A Comparative Study of the effects of Growth and Differentiation Factor 5 on Muscle Derived Stem Cells and Bone Marrow Stromal Cells in an in vitro Tendon Healing Model

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Disclosures:

Introduction: Hypocellularity of flexor tendons slows intrinsic healing and often requires prolonged protection of the injury site after repair, which causes adhesion formation. To address this problem, stem-cell-based therapy has been recently introduced with encouraging outcomes in preclinical evaluations. The cells most commonly used for tendon repair are bone marrow stromal cells (BMSCs) (1). However, our recent data has shown that muscle derived stem cells (MDSCs) have improved potential to differentiate into tenocyte-like cells compared to BMSCs (2). Furthermore, Growth Differentiation Factor-5(GDF-5) has been shown to promote tendon healing in several animal models (3). In this study, we investigated the ability of MDSCs, supplemented with GDF-5, to improve tendon healing compared to that provided by BMSCs with similar treatment in an in vitro tendon culture model.

Methods: A total of eighty canine flexor digitorum profundus (FDP) tendons from hind paw digits of 10 dogs, used in other Institutional Animal Care and Use Committee (IACUC) approved studies, and harvested immediately postmortem, were assigned into 5 groups: 1) repaired tendon without gel patch interposition (no cell group), 2) with BMSC seeded gel patch interposition (BMSC group), 3) with MDSC seeded gel patch interposition (MDSC group), 4) with GDF-5 treated BMSC seeded gel patch interposition (BMSC+GDF-5 group) and 5) with GDF-5 treated MDSC seeded gel patch interposition (MDSC+GDF-5 group).

MDSCs were isolated using a modified pre-plate technique (4). BMSCs and MDSCs were seeded into a collagen gel patch. Each patch included 2.0 X 10^5 cells. Tendons were shortened by cutting them to a standard length of 30 mm, and then lacerated centrally. Two identical gel patches were implanted between the tendon ends and tendons were repaired with two simple suture of 6-0 Prolene (Ethicon). After culturing for 2 or 4 weeks, the failure strength and stiffness of the healing tendons from zone II were measured with a custom-designed micro tester (1). The tendon repair sutures were cut, and the specimen was distracted at a rate of 0.1 mm/second. Stiffness was determined from the slope of the linear region of the force/displacement curve. Repaired tendons from zone III were assigned to quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR) and histological study. The gene expression levels of collagen I, III and tenomodulin were measured. All data were reported as a mean ± standard deviation. One-way analysis of variance (ANOVA) and the Tukey-Kramer post hoc test were performed to compare means of the failure strength, stiffness and gene expression. The significance level was set to P<0.05 in all cases.

Results: After 2 weeks in tissue culture, the mean failure strength in the MDSC+GDF-5 group was significantly higher than that of the no cell group, the BMSC group and the BMSC+GDF-5 group (P<0.05). After 4 weeks in tissue culture, the mean failure strength of the MDSC+GDF-5 group remained significantly higher than that of the no cell group, BMSC group and MDSC group (P<0.05 ) (Figure 1). At both 2 and 4 weeks in culture, the mean repair site stiffness of the MDSC+GDF-5 group was significantly higher than that of the no cell group (P<0.05). The expression of collagen I, III and tenomodulin mRNA was not significantly different among the groups. Under confocal microscopy, implanted cells became incorporated into the original tendon in all four cell-seeded groups (Figure 2).

Discussion: Muscle has been shown to be a rich reservoir of mesenchymal stem cells. These cells have the ability to differentiate into several cell lineages. Considering the potential for local harvest and given the anatomical continuity of muscle with tendon, we chose MDSCs as a novel stem cell source for tendon repair. In the present study, we demonstrated that implantation of MDSCs in combination with GDF-5 accelerated tendon healing at both 2 and 4 weeks in cell culture. This study has several limitations. First, this is an in vitro study. Second, a significant difference in mRNA expression of tenomodulin was not observed, although this may be related to the fact that we measured tenomodulin in a segment of the repaired tendon, including the gel patch with the seeded cells, and not just the patch itself. Third, we did not assess expression of mesenchymal stem cell markers, such as CD44.

Significance: MDSC implantation and administration of GDF-5 may improve the outcome of tendon repair in vivo.

Figure Legends:
Figure 1. Mean failure strength of the repaired tendons. Error bars represent standard deviation. An asterisk indicates a significant difference (P<0.05).
Figure 2. The labeled BMSCs and MDSCs with Vibrant DiI cell labeling solution were observed under confocal microscopy with red fluorescence at 4 weeks. Blue fluorescence indicates nuclei. (Original magnification x100. Scale bar represents 100μm).

Acknowledgments: This study was supported by grants from the American Foundation for Surgery of the Hand (J. Leonard Goldner Pioneer Research Award and AFSH Basic Science Grant).

(4) Chirieleison SM et al. Tissue Eng Part A 2012;183(4):232-