ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.,* DOI: 10.1002/adma.201501099

3D Printing of Highly Stretchable and Tough Hydrogels into Complex, Cellularized Structures

*Sungmin Hong, Dalton Sycks, Hon Fai Chan, Shaoting Lin, Gabriel P. Lopez, Farshid Guilak, Kam W. Leong, and Xuanhe Zhao**

WILEY-VCH

Supporting Information

Three-Dimensional Printing of Highly Stretchable and Tough Hydrogels into Complex, Cellularized Structures

Sungmin Hong[†], *Dalton Sycks*[†], *Hon Fai Chan*[†], *Shaoting Lin*, *Gabriel P. Lopez*, *Farshid Guilak*, *Kam W. Leong*, *and Xuanhe Zhao*[*]

Fracture energy of the hydrogel measured with the pure-shear test

A pure-shear test was used to measure the fracture energy of the hydrogel. As illustrated in **Figure S1,** we separately stretched two identical samples with the same thickness T_0, width W_0, and initial gauge length L_0, where $W_0 >> L_0 >> T_0$. One sample was notched with a crack with length of $\sim 0.5W_0$ and the other was un-notched. The notched sample was stretched to a critical distance L_c (length at which crack propagation began), while the un-notched sample was stretched to measure the force-displacement curve. The fracture energy of the gel can be calculated by $\Gamma = \frac{\int_{L_0}^{L_c} Fdl}{W_0 T_0}$.

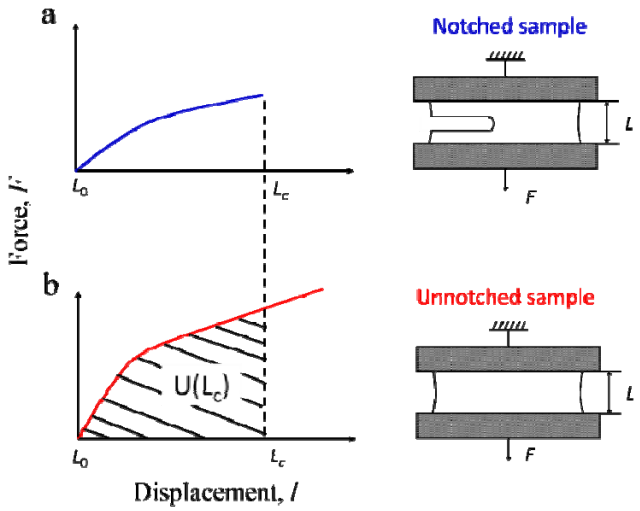

Figure S1. Schematic of the pure-shear test for measuring fracture energy of hydrogels. **a,** notched samples are stretched to a critical distance L_c, at which point crack propagation occurs; **b,** un-notched samples are stretched to L_c, with the force F recorded and the fracture energy of the hydrogel calculated as $\Gamma = (\int_{L_0}^{L_c} Fdl)/(W_0 T_0)$, where W_0, T_0 and L_0 represents width, thickness and initial gauge length of the sample, respectively.

Effect of Ca^{2+} and molecular weight of PEG on fracture energy and hysteresis

Mechanical properties were measured for PEG-alginate hydrogels with PEG of various molecular weights with or without Ca^{2+}. Each molecular weight of PEG (6,000, 10,000, or 20,000 Da, all held at 20 wt%) was mixed with the same concentration of alginate (2.5 wt%) and crosslinked under 365nm UV for 10 min.

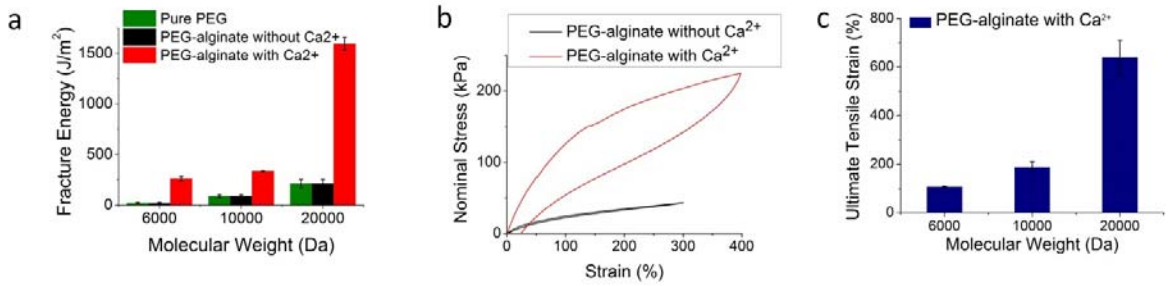

Figure S2. a, Comparison of fracture energies of PEG-alginate hydrogels without and with Ca^{2+} (50µl of 1M $CaSO_4 \cdot 2H_2O$ per 2mL of pre-gel solution) and pure PEG hydrogels as a function of molecular weight of PEG. **b,** stress-strain hysteresis loop of 20 kDa PEG-alginate hydrogel with and without Ca^{2+}. **c**, ultimate tensile strain as a function of PEG molecular weight.

