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Abstract for BMES

Introduction

The use of biomaterials with tissue-like mechanics is important in the fields of drug delivery and tissue engineering due to the impact of cellular mechanosensing in cell growth, development and differentiation. Precisely, the viscoelastic behavior of the biopolymeric network should assimilate that of the biological tissues. Previous work in the Janmey Lab has shown biopolymeric fibrin networks alone soften under compression, whereas tissue stiffens under compressive forces. However, after the addition of inert particles, the viscoelastic properties of the fibrin hydrogel assimilate the behavior of the tissue. Several deformation mechanisms have been proposed to explain this change in mechanical properties, although no exact mechanism has been established. This project investigates the role of spherical polyacrylamide (PAAm) microparticle inclusion in the viscoelastic properties of fibrin hydrogels. Our goal is to explore which particle parameters may cause the observed changes in rheological and other mechanical properties of the fibrous biopolymer gels after large (58 ±13 μm) or small (5 ± 2 μm) microparticle inclusion and shed light on the phenomenological accuracy of the three current viscoelastic deformation models. Additionally, particle diffusion through the fibrin network will be evaluated using fluorescent nanoparticles in the presence of the PAAm microparticles.

Materials and Methods

The polyacrylamide microparticles are synthesized using an inverse emulsion of a polar phase consisting of acrylamide (monomer), bis-acrylamide (cross-linker), aluminum persulfate (initiator), DPBS (buffer), and TEMED (catalyst) in an organic phase made from cyclohexane and 2% Span 85 (surfactant) under two different conditions. The first group includes large microparticles made with a stir bar, while the second group has the same chemical composition and stiffness, but the particles are made with a homogenizer to yield smaller particles. The polar phase is added dropwise into the stirring or homogenizing organic phase in an inert nitrogen atmosphere after removing the oxygen, followed by three washing steps. After a final centrifugation step, the PAAm particles are incorporated into the fibrin hydrogel networks at four different particle volume fractions.

The fibrin hydrogels are synthesized through the biochemical coagulation reaction that converts fibrinogen into fibrin. Specifically, PAAm microparticles in Tris 1X are mixed with salmon fibrinogen and $CaCl_2$, and the solution is added to thrombin, yielding a 5 mg/mL fibrin network. The viscoelastic properties of the fibrin gel are determined using the G' (elastic modulus) and G" (viscous modulus) obtained with stepwise compressions on a rheometer. Similarly, the effect of compression on nanoparticle diffusion through the network is determined by fluorescent and bright field microscopy with a compressive device built in-house and analyzed through ImageJ and Python processing software developed for this project. This software tracks the velocity, distance traveled, and displacement of PEGylated 200 nm Polystyrene fluorescent particles.

Results, Conclusions & Discussion

Microscopic evaluation of large and small PAAm beads in fibrin hydrogels yielded several findings. First, the size of the PAAm particles was determined using ImageJ. Large particles of 58 ± 13 μm were obtained when stirring, while 5 ± 2 μm were made by homogenizing. The latter size resembles the dimensions of mammalian erythrocytes while the former size resembles adipocytes or megakaryocytes (dimensionally). Second, it showed the fluorescent particles are free to perform Brownian motion, which is restricted as the hydrogel is compressed. Subsequent decompression resulted in decreased Brownian motion restriction, indicating mesh sizes decrease under compression and relax after decompression. Third, compression was shown to stretch and align the fibrin network in a parallel fashion. Fluorescent particle tracking inside PAAm-fibrin hydrogel during compression showed most of these polystyrene beads moved with speeds <0.01 μm/s, displacement and distance <5 μm, shedding light on fibrin network mesh sizes.

For both the elastic and viscous moduli, rheological measurements of the fibrin hydrogel with PAAm microparticles show compression-stiffening whereas pure gel compression-softened, in agreement with previous results. Fibrin networks with large diameter (58 μm) PAAm particles stiffened under compression to a greater extent than with small diameter (5 μm) PAAm particles. Similarly, fibrin hydrogels with larger particle volume fractions increased the elastic and viscous moduli to a greater extent. These findings can be implemented for the generation of fibrin hydrogels with tunable stiffness and compositions. In turn, this will help build better tissue engineering scaffolds, where the viscoelastic properties match the cellular requirements.

