Compression-Induced Densification and Its Role in Tissue-Like Compressive Responses

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Introduction: Most biopolymer and fiber networks, such as collagen, fibrin, and actin, are known to soften under compression and stiffen under shear strain. However, many soft biological tissues display conflicting mechanics as they are compression stiffening and shear strain softening, despite being primarily composed of these same biopolymer networks. Interestingly, the addition of cells or cell-like particles to biopolymer networks has been shown to result in behavior that mimics that of biological tissues [1]. A recent computational study proposed that the non-affine displacement of particles under compression led to local fiber stretching within the network, causing stiffening of the composite [2]. While this study assumed that there was uniform compression throughout the network, both prior findings and our recent data show that the tissue deformation under compression is instead non-uniform. Compression induced local densification, where the region closest to the compressive load compacts significantly while distant regions remain relatively less affected [3]. The objective of this study is to characterize this local densification behavior during compression and evaluate its role in compressive responses in tissue-mimicking biopolymer networks. These findings may provide new information and insights into how tissues behave under loading, with potential implications in biomaterial design and disease management.

Materials and Methods: Sephadex G-25 fine microparticles were used as cell-mimicking inclusions. These particles were swelled overnight in Tris buffer and subsequently filtered to obtain a size range of 45-65 μm in diameter. The particles were size measured and counted to obtain a stock solution with a volume fraction (V/V) of 21.5% (Fig. 1A). To measure the compressive and shear responses, pre-gelled solutions of fibrinogen (5 mg/mL), thrombin (2 ug/mL), $CaCl_2$ (2mM) and varying particle V/V ratios were placed on a Kinexus rheometer to form fibrin gels with a diameter of 8 mm and height of 1 mm. Polymerized gels were uniaxially compressed step wise in 3% increments with 1%/s strain rate, allowed to equilibrate after each step, until 60% total compression. Shear amplitude tests were also performed on gels with or without prior compression. Densification of fibrin gels was measured by the addition of 3 μm fluorescent beads inside a custom glass chamber (10 mm x 1mm) and gels were compressed horizontally rather than vertically on a fluorescent microscope. The fluorescent intensity was used to demonstrate the density of the gel, indicating densification. The microparticles were also imaged and tracked to determine their displacement in regions with different densifications.

Results/Discussion: Fibrin gels containing 0% and 10% V/V particles display consistent compression softening behavior as evident by a decrease in shear elastic modulus (Fig. 1A). However, when the V/V is increased to 20% and 30% both softening and stiffening regimes are observed, and finally with 40% and 50% V/V only compression stiffening occurs (Fig. 1B). Under different shear strain amplitudes, pure fibrin is shear stiffening, while the addition of 30% V/V particles decreases the stiffening magnitude. Additionally, when 30% axial compression is applied the gel with 30% V/V particles transforms from shear stiffening to softening, mimicking biological tissues (Fig. 1C). Overall, these results show that the inclusion of microparticles reproduces tissue-like mechanical responses and allow us to study how stress may be propagated through tissues in the form of

densification. Understanding how different volume fractions of particles react to compressive/shear strains is important for determining whether fiber stretching and particle displacement dominate tissue mechanics.

We next measured densification of the composite networks. The compression of gels with 0% V/V of particles reveals a very thin, highly densified region with a steep transition to the non-densified region as compression increases. As the V/V of particles is increased we observe a wider area of densification with a more gradual transition towards the rarified area. At 50% V/V, densification was reduced overall, and there is no discernable boundary between the densified and rarified regions (Fig 2). During compression the densification profile separates the gel into three distinct regions. Imaging of microparticles during a 9% compression reveals minimal motion within both densified and non-densified regions, whereas the densifying region exhibits large particle displacements (Fig 3). In conclusion, these findings demonstrate that stress is not propagated uniformly throughout a sample. Instead, densification occurs near the compressive front, and particle motion is greatest in regions undergoing densification. Our study provides a more precise view of how tissues respond to mechanical loading and highlights cell density as an important factor in determining how mechanical stress is propagated through tissues. This work also offers a new perspective for engineering biopolymer-based biomaterials with physiologically relevant mechanical responses.

