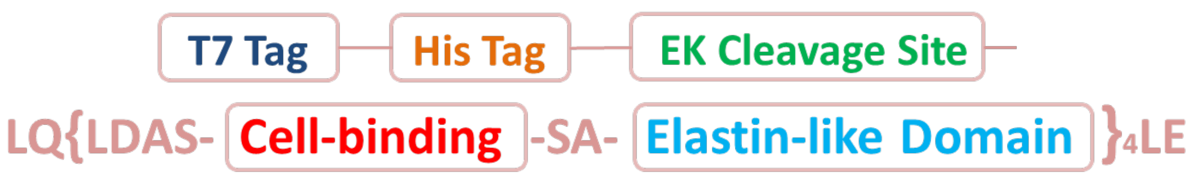
Supporting Information

Hybrid Elastin-like Polypeptide-Polyethylene Glycol (ELP-PEG) Hydrogels with Improved Transparency and Independent Control of Matrix Mechanics and Cell Ligand Density

Huiyuan Wang, Lei Cai, Alexandra Paul, Annika Enejder, Sarah C. Heilshorn *



Module name	Amino acid sequence
T7 Tag	MASMTGGQQMG
His Tag	HHHHH
EK Cleavage Site	DDDDK
Adhesive-RGD	TVYAVTG**RGD**SPASSAA
Non-adhesive-RDG	TVYAVTG**RDG**SPASSAA
Elastin-like Domain	$[(VPGIG)_2VPGKG(VPGIG)_2]_3VP$

Figure S1. Amino acid sequences of RGD-ELP and RDG-ELP.

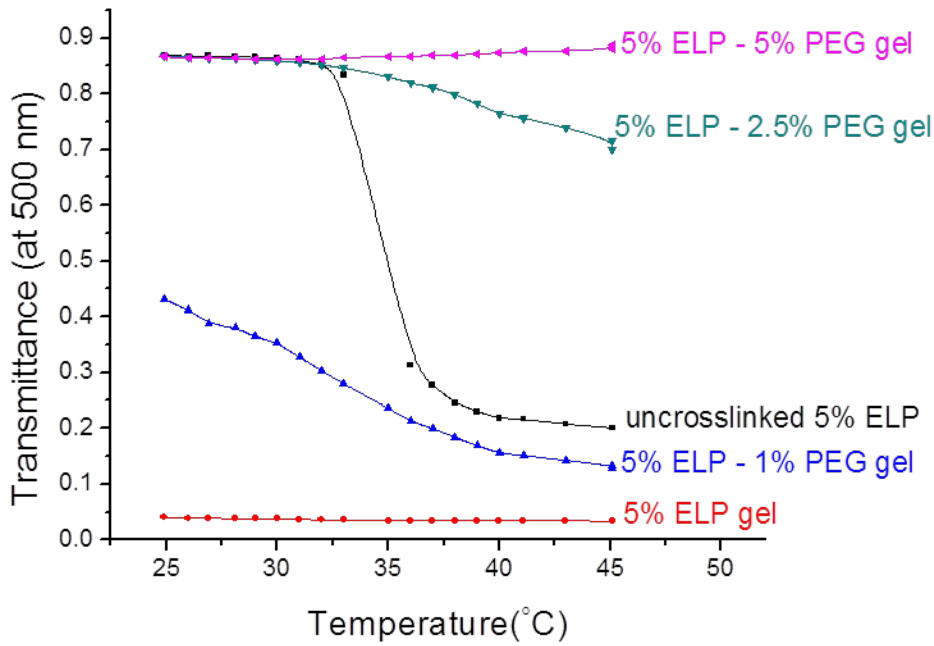


Figure S2. Transmittance (λ = 500 nm) versus temperature for different formulations: uncrosslinked 5 wt% ELP solution in PBS (1X, pH 7.4), or ELP-PEG hydrogels with the same ELP (5 wt%) and THP (1.61 mg/mL) concentrations but different PEG concentrations (0 wt%, 1 wt%, 2.5 wt%, and 5wt%). For gel preparation, 50 μL gel solution was pipetted into a 96 well-plate and left at room temperature overnight to ensure complete gelation. The temperature was increased from 25 to 45 °C in 1 °C increments, with a 1-min equilibration time, and absorbance at 500 nm was recorded. The transition temperature, *Tt*, was defined as the temperature where the first derivative reached a maximum absolute value on the transmittance versus temperature graph. Uncrosslinked ELP had a *Tt* of 33.9 ˚C. As PEG concentration increased, the hybrid ELP-PEG hydrogels displayed increasing *Tt* and higher light transmittance at physiological temperature.

