Tissue-engineered constructs with sustained dexamethasone release as a strategy for osteochondral defect restoration in the knee

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INTRODUCTION: Osteochondral allografts (OCAs) are the gold standard for the treatment of large, symptomatic articular defects in the knee of young, active patients, as marrow stimulation and cartilage procedures do not consistently produce durable results and total knee arthroplasty is not ideal for this patient population [1]. OCAs are used to resurface large (>2 cm^2) full-thickness defects and to address complications associated in the underlying bone. The utility of OCAs, however, is limited by the lack of available donor tissue and the challenges related to allograft bone osseointegration that are influenced by inflammatory-immune system responses to donor tissues [2]. As a result, there remains a clinical need to develop strategies to enhance graft availability and timely integration for functional joint-preserving osteochondral restoration surgeries. In this study, dexamethasone (DEX)-loaded polymeric microspheres were embedded in tissue-engineered osteochondral grafts and assessed for graft-cartilage quality, integration into recipient bone, and related joint inflammatory responses using a preclinical canine model.

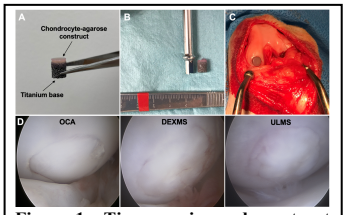

Figure 1. Tissue-engineered construct fabrication and implantation. DEXMS-construct (A) was press-fit into a canine recipient site (B, C). Arthroscopic evaluations at 3 months (D).

METHODS: *Microsphere Fabrication:* Dexamethasone-loaded microspheres (DEXMS) were fabricated as previously described [3]. Drug-free, unloaded microspheres (ULMS) were similarly fabricated without the addition of DEX. *Osteochondral Construct Preparation:* Articular cartilage was aseptically recovered from adult purpose-bred canine tissue donors. Chondrocytes were isolated and expanded. Passage 2 chondrocytes from multiple donors (N = 3) were pooled and mixed with 4% (w/v) type VII agarose (30×10^6 cells/mL) containing either 5 mg/mL of DEXMS or ULMS. 100 µL of the chondrocyte-agarose mixture was pipetted into a custom mold to create a tissue-engineered chondral portion of the graft (∅ 6 mm × 1 mm). Porous titanium bases (∅ 6 mm × 6 mm, Stryker (Mahwah, NJ)) were placed on top of molten chondrocyte-agarose mixture to form tissue-engineered osteochondral grafts (∅ 6 mm × 7 mm, **Fig. 1A**). Osteochondral bases were cultured in chondrogenic medium supplemented with 50 µg/mL ascorbic acid, 100 nM DEX, and 10 ng/mL TGFβ-3. Mature DEXMS- and ULMS-loaded osteochondral constructs were implanted 50-60 days following initial casting. *Surgical Implantation:* Adult purpose-bred mongrel dogs were anesthetized and prepared for aseptic surgery of the right stifle (knee) (University of Missouri, IACUC #9167, #9961, #16680). Experimental groups were divided into osteochondral allografts (OCA, N = 12), DEXMS-loaded constructs (DEXMS, N = 14), and ULMS-loaded constructs (ULMS, N = 14). OCAs (∅ 6 mm) were obtained from size-matched canine tissue donors and press-fit into corresponding recipient sites. DEXMS- and ULMS-loaded constructs were similarly press-fit into recipient sites (**Figs. 1B & 1C**). Dogs were monitored daily by veterinarians for any post-operative complications. *Clinical Assessment and Histopathological Scoring:* Assessments were made at 0-, 3-, and 12-months post-surgery. Clinical measures including comfortable range of motion (CROM), function, pain, and lameness, as well as assessment of graft integrity through arthroscopic evaluations were conducted (**Fig. 1D**). Dogs were sacrificed at the 3- and 12-month timepoints. At the terminal timepoints, the condyles as well as the adjacent synovia and menisci were harvested and fixed in formalin for histopathological analysis. Formalin-fixed condyles were first analyzed for bone fill and microstructure using micro-computed tomography (µCT). Sections of the osteochondral graft, synovia, and menisci were histopathologically scored by two blinded board-certified veterinarian pathologists [4]. Statistics: Measures of range of motion and microstructure of bone structures quantified using micro-CT were analyzed using ANOVA and Tukey's post-hoc test for multiple comparisons. Semiquantitative data, such as the histopathological scores and clinical function measures, were assessed using the Kruskal-Wallis non-parametric test and Dunn's Correction for multiple comparisons (α = 0.05).

