Effect of Polyacrylamide Microparticle Rigidity on Fibrin Gel Viscoelastic Behavior

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Introduction (250)

To function, biomaterials must be compatible with the human body and closely mimic the properties of living tissue. Hydrogels are polymeric biomaterials with various applications including wound healing, drug delivery, and biosensing [1]. However, they exhibit contrasting viscoelastic behavior to living tissue. Living tissue stiffens in compression, while polymer networks soften. Likewise, polymer networks stiffen in extension, while living tissue does not [3].

Fibrin polymer networks occur naturally in the human body [5], making them excellent candidates for biocompatible materials [1]. In this experiment, the fibrinogen polymerization reaction was carried out *in vitro* to form fibrin hydrogels. According to past research, these *in vitro* hydrogels lack the mechanical properties of true living tissue. Still, addition of spherical polyacrylamide (PAAm) microparticles causes the assimilation of compression stiffening trends seen in living tissue [2,4]. However, the reason behind this mechanism of mechanical change remains undiscovered. This project focuses on the role of spherical PAAm microparticle rigidity, and evaluates how this changes hydrogel viscoelastic properties via rheometry. We hypothesize that increased particle stiffness specifically drives compression stiffening of *in vitro* fibrin networks.

Adding PAAm microparticles is a promising solution for synthesizing hydrogels that follow the same elasticity trends seen in living tissue. Determining the specific reason for compression stiffening in PAAm microparticle hydrogels is crucial for understanding how biomaterials can be made more biocompatible with the human body. This can directly impact patient outcomes by advancing our ability to heal and treat diseases.

Materials and Methods (250)

PAAm microparticles were synthesized using inverse emulsification polymerization [2]. The organic phase was prepared using cyclohexane and 2% polysorbate 85 (surfactant). The aqueous phase was prepared using acrylamide (monomer), 2% bisacrylamide (crosslinker), 10% ammonium persulfate (initiator), N,N,N′,N′-tetramethylethylenediamine (catalyst), and DPBS (buffer). The aqueous phase was added dropwise to the organic phase using a 20-gauge syringe. The emulsion was stirred in a nitrogen atmosphere to prevent free radical reactions that would occur in an oxygen atmosphere. Stiffer particles were synthesized using a higher concentration of crosslinker.

To polymerize fibrin hydrogels [1], purified salmon fibrinogen was crosslinked by 0.1M calcium chloride, and mixed with pure particles pelleted in TRIS buffer (pH 7.4). This mixture

was then added to a smaller volume of thrombin, causing gelation within 15 seconds. The overall concentration of the fibrin network was 5 mg/mL. Hydrogels with varying concentrations of particles (0%, 10%, 40%, and 60%) were synthesized by adding the respective percent of the total gel volume from the particles stored in TRIS. Particles were visualized using light microscopy to confirm uniform size and shape (Figure 1).

The acquired viscoelastic properties were characterized using stepwise compression testing on a Kinexus rheometer and parallel plate. The trends in elastic modulus (denoted as G') were analyzed to determine the changes in viscoelastic behavior. A Python script was written to calculate and plot the average elastic modulus at each compression point in each treatment group. Data was normalized to 1 to show overall compression trends.