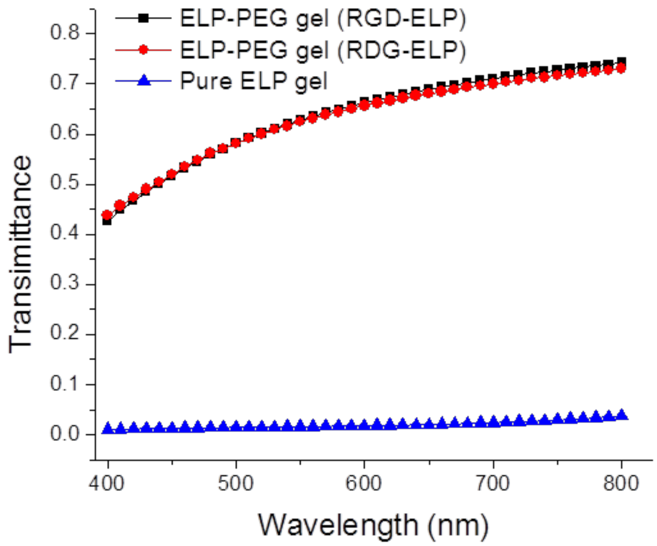


Figure S3. Transmittance spectra of pure ELP (5 wt% RGD-ELP plus 2.5 wt% RDG-ELP, X=2.20) and ELP-PEG hydrogels (5 wt% RGD-ELP or RDG-ELP, 2.5 wt% PEG, X=2.20). Briefly, a hydrogel solution (15 μL) was pipetted into 384 well-plates, resulting in a thickness of ~ 2000 μm. All hydrogel samples were allowed to crosslink for 15 min at room temperature and 10 min at 37 ºC prior to evaluation. The microplate reader was pre-heated to 37 ºC, and absorbance values of the samples were measured from 400 to 800 nm with a step-size of 10 nm. The absorbance values were converted to transmittance through the Beer-Lambert Law. Three replicates were tested for each type of hydrogel.