Fracture energy of the hydrogel as a function of PEG concentration

The effect of PEG concentration on fracture energy of the hydrogel was investigated. Various concentrations of PEG-diacrylate (molecular weight 20,000Da) were mixed with a fixed concentration of alginate (2.5 wt%) in the pre-gel solutions. As shown in **Figure S3**, higher concentrations of PEG increase the fracture energy of the hydrogel. We chose 20 wt% PEG for the pre-gel solutions of PEG-alginate hydrogels in the current study, since the corresponding hydrogel readily exhibits fracture energy values surpassing 1,000 Jm^{-2}. As a control study, we also measured the fracture energy of hydrogels made from PEG-diacrylate (molecular weight 20,000Da) without alginate.

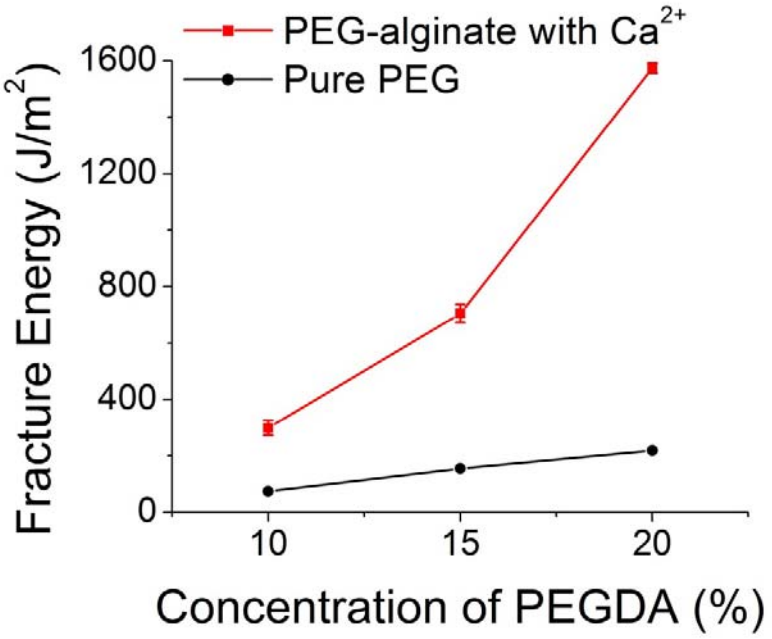

Figure S3. Fracture energy as a function of PEGDA concentration in the pre-gel solution.

Effect of photo initiator density on the fracture energy of PEG-alginate hydrogel

Various concentrations of photo initiator (I-2959) were mixed in the PEGDA-alginate pre-gel solutions. The concentration of 20 kDa PEGDA and alginate was fixed to be 20 wt% and 2.5 wt%, respectively. The fracture energy of the resultant PEG-alginate hydrogel varies with the concentration of I-2959 as shown in **Figure S4**.