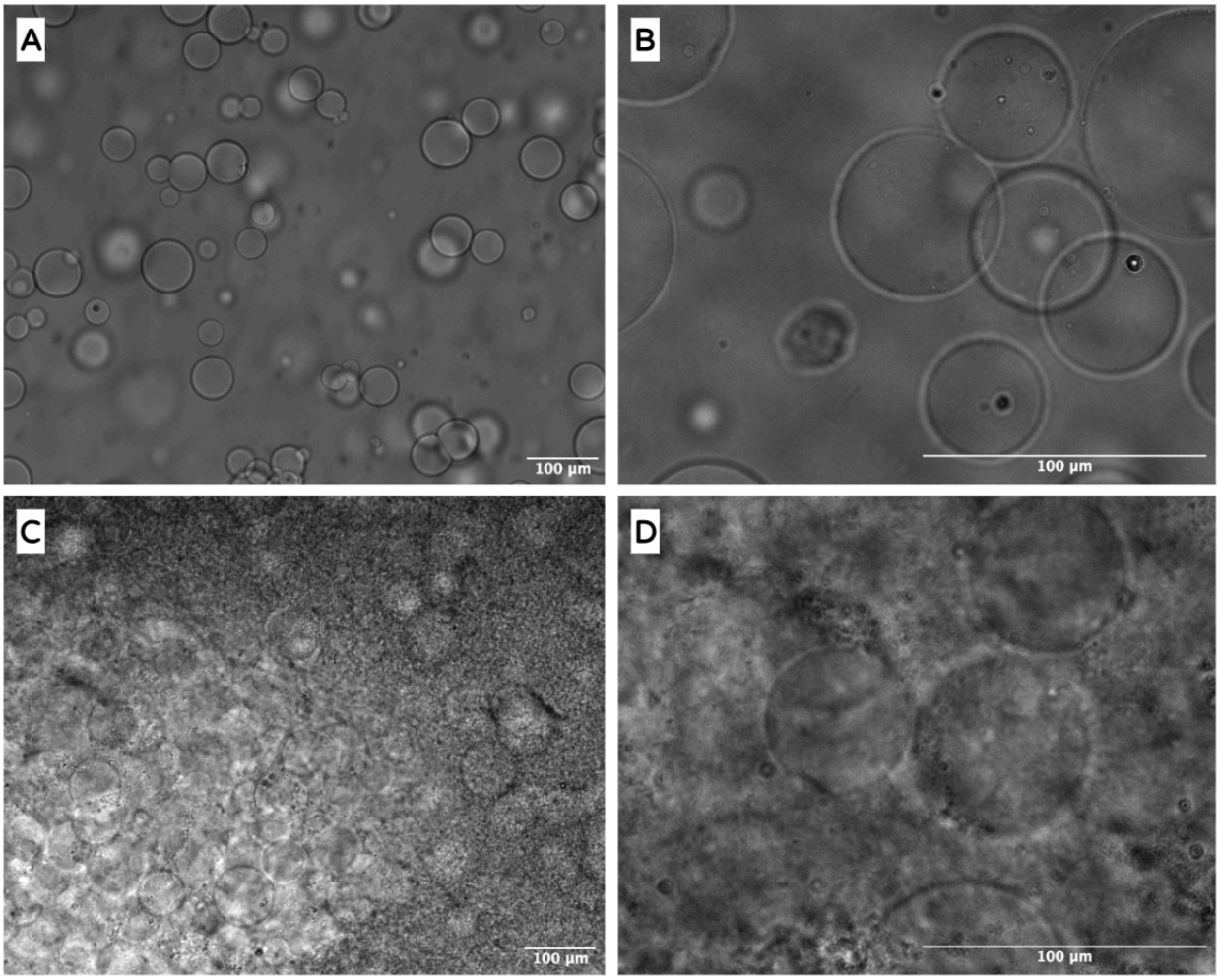

Figure 1. Polyacrylamide particles in PBS solution at 10x (A) and 40x (B) magnification. Polyacrylamide particles in a fibrin network at 10x (C) and 40x (D) magnification.

Results, Conclusions, and Discussions (350)

G' is a measure of the storage modulus, or the response of the elastic part of the network to deformation. An increased G' would indicate that the polymer network itself is more rigid, or stiff. When the control (fibrin hydrogel with 0% particles) is compressed, G' initially decreases and then stabilizes as the compression percentage increases (Figures 2A and 2B). This is expected as polymer networks are known to soften in compression due to the buckling of individual polymers.

For hydrogels containing soft particles, as particle concentration increases (10%, 40%, 60%), the G' also increases with compression (Figure 2A). This can be attributed to the fact that more particles in the hydrogel prevents fiber buckling, and stops the network from collapsing under compression.

Interestingly, there is little compression stiffening seen in fibrin hydrogels with 10% and 40% stiff particles compared to soft particles (Figure 2B). The elastic modulus of the hydrogels containing soft particles begins to increase at a lower compression percentage and increases more dramatically with compression. This indicates that softer particles cause the hydrogel to become more rigid under compression, which is more consistent with the behavior of living tissue.

Previous research suggests that the addition of spherical PAAm microparticles causes fibrin hydrogels to assimilate the same compression stiffening trends seen in living tissue [2,4]. The findings of this experiment confirm this observation and uncover more about the direct reason behind these trends. Stiffer PAAm microparticles may not be the driving factor for compression stiffening acquired by PAAm microparticle fibrin hydrogels. Therefore, other avenues should be explored for fabricating hydrogels that more closely mimic tissue viscoelastic properties.

