

Supporting Information

Mechanical response of collagen networks to nonuniform microscale loads

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Supplemental Methods

To investigate the decay power n as a metric to quantify the nonlinear mechanics of fibrous materials, we directly conjugated a photostable fluorophore (AlexaFluor carboxylic acid, 2,3,5,6-tetrafluorophenyl ester, Thermo Fisher) to commercially available rat tail collagen I (BD Bioscience). This resulted in a bright, photostable fibrous network with minimal background and high signal to noise suitable for analysis by digital image correlation. The protocol is written here.

To begin, take 30–40 mg of collagen I and place in a 50 ml conical tube. Proceed to precipitate the collagen with a 1 M NaCl solution. Gently centrifuge turbid solution, discard the supernatant, and resolubilize the pellet with 2 ml of 2 mM HCl. Gently mix by swirling and slowly vortexing. Allow to fully dissolve on ice before proceeding further. If necessary, remove excess air bubbles by degassing with vacuum for several minutes on ice. Once fully solubilized, estimate volume with a pipet.

Neutralizing and labelling collagen fibrils. To begin the labelling process, neutralize acidified collagen with neutralization buffer (0.5 M NaCl, 0.1 M NaHCO₃, pH = 8.2). Aim for a 4:1 neutralization buffer to acidified collagen ratio. Concurrently, dissolve 1 mg of AlexaFluor 5-TFP in 1 ml of neutralization buffer. Add and mix to neutralized collagen. Wrap 50 ml conical with aluminum foil and allow labelling reaction to proceed for 1-1.5 hrs at room temperature. Periodically mix by inverting and swirling. After 1-1.5 hours, stop the labelling reaction with 1:1 stop buffer (1.5 M NaCl, 1 M acetic acid). Mix thoroughly. Gently centrifuge, discard supernatant, and dissolve labelled pellet on ice in 3 ml of 0.02 M acetic acid. Once dissolved, desalt and remove unreacted fluorophore via dialysis. The labelled collagen was dialyzed against 0.02 M acetic acid in a 10K MWCO dialysis cassette (ThermoFisher). Dialyze at 4°C and protect from light. Exchange dialysate a total 5 times with a minimum of 2 long incubations.

Generation of Poly N-isopropyl polyacrylamide (PNIPAAm) microactuators To generate the temperature-responsive microspheres used in this study, a simple water/oil emulsion was used. The organic solvent base was kerosene supplemented with 3.5% Span 80. The solution was mixed and then de-gassed under vacuum for 1 hour to remove residual dissolved oxygen. After de-gassing, place under a positive nitrogen atmosphere and mix at 350-400 rpm on stir plate at 25°C. Allow to mix for 15 minutes, and then add the water components of the emulsion. The water component was comprised of 1 g PNIPAAm (Sigma), 7.5 ml of 2% Bis-acrylamide (Bio-Rad), 0.05 g Ammonium persulfate (Bio-Rad), and 1.5 ml 1x TBS. Components were mixed and de-gassed under vacuum for 10 minutes. Once de-gassed, the water component was added to the oil component under continuous nitrogen atmosphere. They were allowed to mix for 15 minutes, and then a mixture of de-gassed TEMED/kerosene (400 μ l / 1000 μ l) was added. Incubate overnight under continuous stirring and in the nitrogen atmosphere.

To isolate and purify PNIPAAm microspheres, remove flask from stir plate and allow to settle for minimum of 10 minutes. Once settled, carefully remove solvent leaving a thin layer above the granular, white particles. Transfer remaining contents to 50 ml conical and gently centrifuge at 4500 rpm for 5 minutes. Remove remaining kerosene solvent. Wash microspheres with acetone and centrifuge at 4500 rpm for 5 minutes. Repeat 3 additional times. After acetone washes, wash with distilled water 4 times. Allow last wash to rest for 10 minutes before final centrifugation and removal. Resuspend and store in PBS for future use.

To prepare the thermosensitive microspheres to become microactuators, the microspheres were treated with sulfo-SANPAH (Proteochem). The process begins by pipetting 25 ml of purified PNIPAAm microspheres into a 40 μ m cell strainer (Fisher Scientific). The microspheres were then washed with 100 ml of PBS. Once

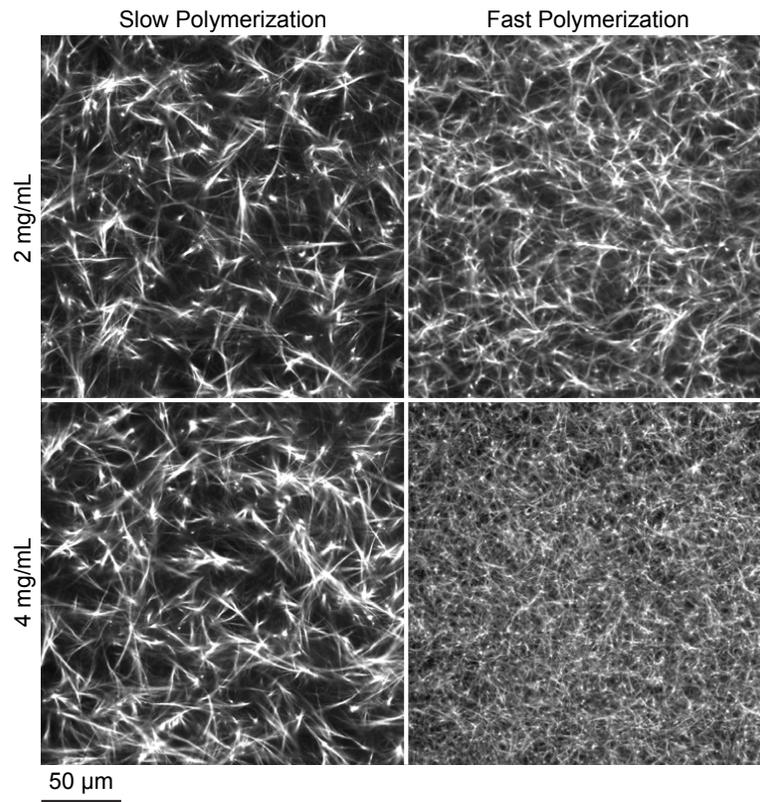
washed thoroughly, dab outside of mesh with a tissue to remove excess moisture. Wrap bottom and sides of the strainer with a piece of Parafilm to form a cup. Set aside and prepare sulfo-SANPAH. Create 40 μl stock solution of sulfo-SANPAH (50 mg/ml in dimethyl sulfoxide). Add sulfo-SANPAH stock to 2 ml of 37°C 0.05 M HEPES solution. Mix thoroughly and add to microspheres in sealed cell strainer. Expose to ultraviolet light of a tissue culture hood (6 inches from lamp) for 10 minutes. After 10 minutes, remove cell strainer, remove Parafilm and wash with 50 ml 0.05 M HEPES. Wash with additional 100 ml of PBS. Temporarily store cell strainer with sulfo-SANPAH treated microspheres in 50 ml conical tube filled with PBS until ready to embed in collagen network.

