Repopulation of Intrasynovial Flexor Tendon Allograft with Bone Marrow Stromal Cells: An Ex Vivo Model

Yasuhiro Ozasa, M.D., Ph.D., Peter C. Amadio, M.D., Kai-Nan An, Ph.D., Andrew R. Thoreson, M.S., Chunfeng Zhao, M.D.
Mayo Clinic, Rochester, MN, USA.

Disclosures:

Introduction: Delayed healing and revitalization are common problems encountered when tendon allografts are used for tendon or ligament reconstruction. Repopulating the allograft with host cells may accelerate tendon regeneration, but cell penetration into the allograft tendon is limited. Processing the tendon allograft surface by introducing slits that guide cells into the allograft substrate may improve healing. However, disruptions to the tendon allograft surface may increase the friction between adjacent structures. The purpose of this study was to investigate a potential processing technique to accelerate allograft tendon regeneration by introducing multiple slits to provide a harbor for cell seeding and promote their survival and proliferation. In addition, a surface modification with carbodiimide derivatized hyaluronic acid gelatin (cd-HA-gelatin) was implemented to reduce the gliding resistance of the slit allograft tendon back to that of normal tendon.

Methods: A total of 84 flexor digitorum profundus (FDP) tendons from the second through fifth hind paw digits of 11 dogs, sacrificed for other Institutional Animal Care and Use Committee (IACUC) approved studies, were randomly divided into four groups: 1) untreated, unprocessed normal tendons, to serve as a control (normal group), 2) tendons decellularized with trypsin and Triton X-100 (decellularized group), 3) tendons decellularized as in group 2 and perforated with multiple, short slits (MS group) and 4) tendons decellularized and slit as in group 3 and treated with a surface modification of cd-HA-gelatin (MS-SM group). In the MS group and MS-SM group, two rows of 2 mm full thickness slits at 2-mm intervals were made with a scalpel. The gliding resistance was measured up to 1000 cycles of simulated flexion/extension motion using a well established method. Following the friction test, the tendon was attached to the clamps of a material testing machine allowing a gauge length of 20 mm. Cross-sectional area was measured before testing. Following preconditioning, the tendon was distracted until failure at a rate of 20 mm/min. The Young’s modulus was calculated from the load and displacement data. To assess tendon repopulation, 100μL of 2x10⁶ cells/1 mL bone marrow stromal cells (BMSCs) suspension were seeded on the surface of the decellularized tendon without slit (decellularized + cell group) or directly into the slits in the MS group tendon (MS+cell group). BMSC-seeded tendons were cultured for 2 weeks. In order to evaluate the cell viability, a Live/Dead Viability/Cytotoxicity kit (Life Technologies, Grand Island, New York) was used for the MS + cell group. The tendon cellularity was assessed by quantifying the tissue concentration of DNA using a PicoGreen DNA assay (Life Technologies), and comparing it to that of normal tendons, non-seeded decellularized tendons, and non-slit tendons with BMSCs in culture. All data were given as means ± standard deviation. One-way analysis of variance (ANOVA) and the Tukey-Kramer post hoc test were performed to compare mean gliding resistance, Young’s modulus and DNA concentration among the groups. The significance level was set at p<0.05 in all cases.

Results: The gliding resistance of the decellularized and MS groups were significantly higher than that of the normal group (p<0.05). There was no significant difference in gliding resistance between the decellularized and MS groups. Gliding resistance of the normal group and MS-SM group was not significantly different (Figure 1). Young’s modulus was not significantly different among the four groups. DNA concentration in the MS group was significantly lower than in normal tendons, but significantly higher than in decellularized tendons, with or without BMSCs (p<0.05) (Figure 2). Viable BMSCs were found in the slits after 2 weeks in tissue culture (Figure 3).

Discussion: In this study, we demonstrated that making multiple short slits improved the ability of BMSCs to penetrate into a decellularized tendon scaffold without compromising the tendon stiffness, and that these cells remain viable. Although the DNA content of the MS + cell group tendons was less than that of the normal tendons, it reached 79% of normal tendon levels. This was higher than the level observed both in this study and by others in decellularized intact tendons cultured with cells. This study has several limitations. First, we did not confirm the post seeding phenotype of the implanted BMSCs and measure the matrix synthesis of the seeding cells. Second, we did not test the cell viability with cd-HA gelatin treatment. Third we did not analyze the mechanical properties of the tendon scaffold after cell seeding.

Significance: A multi-slit tendon reseeded with BMSCs, with a surface treatment applied to restore gliding properties, may potentially promote tendon revitalization and accelerate healing for tendon or ligament reconstruction applications without compromising gliding properties.

Figure Legends:
Figure 1. Mean gliding resistance of the first and 1,000th cycles for the normal group, decellularized group, MS group and MS-SM group. Error bars represent standard deviation. An asterisk indicates a significant difference (p<0.05).
Figure 2. Mean DNA concentration of normal group, decellularized group, decellularized + cell group and MS + cell group. Error
bars represent standard deviation. An asterisk indicates significant difference (* p<0.001, ** p=0.02).

Figure 3. Live/Dead cell viability assay showing live cells stained with Calcein-AM and dead cells with EthD-1 (Original magnification x100. Scale bar represents 100μm). The viability of most seeded BMSCs was maintained in the slits of the decellularized tendon.

Acknowledgments: This study was supported by grants from NIH/NIAMS (AR57745).

(4) Woon CY et al. Tissue Eng Part A. 2012;18:2406-
Figure 2

DNA concentration (ng/mg dry weight)

595.3 ± 136.4

470.6 ± 118.1

11.5 ± 10.1

18.5 ± 16.5

- Normal
- Decellularized
- Decellularized+cell
- MS+cell

Figure 3

Green: Live cells (Calcein-AM)
Red: Dead cells (EthD-1)

ORS 2014 Annual Meeting
Poster No: 1381