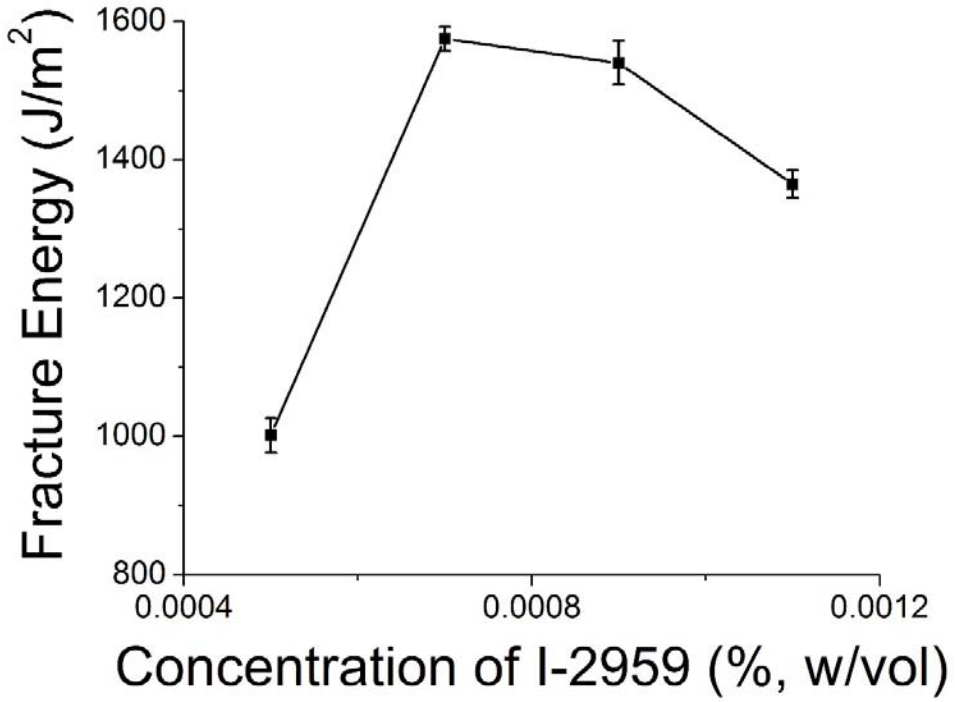

Figure S4. The effect of I-2959 concentration on fracture energy of the hydrogel

Digital image correlation to measure the strain field of the deformed hydrogel

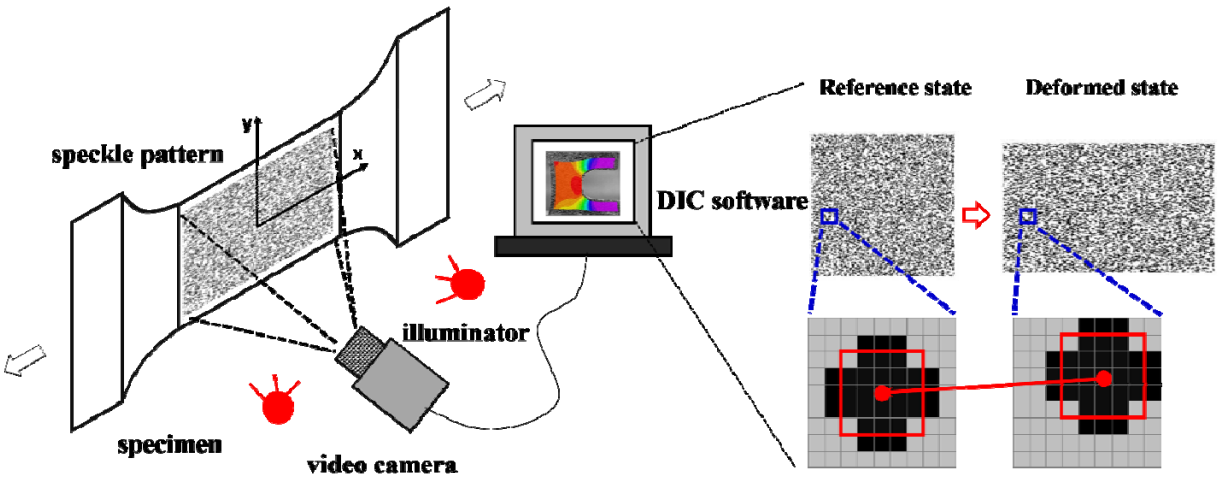

Figure S5. Schematic illustrating the digital image correlation technique. A random speckle pattern was spray painted onto the surface of a sample. Images of the speckle patterns at both the reference state and deformed state were recorded by a standard video camera throughout sample extension. The surface strain was measured by matching the digitalized images before and after deformation via VIC-2D software.

Viscosity of pre-gel solutions with various concentrations of nanoclay

The viscosity of pre-gel solutions with various concentrations of nanoclay was tested in an AR G2, 2° cone and plate viscometer (TA Instruments, New Castle, DE). Each sample was tested in a shear ramp test running from $0.01s^{-1}$ through $100s^{-1}$ shear rate over the course of two minutes. At low shear rates, increasing the concentration of clay as well as adding 50µl of 1M Ca^{2+} per mL of pre-gel solution raises the viscosity; this allows the printed pre-gels to maintain their shapes before the PEGDA is crosslinked. The hydrogel begins to shear thin at a critical shear rate; this results in an inverse relationship between viscosity and shear rate.

