A Drug-Loaded Collagen Wrap For Augmented Primary Repair Of The Anterior Cruciate Ligament

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DISCLOSURES: JR (N), LAM (N), AJL (N), RMS (N), CP (N), CS (N), CNF (), KGM (N), GAA(N), CCB (N), JLC (1-Arthrex; 3B-Collagen Matrix Inc, Trupanion; 5- AANA, AO Trauma, NIH, ARPA, OREF, Orthopaedic Trauma Association, PCORI, Regenosine, SITES Medical, US DOD; 8-J of Knee Surg; 9-MTN, MTF), CTH (1-MTF. 8-JOR, ORR)

INTRODUCTION: The anterior cruciate ligament (ACL) is essential for knee stability and function. ACL tears cause pain and dysfunction, such that surgical treatment is typically recommended. Unfortunately, current surgical techniques, including reconstructions and repairs, fail to restore native ACL structure and function such that retear poses a significant risk and post-traumatic osteoarthritis (PTOA) is a common sequela [1]. As such, strategies that promote intrinsic healing have gained interest, particularly those that use an "internal bracing" concept to augment ACL repair [2]. However, the post-injury intra-articular environment impairs healing of the ACL due to exposure to the inflammatory and catabolic synovial milieu. Biomaterial wraps offer a promising addition to an effective ACL repair strategy by shielding the repair site and enabling local targeted drug delivery. Collagen vitrigel, a tunable biocompatible scaffold, has shown potential for promoting cellular infiltration and organized ECM deposition. Dexamethasone (DEX), a potential disease-modifying OA drug (DMOAD), can further improve outcomes by mitigating the post-injury inflammation that accelerates cartilage degradation. Local, sustained, low dosage delivery of DEX through encapsulation in microspheres (MS) has been shown to be anti-inflammatory and chondroprotective such that incorporation of DEXMS into a biomaterial may serve to support ligament healing and mitigate PTOA [3]. We hypothesize that *in vivo* application of a DEX-loaded collagen wrap, to augment primary ACL repair in a canine model, will enhance healing outcomes and promote joint health.

METHODS: Fabrication of Biomaterials: DEXMS were made by encapsulating 100mg DEX in 75:25 PLGA. The DEXMS were then mixed into a collagen type I solution, gelled in a mold at 37°C for 2hr, then vitrified at 25°C overnight to create a flat sheet. The collagen sheet (3mg/cm^2) was rehydrated in PBS, then wrapped to create a hollow, cylindrical sleeve (⌀ = 8mm), or vitrified collagen wrap (VC-Wrap). Initial mechanics of the biomaterial were measured using tensile testing and fluorescent recovery after photobleaching (FRAP) to determine diffusion coefficient. To contextualize outcomes, FRAP measurements were also made of juvenile bovine ACLs collected from a local abattoir (IACUC-exempt). Samples were saturated in FITC-dextrans of multiple sizes (20, 70, and 2000kDa) prior to photobleaching. DEX release was evaluated over 10 weeks by culturing VC-Wraps in PBS and assaying the absorbance (242nm). Preclinical Animal Model: Adult, purpose-bred research hounds (n=12; >20kg, female) underwent aseptic arthroscopic surgery of the right stifle (knee) (IACUC #42907). The native CCL (ACL) was debrided of its synovial sheath and transected at the midpoint. Using arthroscopic guides, femoral and tibial tunnels were drilled to allow for a spanning internal brace (FiberTape, FT, Arthrex) to be placed, tensioned to bring the "torn" ends of the ACL into apposition, and secured over cortical buttons. In 6 dogs, a VC-Wrap was delivered into the joint, wrapped circumferentially around the repaired ACL and internal brace, and secured with suture; the other 6 dogs did not receive the wrap (Controls). 12 weeks after ACL repair, arthroscopic and radiographic assessments and scoring, as well as clinical assessments of anterior drawer, comfortable range of motion (CROM), and pain, were performed. Animals were then humanely euthanized for gross and histologic OARSI scoring. Statistics: A post-hoc power analysis determined $(1 - \beta) = 0.407$. Data was reported as mean ± standard deviation. Multiple comparisons were analyzed with One-way ANOVA with Tukey's HSD post-hoc test and paired data analyzed with t-test or Mann-Whitney test ($\alpha = 0.05$).

