Compression-induced structural and mechanical changes of fibrin-collagen composites

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Abstract

Fibrin and collagen as well as their combinations play an important biological role in tissue regeneration and are widely employed in surgery as fleeces or sealants and in bioengineering as tissue scaffolds. Earlier studies demonstrated that fibrin-collagen composite networks displayed improved tensile mechanical properties compared to the isolated protein matrices. Unlike previous studies, here unconfined compression was applied to a fibrin-collagen filamentous polymer composite matrix to study its structural and mechanical responses to compressive deformation. Combining collagen with fibrin resulted in formation of a composite hydrogel exhibiting synergistic mechanical properties compared to the isolated fibrin and collagen matrices. Specifically, the composite matrix revealed a one order of magnitude increase in the shear storage modulus at compressive strains > 0.8 in response to compression compared to the mechanical features of individual components. These material enhancements were attributed to the observed structural alterations, such as network density changes, an increase in connectivity along with criss-crossing, and bundling of fibers. In addition, the compressed composite collagen/fibrin networks revealed a non-linear transformation of their viscoelastic properties with softening and stiffening regimes. These transitions were shown to depend on protein concentrations. Namely, a decrease in protein content drastically affected the mechanical response of the networks to compression by shifting the onset of stiffening to higher degrees of compression. Since both natural and artificially composed extracellular matrices experience compression in various (patho)physiological conditions, our results provide new insights into the structural biomechanics of the polymeric composite matrix that can help to create fibrin-collagen sealants, sponges, and tissue scaffolds with tunable and predictable mechanical properties.

Introduction

Collagen and fibrin are two major components of extracellular matrix providing structural integrity and mechanical strength to various tissues in the body, and they are present together in wounds. These biopolymers are also employed medically as safe, versatile and clinically applicable biomaterials. They have been effectively used separately [1–6] as well as in combination as fibrin-collagen composites [7–14] for various biomedical applications [2–4,15–17]. Studying mechanical and structural properties of fibrin-collagen composites has a great practical significance, as they were shown to serve as effective hemostatic seals [13].

Addition of collagen to the fibrin hydrogel was shown to provide extra stiffness and durability [8,18].

In addition to improved elasticity, composite fibrin-collagen matrices were shown to be permissive to endothelial network formation in vitro [8] and in vivo [9], providing support for their use instead of purified collagen and fibrin.

Individual collagen and fibrin matrices have been extensively characterized in terms of their mechanical response to shear and tensile load, yet little work has been done on studying their compressive behavior [19–25]. Both biopolymers self-assemble into thick fibers via hydrophobic and electrostatic interactions [26] to form three-dimensional filamentous networks with mechanical properties that are substantially different from those of synthetic polymers [25,27]. Both biopolymers are known to exhibit a highly nonlinear stress–strain response known as strain-stiffening when exposed to tension or shear [22,28–33]. This nonlinearity was suggested to originate from stiffening of individual fibers and redistribution of the load over the entire network, as well as some structural rearrangements [28,34–36]. In contrast to the hydrogels made of pure proteins, much less is known about the mechanics of fibrin-collagen composites. It was shown that the fibrin-collagen mixture was several-fold stiffer than its components [37] and the interactions between components can be described in terms of series and parallel-type of network connections with transition to parallel type as the collagen content increased [18]. The linear modulus and toughness of tubular vascular constructs made of fibrin-collagen mixtures was also increased compared to collagen and fibrin alone [7].

There are only a few studies of compressive mechanics and structural rearrangements of these bio-composites, although compression of fibrin-collagen biomaterials occurs under various in vivo conditions, such as heart or limb muscle contraction, blood clot retraction, tendon or ligament reconstruction and controlling hemostasis in cardiovascular and hepatic surgery. Both unconfined and confined compression were previously used to increase the density and elasticity of separate collagen and fibrin matrices in order to assist in fabrication of tissue analogous implants [38]. In particular, plastic compression of collagen was used to rapidly generate tissue scaffolds of optimal density to support myocyte contractility [39]. Viscoelastic properties of compressed fibrin were studied in [40,41], highlighting a non-linear mechanical response to compression attributed to structural alterations of the matrix.

In the current paper, we studied structural and mechanical changes in the combined fibrin-collagen polymeric networks in response to controlled compression. We have shown that the composite fibrin-collagen matrix exhibits compressive viscoelastic and poroelastic properties that are different from the properties of its individual components. This distinction was attributed to structural alterations during simultaneous gelation of collagen and fibrin, leading to increased connectivity of the resultant network. These results provide mechanical and mechanistic bases for better understanding both the similarities and distinctions of the combined fibrin-collagen networks from their pure components to advance their usage as versatile biomaterials.

Results

Mechanical responses to compression of fibrin-collagen composite and its separated fibrin and collagen components

Changes in viscoelastic properties of the fibrin-collagen composite during compression

Mechanical response of fibrin-collagen composite exhibited remarkable softening-stiffening transitions as the compressive strain increased (Fig. 2A). First, its elastic modulus decreased 4.6-fold at $\gamma = 0.3$ (softening), remained relatively constant up to a compressive strain $\gamma = 0.82$ (plateau), and increased 4.5-fold (stiffening) at a maximal compression at $\gamma = 0.99$. There were significant differences in the elasticity and viscosity of fibrin-collagen matrices compared to pure collagen and fibrin gels. The shear storage modulus (G') of a fibrin-collagen construct was larger than that measured for its components alone over the whole range of compression degrees. The initial stiffness (G') of the fibrin-collagen composite was 1.5-times higher than the sum of the elastic moduli for pure collagen and fibrin gels. This difference decreased as the compressive strain increased up to $\gamma = 0.8$, but then displayed a marked (more than 7-fold) increase at $\gamma > 0.9$. Thus, the fibrin-collagen composite was initially stiffer than its individual components and was more responsive to compression than either fibrin or collagen alone.

Changes in the loss modulus (G'') of the fibrin-collagen constructs followed the same trend as the shear storage modulus (Fig. 2B). First, it decreased 3-fold at $\gamma = 0.2$, revealed a relative plateau up to $\gamma = 0.82$, and displayed a marked 8.2-fold increase at a maximal compression degree. However, the composite material had a higher loss modulus than collagen and fibrin alone over the entire range of compression degrees. It was initially 2.5-fold larger than the sum of fibrin and collagen loss moduli. This difference was minimal in a plateau regime at $\gamma = 0.7$ and increased 4.3-fold under a high compression degree at $\gamma > 0.9$.

To assess relative viscosity and elasticity of compressed fibrin-collagen composite matrices, the phase angle, $\tan(\delta) = G'' / G'$, was calculated. As shown in Fig. 2C, the phase angle of the composite as well as its components (fibrin, collagen) revealed a slight or no increase up to $\gamma = 0.85$ of compressive