

Results: PBS resulted in disc height loss, decrease in MRI Index and mild degenerative changes on histological examination. SVF resulted in severe inflammatory responses, resulting in severe degenerative changes in all parameters. Histologically, round cellular infiltration and osteoclastic activity were observed. This inflammatory response was observed in neither cultured nor Optiprep® “treated” discs. The disc height and MRI index were decreased comparable to PBS-injected discs.

Conclusion: Removal of RBCs from SVF prevented a negative, inflammatory response in degenerated IVDs, implying that RBC removal from SVF is crucial for IVD regeneration.

(OP 169) Intra-Individual Comparison of Human Ankle and Knee Chondrocytes *In Vitro*: Relevance for Talar cartilage Repair

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As compared to knee chondrocytes (KC), talar chondrocytes (TC) have superior synthetic activity and increased resistance to inflammatory stimuli. We thus investigated whether these properties are maintained when TC are isolated from their own environment and de-differentiated *in vitro*.

TC and KC ($n=10$) were expanded in monolayer for 2 passages and then cultured in pellets for 3 and 14 days or in hyaluronan meshes (Hyaff®-11) for 14 and 28 days. The generated tissues were assessed biochemically [glycosaminoglycans (GAG), DNA, collagen I and II], histologically (Safranin-O) and by RT-PCR (collagen I and II). The proteoglycan and collagen synthesis of the pellets were measured following or not exposure to IL-1b.

Following 14 days of pellet culture, TC and KC expressed similar amount of collagen I and II mRNA and produced tissues with comparable amount of GAG and collagens. Proteoglycan and collagen synthesis increased between 3 and 14 days of culture to a similar extent for TC and KC. The drop in synthetic activity in response to IL-1b was similar among TC and KC.

Following 14 days of culture in Hyaff®-11, TC and KC generated tissue with similar amounts of GAG and collagens. The increase in the contents of these macromolecules from 2 to 4 weeks culture was larger (up to 2.2-fold) in tissues generated by KC.

The superior synthetic activity of TC as compared to KC is lost when chondrocytes, isolated from their original matrices, are de-differentiated and subsequently induced to re-differentiate, suggesting a critical role of the tissue environment in determining the properties of KC or TC.

(OP 170) Intracellular Fate Investigation of Bio-Eliminable Polymeric Nanoparticles by Confocal Laser Scanning Microscopy (CLSM)

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The present contribution is reporting on the assessment of *in vitro* cytotoxicity and intracellular fate of poly[(glycylglycine methacrylamide)*x*-co-N-(2-hydroxypropylmethacrylamide)] bio-eliminable polymer samples and relevant nanoparticles in Balb/c 3T3 cloned A31 mouse embryo fibroblasts cell line by using Confocal Laser Scanning Microscopy (CLSM). Nanoparticles were prepared by co-precipitating the polymers with fluorescein labeled human serum albumin (HSA-FITC) as the fluorescent probe and as the model protein drug. The toxicity of the polymer samples consisting of 25, 50 and 100%, respectively, of glycylglycinemethacrylamide monomeric units (GGMA), was investigated in terms of cytoskeleton morphology by exposing cell cultures to various concentrations of polymers for 24 h. Under normal culture conditions, fibroblast cells exhibit characteristic spreading and shape, however, when the cell cultures were subjected to chemical, metabolic or physical stress, their morphology changed in terms of cytoskeletal architecture and reducing their visibility. The co-polymer samples at 25% GGMA monomeric units showed a lower toxicity even at high concentrations [8.5 mg/mL]. The cellular uptake of polymeric nanoparticles was determined by comparing incubation of fibroblasts with HSA-FITC loaded particles to HSA-FITC alone at three different time end-points. The results indicate that the nanoparticles were up-taken by the cells in a time dependent fashion. Computer assisted analysis of nanoparticles fluorescence emission suggests a possible lysosomal escape as intracellular fate.

(OP 171) Isolation and Characterisation of Stem Cells from Different Human Salivary Glands

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There is continuously growing interest in adult stem cells as a source for regenerative medicine and tissue engineering applications. The characterisation of novel stem cell sources is therefore of utmost concern.

Recently we reported for the first time the presence of adult stem cells with mesenchymal characteristics in human parotid gland tissue. In this study we isolated stem cells from human submandibular glands and compared these cells to cells from parotid origin and characterised both in more detail.

Cells were isolated from normal submandibular and parotid glands by enzymatic digestion. Following initial proliferation cells were characterized by flow cytometry. For differentiation specific induction media and growth conditions were used with the purpose to generate adipogenic, osteogenic and chondrogenic cells. Differentiation was assessed by histochemical and immunocytochemical stainings as well as by the demonstration of specific mRNA using RT-PCR.

Cells from both gland types had surface characteristics very similar to mesenchymal stem cells. They were e.g. positive for CD13, CD29, CD44, and CD90 and negative for CD34 and CD45. Cells could be induced into adipogenic, chondrogenic and osteogenic celltypes, demonstrating their differentiation capability.