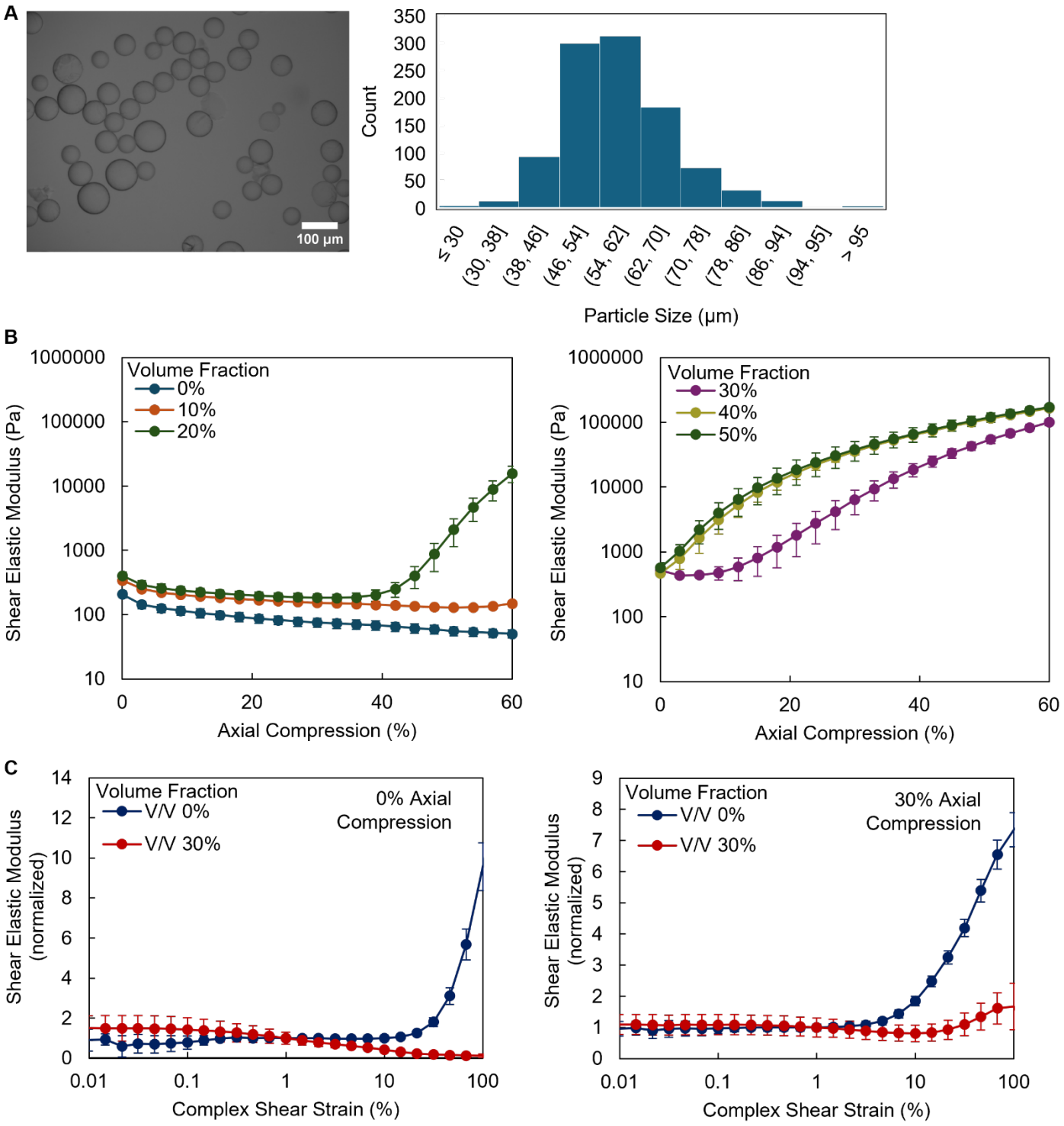

Figure 1: Microparticle volume fraction determines compressive and shear stress response. (A) Size distribution of filtered particles. **(B)** Shear elastic modulus of fibrin gels with different volume fractions of microparticles under axial compression. **(C)** Shear elastic modulus of fibrin gels with different volume fractions of microparticles under complex shear strain with and without initial compression.