Figure 1. Particle Size & Volume Fraction Affect the Elastic Modulus (only 20% and 60% V/V are shown for clarity)

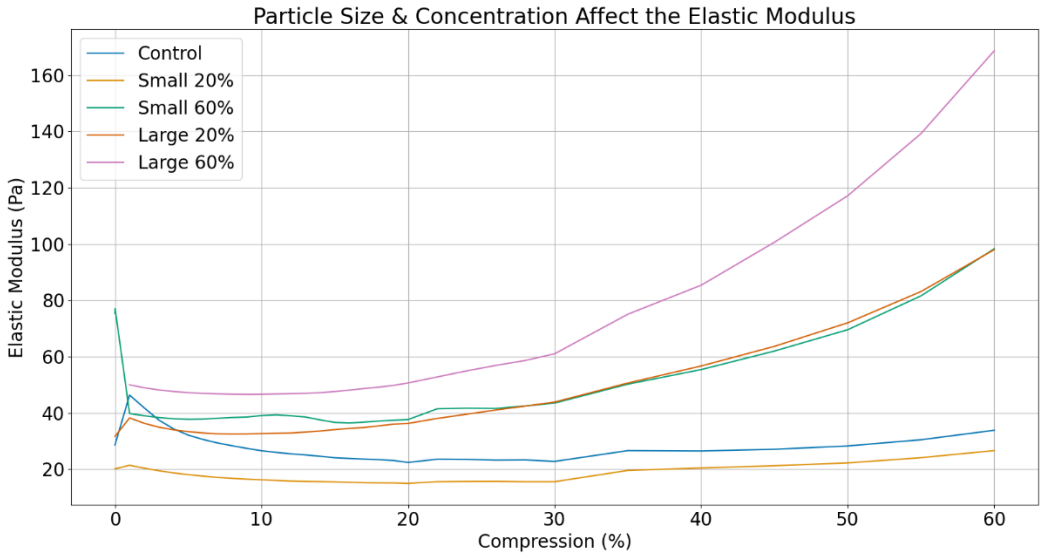

Figure 2. Particle Size & Volume Fraction Affect the Viscous Modulus (only 20% and 60% V/V are shown for clarity)

