Arthroscopically harvested, minimally manipulated synovium is a source of chondrogenic tissue and comparable to single cell isolation as determined by Safranin-O staining and immunohistochemistry

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Introduction

The synovium or synovial membrane is a thin, weak layer of tissue, which lines the non-cartilaginous surfaces within the joint space, sealing it from the surrounding tissue. Previously a number of groups have reported on the potential of synovium from the lining of the knee joint containing a source of progenitor cells that are capable of forming multiple tissues types. It has been suggested that between 3-10% of cells isolated after enzymatic digestion of synovium have multi-lineage potential and have been shown to be recruited from the synovial membrane into cartilage defects. Furthermore Sakaguchi et al showed that synovium-derived MSCs had great proliferation potential and had multi-lineage differentiation potential in vitro.

Current efforts for cell based therapies for focal defect repair such as ACI focus on in-vitro cell isolation and proliferation from terminally differentiated tissue and involve a multi-step two surgical procedure that is costly and time consuming.

The aim of our investigation was to determine:

i. if minimally manipulated synovial tissue fragments maintained a viable cell population post harvest
ii. could viable tissue be harvested arthroscopically
iii. did the tissue fragments have a chondrogenic capability

Methods

Preliminary work was carried out in the porcine knee; subsequent work with human tissue was harvested from surgical surplus from total knee replacement patients. Joint tissue was harvested using a Dyonics ® Shaver system and an ultraturbowhisker blade set at specific parameters determined to be optimal for providing a highly minced tissue.

Single cell isolates were prepared by resecting tissue from the same donor joint via pellet assay and used as a control for comparison to arthroscopically harvested tissue. Resected tissue was treated with enzymatic digestion, and cultured for 24 hours.

Cell viability was determined using live/dead stain and a Guava ® EasyCyte. Chondrogenic capacity was assessed via cartilage pellet assays culturing in basal media and chondrogenic media for 28 days and visualized for GAG content with Safranin-O and Collagen I, II and X via immunohistochemistry.

Results

Cell viability

Figure 1. Cell viability of four human donors each assessed in triplicate

There was no statistical difference in cell viability between the tissues isolated via arthroscopy (65.92% +/-16.4) or the control cell isolate group (72.8% +/-10.3).

Chondrogenic capacity of the harvested tissue

Figure 2. Cartilage pellet assays comparing arthroscopically harvested porcine tissue fragments against single cell isolation from the same donor.

A comparison between arthroscopically harvested minimally manipulated synovium and cells isolated from the same tissue using a pellet assay confirmed that both maintained a stable chondrogenic phenotype. Pellets were assessed via Safranin-O and immunohistochemistry (Col I, II and X) known chondrogenic markers in porcine and human tissue.

Discussion

Utilising both porcine and human tissue it has been demonstrated that the isolation via arthroscopy of minimally manipulated synovial tissue fragments provide a viable source of tissue. Furthermore comparing a number of indicators of cartilage formation the data confirms that synovium tissue fragments have the potential to become cartilage-like under the right conditions.

This study suggests that without the use of enzymatic cell isolation or cell proliferation that minimally manipulated joint tissues such as synovium have an inherent chondrogenic capacity and could be considered as a tool for the repair of articular cartilage focal defects.

References:


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