Rupture of Fibrin Clots: Structural and Thermodynamic Mechanisms

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Statement of Purpose: Fibrin, a naturally occurring polymer, is the major mechanical component of blood clots and plays a critical role in 1) the formation of a strong, hemostatic seal to prevent bleeding and 2) the propensity for thrombotic embolization. While the viscoelastic mechanical properties of fibrin have been well studied, these properties are distinct from the ability of a material to resist rupture. Determining the structure-function relationship of fibrin rupture is critical for understanding mechanisms involved in the formation of a hemostatic blood clot to prevent excessive bleeding following traumatic injury. This information also provides fundamental knowledge needed for the developing of mechanically tunable fibrin biomaterials and hemostats.

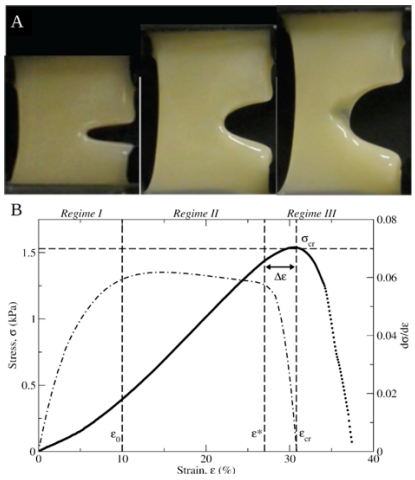

Figure 1. Experimental stress-strain analysis of the dynamics of deformation and rupture of a fibrin clot with single-edge crack.

Methods: To assess the dynamics of mechanical deformations/resistance to rupture, we performed tensile testing experiments on fibrin gels with a single edge crack ranging from 7-35% of the sample width (Figure 1A). Fibrin gels were formed from platelet poor plasma pooled from 25 distinct donors. Clotting was initiated through the addition of 25mM $CaCl_2$ and 1:80 dilution of thromboplastin. Sample ultrastructure was analyzed for fiber density, diameter, and length in unstretched and stretched samples, utilizing scanning and transmission electron microscopy. To interpret the stress-strain curves, we developed a Fluctuating Spring model, which maps the changes in fiber alignment at the characteristic strain, stretching of the elastic network, and sequential rupture of coupled fibrin fibers with cooperativity on the strain-scale into a theoretical framework. We used this theoretical framework to calculate critical stresses/strains and determine the free energy changes in fiber deformation and rupture. This combination of experimental and theoretical approaches allowed us to determine mechanisms of fibrin rupture.

Results: Through examination of experimental stress-strain curves and the fibrin ultrastructure, we determined that fibrin undergoes three distinct deformation regimes during the rupture process (Figure 1B): 1) a weakly non-linear regime at low strain levels (<15%), which occurs due to fiber alignment 2) linear regime of elastic deformation for intermediate deformation related to the reversible stretching of fibers, and 3) a rupture regime when irreversible breaking of fibrin fibers ahead of the crack tip occurs at high strains (>30%). We show that for fibrin gels formed under these conditions, the critical strain is 34–45% and the critical stress is 1.5–22 kPa. Our theoretical framework reveals that the presence of a crack or defect in the fibrin network renders them more stochastic. We show that the free energy associated with the fiber deformation and rupture is inversely related to the crack length; therefore, increasing crack length makes network rupture more spontaneous. By contrast, inter-fiber connectivity reinforces the fiber network, making rupture less likely to occur. Microscopy (Figure 2) and theoretical results reveal that the fibrin network forms multifiber threads during the extension process; when strain exceeds the limits of the thread extensibility, the fibrin begins to rupture cooperatively.

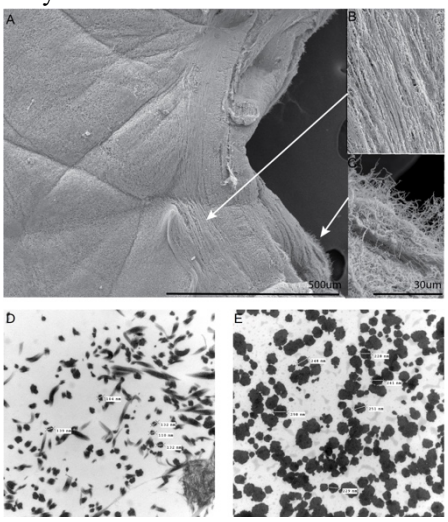

Figure 2. Scanning and transmission electron microscopy of fibrin gel

Conclusions: Study of the ability of fibrin gels to withstand breaking helps us to understand mechanisms of mechanical stability in fibrin-based engineered tissues and hemostatic sealants, which are used widely in surgery and in treating traumatic injury. In this work, we coupled experimental and theoretical approaches to 1) uncover the structure-mechanical characteristics of rupture, and 2) resolve the thermodynamics of forced deformation/rupture. This theoretical framework can be applied to other protein networks to understand rupture of biological materials. In addition, further fibrin-based studies can help to reveal how the structural architecture of the fibrin network influences the resistance to rupture.