Engineering Large Meniscal Tissues via 3D-Printed Multipart Molds

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Introduction: The knee menisci are highly aligned fibrocartilaginous tissues that distribute load, absorb shock, and enable near frictionless movement of the tibia with the overlying femur. Meniscal injuries are common and may lead to pain and early onset of osteoarthritis (OA) if left untreated. While removal of damaged meniscal tissue with either a partial or complete meniscectomy may be used for treatment, excision of the meniscus alters joint loading and increases contact stresses, which may accelerate OA (*1*). This motivates the development of new therapies to replace damaged meniscal tissue, which requires implants that are centimeters in length. There are only a few examples where meniscal tissue constructs of such sizes have been produced (*2–4*), but these rely upon custom mechanical devices that are not easily adaptable, making tuning, adoption, and scale-up within and across research groups challenging. Towards this, we recently demonstrated the use of digital light processing (DLP) 3D printing to scale-up both the production and size (>2 cm length) of hydrogel pillar molds that support the growth of large anisotropic meniscal tissue constructs over short term cultures (7 days) by providing boundary constraints that direct meniscal fibrochondrocyte (MFC) alignment (*5*). Further, computer aided design (CAD) models for these molds can be easily edited and shared, making this platform especially amenable to widespread adoption and scale-up. Here, we build on this work by using an inexpensive (< $300 USD) commercial LCD-based DLP printer to fabricate larger multipart hydrogel molds that support the development of robust engineered meniscal tissues during 28-day cultures, as assessed via mechanical testing, histological staining, and biochemical assays. With these results, these engineered tissues are now ready for pre-clinical translation.

Methods: Mold and tissue fabrication. Hydrogel molds of varying depths (3, 5, 7 mm) and with removeable walls were DLP 3D printed using a poly(ethylene glycol)-based resin (**Fig. 1A**). Molds were assembled, and MFCs (0.5×10^6 cells/mL) were mixed with collagen (4.8 mg/mL) and injected into the molds. After collagen gelation, tissue constructs were immersed in DMEM (growth media). On day 3, mold walls were removed and growth media was replaced with chondrogenic media (+TGF-β3) (**Fig. 1B**). Brightfield images were acquired weekly to monitor construct dimensions. Mechanical testing. Engineered tissues underwent uniaxial compression testing (0.05 N/min) or tensile testing (preconditioning for 10 cycles of 0.5%-2% strain, re-equilibration, and test to failure at 0.1% strain/s) to determine compressive and linear tensile moduli. Biochemical analysis. Tissues were de-molded, enzyme-digested (24-48 hrs.), and assessed via Picogreen dsDNA (DNA content), dimethylmethylene Blue (DMMB) (glycosaminoglycan (GAG) content), and hydroxyproline collagen (collagen content) assays. Histology. Paraffin-embedded samples were sectioned (7 µm), and stained to visualize cells, deposited GAGs, and collagen. Integration. Juvenile bovine meniscus tissue was cut into strips matching the geometry and orientation of engineered tissues. Engineered tissues (day 14) were removed from molds. Next, engineered and native tissues were held in contact with native tissues via DLP-printed hydrogel clamps and cultured for an additional 14 days. After 14 days in contact, lap shear testing was conducted (0.1 mm/s) to assess integration strength. Statistics. 1-way ANOVAs followed by Tukey's post-hoc test, and unpaired t-tests were performed. $*p < 0.05$, $***p < 0.001$, $****p < 0.0001$.

Results: Tissues cultured in hydrogel molds with greater depths maintained greater volumes throughout culture (not shown), motivating the use of the 7 mm mold for subsequent studies (**Fig. 1C**). Tissues compacted rapidly during the first two weeks of culture followed by gradual volume growth during the final two weeks of culture (**Fig. 1D**). Between days 0 and 28, the compressive moduli of tissues increased nearly seventy-fold from 0.55 ± 0.04 to 37.93 ± 1.75 kPa (**Fig. 1E**) and day 28 engineered tissues achieved a tensile modulus of 12.0 ± 0.9 MPa (**Fig. 1F**). As expected, dsDNA, GAG, and collagen content per construct each increased and wet mass decreased between days 0 and 28 (**Fig. 1G**). Extensive cell, GAG, and collagen alignment (along the longitudinal direction) were visible via histological analysis by day 28 (**Fig. 1H**). Furthermore, integrated engineered tissues exhibited a 10× greater lap shear strength (8.59 ± 2.29 kPa) than native tissue controls (0.78 ± 0.48 kPa) (**Fig. 1I**).

