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Cartilage, synovium and osteoimmunology

FRI0001 ALL STAGES OF SYNOVIAL MESENCHYMAL STEM CELL ACTIVITY INCLUDING ADHESION, PROLIFERATION, MIGRATION AND CHONDROGENIC DIFFERENTIATION ARE SUPPORTED BY HUMAN PLATELET LYSATE- IMPLICATIONS FOR NOVEL ONE STAGE JOINT REGENERATIVE PROCEDURES

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Background: Both synovial and related synovial fluid-derived mesenchymal stem cells (SF-MSC) are highly proliferative and chondrogenic and have direct access to superficial cartilage injuries [1]. Human platelet lysate (PL) is being increasingly used for joint regenerative approaches [2,3] but the biological basis for in vivo effect, if any is lacking.
Objectives: The study evaluated the effect of PL on adhesion, proliferation, migration and trilineage differentiation of synovial cavity derived MSCs in comparison to "gold standard" foetal calf serum (FCS) based stem cell media.
Methods: MSCs were derived from joint cavity washout in patients undergoing anterior cruciate ligament reconstruction or meniscal repairs. Cells were plated in StemMACS (FCS media) or with DMEM Supplemented with 10% Stemulate (PL media, Cook Regentec). Standard MSC proliferation, trilineage differentiation assays, and transwell migration assays were performed for both conditions.
Results: PL enhanced MSC colonies sizes (CFU-F) by 2.5 folds compared to FCS (p=0.0023, n=5). PL shortened MSC doubling times (p=0.0411, n=6). However, PL had no impact on CFU-F numbers (p=0.5973, n=5). In transwell migration assays, MSC migration was significantly increased toward 10%PL compared to 10%FCS (p=0.0286, n=6). Under standard chondrogenic induction conditions, PL-expanded MSC showed 30% more sulphated GAG (sGAG) production compared to FCS-expanded cells (n=4). In osteogenic and adipogenic induction, Alizarin red staining Ca++ assay and Nile Red showed no significant difference compared to FCS expanded cells (p=0.3429, n=4). Interestingly, replacing TGFβ with 20% PL was as good as standard chondrogenic media and with increasing PL percentage sGAG production increased (n=4). In same time using PL with high glucose media in 1:1 ratio was efficient to produced sGAG as compared to negative control (media only) (p=0.0286, n=4, figure 1). Moreover, substituting FCS with PL in the osteogenic media resulted in increased Ca++ deposition in both PL and FCS expanded cells by 90% and 100% compared standard osteogenic media.

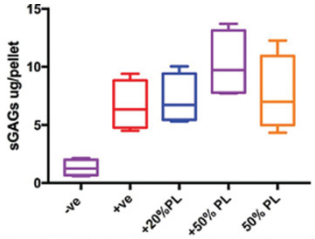

Figure 1: Evaluation the use of PL as an alternative TGFBeta in chondrogenic media. A: sGAGS activity of SF-MSC in different chondrogenic media; -ve: high glucoase+10% FCS, +ve: complete chondrogenic media, +20%PL: complete media with no TGFBeta and with 20%PL, +50%PL: complete media with nc TGFBeta and with 50%PL, 50%PL: high glucose DMEM with 50%PL. (N=4), Mann-Whitney test.

Conclusions: This is the first study that assessed joint cavity MSC responses to PL. All aspects of joint cavity derived MSC biology towards cartilage repair were augmented in vitro with PL based media over "gold standard" MSC media. This work provides biological plausibility for the combination of PL with joint resident MSC towards endogenous joint repair strategies.
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FRI0002 DEVELOPMENT OF AN IN VITRO MULTI-COMPONENT 3D JOINT MODEL TO SIMULATE THE PATHOGENESIS OF ARTHRITIS

