Augmentation of In Vitro Flexor Tendon Repair Using Freshly Isolated Bone Marrow Mononuclear Cells

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Introduction: Despite advances in surgical repair techniques, healing of flexor tendons remains problematic because of poor vascularization and hypocellularity [1-2]. Since extrinsic healing processes are usually predominant in flexor tendon healing, augmentation of intrinsic healing using cells, scaffolds, growth factors, or a combined solution, is warranted. Mesenchymal stem cells (MSCs), expanded over time in in vitro culture, are effective in enhancing wound healing [3]. Such an approach will not be practical in patients with acute tendon injuries, since the tendons require immediate repair. Hence, investigation into alternative cells sources which can be isolated without the need for in vitro culture and which have the capacity to accelerate repair is required. Bone Marrow-Mononuclear Cells (BM-MNCs) are an ideal cell source, because they are easily isolated from bone marrow in a closed, sterile system in a short period of time (~30 mins). Cell therapies using BM-MNCs have been used to treat myocardial infarction, limb ischemia, bone defect reconstruction, ischemia-reperfusion muscle injury and collagenase induced tendinitis [4-7]. To our knowledge, these freshly isolated BM-MNCs have not been utilized in treatment of acute flexor tendon lacerations. Therefore, we proposed to 1) investigate the ability of the BM-MNCs to improve flexor digitorum profundus (FDP) tendon healing compared to BMSCs in a canine tendon in vitro culture model and 2) determine if the addition of Growth and Differentiation Factor-5 (GDF-5), would further enhance the results.

Methods: Mixed-breed dogs were used for this study, in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines. A total of 96 FDP tendons from the 2nd to 5th front paw digits of 12 dogs were harvested. The tendons were divided into six groups: 1) Suture (control) 2) Sponge (control), 3) BM-MNC, 4) BMSC, 5) BM-MNC+GDF5 6) BMSC+GDF5. Tendon tissue from zone II was used for biomechanical testing and that from zone III was used for histological assessment. After 2 or 4 weeks tissue culture, mechanical strength, gene expression, and histology of the tendons were assessed. Bone marrow stromal cells (BMSCs) were isolated from harvested tissue using established procedures. Mononuclear cells from bone marrow were isolated from the same dogs from which BMSCs were sourced, through the ficoll-gradient centrifugation method. For both cell types, 0.5 x 10⁶ cells were seeded into collagen sponges. For the growth factor stimulation group, the BMSC- or BM-MNC-seeded collagen sponges were supplemented with rhGDF-5 at a concentration of 100 ng/mL. The collagen patch was placed between the lacerated tendon ends and the repair was sutured. Tendons were placed in a custom frame and cultured in an incubator for either 2 or 4 weeks. The biomechanical properties (failure strength and stiffness) of the repairs were tested using a custom uniaxial test system. The sutures were cut prior to testing, in order to test the strength of the healing tissue alone. Gene expression of Col1, Col3, FN and Tenomodulin were analyzed using RT-PCR. H&E staining was also performed. Differences in mean maximum healing strength, stiffness and gene expression between groups were analyzed by two-way analysis of variance (ANOVA). The Tukey-Kramer post hoc test was performed for each pairwise comparison, with significance set to p<0.05.

Results: At two weeks, no difference was seen in failure strength and stiffness between controls and cell-sponge groups. By four weeks all the cell-sponge group exhibited significantly higher failure strength and stiffness than that of controls (Figures 1 & 2). The failure strength and stiffness of the BM-MNC group with or without the GDF-5 were similar to that of the cultured BMSCs groups at both 2 and 4 weeks (Figures 1 & 2). GDF-5 did not augment the strength or stiffness at either 2 or 4 weeks. Gene expression analysis revealed that Col1, Col3, FN (not shown in figure) and tenomodulin were similar among the cell-sponge groups at 4 weeks (Figure 3). Tenomodulin was higher in BM-MNC groups at 4 weeks.

Discussion: In this study, we demonstrated the ability of freshly isolated BM-MNCs to augment in vitro flexor tendon repair, and this improvement was similar to that obtained by using expanded BMSCs. Hence these BM-MNCs would be an ideal cell source for acute needs, such as to augment a flexor tendon repair. Our study has some weaknesses and limitations. This in vitro healing model does not replicate the healing in vivo. This model is useful in screening various conditions before expensive in vivo studies are enacted. The in vitro failure strength is very weak, and modest improvements in the cell-sponge group were observed. However, it is important to note that this model examines the healing strength of the tendon itself, and small improvements in vitro would, likely, translate to larger effects in vivo. Based on these results, we anticipate pursuing future large animal and, eventually, clinical studies involving immediate harvest and seeding of BM-MNCs to augment tendon repair after injury, using these freshly isolated cell-seeded tendon patches, with the ultimate goal being production of a strong tendon repair. Significance: Methods for substantially improving intrinsic tendon healing are still needed in order to improve clinical outcomes. Current regenerative medicine strategies are inefficient, expensive, and time consuming requiring many weeks of in vitro culture.
to isolate and expand MSCs, which hampers translation of such therapies to clinical practice. The methods proposed here using freshly isolated BM-MNCs could accelerate the translation of regenerative medicine to clinical practice.

Figure Legends:
Figure 1. Failure strength at 2 and 4 weeks. N=8. Error bars represent standard deviation.
* Significantly different from Sponge (p<0.05). , ** Significantly different from Suture (p<0.05).
Figure 2. Stiffness at 2 and 4 weeks. N=8. Error bars represent standard deviation.
* Significantly different from Sponge (p<0.05). , ** Significantly different from Suture (p<0.05).
Figure 3. Tenomodulin expression at 4 weeks. N=5. Error bars represent standard deviation. * Significantly different from Sponge (p<0.05). , ** Significantly different from Suture (p<0.05)

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References: