Acta Biomaterialia 60 (2017) 275–290

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Full length article

Phase transitions during compression and decompression of clots from platelet-poor plasma, platelet-rich plasma and whole blood

Xiaojun Liang [a,1], Irina Chernysh [b,1], Prashant K. Purohit [a,*], John W. Weisel [b]

[a] Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

ARTICLE INFO

Article history:
Received 14 March 2017
Received in revised form 28 June 2017
Accepted 5 July 2017
Available online 8 July 2017

Keywords:
Phase transitions
Platelet poor plasma clots
Platelet rich plasma clots
Whole blood clots
Fibrin network
Red blood cells

ABSTRACT

Blood clots are required to stem bleeding and are subject to a variety of stresses, but they can also block blood vessels and cause heart attacks and ischemic strokes. We measured the compressive response of human platelet-poor plasma (PPP) clots, platelet-rich plasma (PRP) clots and whole blood clots and correlated these measurements with confocal and scanning electron microscopy to track changes in clot structure. Stress-strain curves revealed four characteristic regions, for compression-decompression: (1) linear elastic region; (2) upper plateau or softening region; (3) non-linear elastic region or re-stretching of the network; (4) lower plateau in which dissociation of some newly made connections occurs. Our experiments revealed that compression proceeds by the passage of a phase boundary through the clot separating rarefied and densified phases. This observation motivates a model of fibrin mechanics based on the continuum theory of phase transitions, which accounts for the pre-stress caused by platelets, the adhesion of fibrin fibers in the densified phase, the compression of red blood cells (RBCs), and the pumping of liquids through the clot during compression/decompression. Our experiments and theory provide insights into the mechanical behavior of blood clots that could have implications clinically and in the design of fibrin-based biomaterials.

Statement of Significance

The objective of this paper is to measure and mathematically model the compression behavior of various human blood clots. We show by a combination of confocal and scanning electron microscopy that compression proceeds by the passage of a front through the sample that separates a densified region of the clot from a rarefied region, and that the compression/decompression response is reversible with hysteresis. These observations form the basis of a model for the compression response of clots based on the continuum theory of phase transitions. Our studies may reveal how clot rheology under large compression *in vivo* due to muscle contraction, platelet retraction and hydrodynamic flow varies under various pathophysiological conditions and could inform the design of fibrin based biomaterials.

1. Introduction

Fibrin forms the structural basis of blood clots that are required to stem bleeding and of thrombi that obstruct the flow of blood, and thus cause heart attacks, ischemic strokes and other cardiovascular pathologies. Much is known about how clotting occurs and the factors that lead to bleeding and thrombosis [1]. Less is known about the mechanical properties of clots. Since clots perform primarily a mechanical function to stem bleeding, their mechanical response to various forces is important [2,3].

The mechanical properties of fibrin networks have been studied at different spatial levels including whole clot, networks, single fibrin fiber, and molecular levels [4–7]. Fibrin networks are composed of semiflexible fibrin fibers, which are extraordinarily extensible [8]. Fibrin networks are nonlinearly elastic and exhibit strain stiffening behavior, which means that clots become stiffer at higher strains [9]. The behavior of fibrin under shear deformation has been most extensively studied; there have also been some studies of extension [10].

Much less is known about the response of fibrin to compression, but recent studies have started to define its basic compressive

* Corresponding author.
E-mail address: purohit@seas.upenn.edu (P.K. Purohit).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.actbio.2017.07.011
1742-7061/

properties. The nonlinear response of the fibrin network to compression can be described as three stages – first a softening response, second a plateau, and finally stiffening [11]. The stress-softening behavior happens at low strain, where the storage and loss moduli of the network gradually decrease, followed by a plateau phase where the stiffness remains almost constant. Stress-stiffening behavior happens at higher strains and results in a steeply rising stress-strain curve. The structural changes of the network in response to compressive stress in the three stages above have been observed using a unique instrument that combines a rheometer and a confocal microscope. In the low strain regime, fibers start to buckle after a critical stress, which is responsible for the softening. Increasing strain causes progressive buckling which decreases the space between fibers and creates more contacts between them, called criss-crossing, and leads to densification of the network, with the combined effect of constant stiffness. At higher strains, there is more criss-crossing and densification of the network, which is responsible for the dramatic strain stiffening [12].