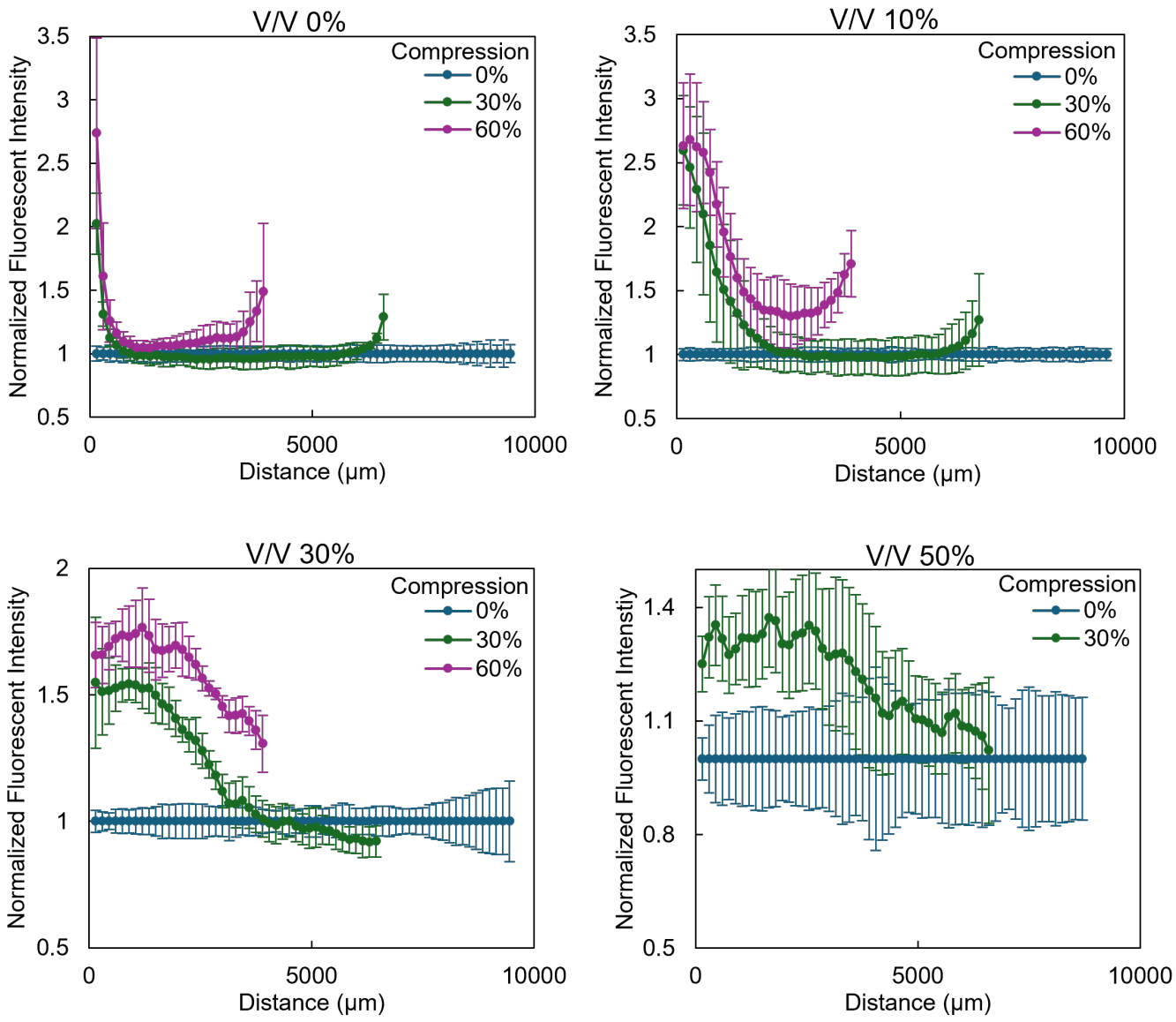

Figure 2: Particle volume fraction changes densification pattern during compression. The fibrin gel samples are in a transparent chamber between two glass slides and two walls for imaging. The moving wall (x=0) is pushed horizontally, compressing the sample against the stationary wall. Densification of gels with varying volume fractions of microparticles under compression is measured using the normalized intensity of fluorescent beads.

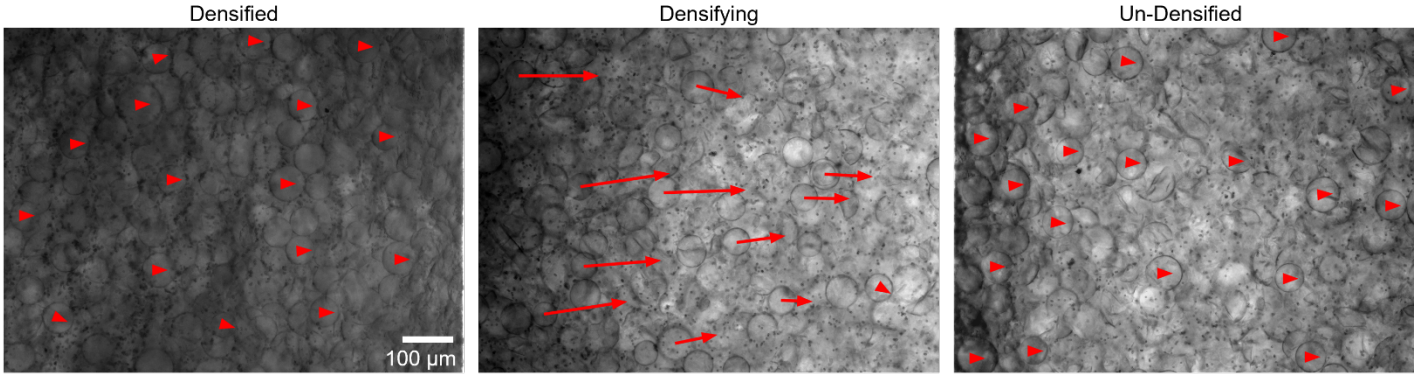

Figure 3. Particle displacement in different regions of a gel containing 20% v/v microparticles during compression. Red arrows indicate tracked particle displacement vectors following an additional 9% compression step. Minimal motion is observed in both the densified and non-densified regions, while the densifying region shows significant particle displacement.

References:

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[3] Liang, X., Chernysh, I., Purohit, P. K., & Weisel, J. W. (2017). Phase transitions during compression and decompression of clots from platelet-poor plasma, platelet-rich plasma and whole blood. Acta Biomaterialia, 60, 275–290. https://doi.org/10.1016/j.actbio.2017.07.011