Fabrication, Maturation, and Implantation of a Composite Tissue-Engineered Total Disc Replacement

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Introduction

Low back pain arising from disc degeneration is one of the most common causes of limited function in adults.[1] Current treatment options are limited, favoring either physical therapy and pain management or surgical methods to fuse the motion segment. Neither approach restores native tissue structure and function, and so a number of tissue engineering strategies have emerged that focus on the creation of a composite tissue engineered total disc replacement,[2,3] with some studies showing promise in vivo.[4,5] To further this line of inquiry, we fabricated a simple composite engineered disc based on the combination of a porous polymer foam annulus fibrosus (AF) and a hyaluronic acid (HA) gel nucleus pulposus (NP). We used these constructs to determine whether the combination of native AF/NP cells or mesenchymal stem cells (MSCs) would mature to a greater extent in vitro and which cell type would best retain their phenotype after in vivo implantation in a rat tail model of disc replacement.[6]

Methods

Porous polycaprolactone (PCL) foams were fabricated by salt-leaching to form the AF regions of the engineered discs. PCL was dissolved in chloroform at a 20% (w/v) concentration and NaCl particles were sieved to yield particulate of ~106 μm that was loaded and mixed into the PCL solution with a PCL/NaCl mass ratio of 1:4 (% w/w). The resultant solidified PCL sheet with entrapped salt particles was 1.5 mm in height and individual plugs were extracted using 4 mm biopsy punch for the outer diameter and 2 mm for the inner diameter; this geometry approximates that of the rat caudal disc. To form the NP regions of the engineered discs, 1% methacrylated HA (MeHA) hydrogels were produced as in Kim, et al.[7] AF cells (AFCs) or MSCs were seeded onto the PCL foam at a density of 2×10^6 cells/construct, whereas NP cells (NPCs) or MSCs were encapsulated in HA at a density of 20×10^6 cells/ml.AF and NP regions were cultured separately in chemically defined media and combined at 2 weeks. At regular intervals over 9 weeks, compressive mechanical, biochemical, and histologic properties were evaluated. Additionally, AF/NP cell and MSC/MSC cell-seeded constructs were implanted into the rat caudal disc space after 5 weeks of pre-culture, as in Martin, et al.[6] After 5 weeks in vivo, disc height, hydration of the nucleus pulposus, and structure were assessed by μCT, fluoroscopy, and quantitative T2 MRI, and structure was evaluated via histological analyses with alcian blue/picrosirius red and collagen type II staining.

Results

By 3 weeks, the NP region of all the groups stained intensely for proteoglycans, while collagen staining in the NP increased with further culture time. In the AF region, staining gradually increased with time, though to a lesser extent than in the NP (Figure 1). There were no significant changes in the compressive modulus over 8 weeks for either group (not shown). After 5 weeks of pre-culture and 5 weeks of implantation, the disc height index (DHI) for implanted constructs was significantly greater than pre-operative levels, with only small differences between groups. Implanted discs did not result in intervertebral fusion (Figure 2A). Alcian blue/picrosirius red staining showed abundant collagen in the disc, but little

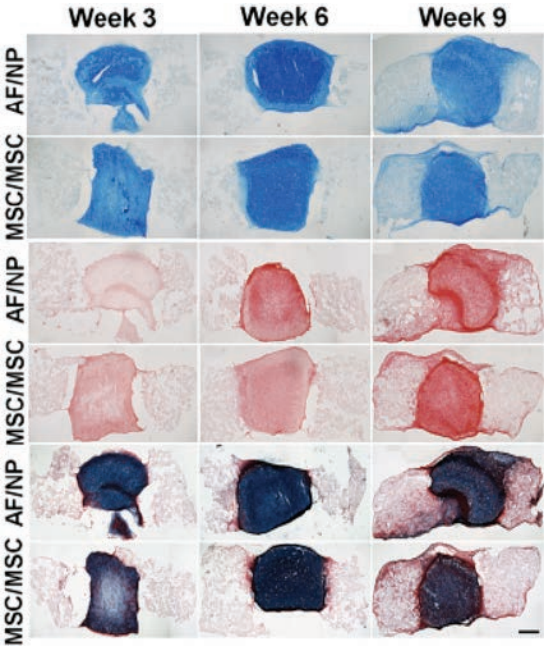

Figure 1. (A) Alcian blue, (B) picrosirius red, and (C) Alcian blue/picrosirius red staining of AF/NP and MSC/MSC engineered disc with time in in-vitro culture. (bar = 500 μm).

