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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.

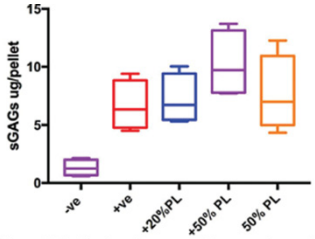

Figure 1: Evaluation the use of PL as an alternative TGFBeta in chondrogenic media. A: sGAGS activity of SF-MSC in different chondrogenic media; -ve: high glucoase+10% FCS, +ve: complete chondrogenic media, +20%PL: complete media with no TGFBeta and with 20%PL, +50%PL: complete media with nc TGFBeta 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