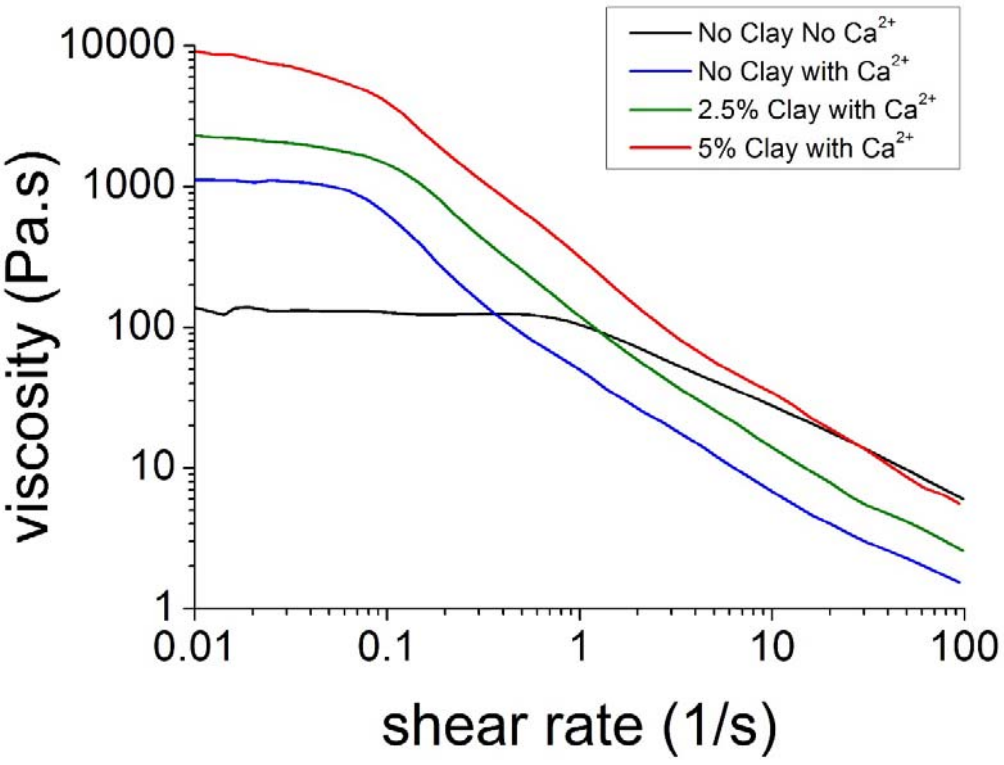

Figure S6. Viscosity of the pre-gel solution with various concentrations of nanoclay as a function of shear rate.

Printed mesh of PEG-alginate-nanoclay hydrogel immersed in collagen

A printed mesh consisting of the previously described PEG-alginate-nanoclay hydrogel was immersed in a cell-laden (HEK cells) collagenous solution. The collagen infiltrates into the mesh of the PEG-alginate-nanoclay hydrogel. At 22 °C, the collagen solution gelled over the course of 20 minutes to form a composite hydrogel composed of collagen gel between the channels of the printed PEG-alginate-nanoclay mesh, as shown below in **Figure S7**. Cell viability in the collagen remained approximately 95% throughout a 7 day study, as shown in Figure 4c.

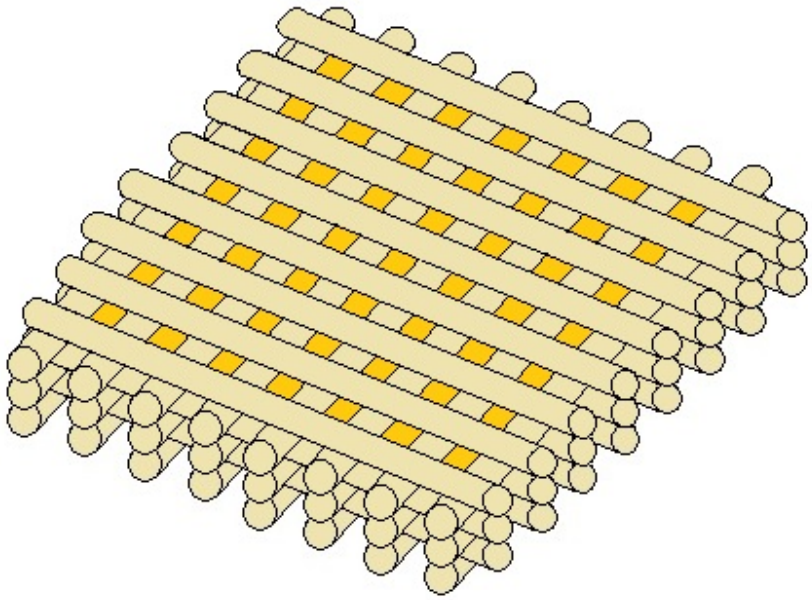

Figure S7. An illustration of a 3D printed PEG-alginate-nanoclay hydrogel mesh (shown above in tan) with collagen gel (gold) formed throughout the interconnected pore network.

Supplementary Movie 1. This movie shows a direct fabrication of a 3D hydrogel cube without the need for support material or post-processing modifications.

Supplementary Movie 2. This movie demonstrates recovery of a printed pyramid of the PEG-alginate hydrogel from compression test. It could withstand 95% compressive strain and nearly recover its original shape when the strain was released.