

Printing Mechanically Tunable Cardiac Decellularized Extracellular Matrix Bioinks for Modeling Cardiac Fibrosis

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Statement of Purpose: Cardiac fibrosis is a devastating form of pathological remodeling in the myocardium, which results in progressive heart failure. Therefore, there is need for an *in vitro* platform that mimics the structural and mechanical properties of *in vivo* conditions to study disease mechanisms. We hypothesize that 3D-printed, cell-laden constructs derived from the cardiac ECM can be used to recapitulate the *in vivo* properties of both healthy and fibrotic cardiac tissues. In this work, we report bioinks composed of decellularized ECM (dECM), Laponite, and poly(ethylene glycol)-diacrylate (PEG-DA) that are extrudable, photo-crosslinkable, and demonstrate good printing fidelity. Furthermore, the shear storage modulus (G') after photocuring can be tuned to model the stiffness of both healthy and fibrotic cardiac tissue.

Methods: *dECM Preparation:* Porcine hearts were decellularized with 1% SDS and 1% Triton-X 100 for 73 h followed by a rigorous wash in PBS for 72 h. Decellularized tissues were collected lyophilized for 72 h. *dECM solubilization:* Lyophilized dECM was digested at 10% w/v in 0.1 M HCl using pepsin (10% mass of dECM) for 72 h at RT. pH was adjusted to 7~7.5 using 6 M NaOH and diluted with 10X PBS or RPMI 1640 media.

Hydrogel Preparation: Gels were prepared by first vortex-mixing all components in a vial for 30 s, then centrifuging at 4000 rpm for 2 min, and finally stirring at 200 rpm for 30~60 min. Final gel formulations consisted of 5% dECM (w/v), 2.5% Laponite-XLG, 0.4% photoinitiator (Lithium phenyl-2,4,6-trimethylbenzoylphosphine), and the required amount (6%, 8.25%, and 10.5% w/v) of PEG-DA

Rheology: All rheological experiments were performed on a TA Instruments Discovery HR-2 hybrid rheometer equipped with a Peltier temperature-control accessory. All experiments utilized a flat-plate 8 mm stainless steel geometry. Blue-light (405 nm) photocuring at 28 mW/cm² intensity was performed using a Mightex BLS-series BioLED analog control module coupled to a Mightex WLS-series WheelLED™ light source.

3D Bioprinting: dECM gels were extruded using a dual-syringe Allevi 2 bioprinter at a pneumatic pressure of 3-4 psi and using a 22G cylindrical needle. The gels were continuously photocured using a 410 nm light source.

Results: Cardiac ECM was decellularized, solubilized as the base material for the hydrogels, and combined with Laponite-XLG and PEG-DA to afford an extrudable and photo-crosslinkable bioink (Fig. 1A). Decellularized cardiac ECM acted as a biomimetic scaffold which represents the cardiac biological environment, Laponite served as a viscosity modifier, and PEG-DA allowed photo-crosslinking of the printed construct into desired matrix stiffness. Standard rheological experiments were performed to determine the viscosity, shear storage (G'),

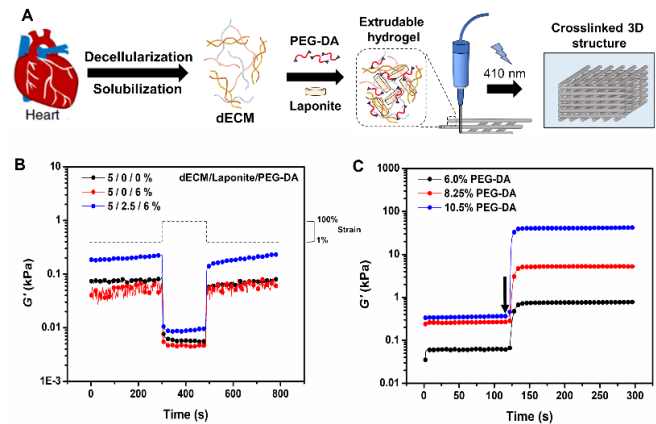


Figure 1. Synthesis and characterization of cardiac dECM bioink with tunable gel modulus (A) Schematic illustration of dECM-Laponite bioink preparation and tissue construct printing. (B) Graph showing changes in storage modulus with applied strain, as well as differences in modulus. (C) Blue-light photocuring of dECM-Laponite bioink. Irradiation was started at 120 s (black arrow).

and loss (G'') moduli for the bioinks (Fig. 1B). The bioinks demonstrated shear-thinning behavior, and in the cyclic strain test, a sudden decrease in moduli at 100% strain (high-shear) and recovered mechanical stability at 1% strain (low-shear) following periods of high oscillatory strain. Furthermore, Laponite increased the modulus of the ink providing the mechanical stability prior to crosslinking. This result indicates the potential for dECM-Laponite bioink to extrude smoothly at high shear and maintain dimensional fidelity after extrusion. Importantly, the inks showed rapid photocuring with blue-light (405 nm) irradiation, reaching saturation moduli of 0.766 kPa, 5.25 kPa and 41.3 kPa (6%, 8.25% and 10.5% PEG-DA, respectively) within 30 seconds of light stimulus. (Fig. 1C). Final G' values were comparable to the physiologically relevant stiffness of healthy heart (5-15 kPa) and fibrotic heart (30-50 kPa), indicating successful modeling of mechanical properties in human myocardium.

Conclusions: In this work, we developed an extrudable, biomimetic cardiac dECM bioink with tunable gel modulus by incorporating Laponite-XLG, and PEG-DA. We found that the mechanical stability of solubilized dECM can be significantly improved by adding Laponite-XLG, which in turn enhances the printability of the pre-gel. Furthermore, precise control of PEG-DA concentration led to fabrication of hydrogels that recapitulate physiologically relevant stiffness in native cardiac tissues. As such, our bioink can be utilized to faithfully model both biochemical and mechanical cues of the human heart, highlighting the possibility of modeling cardiac fibrosis. In our on-going study, we utilize this technology to investigate the disease mechanism and fibrotic activity in cardiac fibrosis.