

Extracellular Matrix Hydrogels Derived from Decellularized Human Synovium as a Tissue Engineering Platform

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INTRODUCTION. The synovium is a soft tissue that lines diarthrodial joints and contributes to joint health and homeostasis by regulating the composition of the synovial fluid, which provides anti-adhesion properties, lubrication, and nutrition to tissues within the synovial joint. The synovium must maintain both mechanical toughness and deformability in order to function under the physically-demanding conditions of the joint.¹ The extracellular matrix (ECM) of the synovium determines its mechanical properties and influences the behavior of the resident cells – such as fibroblast-like synoviocytes (FLS) – which in turn modulate the synovial fluid. In diseases such as rheumatoid arthritis and osteoarthritis, biological and mechanical changes occur within the synovium, along with concomitant changes to the ECM composition and structure^{2,3}. Studying the interplay between cells and ECM in both healthy and diseased states is difficult *in vivo* due to limitations in controllability and feasibility of experiments, and current *in vitro* models are limited. Explant models retain native ECM but are irregular in geometry and availability.⁴ Current tissue-engineered models are easier to manipulate and are useful for exploring some aspects of synovial structure and function, but they lack the native ECM composition. Modulable hydrogels derived from decellularized ECM (dECM) may address these shortcomings, providing an improved *in vitro* model for study of the synovium. In the current work, we develop a process for producing dECM hydrogels from synovial membrane, characterize their biochemical compositions, and assess their biocompatibility using a relevant cell type.

METHODS. Tissue sources and decellularization: Human synovial tissue was recovered from the joint capsule of two cadavers (donors 4056 and 4240) without known joint pathology (MTF Biologics) and was decellularized as previously reported.⁵ Briefly, 280 μ m-thick sections of tissue were treated with 1% Triton X-100 for two days to remove cellular material, DNase overnight to eliminate released DNA, and 0.1% peracetic acid to sterilize the resulting dECM. **dECM pre-gel preparation:** dECM samples were lyophilized, minced, and digested with pepsin (1 mg/mL in 0.01N HCl, Sigma-Aldrich) at 10 mg tissue/mL for 72 hrs. Digestion pH was monitored, and 2N HCl was used to maintain a pH of ~1.5–2.5. Samples were taken at 48 and 72 hours for analysis and centrifuged. At 72 hours, spontaneously-formed gel and the soluble fraction of the digestions were retained. dECM samples were neutralized and brought to isotonicity by the addition of NaOH and 10X phosphate-buffered saline prior to assaying. **Biochemistry:** Spontaneously-formed hydrogels were lyophilized and digested with proteinase K prior to assaying. Total protein was measured with the bicinchoninic acid (BCA) assay. Collagen content was measured using the orthohydroxyproline (OHP) assay. Glycosaminoglycan (GAG) content was measured using the DMMB assay. **Cytocompatibility:** Human FLS derived from normal synovium (MTF Biologics) were grown in a 24-well plate for 24 hours with gelled ECM suspended in a transwell or supplemented with neutralized soluble-fraction ECM (5% v/v). Cells were then used in an MTT assay to assess cellular metabolism. **3D culture:** FLS (300k) were seeded onto neutralized, spontaneously-formed hydrogels and were cultured for 48 hours prior to live/dead staining and confocal imaging. **Statistics:** BCA results were analyzed by 2-way ANOVA with Tukey's post-hoc test. MTT results were analyzed by ANOVA with Tukey's post-hoc test. Biochemistry results were analyzed by t-test.

RESULTS. Characterization of dECM digests and hydrogels: At 48 hours, pepsin digestion of dECM produced a viscous liquid containing 0.956 ± 0.120 mg/mL soluble protein, with evidence of early gelation (Fig. 1A). Though the suspended gels were not stable enough to handle, they sedimented out of suspension upon centrifugation. At 72 hours, the soluble fraction contained 2.22 ± 0.390 mg/mL protein (Fig. 1B) across both tissue sources, and a gel formed that was robust enough to handle and required a scalpel to cut (Fig. 1A). These gels were comprised of $32.1 \pm 6.8\%$ (n=6) collagen by dry weight and $0.27 \pm 0.16\%$ (n=6) GAG by dry weight. dECM properties were dependent upon tissue source: At 72 hours, the dECM hydrogels were notably different physically between the two donors. In particular, the ECM from donor 4056 was stronger and more rigid than that from donor 4240. Interestingly, both collagen content and GAG content as percentage of gel dry weight were not different between the two donors (p = 0.46 and 0.53, n=6). However, the proportion of dry weight vs. wet weight was significantly greater in gels from 4056 ($4.2 \pm 0.5\%$ vs. $1.8 \pm 0.3\%$, p = 0.002, n = 3) despite identical processing conditions. Likewise, the soluble digest fractions differed in total protein content (Fig. 1B) and soluble collagen content (55.6 ± 18.1 vs. 94.4 ± 5.8 μ g/mL, n = 4), with 4240 having higher content in each case. **dECM gels and soluble digest fractions are not cytotoxic and support cells in 3D culture:** After 24 hours in culture with dECM hydrogels and supplemented with soluble digests, FLS metabolism was not negatively affected, while cells treated with a neutralized pepsin solution identical to that used to perform the dECM digestion showed lower metabolic activity (Fig. 1C). Cells seeded onto dECM hydrogels attached and assumed an elongated, fibroblast-like morphology while retaining high viability in gels derived from both tissue sources. (Fig. 1D).

DISCUSSION. We report the production and early characterization of a hydrogel derived from human synovial membrane that supports 3-dimensional tissue culture and may become a useful model for human diseases of the synovium. Using a

previously-reported method for decellularizing synovial tissue along with a common technique for producing ECM-derived hydrogels, we find that robust synovial ECM-derived hydrogels form spontaneously even under conditions that should inhibit collagen crosslinking.^{5,6} Both the hydrogel and the soluble product of the dECM digestion are non-cytotoxic to fibroblasts derived from the synovium, and the hydrogel supports 3D culture without the addition of any non-native matrix constituents. As the source material for these products is explant tissue, variability is expected. Indeed, soluble protein liberated by digestion varied significantly between the two donors after 72 hours, as did the characteristics of the hydrogels that formed. Interestingly, the tissue sample that yielded higher solubilized protein and soluble collagen content generated a weaker gel with less solid constituents, despite the similarity in composition with respect to dry weight when compared to the other sample. These results indicate that the formation of these hydrogels involves more than just the reassembly of solubilized collagen and is influenced by the components present in the specific tissue source used. While this complexity introduces technical challenges, it is also a reminder of the inhomogeneity of the human synovial ECM, both within and across individuals, which necessitates tissue- and donor-specific models such as this one. While researchers have shown the utility of dECM in models of other tissues, further work is required to establish the value of a tissue-engineered synovium based on the synovial dECM hydrogel presented here.⁷

CLINICAL RELEVANCE. The ECM determines the mechanical properties of the synovium and influences the behavior of resident cells, and its structure and composition are altered in disease. This model incorporates native ECM, which will allow researchers to probe the effects of different ECM compositions and test therapeutics on cells in a more biofidelic context.

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