Hyaluronan and Elastin-Like Protein (HELP) Gels Significantly Improve Cargo Retention in the Myocardium

Meghan E. Hefferon[1,2], Riley A. Suhar[1], Vanessa M. Doulames[1,2], Yueming Liu[1], Oscar Figueroa III[3], Hana Buabbas[4], Sarah C. Heilshorn[1].

[1]Department of Materials Science and Engineering, Stanford University, Stanford, California, 94305, USA.
[2]Department of Neurosurgery, Stanford University School of Medicine, Stanford, California, 94305, USA.
[3]Unaffiliated
[4]Department of Biology, Stanford University, California, 94305, USA.

Statement of Purpose: The delivery of therapeutics (e.g., cells, drugs, gene therapies) into the myocardium is an important clinical goal to promote regeneration following myocardial infarction. Current delivery media being explored in pre-clinical and clinical settings, such as saline and Matrigel, exhibit poor retention following direct injection into the contracting heart primarily due to immediate material evacuation and rupturing of blood vessels (**Fig. 1A**). Since cardiac function has been correlated with retention and most cargo loss is observed at the time of injection, *improving acute cargo retention at sites of injection is paramount*. In this study, we synthesized and characterized a family of recombinantly engineered hydrogels (**Fig. 1B**), consisting of chemically modified hyaluronan (HA) and elastin-like protein, named HELP. HELP's independently tunable mechanical and biochemical properties allow for more refined control over matrix properties than many naturally-derived biomaterials. In addition, HELP gels are cell-compatible and injectable.[1] Here, we demonstrate that tuning of the HA component enables direct control of the hydrogel stiffness, stress-relaxation rate, fracture stress, and injectability. Through use of a preclinical rodent model, we show that HELP significantly improves the retention of delivered cargo within the contracting myocardium.

Methods: HELP hydrogels consist of a recombinantly engineered elastin-like protein that is functionalized with hydrazine, and hyaluronan that is functionalized with either an aldehyde or benzaldehyde moiety through an oxidation or bioconjugation reaction. Three of the HELP variants were synthesized by oxidation of 1.5-MDa HA component for 8, 16, and 24 hours (HA8, HA16, HA24). Two other variants were developed using a two-part bioconjugation reaction of a small molecule with either a pendant aldehyde or benzaldehyde group to 100-kDa HA (HA-A and HA-B, respectively). Shear rheology experiments were performed to assess stiffness, stress-relaxation rate, and fracture stress properties. The variants were also screened and rated for their relative ease of injectability. As representative cargo, fluorescent microspheres were encapsulated within HELP gels and, as a control, Matrigel. To evaluate *in vivo* retention, Sprague Dawley rats (n=38; mixed gender) were injected with 50 μL of microsphere-containing gel and sacrificed at either a one- or seven-day time point. Heart samples were explanted and fluorescently imaged using a Leica Thunder Inverted microscope to assess both microsphere and hydrogel retention. Quantification of retention was performed through a combination of Fiji image analysis and an automated Python script.

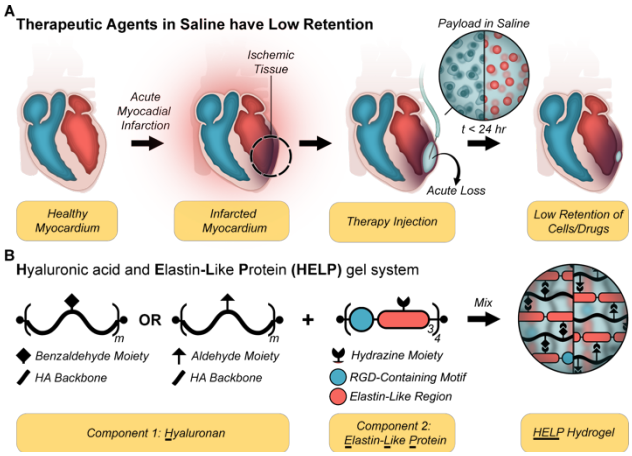

Figure 1. *Injectable hydrogel strategy to improve cargo retention in the myocardium*. **A.** Timeline of acute cargo loss using saline as delivery medium. **B.** Chemically modified HA and ELP components form dynamic covalent bonds in crosslinked hydrogel network.

Results: Initial mechanical characterization of HELP variants using oscillatory shear rheology demonstrated that all five HELP variants formed gels in less than 30 seconds and were fully crosslinked by 30 minutes. Altering the HA component alone affected both stiffness (G' ~ 500-3,000 Pa) and stress relaxation rate ($t_{1/2}$ ~ 0.5-13.5 hr). Additional mechanical characterization showed that gel fracture stress is likely a better indicator of relative injectability than stiffness or stress relaxation rate for this system. HA16 and HA24 both had fracture stress values less than ~1300 Pa and could be extruded easily through a 30-G needle post gelation with one hand. In contrast, HELP formulations with higher fracture stress were qualitatively assessed by our surgeon as being difficult to inject. In an *in vivo* retention study, HA16 significantly improved the uniform distribution of delivered cargo relative to Matrigel within the rat myocardium. Cargo retention was significantly improved using the HELP gel compared to Matrigel (retention of 70.4% *vs.* 35.2% at 7 days post-injection, respectively, $p < 0.05$)

Conclusions: Tuning of the HA component within HELP allows formulation of gels that are easily injectable by hand. Compared to Matrigel, HELP significantly improved acute cargo retention in the contracting myocardium.

References: [1]Suhar RA. bioRxiv. 2021.