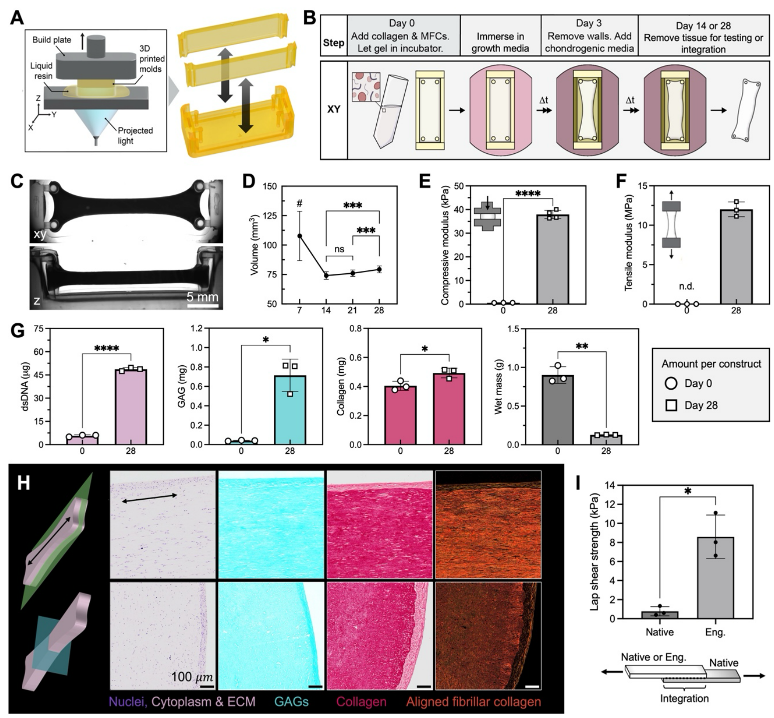

Figure 1. Engineering large meniscal tissues. (A) 3D printing of hydrogel molds with removable walls. (B) Culture timeline. (C) Images of day 28 engineered (eng.) tissue in mold (xy: top, z: side). (D) Tissue volume over time (n = 6). # indicates $*p < 0.05$ between day 7 and each other time point. (E) Compressive and (F) tensile moduli of tissues on days 0 and 28. (G) Biochemical content (dsDNA, GAGs, collagen) and mass of tissues. (H) Histological staining of day 28 tissues in the longitudinal and radial directions (Brightfield H&E, Alcian blue, & Picrosirius red (PSR) and Polarized light PSR). (I) Lap shear strength of integration interface (native + native or eng. + native) after 14 days of culture. For panels E, F, G, and I, each data point represents one tissue (n = 3-4).

Discussion: To rapidly fabricate hydrogel molds, an inexpensive (< $300 USD) commercial LCD-based DLP printer was used. We increased the size and complexity of hydrogel molds (e.g., incorporating removable parts), allowing for temporal control over boundary conditions and increased nutrient diffusion to support large-scale construct growth. Mechanical properties of engineered tissues increased dramatically between days 0 and 28, with the tensile modulus approaching that of 1 week old bovine menisci (13-26 MPa, gestation period of ~40 weeks) (*3*) and achieving a multifold increase in tensile modulus over prior collagen-based day 28 engineered meniscal tissues. Furthermore, our engineered tissues hold promise for clinical translation with improved integration capacity with native tissue relative to a native tissue control.

Significance: Excitingly, we have produced large meniscal tissues with robust mechanical properties and biochemical composition after just four weeks of culture and using a relatively inexpensive and highly adaptable platform. Furthermore, preliminary mechanical testing reveals that these engineered tissues promote improved integration with native tissue ex vivo.

References: (1) A. J. S. Fox, et al., Clinical Anatomy. **28**, 269–287 (2015). (2) J. L. Puetzer et al., J Biomech. **48**, 1436–1443 (2015). (3) M. E. Bates et al., Acta Biomater. **160**, 98–111 (2023). (4) J. L. Puetzer et al., Biomaterials. **269**, 120527 (2021). (5) A. DeFoe et al., APL Bioeng. **9** (2025).

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