To embed, add desired amount of PNIPAAm particles into unpolymerized collagen of the appropriate concentration. The unpolymerized collagen should be doped with labelled unpolymerized collagen at a ratio of 1:20. This concentration will have to be modified depending on the labelling efficiency of the particular batch of labelled collagen. 1:20 is only the starting point; it could be increased to 1:10, 1:5 or until desired brightness is achieved. Allow collagen solution to polymerize for a minimum of 1 hour before imaging. 200 μm tall PDMS microchannels were used to minimize the amount of collagen required and to maximize to the contrast of the optical sections by reducing the thickness, thereby reducing loss of contrast due to out-of-focus light.

Supplemental Note

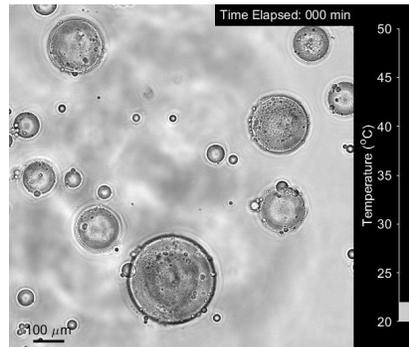
Here we show that classical linear elasticity predicts the scaling $u = Ar^{-n}$ with $n = 2$ for a contracting/expanding spherical inclusion in a matrix of any modulus. We work in spherical coordinates with radial position r having a value of zero at the center of the inclusion. All equations are presented assuming spherical symmetry. Angular displacements in the matrix are zero; let radial displacements in the matrix be denoted by u . Then radial normal strains are $\epsilon_{rr} = \frac{du}{dr}$, and angular normal strains are $\epsilon_{\theta\theta} = \epsilon_{\phi\phi} = \frac{u}{r}$. Note that partial derivatives have been converted to total derivatives, because u is a function of r only. Substituting these into Hooke's law gives normal stresses $\sigma_{rr} = \lambda \left(\frac{du}{dr} + 2\frac{u}{r} \right) + 2\mu \frac{du}{dr}$ and $\sigma_{\theta\theta} = \sigma_{\phi\phi} = \lambda \left(\frac{du}{dr} + 2\frac{u}{r} \right) + 2\mu \frac{u}{r}$, with λ and μ being the Lamé and shear moduli of the matrix. Substituting into the differential equation of equilibrium in the radial direction, $\frac{d\sigma_{rr}}{dr} + \frac{1}{r}(2\sigma_{rr} - \sigma_{\theta\theta} - \sigma_{\phi\phi}) = 0$, gives the general solution $u = \frac{A}{r^2} + Br$, where A and B are constants. For stresses to decay over distance, $B = 0$, leaving $u = Ar^{-2}$. Note that the power -2 is independent of the moduli of the matrix and inclusion.

Supplemental Figures

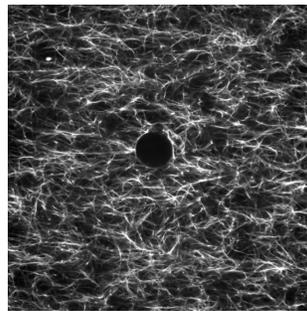


Supplemental Figure S1: Representative images of collagen fibers at two different concentrations, 2 and 4 mg/mL, and two different polymerization rates, “slow” (at 22°C) and “fast” (at 30°C).

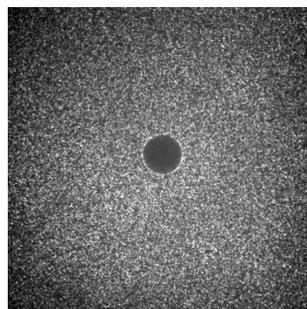
Supplemental Movie Legends



Supplemental Movie 1: Effect of temperature on PNIPAAm particles. PNIPAAm particles are in water inside a temperature-controlled incubator on the microscope stage. The temperature bar on the right shows the temperature of the incubator. The particles decrease in size as the temperature is increased, and they recover to their initial size when the temperature is decreased. The delay between changes in temperature and changes in particle size are due to the time for the heat to transfer from the incubator into the fluid surrounding the particles.



Supplemental Movie 2: PNIPAAm particle contracting in a fibrous collagen network.



Supplemental Movie 3: PNIPAAm particle contracting in a homogeneous, linear polyacrylamide gel.