A number of models have been developed to quantify and help understand the mechanical properties of fibrin networks. All these models incorporate the stiffness of the individual fibers in the network and the molecular properties of fibrin molecules, as well as the network structure. For extension, these models are based on the phenomena of fiber alignment in the direction of strain at low strains, with a transition to stretching fibers and unfolding molecular domains at higher strains [10]. For compression, it was demonstrated that fibrin networks exhibit foam-like behavior [12]. The compression response of foams is also characterized by the three stages described above [13], and the properties of foams can be described in terms of those of the individual struts and those of the network (which could be triangular, square, honeycomb, etc.). Indeed, stress-strain curves with the three stages described above are commonly observed in materials that undergo phase transitions [14]. Just as in such phase changing materials, the fibrin network at low strains with unbuckled fibers can co-exist with a network at high strains with buckled fibers across a phase boundary [12] while the stress is almost constant (e.g. liquid water can co-exist with ice at constant atmospheric pressure at 273 K as it undergoes a phase change). In this paper we will show that the phase transition in fibrin networks is reversible, like any other material.

Most studies of clot mechanics and all studies of compression of clots have been carried out using clots made only of fibrin [15]. However, the fibrin network is only a part of the blood clots formed *in vivo* at sites of vascular injuries to prevent blood loss. These clots also contain varying amounts of platelets, red blood cells (RBCs), other cells and plasma proteins. Furthermore, such blood clots formed in a vessel are subject to external and internal mechanical forces. Internal mechanical forces are generated in the vasculature by blood flow, created by cardiac contraction producing the hydrostatic force of the blood within the vessel. There is also internal mechanical deformation generated by platelets pulling on fibrin in clot contraction (retraction). External forces are created as a result of vessel wall contraction, cardiac muscle and striated muscles adjacent to the blood vessels, especially in veins of the lower limbs. Thus, there are multiple forces acting on blood clots, including shear, tensile, and compressive forces.

There are only a few studies of the effects of platelets on clot mechanical properties, although they affect fibrin structure [16]. It is likely that platelets pulling on fibrin induce the fibers to go beyond the low strain linear viscoelastic limit and exhibit strain stiffening [17,18]. Since much thrombin is generated on the platelet surface, many fibrin fibers originate from platelets or platelet aggregates, affecting the overall structure of the clots [16].

Similarly, not much is known about the effects of RBCs on clot mechanical properties. Although they have long been viewed as passive participants in clotting, there is increasing evidence that they play a more active role [19]. For example, in clot contraction, RBCs are compressed to form a tightly packed array of polyhedral cells, which forms a very tight seal that is largely impermeable [20]. Thus, it is very important to understand how these various components of the clot, the fibrin network, RBCs and platelets, interact with each other in response to deformation. This knowledge can be important for understanding how blood clots and thrombi respond to mechanical forces *in vivo*. In addition, fibrin is increasingly being used as a biomaterial, including with composite materials and various cells, so its responses to compression are also important in this context.

Here, we investigate the mechanical response of human whole blood clots, platelet-rich plasma (PRP) clots, and platelet-poor plasma (PPP) clots, to cycles of compression and decompression, and correlate the effects with structural changes in all the components (see also [22,23]). These mechanical responses show unique characteristics that were unexpected, but can be interpreted in terms of a continuum phase transition model. In addition, we also investigate the viscoelastic properties of the clots and how they change with compression/decompression and a structural basis of these properties.

2. Experiment

2.1. Materials

Whole blood from healthy volunteers was collected according to an IRB protocol at the University of Pennsylvania with informed consent. The blood was collected into 12 mM sodium citrate (final concentration) as an anticoagulant, and then centrifuged at 130 g for 15 min to obtain PRP, which was again centrifuged at 10,000 g for 15 min to obtain PPP. Clots were made by adding $CaCl_2$ (25 mM final concentration) and 1 U/mL (final concentration) human α-thrombin (American Diagnostica Inc. Stamford, CT USA).With this high thrombin concentration, essentially all clotting reactions were completed within 40 min and both the extrinsic and intrinsic coagulation pathways are bypassed. Clots of 600 μm thickness were formed at 37 C directly between the stainless steel rheometer plates (ARG2; TA Instruments, New Castle, DE) for all whole blood, PRP and PPP clots. The fibrin component of all clots is strongly adherent to the rheometer plates.

2.2. Compression experiments

Clots were compressed continuously at the rates of 10 or 100 μm/s, as the upper rheometer plate moved down to exert an axial force on the upper surface of the clot. When the maximum compressive strain was achieved, the upper plate was moved back to its original position at the same rate to forcefully decompress the clot. During the compressive part of the cycle, serum for PPP and PRP clots and serum with RBCs for whole blood clots was forced out and it was pulled back into the clots during the decompressive part of the cycle (see Supplemental Video). Three cycles of compression and decompression were performed on the same clot. The clots were compressed and decompressed vertically up to compressive strain $\varepsilon = 0.33$ or 0.5, i.e., compression of 1.5X or 2X. The compressive strain (degree of compression) was defined as the absolute fractional change in fibrin clot thickness $\varepsilon = |\Delta h/h_0|$, where $\Delta h = h - h_0$, and h_0 and h are the thickness dimensions of the uncompressed and compressed clots, respectively. During compression and decompression, the normal stress was measured and stress-strain curves were plotted for further