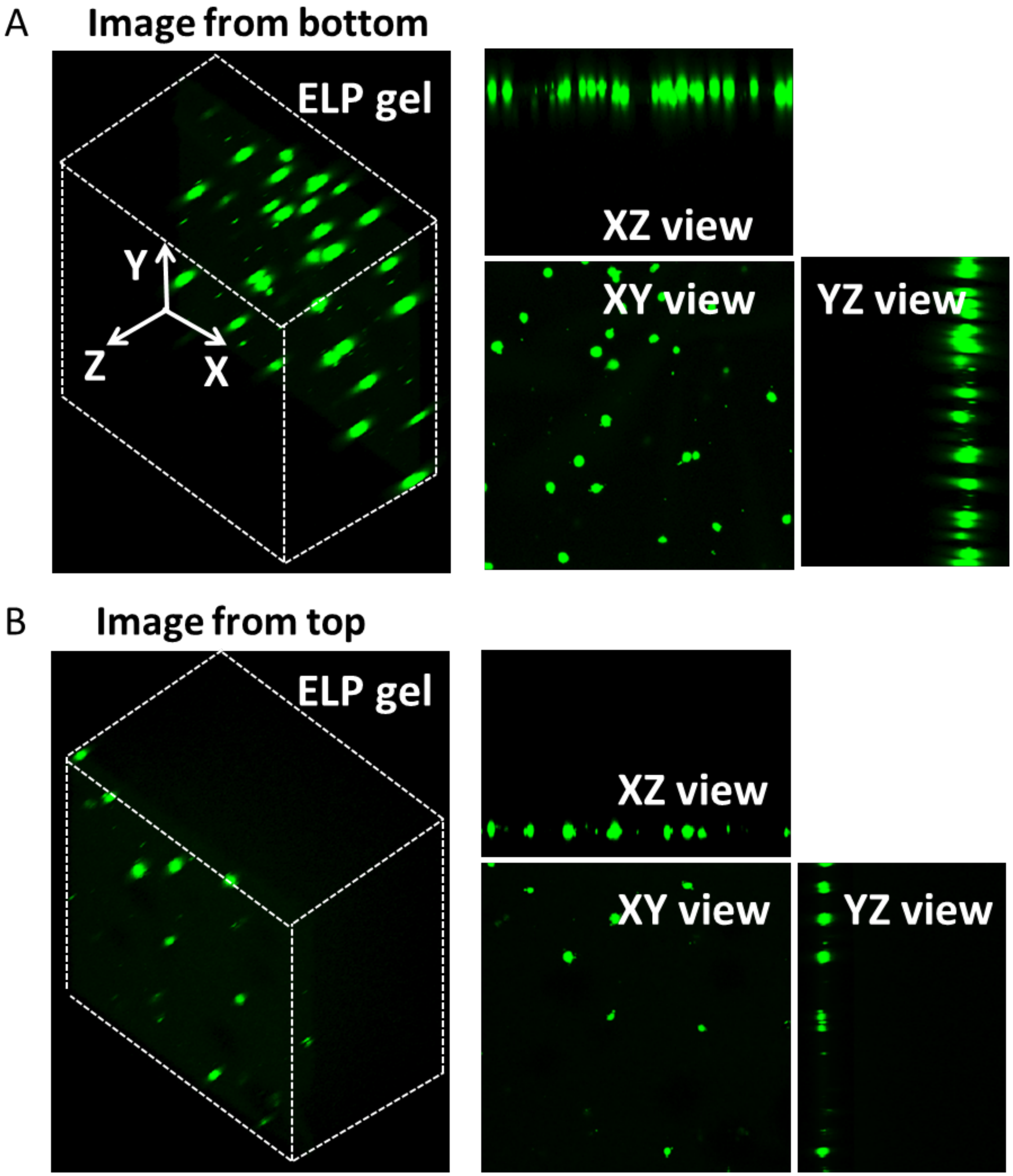


Figure S4. Observation of encapsulated human fibroblasts from bottom (A) and top (B) surfaces of a 5 wt% ELP hydrogel with crosslinking ratio, X, of 2.20. Cells were stained by calcein AM, showing that they were dispersed throughout the gel, but their observation was limited by the low light transmittance of the hydrogel.

