Woven Collagen Biotextiles for Rotator Cuff Tendon Repair - An *In Vivo* Pilot Investigation

Greg D. Learn[1]; Phillip E. McClellan[2]; Derrick M. Knapik[3]; Jameson L. Cumsky[4]; Robert J. Gillespie[3]; Ozan Akkus[1,2,3]
[1]Dept of Biomedical Engineering; [2]Dept of Mechanical/Aerospace Engineering; [3]Dept of Orthopaedics; [4]School of Medicine
Case Western Reserve University (CWRU) / University Hospitals, Cleveland, OH 44106

STATEMENT OF PURPOSE: Tendon injuries are a common cause of pain and disability. Tendons have low healing capacity and surgical repair failures are prevalent, especially for the rotator cuff (RC) tendons [1], with (w/) defect size and soft tissue degeneration being risk factors for re-tear. While surgical techniques have progressed, the ability to replace missing/defective tendon tissue would benefit repair outcomes. This study explores the use of robust, biomimetic, woven collagen (COL) scaffolds (WCSs) for RC tendon repair/regeneration (R/R) *in vivo*.

METHODS: *WCS Fabrication*: Acid-soluble Type I COL (Collagen Solutions) was dialyzed and compacted between 2 stainless steel wires (30V, 90s) to form electrochemically aligned COL (ELAC) threads. Threads were combined into 3-ply yarns, cross-linked (2% wt/vol. genipin), and manually woven [2] to produce scaffolds ~14x5x2 mm^3 (Fig 1). WSCs were sterilized in peracetic acid / ethanol solution.

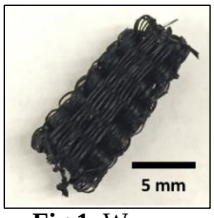

Fig 1. Woven ELAC scaffold.

Animal Surgeries: Procedures were performed as approved by the IACUC at CWRU. 15 adult NZW Rabbits (Charles River, 3-5kg) underwent open surgical creation of a right infraspinatus (IS) tendon (IST) defect, while the left IST served as an intact control (IC, Fig 2a) for each rabbit. ISTs were detached at the bone, and either reattached directly w/ suture ('DR') as a clinical standard (Fig 2b), or a critical 5mm IST defect was created and the gap was bridged w/ WCS (Fig 2c) either by itself ('SR') or pre-seeded w/ P5 allogeneic rabbit MSCs ('SCR'). The tendon/WCS were sutured (3-0 Ethibond, Krakow stitch) and re-anchored to the insertion via bone tunnels to the bicipital groove (Fig 2 b,c). Scapulohumeral complexes (SHCs) were harvested at 3 months, and for a subset of rabbits, imaged w/ micro-CT (µCT) to assess fatty infiltration (FI) of RC muscles. IS muscles (ISMs) were then dissected away from scapulae to isolate humerus-IST-ISM units. 4 rabbits/group were used for biomechanical testing (BMT) of repair stiffness and peak load (PL). Histology was performed on remnant tissues after BMT plus 1 extra rabbit/group (3/group total).

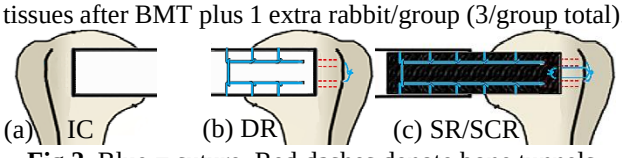

Fig 2. Blue = suture. Red dashes denote bone tunnels.

µCT: SHCs were imaged (70kV, 470µA, 800ms, 42µm / pixel) in a Siemens Inveon PET/CT scanner.

BMT: Humeri were potted/clamped in aluminum blocks, and the ISM was pulled at 10mm/min to repair failure [3]. Stiffness and PL were extracted from load-displacement curves (LDC) and normalized to IC values. Regression was performed on elastic regions of LDC from 40-60% PL, and slope of the best-fit line ($r^2 \geq 0.95$) was taken as stiffness. Signif. differences (SDs) were assessed using Kruskal-Wallis test (SD at $p \leq 0.05$), followed by pairwise comparisons using Mann-Whitney U test ($p \leq 0.05$).

Histology: Samples were embedded, sectioned, stained w/ Masson's Trichrome (MTc), and scanned in brightfield.

RESULTS: BMT revealed SD in stiffness (Fig 3a) but not PL (Fig 3b) among SCR and SR. No other SDs were found.

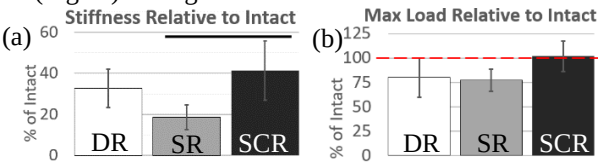

Fig 3. (a) Stiffness and (b) PL of IST repair specimens (n=4/group). Mean ± st.dev. Red dashes = IC value.

IC and DR samples failed at the bone interface (BIF), SCRs failed midsubstance (MS), and SRs failed both ways (Table 1). Extra SR and IC samples are due to a mid-test slip for 1 animal (excluded from stiffness/PL data).

Table 1. Failure Locations by Group

Site \ Group	IC	DR	SR	SCR
BIF	13	4	3	0
MS	0	0	2	4

µCT revealed moderate FI of the operative (op) ISM for DR rabbits (Fig 4b); FI was less severe for the op SR ISM based on 1 rabbit (Fig 4d). FI was not observed in ICs (Fig 4 a,c) or neighboring RC muscles.

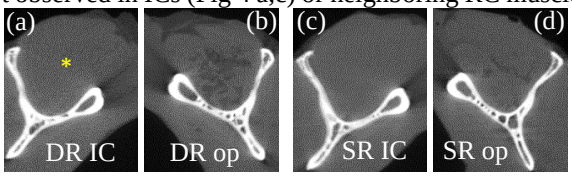

Fig 4. µCT from (a,b) DR rabbit (represents n=3), and (c,d) SR rabbit (n=1). White = bone. Light gray = muscle. Dark gray = fat. Black = air. Star = ISM belly.

SCR histology showed increased cell density and COL deposition (COL-dep) throughout WCS network relative to SR sections (Fig 5). COL-dep follows ELAC contours.

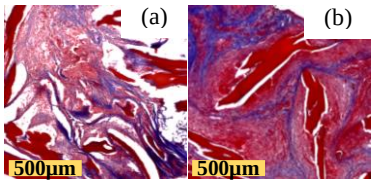

Fig 5. MTc sections showing WCS-tissue integration. (a) SR. (b) SCR. Navy = *de novo* COL. Pink = cells. Crimson = ELAC.

CONCLUSIONS: This study presents a novel approach which may hold merit for tendon R/R, though further study is needed. Results suggest that IST repairs w/ WCSs: are comparably stiff/strong to DRs, are made stiffer/stronger w/ MSCs, and may halt FI of the attached ISM. Findings herein support use of WCSs, possibly combined w/ patient-derived MSCs, for functional repair of RC tendon defects.

ACKNOWLEDGMENTS: This work was supported by grants from the NIH NIAMS: R01 AR063701 to O.A. and Ruth L. Kirschstein NRSA T32 AR007505 to G.L.

REFERENCES

[1] Galatz LM. *J Bone Joint Surg*. 2004;86-A(2):219-24.
[2] Younesi M. *Adv Func Mat*. 2014;24(36):5762-5770.
[3] Islam A. *Clin Biomech*. 2015;30(7):669-675.