cultured in DMEM medium supplemented with either FGF2 & TGFβ1 or 10% human apheresis PRP for 3 weeks. Gene expression of lysel oxidase-like 1 (LOXL1), Integrin α3 (ITGA3), collagen type X (ColX) and chondromodulin 1 (Chm1) was analyzed using qRT-PCR. LOXL1 and ITGA3 protein localisation was analyzed using immunostaining. General histology was performed by H&E staining.

RESULTS: The application of PRP had a similar effect as the combination of FGF2 & TGFβ1, in that it increased the gene expression of LOXL1 (Fig. 1A) and ITGA3, while decreasing the expression of Chm1 & ColX. PRP was also similar to FGF2 & TGFβ1 treatment in LOXL1 localisation, which was predominantly found at superficial & middle zones of cartilage (Fig. 1B). However, PRP increased ITGA3 labeling at the superficial & middle zone while FGF2 & TGFβ1 increased protein labeling in the middle zone. The effect of PRP on cartilage thickness or water content was weaker compared to that seen with FGF2 & TGFβ1 treatment.

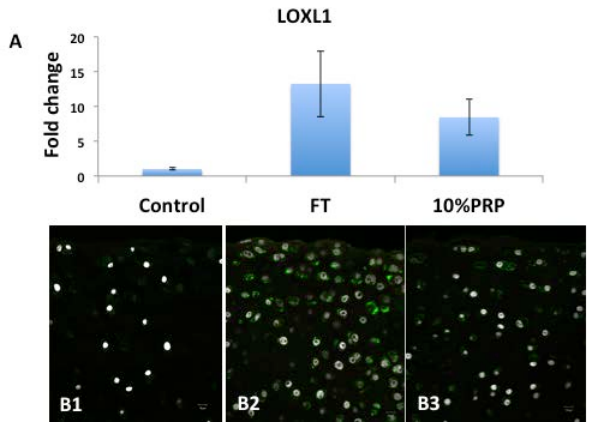

Fig.1 Comparison of PRP with FGF2&TGFβ1 on the gene expression and localization of LOXL1 in immature bovine articular cartilage cultured for three weeks. LOXL1 gene expression was up-regulated by 10%PRP which is similar to FGF2 & TGFβ1 treatment (A). Control sample without treatment (B1). Localization of LOXL1 was increased by 10%PRP (B3) but less than with FGF2 & TGFβ1 treatment (B2).

DISCUSSION & CONCLUSIONS:
PRP was able to functionally substitute for FGF2 and TGFβ1 growth factors used in the standard *in vitro* model of articular cartilage maturation. LOXL1 gene expression and protein localisation was similar in pattern but weaker in magnitude compared to growth factor stimulation. PRP may play a positive role in cartilage repair by inducing maturation in damaged and diseased cartilage. Therefore, PRP can potentially be used as an alternative to growth factors in cartilage repair.

REFERENCES: [1]BC. Halpern, S. Chaudhury and SA. Rodeo (2012) *HSSJ* 8:137-145; [2]MP. Hall, PA. Band, RJ. Meislin, *et al.* (2009) *J Am Acad Orthop Surg* **17**(10):602-8; [3]IM. Khan, SL. Evans, RD. Young, et al. (2011) *Arthritis & Rheumatism* **63** (11): 3417-3427.

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