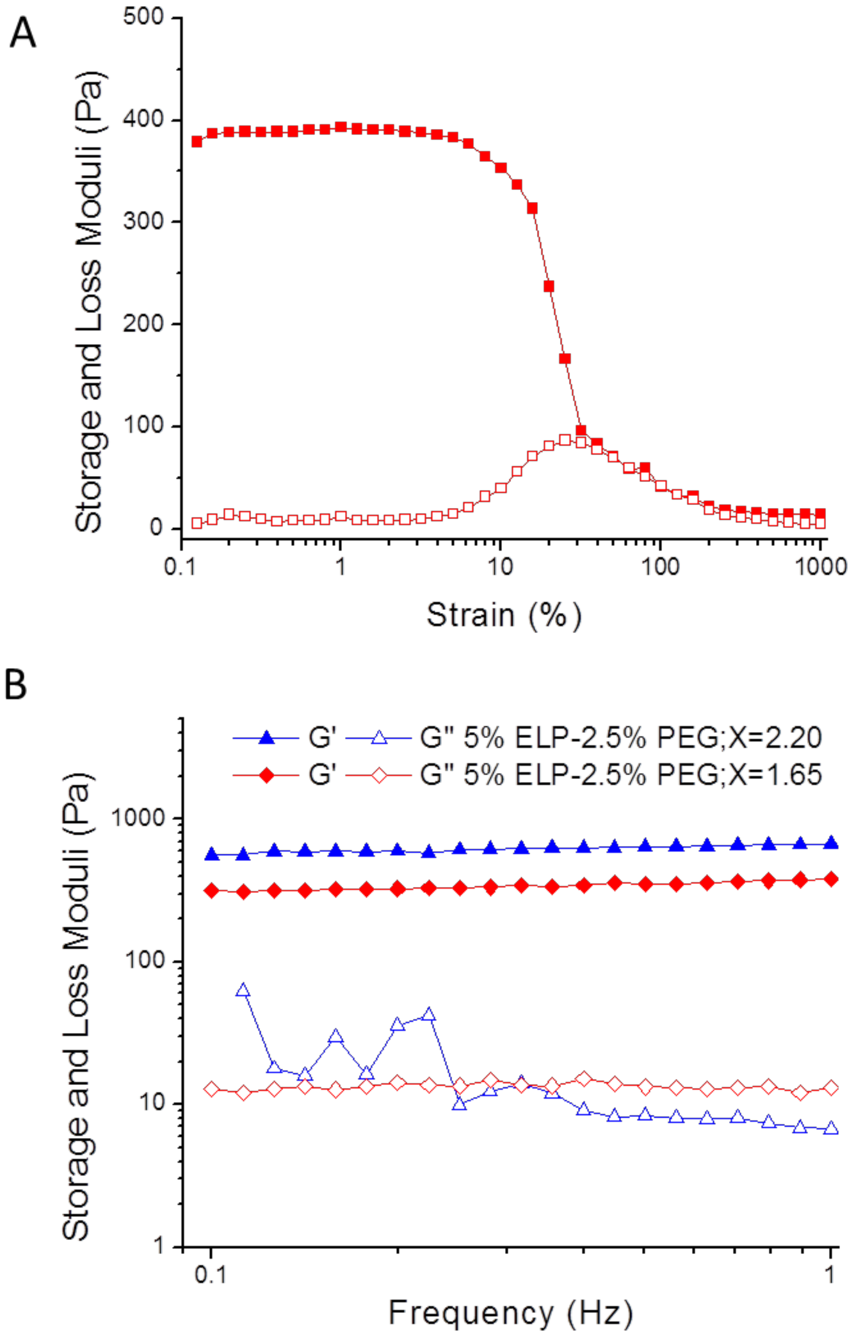


Figure S5. Strain sweep and angular frequency sweep rheology. (A) Strain sweep of ELP-PEG hydrogels (5 wt% RGD-ELP, 2.5 wt% PEG, X=1.65). (B) Angular frequency sweeps of ELP-PEG hydrogels (5 wt% RGD-ELP, 2.5 wt% PEG, X=1.65 and 2.20).

