214 ROLE OF INSULIN IN SOX9 MEDIATED COL2A1 EXPRESSION IN PASSED ARTICULAR CHONDROCYTES

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Purpose: One of the factors preventing clinical application of regenerative medicine to degenerative cartilage diseases is a suitable source of cells. Cartilage tissue is sparsely cellular and chondrocytes are the only cell type but under in vitro culture conditions they lose their phenotype along with the ability to generate hyaline cartilaginous tissue. Here we describe a reliable serum and growth factor free culture system to reverse this change in phenotype.

Methods: Bovine articular chondrocytes (BAC) were passaged twice to allow for cell number expansion (P2) and cultured at high density on 3D collagen type II-coated membranes in media supplemented with ITS+ with 4.5g/l glucose, proline and 10-8 M dexamethasone (SF3D). Cultures were grown for up to 4 weeks. Inhibition studies were initiated on day 2 with 100μM HNMPA-(AM3). For FACS cells were harvested with Trypsin, stained with antibodies reactive with CD105-PE or CD44-PE (eBioscience, US) and analyzed by EPICS XL FACS and Kaluza analysis software (Beckman Coulter, US). ChIP-qPCR was carried out after in-situ cross-linking with 0.75% formaldehyde. Cells were sonicated in 1% Triton-100 and 1% SDS lysis buffer (Vibracell 30sec ON and 60sec OFF, 20x) and 250-900 bp DNA fragmentation was confirmed by agarose gel electrophoresis. Purified DNA was analyzed by qPCR using primers within the enhancer region of COL2A1. For immunoprecipitation cells were extracted in RIPA buffer and incubated with Sox9 antibody, the immune complex was harvested with Protein A/G beads. Extracts were immunoblotted with antibodies reactive with Sox6 and Med12.

Results: 99% of P2 cells in monolayer culture were CD44+ and 40% were CD105+. They showed differential gene expression with high type 1 collagen and low type II collagen. Gene expression of other cartilage associated genes was lower in P2 cells when compared with the primary P0 chondrocytes. When placed in SF3D the P2 cells expressed chondrogenic genes and accumulated extracellular matrix (ECM) rich in proteoglycans (PGs) and type II collagen. Decreasing insulin receptor (IR) with HNMPA-(AM3) inhibited collagen and PGs synthesis and reduced expression of col II, col XI, IR and IGF1R genes as well as Sox6 and 9 proteins. Co-IP and ChIP analysis on inhibited cells showed binding of co-activators Sox6 and Med12 with Sox9 but reduced Sox9-Col2A1 binding.

Figure 1. Photomicrographs of the tissue formed by P2 when cultured in SC3D culture show no matrix accumulation (far left panel) SF3D show accumulation of matrix rich in PGs evident by Toluidine Blue staining (second panel). SF3D tissue stained positively for collagen type II (red) (third panel) similar to native cartilage tissue stained with the same antibodies (fourth panel).

Conclusions: Here we describe a novel serum/ xenogeneic compound/ growth factor- free culture system which promotes hyaline-like cartilage tissue formation by insulin mediated regulation of Sox9-Col2A1 binding. This is a critical step towards the ultimate goal of developing autologous patient specific tissue engineered hyaline-like cartilage tissue suitable to use for repair/replacement of damaged articular cartilage. The suitability of this system for use in regenerative medicine approaches needs to be examined in vivo.

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215 JOINT DISTRACTION RESULTS IN CLINICAL AND STRUCTURAL IMPROVEMENT OF HAEMOPHILIC ANKLE ARTHROPATHY: A SERIES OF THREE CASES


Purpose: Joint distraction is extensively studied in osteoarthritis and found to be very effective in diminishing pain and improving joint function. Uniquely, this treatment also results in actual joint tissue repair as proven by MRI, X-ray and biomarker analyses (Intema et al., Ann Rheum Dis 2011). With this treatment a prosthesis or arthrodesis can be delayed which is especially of value for younger patients (<65 years). This promising treatment can be a good alternative treatment for patients with haemophilic arthropathy who regularly need joint replacement of their affected joint or arthrodesis on a very young age (<40 years).

In severe haemophilic ankle arthropathy, the standard surgical treatment options are fusion of the tibiotalar joint and total ankle replacement. Both treatments have complications and therefore an alternative treatment as joint distraction is desired. In this study, treatment of haemophilic ankle arthropathy with joint distraction was explored for the first time.

Methods: Three patients with haemophilic ankle arthropathy were treated with joint distraction using an Ilizarov external fixator for 10–15 weeks. After 1.7–3.4 years clinical outcomes like function, participation and pain were evaluated in retrospect with different questionnaires: haemophilia activities list (HAL), impact on participation and autonomy (IPA) and the Van Valburg questionnaire. Post-operative ankle joint mobility was measured. Structural changes were assessed blinded on X-ray by the Pettersson score and ankle images digital analysis (AIDA) and by an MRI score.

Results: All three patients were very satisfied with the clinical outcome of the procedure. They reported a clear improvement for self-perceived functional health (mean HAL sum score improved from 44±28.4% to 73±17.7% (p=0.04)), impact on participation and autonomy (statistically significant improvement on the domains family role, autonomy outdoors and work & participation (p=0.014; p=0.004 and p=0.046 respectively)) and pain (mean VAS score decreased from 80±5.0% before distraction to 23±15.3% after distraction (p=0.016)). Partial ankle joint mobility was preserved in the three patients. The Pettersson score remained the same in one patient and slightly improved in the two other patients. The mean joint space width measured by AIDA increased from 2.3±1.8 mm to 3.4±1.0 mm (p=0.07) and the MRI score demonstrated improvement for all three patients after ankle distraction (pre-treatment 12.0±3.5; post-treatment 8.0±4.4 (p=0.020)).

Conclusion: Although retrospective in nature, this study suggests that joint distraction is also a promising treatment for individual cases of haemophilic ankle arthropathy, without additional risk of bleedings during treatment. These results warrant further prospective studies on joint distraction as treatment for haemophilic arthropathy.

216 PROMOTION OF HUMAN MSC OSTEOGENESIS BY PI3-KINASE/AKT SIGNALING AND POTENTIAL INFLUENCE OF CHOLESTEROL

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Purpose: Stem cells are an important resource for tissue repair and regeneration. The organization of signaling molecules in the stem cell membrane likely plays an important, but incompletely understood, role in governing stem cell phenotype and responsiveness to external stimuli. We have recently demonstrated that caveolin-1 (Cav-1) acts to inhibit osteogenesis of human bone marrow derived mesenchymal stem cells (MSCs). Cav-1 is a scaffolding protein of caveolae lipid rafts of the plasma membrane with an affinity for many signaling molecules. Others have shown that caveolar endocytosis of activated integrins contributes to the repression of rat MSC osteogenesis on soft matrices, at least partially by the co-internalization of BMP receptors. Given that caveolae are capable of integrin internalization, integrin focal adhesion signaling activates Akt, and Cav-1 is known to bind to Akt, we hypothesize that Cav-1 inhibition of osteogenesis may be mediated via