Mesenchymal Stem Cell Delivery via Topographically Tenoinductive Collagen Biotextile Enhances Regeneration of Segmental Tendon Defects

Phillip McClellan, PhD[1], Jason G. Ina, MD[2], Derrick M. Knapik, MD[2], Ilaha Isali, MD[1], Alexis Valente[3], Yujing Wen[4], Cindy Wu[4], Ruiqi Wen[4], James M. Anderson, MD, PhD[4,5], Robert J. Gillespie, MD[1], Ozan Akkus, PhD[3,4]

Dept. of Urology[1], Case Western Reserve University, University Hospitals Cleveland Medical Center[2], Dept. of Mechanical & Aerospace Engineering[3], Biomedical Engineering[4], Dept. of Pathology[5], Case Western Reserve University

Introduction: Injuries to the rotator cuff (RC) tendons remain a common issue encountered by orthopedic surgeons with approximately 330,000 rotator cuff surgeries conducted on individuals each year in the United States alone [1]. For massive RC tears (≥ 5 cm tendon retraction or involvement of ≥ 2 tendons), recurrent tears after surgical repair are a major concern resulting from inferior tissue quality or excess mechanical stress at the repaid site [2].Tissue-engineered scaffolds exhibit significant benefits in RC tears where bulk tendon tissue is lost due to muscle atrophy, tissue fibrosis, or fatty infiltration. Scaffolds composed of biological molecules, such as collagen, can guide regeneration, but collagen use alone is limited as many strategies rely on extended culture periods *in vitro* to achieve the mechanical robustness required to bridge large tendon defects. Our laboratory developed a novel method to fabricate mechanically robust, molecularly-aligned collagen fibers by means of electrochemical compaction that can be woven together to form scaffolds capable of sustaining mechanical loads of in a rabbit infraspinatus tendon defect model over the course of six (6) months *in vivo*. Additionally, scaffolds were capable of delivering mesenchymal stem cells (MSCs) and inducing tenogenic differentiation of said MSCs by way of topographical cues.

Materials and methods:

Scaffold Fabrication: Scaffolds were fabricated from electrochemically aligned collagen (ELAC) threads as described previously [3]. Individual fibers were combined to form 3-ply yarns and crosslinked using genipin (2% w/v in 90% ethanol). Crosslinked yarns were woven into scaffolds via a stainless-steel pin array.

Cell Seeding: Mesenchymal stem cells were isolated from bone marrow of New Zealand White (NZW) rabbits and adherent cells were cultured, flow-sorted (CD44+, CD45-, and CD90-), seeded onto ELAC scaffolds, and cultured for 3 days prior to surgical implantation.

Surgical Implantation: Thirty-four (34) NZW rabbits were divided into four groups (gap = no repair, direct repair "DR" = clinical standard, ELAC = scaffold alone, ELAC+MSCs = MSC-seeded scaffold) prior to surgical creation of a massive (≥ 5 mm) defect in the infraspinatus tendon which was then left unrepaired (gap) or repaired (DR, ELAC, and ELAC+MSCs).

Analysis: Shoulders were harvested at 6 months following surgery and analyzed by microCT, biomechanical testing, histology, and immunohistochemistry (IHC).

Results: Based on microCT analysis, specimens treated with ELAC showed reduced tendon retraction as compared to DR ($p < 0.05$). Biomechanical testing revealed shoulders in the ELAC + MSCs group had maximum load to failure values (178 ± 50 N), comparable to intact, contralateral control shoulders (199 ± 35 N; $p > 0.10$) (Fig.1).

Histological analyses using picrosirius red and Masson's trichrome stains revealed abundant, well-aligned de novo collagen around ELAC threads in both ELAC and ELAC + MSC shoulders, with ELAC + MSC specimens demonstrating increased ELAC resorption (7% versus 37%, respectively; $p < 0.01$) (Fig.2A-B). IHC staining for collagen type I and tenomodulin (Fig. 2C), indicated tendon-like tissue formation, in ELAC and ELAC + MSC groups. Tenomodulin was absent in DR and gap groups, suggesting ELAC provided a topographically tenoinductive effect for up to 6 months under functional load-bearing conditions *in vivo.*

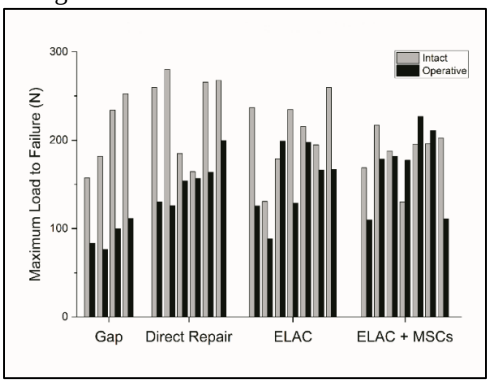

Figure 1. Maximum load to failure of individual shoulders from each group (operative and contralateral "intact" assessed as pairs from each rabbit).

Figure 2. A) Collagen alignment visualized with PSR staining under polarized light. B) MT staining showing de novo collagen between ELAC fibers. C) Tenomodulin staining in ELAC + MSC specimen. Scale bar = 100 um.

Conclusions: MSCs delivered locally by way of mechanically robust ELAC scaffolds enhance biomechanical and histological outcomes when compared to a current clinical standard approach. The presence of a key marker of native tendon tissue, tenomodulin, in the MSC-seeded group suggests ELAC represents a suitable platform for regeneration of bulk tendon tissue *in vivo*. Future work will focus on regeneration of the enthesis via addition of mineralized ELAC to faciliate bone-scaffold integration.

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