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Background: Our ultimate goal is to develop a valid *in vitro* 3D model to simulate the immune mediated pathogenesis of arthritis in order to present an alternative experimental setup for the traditional animal models. Therefore, we work to establish an *in vitro* simulation of a healthy joint, followed by the depiction of an inflamed arthritic joint to finally study the efficacy of drug treatment. The *in vitro* 3D joint model consists of different components including an (1) osteogenic and (2) chondrogenic part, (3) the joint space with synovial fluid and (4) the synovial membrane. The model is suggested to include all involved cell types and thus, to allow interactions between cells by cell contacts and signaling molecules. To our knowledge, there is currently no valid 3D model which is able to mimic an inflamed arthritic joint.
Objectives: Here, we aim to mimic the (1) osteogenic and (2) chondrogenic part of the joint for our *in vitro* multi-component 3D joint model.
Methods: We used β-tricalcium phosphate (TCP) particles as a mineralized 3D bone scaffold and human bone marrow derived mesenchymal stem cells (hMSCs), non- and pre-differentiated towards osteoblastic lineage. Both were cultured up to 21 days on β-TCP. Osteogenic differentiation was performed in the presence of osteogenic supplements under normoxic conditions (37 °C, 18% O2). Adhesion and proliferation of hMSCs on β-TCP were evaluated by immunofluorescence and histological analysis. To confirm cell attachment and biocompatibility of β-TCP particles cellular release of LDH was assessed. Osteogenic differentiation was analyzed on gene expression level using qRT-PCR. The chondrogenic model, a scaffold-free 3D cartilage construct (fzmb GmbH) was generated using hMSCs. Chondrogenic differentiation was performed under hypoxia (37 °C, 1% O2) with intermittent mechanical stimulation and analyzed by histology.
Results: We developed an *in vitro* 3D trabecular bone model by seeding hMSCs on β-TCP scaffold after pre-incubation for 24 hours. The analysis of cell viability via LDH detection showed no toxic effects on the cells seeded as compared to the corresponding control. Furthermore, we assessed cell attachment and proliferation by measurement of LDH activity after scaffold crushing. As a result, samples showed higher LDH activity compared to the controls. Histological and immunofluorescence analysis based on DNA and actin staining demonstrated cell attachment until day 21. After 21 days, cells were located more inside the scaffold compared to day 1. qRT-PCR expression of bone-related genes such as RUNX2, SPP1 and COL1A1 confirmed the phenotypic change during osteogenic differentiation on the scaffold. Furthermore, the scaffold-free 3D chondrogenic structure was confirmed by HE staining representing the different zones. Cartilage phenotype was confirmed by the reduced expression of Col1a1, an abundant expression of Col2a1 and Aggrecan.
Conclusions: The initial results from our *in vitro* 3D osteogenic and chondrogenic model confirm good cell vitality which indicates successful progression. To confirm the exchange of β-TCP through cellular matrix, we will now extend the assay co-cultivation time for up to 6 weeks. This 3D multi-component joint model should enable us to simulate arthritis and to study the efficacy of drug treatment *in vitro.*
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FRI0003 MODULATION OF ARTICULAR JOINT TISSUE TURNOVER IN BOVINE EXPLANT MODELS OF CARTILAGE AND SYNOVIAL MEMBRANE: DEVELOPMENT OF NOVEL DRUG DEVELOPMENT TOOLS

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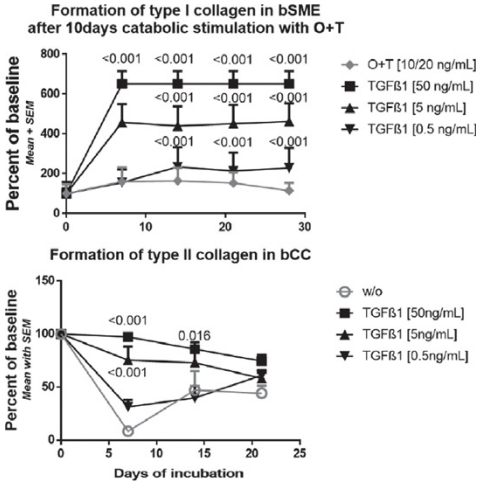
Background: There is currently a lack of disease modifying OA drugs approved by the drug administration agencies. One of the obstacles in the development of DMOADs that can effectively halt or reverse cartilage degradation is the lack of robust and reproducible model systems for early drug testing.
Objectives: To develop inducable anabolic bovine ex vivo models of 1) synovial membrane explants and 2) synovial membrane explants in co-culture with cartilage explants.
Methods: Synovium (bSME) from healthy bovine (<24months old) hind knees were isolated and cut into explants of 30±5 mg. Bovine cartilage (BEX) was cut into equally sized explants using a 6mm diameter biopsy puncher. bSME and BEX were cultured together (bCC) or alone for 14–35 days in DMEM-GlutaMAX™ with or without continuous stimuli in 4 replicates per treatment: Oncostatin M [10 ng/mL] + TNF-α [20 ng/mL] (O+T), O+T + GM6001 [1uM], IGF-1 [100ng/mL] or TGFβ-1 [50–0.5 ng/mL]. Media were changed 3 times a week and the viability was assessed with Alamar Blue every week. The reversibility of synovial membrane degradation was investigated by 10 days incubation with O+T followed by 21 days of TGFβ-1 [50–0.5 ng/mL] treatment. The following biomarkers were assessed in the conditioned media by competitive ELISA: Type I, II and III collagen degradation (C1M, C2M and C3M), formation of type I and II collagen (P1NP