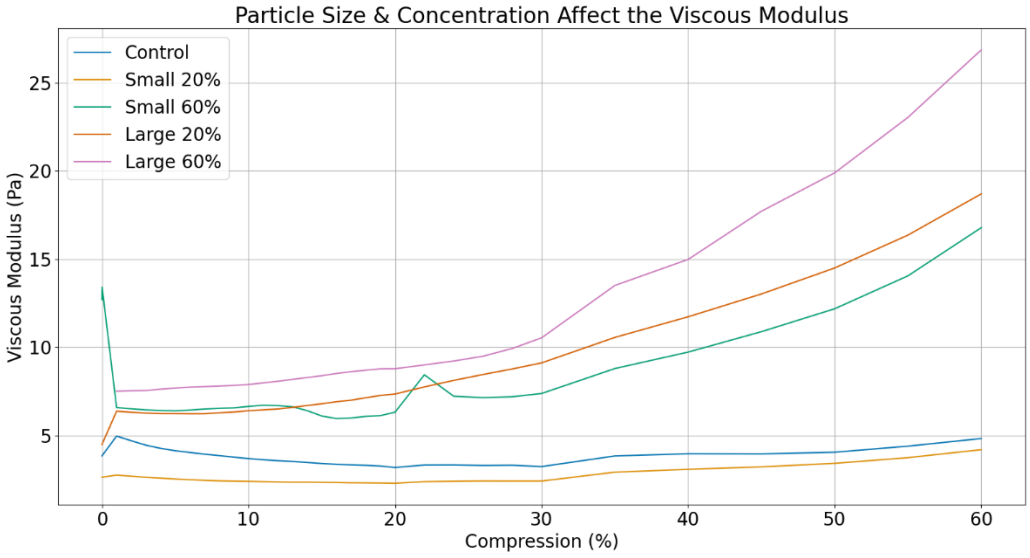

Figure 3. Polyacrylamide Size Distributions

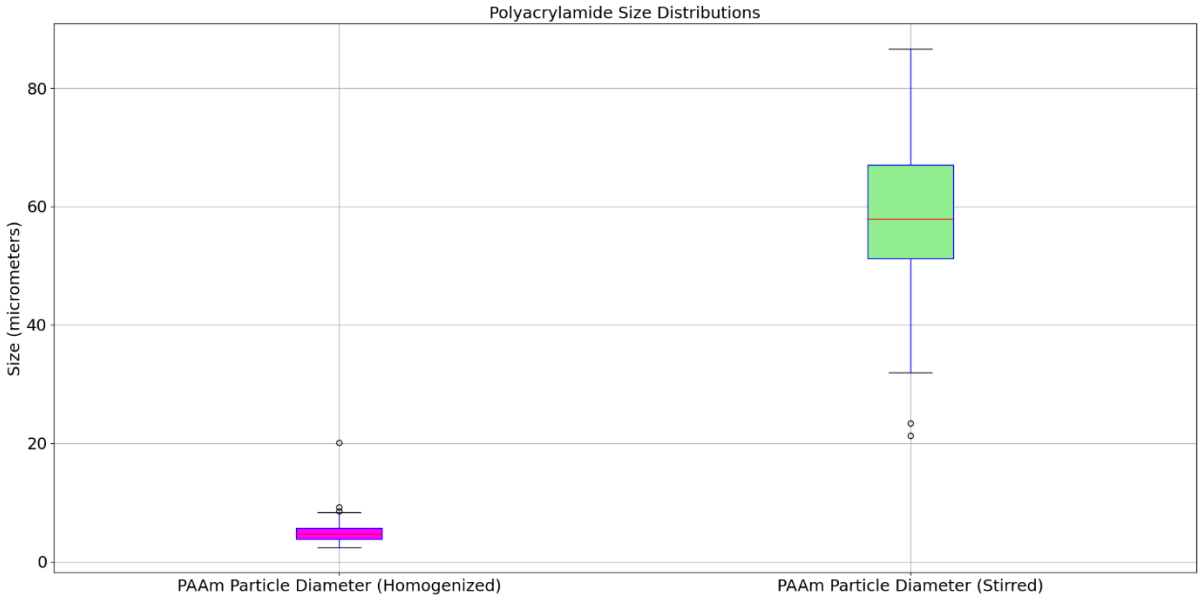

Figure 4. Hydrogel Compression Stretches Fibrin Fibers: Left) Uncompressed, Right) 80% Compression

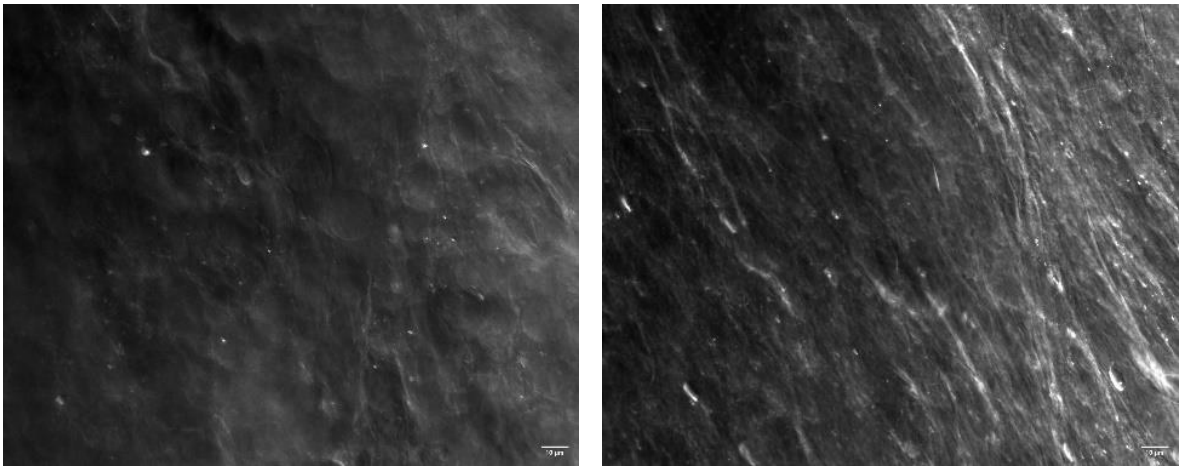

Figure 5. Fluorescent particles exhibit bulk motion under compression and Brownian motion inside the fibrin gel network