present. It is quite possible that these sMSCs are extremely sensitive to changes/damage to the joint environment and therefore may be exploited as a sentinel for early detection of OA.

**89 NORMAL AND OSTEOARTHRITIC SYNOVIAL STEM CELL- DERIVED TISSUE-ENGINEERED CONSTRUCTS RESPOND TO MECHANICAL STIMULUS FOLLOWING CHONDROGENIC DIFFERENTIATION**


**Purpose:** Tissue-engineering (TE) comprises cells, a scaffold, and appropriate signals. In the context of TE of articular cartilage for treatment of osteoarthritis (OA), synovial tissue-derived mesenchymal stem cells (sMSCs) have been demonstrated to be particularly suitable. These MSCs are present in the synovial fluid and their numbers increase dramatically with the onset of OA. Recently scaffold-free tissue-engineered constructs (TECs) from sMSC monolayers treated with ascorbic acid (AA) were developed in our laboratory. These TECs retained their chondrogenic potential after transplantation into chondral defects within a porous model. However, the implanted TECs exhibited suboptimal healing as they failed to take on a completely hyaline phenotype based on the presence of fibrous tissue at the surface of the repair sites. Therefore, the objective of this study is to modify the present TEC technology through the induction of chondrogenic differentiation using biochemical and mechanical signals.

**Methods:** Human sMSCs were derived from donor synovial tissue and synovial fluid and isolated using magnetic purification. When the cells reached confluence they were treated with either chondrogenic media containing TGF-β3, BMP-2, AA, and dexamethasone, media supplemented with only AA (TEC), or control media, for a period of 14 days. The chondrogenic TECs (cTECs) and AA-treated TECs were then subjected to mechanical compression for 24 hours with a loading protocol of 1 MPa at 1 Hz for 1 minute and 14 minutes of no load using a Flexcell system. Non-loaded TECs were maintained in parallel as controls. After loading, the remaining TECs and cultures were harvested for analysis. Analysis included RT-qPCR, histology & immunohistochemistry, and mechanical testing.

**Results:** Mechanical loading differentially regulated several genes commonly used as markers of chondrogenic differentiation in both the cTECs and AA-treated TECs (Fig. 1). Following mechanical compression, mRNA expression of SOX-9 and aggrecan are upregulated, while type-II collagen expression is highly variable. As well, histological assessment indicated significant differences in composition between the cTECs and AA-treated TECs (Fig. 2). The cTECs appear to have increased collagen content and are more sparsely populated with cells. Similar observations were made for TECs formed using MSCs from OA tissue, however the OA TECs tended to be quite variable in terms of their various properties.

**Conclusions:** The results indicate that the modified TEC protocol is more effective than the original protocol for producing TECs that exhibit characteristics similar to that of native articular cartilage, such as increased collagen content and relatively low cell density. Optimization of the protocol (e.g. loading regimen, timing of biochemical and mechanical cues, etc.) could lead to further improved TEC properties. However, additional modifications are necessary to achieve similar results with OA TECs.

![Fig. 1. Relative mRNA expression of chondrogenic markers in loaded and unloaded cultures treated with either chondrogenic media (left) or media containing AA (right), compared to initial controls.](image)

**Fig. 2. Sections of cTECs (A,B) and AA-treated TECs (C,D) stained with Masson’s trichrome (blue: collagen; red/purple: cytoplasm; black: nuclei). TECs were either loaded (A,C) or unloaded (B,D).**