Controlled Delivery of Mesenchymal Stem Cells and Growth Factors Using a Tendon-Specific Nanofiber Scaffold

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SIGNIFICANCE: Despite advances in surgical techniques and rehabilitation protocols, tendon repairs remain prone to poor clinical outcomes [1]. We have developed a novel, tendon-specific scaffold capable of delivering cells and growth factors for improved tendon healing.

INTRODUCTION: Recent efforts to enhance tendon healing have focused either on cell-based or growth factor-based therapy. Few studies have examined the combination of cells and growth factors for tendon repair. We developed a tendon-specific scaffold for delivery of stem cells and growth factors concurrently. Our scaffold combines a heparin/fibrin-based delivery system (HBDS) with an electrospray nanofiber poly-lactic-co-glycolic acid (PLGA) backbone. The HBDS allows for the delivery of stem cells and tenogenic growth factors (e.g. BMP-12 or BMP-14) in a controlled manner [2, 3], while the PLGA backbone provides scaffolding, providing a platform for the scaffold properties of the scaffold. The PLGA can be electrosprayed in an aligned fashion to mimic collagen fibers found in the native tendon. We hypothesized that a HBDS/Nanofiber scaffold will be tolerated in vivo and will be capable of delivering both mesenchymal stem cells and growth factors in a controlled manner.

METHODS: HBDS/Nanofiber scaffold fabrication: PLGA nanofiber mats were electrosprayed in an aligned fashion using a mandrel collector. The mats were then layered with either a fibrin gel or a HBDS. The number of layers was adjusted based on the specific use of the scaffold.

In vitro studies: Although the release kinetics of the HBDS alone is well characterized [3], the effect of the layered PLGA on those release kinetics was unknown. Therefore, 3-layer scaffolds (i.e. 3 layers of PLGA and 2 layers of fibrin) containing 50 ng of platelet-derived growth factor (PDGF-BB) were made with and without the HBDS (N=4). HBDS gels were also made as controls. The scaffolds and the gels were washed daily for 9 days and an enzyme-linked immunosorbent assay (ELISA, R&D Systems) for PDGF-BB was performed on all wash volumes. In vivo studies: A series of surgeries were performed in order to: 1) ensure that the scaffold did not elicit a negative response, 2) determine cell viability post-operatively, and 3) determine the residence time of the scaffold. During fabrication, a small amount of FITC was incorporated into the PLGA in order to identify the scaffold post-operatively using fluorescent imaging. Similarly, the ASCs were labeled with Di-I, a fluorescent membrane dye, prior to scaffold assembly. 6-layer scaffolds containing 1x10^6 adipose-derived mesenchymal stem cells (ASCs) were implanted into the intrasynovial flexor tendons of canines (n=7) at the time of repair [4]. Flexor tendons were transected sharply and longitudinally oriented horizontal slits were created in the center of each tendon stump. The HBDS/Nanofiber scaffold was secured within the repair site by the core suture and sealed in that location by a running epitendon suture. Acellular scaffolds were implanted into a second digit of each dog to serve as a paired control. The animals were sacrificed 9 days post-operatively. The operated tendons were removed by dissection and prepped for either histology (N=2) or Total DNA (N=5). A “time-zero” control was also performed on a cadaver animal.

RESULTS: In vitro studies: Sustained delivery of PDGF-BB was achieved from the HBDS/Nanofiber scaffold (Figure 1). The HBDS/Nanofiber scaffold released an initial burst of growth factor amounting to ~40% of the total dosage on the first day. The remaining growth factor was released slowly over the course of the next 8 days for a total release of ~90%. Compared to the HBDS alone, the HBDS/Nanofiber scaffold released a greater initial burst of growth factor. Fibrin/Nanofiber scaffolds released ~70% of the growth factor on day 1 and released very little growth factor thereafter. In vivo studies: Based on gross observations at the time of dissection, the HBDS/Nanofiber scaffold did not elicit any negative response. The tendons were intact and had minimal gapping and adhesions (Figure 2). Using fluorescent labeling and imaging techniques, the scaffold at the repair site was easily identifiable (Figure 3). Fluorescent imaging also verified the viability of the implanted cells. 9 days post-operatively, fluorescently labeled cells were clearly evident in the 9 day cellular group, but not in the 9 day acellular group (Figure 4). Similarly, there was a significant increase in total DNA in the cellular group compared to the acellular group and normal/uninjured controls (Figure 5).

DISCUSSION: We developed a novel scaffold that allows for the delivery of both cells and growth factors. We showed that:
1) The delivery of growth factors can be controlled using a layered HBDS/Nanofiber scaffold.
2) Implantation of the HBDS/Nanofiber scaffold is well tolerated in the intrasynovial flexor tendon environment.
3) ASCs delivered using the HBDS/Nanofiber scaffold remain viable for at least 9 days post-operatively.

Future studies will implement delivery of ASCs in conjunction with tenogenic growth factors in an effort to enhance flexor tendon repair.

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