INTEGRATION ACROSS A CARTILAGE-CARTILAGE INTERFACE

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INTRODUCTION: The integration of native articular cartilage with allografts or tissue engineered constructs faces many challenges including; chondrocyte death adjacent to the cut surface [1], and a low number of chondrocytes migrating into the defect site [2]. Digestive enzymes allow chondrocytes to escape their dense matrix [3], but the ability of the freed chondrocytes to generate matrix that can span a fissure and reestablish a long-term stable interface is unclear. A filler material that could act as a conduit for chondrocyte movement whilst also supporting cell proliferation and matrix generation could circumvent some of these challenges.

We hypothesized that a biodegradable nanofibrous filler material could enhance the interface strength across a cartilage defect. In this study a commercially available hydrogel was injected into an in vitro cartilage gap model. The effects of cell incorporation within the hydrogel and supplementation with transforming growth factor (TGF-ß3) on cartilage integration were examined.

METHODS: Cartilage plugs (8mm diameter) were harvested from the femoral condyles and patella grooves of immature bovine knees (age of animals 3-6 months). The superficial and deep zones were removed, and samples were sliced to a thickness of 2.3 mm using a custom designed slicing apparatus. Samples were randomized to the following groups:
(1) Hydrogel, no cells, +TGF-ß3; (2) Hydrogel, cells, +TGF-ß3; (3) Hydrogel, cells, -TGF-ß3

Isolated chondrocytes were suspended in 10% sucrose solution at twice their final concentration, and mixed at a ratio of 1:1 with 1% Puramatrix™ (BD Biosciences, Franklin Lakes, NJ) to form a cell/gel solution of 0.5% Puramatrix™ containing 30 million cells/ml. Each piece of cartilage tissue was spot-dried and sequentially cored with 3.5 mm and 4.0 mm diam. biopsy punches to construct an inner core/outer ring with a peripheral gap of 0.25 mm. The inner core was removed, the outer ring was filled with either the hydrogel alone or the hydrogel/cell mixture and the corresponding inner core was replaced. Excess gel was removed and the Puramatrix™ was induced to self-assemble by exposure to 3 washes of DMEM for 5 min. each. The assembled construct placed into a 12 well plate which was filled with 3ml of chondrogenic medium (CM) consisting of DMEM supplemented with 0.1 µM dexamethasone, 50 µg/ml ascorbate, 40 µg/ml L-proline, 100 µg/ml sodium pyruvate, 1X IT5+ premix, 1X PSF [4] supplemented with 1% antibiotic/antimycotic, either with or without 10 ng/ml TGF-ß3 (R&D Systems, Minneapolis, MN) supplementation. Medium was changed twice weekly.

After 21 days of incubation 5 samples from each group were subjected to a push out test using an Enduratec ELF3200 testing machine (Enduratec Systems, Minnetonka, MN). Each sample was loaded at a rate of 0.2 mm/s until complete push out of the inner core occurred. The maximum stress (maximum load divided by surface area of outer ring) was computed. Two samples from each group were sequentially dehydrated and embedded in paraffin, sectioned into 8 micron thick slices, stained with Alcian Blue and Eosin, and microscopically examined.

RESULTS: The cell seeded hydrogels exhibited cell viability and matrix generation within the interface, and the other group remained in intimate contact with cartilage on either side of the gap (Fig 1a). Interface matrix generation within the group that was not supplemented with TGF-ß3 was not as robust (Fig 1b). Cells were found within the initially acellular group, however little matrix was generated by these cells (Fig. 1c).

A five-fold increase in maximum push-out stress was found for the cell seeded, TGF-ß3 supplemented group; and was statistically significantly higher than that of both other groups (Student’s t-test, p<0.001). There was no significant difference between the acellular or the cell seeded –TGFß3 groups.

DISCUSSION/CONCLUSION: This study demonstrated the potential application of a commercially available nanofibrous cell seeded hydrogel to foster integration across a cartilage gap. The cell seeded hydrogel that was supplemented by TGF-ß3 (a known regulator of cell growth and differentiation) exhibited abundant matrix generation within the hydrogel and a corresponding increase in maximum push-out stress as compared to all other groups.

While this study does not allow us to establish the origin of all of the cells within the hydrogel, the presence of some cells within the acellular group suggests that a small percent of cells can actually migrate into this material, which could further assist integration. Lack of TGF-ß3 supplementation detrimentally affected the histological appearance and mechanical strength of the interface. This suggests that to replicate the response seen in the cell seeded, TGF-ß3 supplemented group in vivo, cell transfection [6] prior to hydrogel incorporation might be required.

In conclusion, we have demonstrated the feasibility of using a nano-fibrous filler material towards integrating fissures in articular cartilage. This approach could be adapted to integrate osteochondral grafts with surrounding cartilage in a clinical setting.