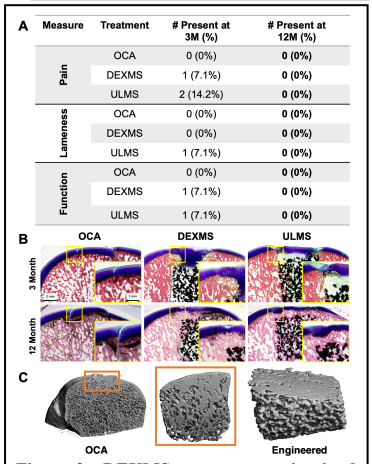

Figure 2. DEXMS constructs maintained clinical function, cartilage surface congruity, and bone architecture. No dogs were clinically impaired at 12-months (A). DEXMS exhibited improved cartilage edge integration and surface congruity relative to OCA (B). Subchondral bone microstructure and trabecular properties were similar between OCA and DEXMS (C).

RESULTS: For all dogs, clinical function assessments returned to pre-operative levels by 12 months post-operatively (**Fig. 2A**) [5-8]. Longitudinal analyses of osteochondral graft scores revealed improved outcomes for both DEXMS and ULMS at 12 months compared to those at 3 months. Interestingly, this same trend did not hold for OCAs, with scores either remaining the same or worsening at 12-months compared to the 3-month timepoint (data not shown). At the clinically relevant 12-month timepoint, both DEXMS and ULMS groups exhibited improved cartilage integration and surface congruity relative to OCA controls (**Fig. 2B**). Bone microstructural properties remained similar between experimental groups (**Fig. 2C**). The local delivery of DEX improved joint inflammation in neighboring joint tissues. Measures of synovial pathology were not different between OCA and DEXMS-treated groups; however, ULMS-treated canines exhibited significant synovial hyperplasia and lymphoid follicles in the subintima, and ultimately an elevated total pathological score of the synovium (**Fig. 3A**). In the meniscus, DEXMS-treated knees exhibited improved outcomes in all categories, with significant improvement due to DEX in the total meniscus pathology score versus ULMS. ULMS menisci exhibited tissue loss and reduced proteoglycan content, which was rescued by DEXMS treatment (**Fig. 3B**).

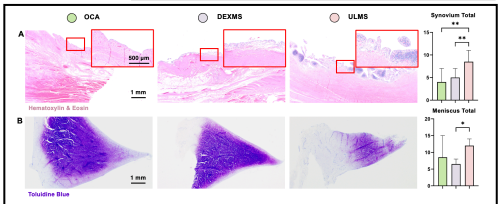

Figure 3. Local sustained delivery of DEX mitigated the local inflammatory response. ULMS synovium exhibited prominent synovial hyperplasia and lymphoid follicles in subintima (A, Inset: Magnified views of synovial lining). ULMS meniscus demonstrated tissue loss and reduced proteoglycan content, which was rescued by DEXMS (B). Statistics: * p<0.05, **p<0.01.

DISCUSSION: Tissue-engineered DEXMS constructs were compared to native OCA controls and an ULMS vehicle control. Over the course of 12 months, we demonstrated not only the maintenance of clinical function using tissue-engineered grafts, but also improvements in joint inflammation as measured through histopathological scoring of the meniscus and synovium. No dogs were clinically impaired by the 12-month timepoint, suggesting the successful use of tissue-engineered osteochondral constructs relative to gold standard OCA controls. Notably, local sustained low-dose delivery of DEX from within engineered cartilage appeared to promote overall joint health, similar to OCA controls. This is one of the first studies to assess tissue-engineered constructs as a vehicle to deliver therapeutic compounds from within a load-bearing region of the knee joint over a clinically relevant 12-month observation period.

SIGNIFICANCE/CLINICAL RELEVANCE: Tissue-engineered DEXMS-loaded constructs maintained clinical function comparable to OCAs and modulated joint inflammation through sustained, low-dose DEX delivery.

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