Future experiments hope to analyze the effect of differently shaped particles on mechanical properties. For example, elongated particles, rather than spherical, may better attach to the fibrin network and therefore strengthen the stiffening on compression. Ideally, real cells may also be added to the network to obtain a more biologically accurate measurement. Overall, this area of research can further quality medical care by advancing the biocompatibility of hydrogels - novel agents of regenerative medicine, therapeutic delivery, and other medical applications.

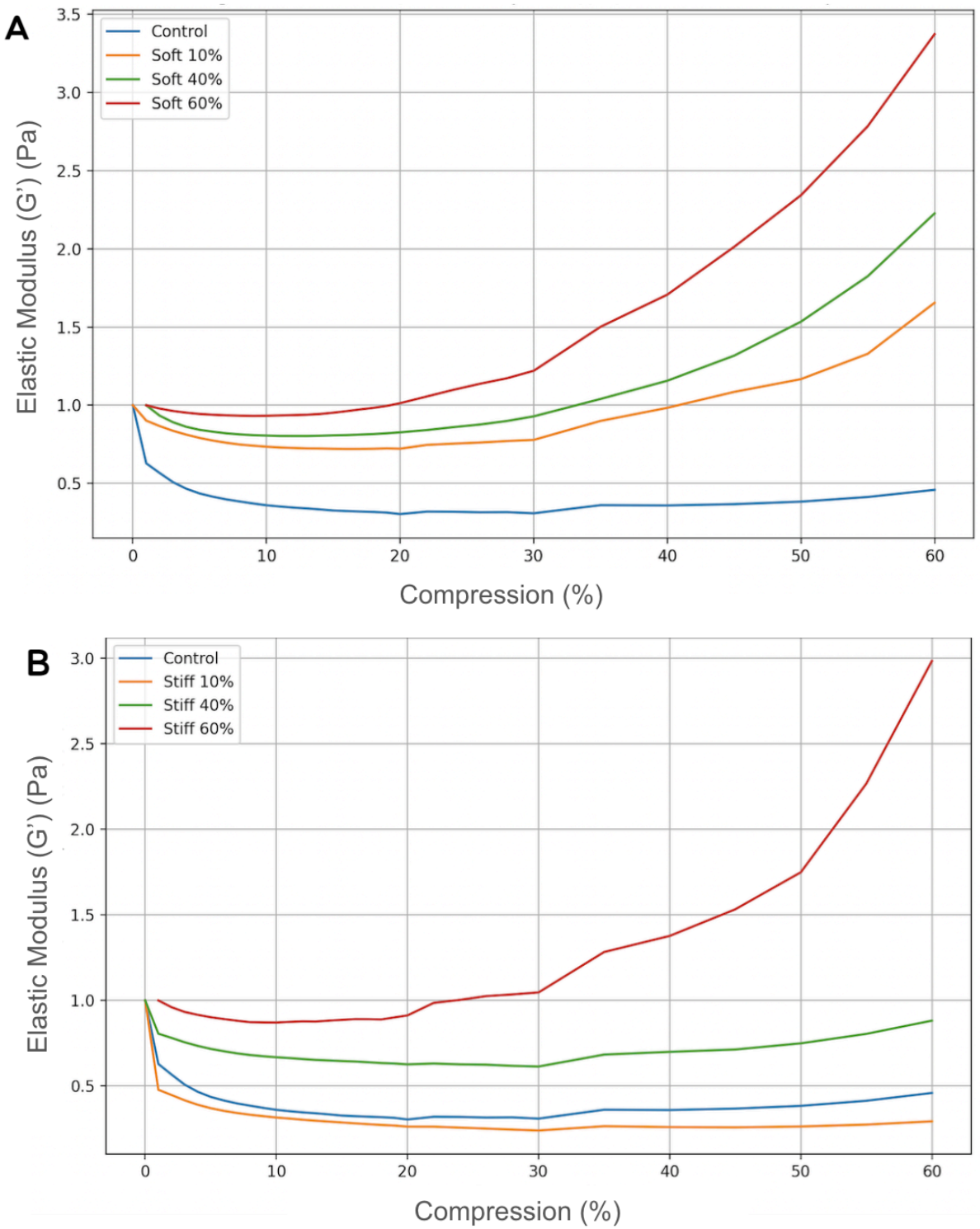

Figure 2. Results of stepwise compression testing. G' for 1mm fibrin hydrogels (5 mg/mL) containing soft (A) and stiff (B) PAAm particles at volume fractions of 0% (control), 10%, 40%, and 60%. Measurements of elastic modulus (G') (n=3) were run through a Python script to calculate the average G' at each compression point in each treatment group.

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