and PRO-C2), aggrecanase degraded aggrecan (AGNxI) and MMP degraded aggrecan (exFFGV). C1M and C3M are synovial membrane biomarkers and C2M, AGNxI and exFFGV are cartilage biomarkers.

Results: Explants were viable throughout the experiments, albeit the bSME lost some viability with time. bSME treated with O+T showed increased C1M and C3M (>400% and >200%) from day 10, compared to w/o, whereas in bCC O+T increased C1M from day 21 and C3M from day 14 (>800%, >1900%). O+T treatment increased C2M was increased from day 21 (>400% and >1000%) in both BEX and bCC. The release was blocked by the generic MMP inhibitor GM6001 which also decreased the C1M and C3M compared to w/o. O+T treatment increased AGNxI at day 7 and 10 (>600%) and exFFGV from day 21 (>650%) in both BEX and bCC. In bSME, TGF-b1 continuously and dose-dependently increased P1NP from day 7 compared to w/o (250%). O+T pre-treatment for 10 days followed by TGF-b1 stimulation increased P1NP after 7 days of TNF-b1 treatment (150%, figure). IGF-1 did not affect the P1NP level at any time point in bSME. In bCC both TGF-b1 (dose-dependently) and IGF-1 sustained the PRO-C2 level at the level of baseline throughout the study periods (figure), whereas O+T decreased PRO-C2 compared to with w/o. The PRO-C2 level in BEX with TGF-b1 was unaltered compared to w/o.



Conclusions: We here show that bovine synovium can be anaobolic and catabolic stimulated, both alone and in co-culture with cartilage. Anabolic stimulation was achieved with TGF-b1 in both bSME and bCC, while IGF-1 only showed an anabolic effect in the bCC. Previous human explant models using human tissuehave lacked the anabolic capacity. These translational explants models may be applied in the early development of anabolic drugs for cartilage degenerative diseases.

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FRI0004 14-3-3ETA AS A NOVEL INVADOSOME REGULATORY MOLECULE IN RHEUMATOID ARTHRITIS

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Background: Progressive cartilage destruction, mostly mediated by invasive fibroblast-like synoviocytes (FLS), is a central feature in the pathogenesis of rheumatoid arthritis (RA). We have reported that the ability of arthritic FLS to degrade the extracellular matrix depends on the formation of actin-rich plasma membrane invadosomal structures detected in cells strategically located at the cartilage-synovial membrane interface. Interference with the formation of invadosomes in RA FLS impeded matrix degradation in vitro and cartilage degradation in a model of collagen-induced arthritis, suggesting that invadosomes are important physiological structures involved in cartilage destruction.

The chaperonin molecule 14–3-3η has been detected in the joints of patients with early and established RA and that its concentration in both serum and synovial fluid correlated with elevated expression of extracellular matrix (ECM) degrading enzymes and erosive damage. Extracellular 14–3-3η has therefore been proposed to be a novel biomarker for joint damage and a potential drug target for the personalized treatment of connective tissue-associated diseases but the direct relationship between 14–3-3η and joint damage remains a key area of research.

Objectives: To evaluate the role of 14–3-3η in the ability of synoviocytes to degrade the ECM.