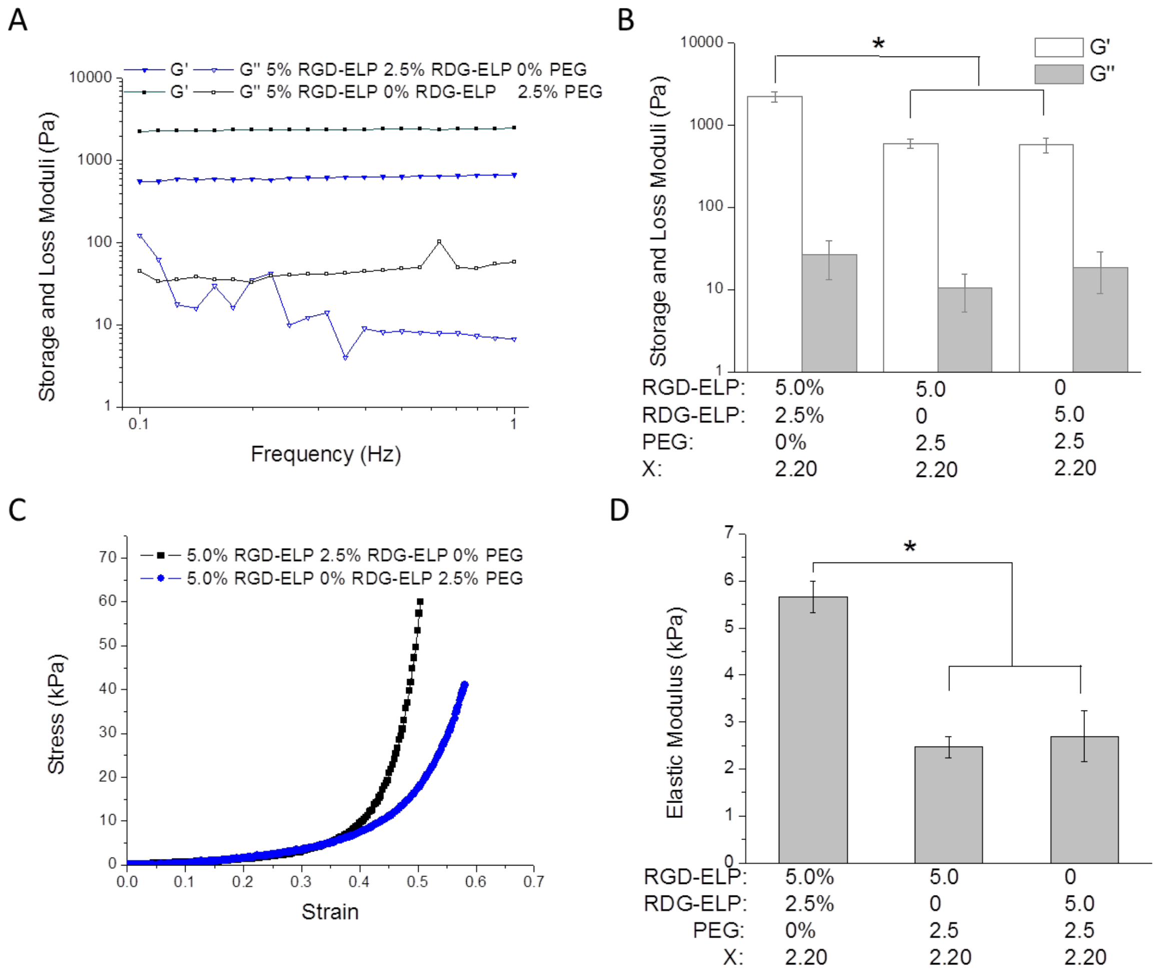


Figure S6. Rheological comparison between pure ELP and hybrid ELP-PEG hydrogels fabricated from RGD-ELP or RDG-ELP. Overall polymer concentration (including ELP and PEG) was kept constant at 7.5 wt%, and crosslinking ratio, X, was kept constant at 2.20. (A) Angular frequency sweep. (B) Storage and loss moduli at 1 Hz. (C) Stress-strain curve obtained from unconfined, uniaxial compression tests. (D) Quantification of elastic moduli derived from stress-strain curves.

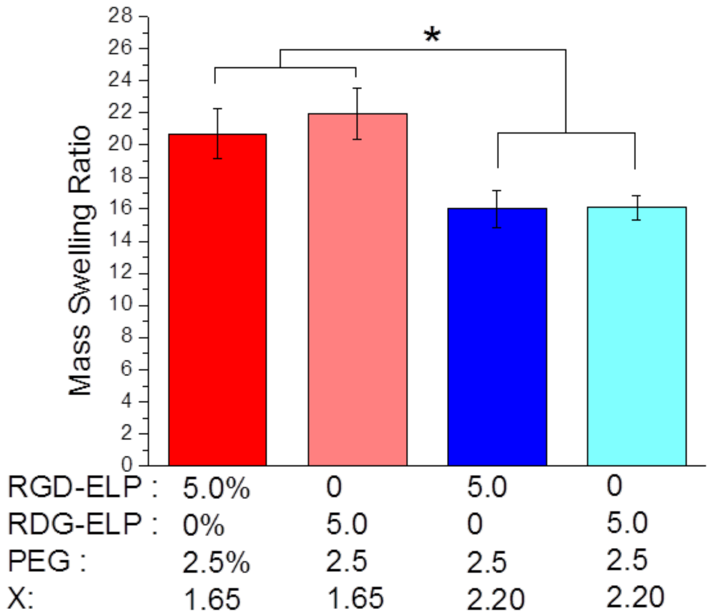


Figure S7. Mass swelling ratio of ELP-PEG hydrogels (5 wt% RGD-ELP or RDG-ELP, 2.5 wt% PEG, X=1.65 and 2.20).