RESULTS: Functionality of the VC-Wrap: After 10 weeks it was determined that 2.5mg/mL DEXMS embedded into the VC-Wrap would deliver the critical dosage of 0.9ng DEX/day after an initial burst release (Fig. 1A). The diffusion coefficients of all dextrans were not significantly different between the VC-Wrap and the ACL sheath (Fig. 1B). The presence of DEXMS in the VC-Wrap did not significantly weaken the material properties of the collagen wrap compared to a MS-free control. Young's Modulus was measured to be 1.90 ± 1.88 MPa vs. 1.16 ± 0.69 MPa and Ultimate Tensile Strength was measured to be 0.39 ± 0.50 MPa vs. 0.070 ± 0.04 MPa for the control vs. VC-Wrap, respectively. Surgical Outcomes: All surgical sites healed fully with no infections. Subjects repaired with the VC-Wrap appeared to have less degradation of the FT than subjects without the augmented repair (Fig. 2A). Significant improvements were seen in anterior drawer, CROM, pain, XRay OA, and cartilage integrity scores, where most areas showed no signs of damage. Evaluation of histology revealed the CCLs were more often fully intact after augmented repair than FT-alone (Fig. 2B). Histology of synovia showed similarly fibrotic ECM in both groups. There were greater overall OARSI scores on the medial side of the stifle compared to the lateral side. CCL and synovia scores were significantly different between surgical groups (data not shown).

DISCUSSION: In this study, the VC-Wrap group showed reduced anterior laxity, pain, and lameness, along with improved CCL tissue integrity. Histological and arthroscopic assessments revealed the wrap maintained a healing-conducive microenvironment while fully degrading by 12 weeks. The VC-Wrap appeared to act as a surrogate synovial sheath, protecting the repair site and supporting ECM deposition and cellular infiltration along the periphery of the CCL. Localized DEX delivery likely improved outcomes by attenuating inflammation and reducing the risk of PTOA. Cartilage across the joint was better preserved in DEX-treated subjects, supporting DEXMS as an effective DMOAD. Longer term studies and those that include both sexes [4] will need to be performed to determine if the encouraging results persist.

CLINICAL RELEVANCE: Under the conditions of the current study, we demonstrate that a DEX-eluting biomaterial wrap can significantly improve ACL healing by reducing inflammation, preserving tissue integrity, and preventing early joint degeneration. This approach may offer a promising, biologically driven adjuvant therapy to current surgical treatments that often fail to restore native ligament function or prevent PTOA.

REFERENCES: [1] Liukkonen+, Bone Joint J., 2023; [2] Smith+, J. Knee Surg., 2019; [3] Stefani+, Acta Biom., 2020; [4] Slauterbeck+, Clin. Orthop. Rel. Res., 2004

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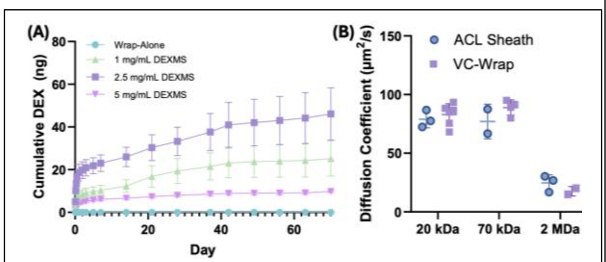

Figure 1. (A) Cumulative DEX release *in vitro* over 10 weeks. **(B)** Diffusion coefficients of 3 FITC-dextran solutes in the bovine ACL sheath vs. VC-Wrap.

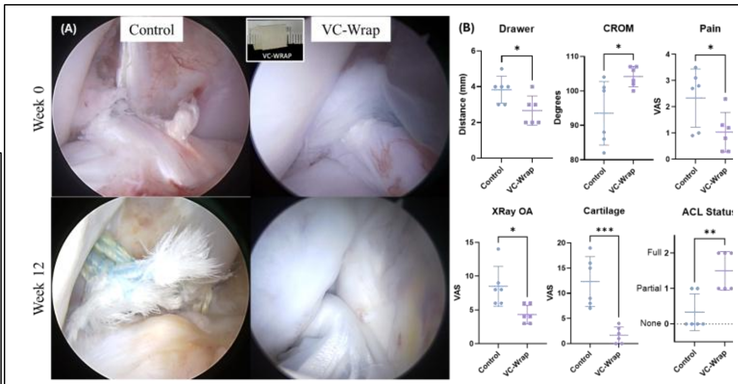

Figure 2. (A) Representative arthroscopic images of the CCL during and 12-weeks after surgery ± VC-Wrap. Inset: VC-Wrap alone. **(B)** Clinical assessments of anterior drawer, CROM, Pain, XRay OA, Cartilage integrity, and ACL Status. Assessments were made on the visual analog (VAS) scale according to OARSI scoring. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$