Methods: mRNA from primary synoviocytes of healthy individuals (N=3) and RA patients (N=5) was extracted and the relative level of 14–3-3 isoforms and MMP gene expression was determined by qPCR. The ability of the synovial cell lines to degrade ECM was assessed by in situ invadosome assays using fluorescent cross-linked gelatine. Confocal microscopy was used to determine the cellular localization of 14–3-3η.

Results: We found a significant increase in 14–3-3η, MMP1 and MMP3 mRNA levels in synoviocytes from rheumatoid arthritis patients compared to cells from non-arthritis individuals. A strong correlation between 14–3-3η expression levels and the ability of synoviocyte cell lines to form invadosomes was observed (r2=0.8299). Knockdown of 14–3-3η decreased the ability of arthritic synoviocytes to form invadosomes indicating a role of 14–3-3η in extracellular matrix degrading ability. Confocal microscopy revealed that 14–3-3η staining was mostly found as small punctated structures in the cytoplasm and at the cell periphery of arthritic synoviocytes where they colocalized with leading edge F-actin and discrete patches of the exocyst component, Exo70.

Conclusions: The finding of the role of 14–3-3η in invadosome formation points to a previously unappreciated facet of how 14–3-3η influences joint ECM remodelling and reinforces its role as a marker of RA progression and joint damage. How 14–3-3η is involved in the regulation of MMP production/secretion and the possible role it plays in remodelling of actin-rich subcellular structures is the subject of ongoing studies.

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FRI0005 TARGETING CARTILAGE AGING AS OSTEOARTHRITIS THERAPEUTICS BY DRUG REPURPOSING

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Background: Effective treatments for Osteoarthritis (OA) are not available. In aging-related diseases, including OA, failure of cellular homeostasis mechanisms, such as autophagy can cause extracellular matrix destruction and cell death (1). With aging, chondrocyte function is diminished, contributing to a cellular senescence phenotype often observed in OA chondrocytes. In addition, a defect in autophagy is observed in both aging and cartilage degeneration (2,3).

Objectives: The objective of this study was to identify anti-senescence and pro-autophagy molecules by a cell-based high-throughput screening (HTS) in human chondrocytes.

Methods: To induce cellular senescence or reduced autophagy, immortalized human chondrocytes (TC28a2) were seeded (2500 cells/well) in 384 well plates, and treated with IL-6 (20 ng/ml) for 72 or 18 hours, respectively. Then, chondrocytes were incubated with Prestwick Chemical Library (1120 approved drugs with chemical and pharmacological diversity, as well as bioavailability and safety in humans) at 10 μM for 72 hours. To identify anti-senescence hits, nuclei was stained with Hoechst 33342 (2,5μg/ml), while β–galactosidase subcellular structures was stained by using Imagene Green C12FDG substrate (30 μM). To evaluate autophagic flux, a reporter cell line was generated by retrovirus transfection of pBABE-mCherry-EGFP-LC3 plasmid in TC28a2 chondrocytes. Plates were imaged by using Operetta® High Content Screening (HCS) system in non-confocal mode using the 20x WD objective. For each well, 4 fields and 4 planes of bright field, Hoechst and fluorescein channels were obtained. Relative intensity of C12FDG in cytoplasm and number of autophagosomes/autolysosomes per area of cytoplasm were determined to quantitate β–galactosidase activity and autophagy flux respectively. Compound validation was performed in TC28a2 chondrocytes and in primary human chondrocytes by evaluating cell senescence, autophagy pathway and cell death by apoptosis.

Results: A primary screening was performed to identify anti-senescence compounds by measurement of senescence-associated β-galactosidase activity. 283 compounds with anti-senescence effects were identified. The anti-senescence hits were analyzed by monitorizing autophagic flux. 29 compounds with both anti-senescence and pro-autophagy effects were selected. Then, one compound was selected for further validation. The compound reduced chondrocyte senescence, increased autophagy ($p<0.0001$) and protected against inflammation and cell death by apoptosis in human chondrocytes ($p<0.05$) in response to IL-6. Interestingly, this protective effect was partially mediated by mTOR inhibition, a proposed mechanism to prevent cartilage aging.

Conclusions: Our screening methodology provides a unique opportunity to repurpose drugs and mechanisms to prevent cartilage aging. Autophagy activation and protection against senescence in chondrocytes may provide benefits for delaying cartilage degeneration.

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