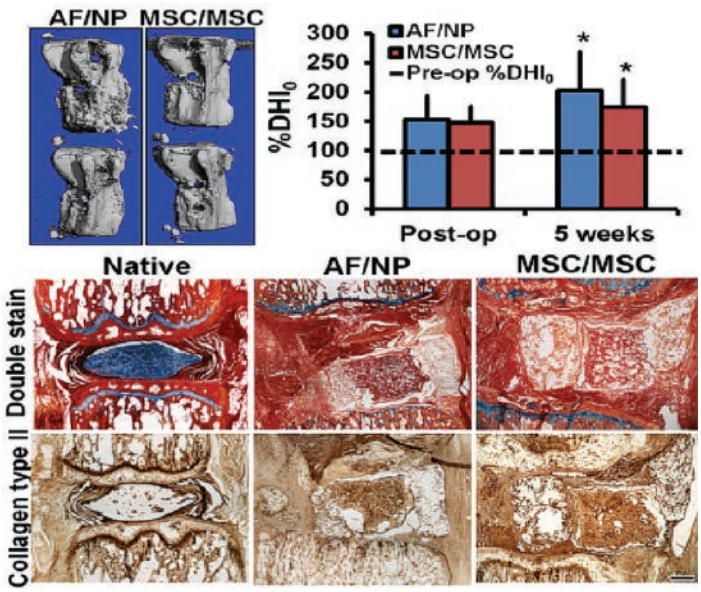

Figure 2. (A) μCT and fluoroscopic analysis of %DHI for implanted AF/NP and MSC/MSC engineered disc and (B) histology and immunostaining at 5 weeks after implantation. (*:$p < 0.05$ vs. pre-op) (bar = 500 μm).

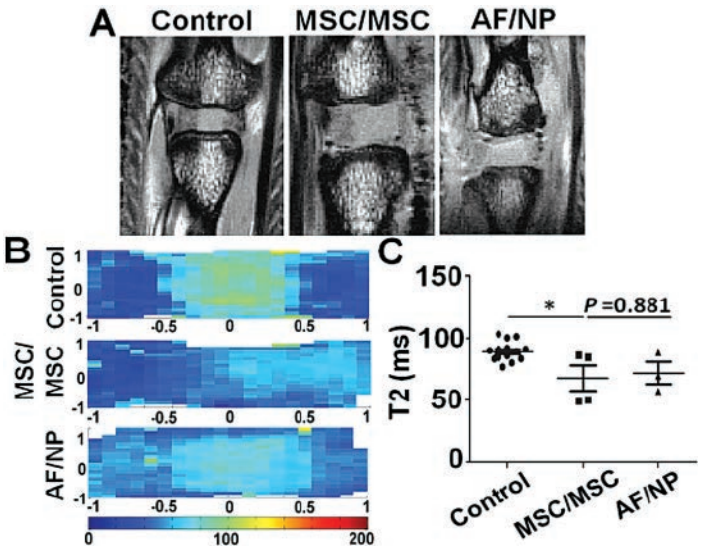

Figure 3. (A) T2 MRI images and (B) T2 maps with (C) quantification at 5 weeks after implantation (*:$p = 0.009$ vs. control).

proteoglycan in the NP region in either group. However, collagen type II staining was intense and localized to the NP at this time point (Figure 2B). MRI showed that implanted discs had a similar structure to native discs (Figure 3A, B). T2 mapping showed reduced signal in the NP for both groups compared to native discs. However, there was no significant difference between the AF/NP group and native discs (Figure 3C).

Discussion

This study demonstrated that a tissue engineered disc composed of a PCL foam AF region and a hydrogel NP region could be fabricated, matured in vitro, and implanted and maintained in the rat caudal spine. Engineered discs comprised of AF/NP cells and MSCs performed similarly, maintaining their structure after 5 weeks in vivo, though loss of proteoglycan was evident in the NP region for both groups. This suggests that, following 5 weeks of implantation, water and proteoglycan content are less than in the native disc, perhaps reflecting the inflammatory nature of the operative site and unwanted remodeling post-implantation.

Significance

This work demonstrates the successful fabrication, maturation, and in vivo function of a composite engineered disc composed of a PCL foam AF and a hydrogel NP using both native disc cells or MSCs.

Acknowledgements

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