Structure-Function Micromechanics of Fibrin Biomaterial Scaffolds

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Statement of Purpose: Normal wound healing in the body includes the coagulation of fibrin clots. The two components that comprise fibrin are fibrinogen and thrombin. Fibrin exhibits a linear relationship between its Young's Modulus and the concentration of fibrinogen. An increase in fibrinogen concentration resulted in a heterogeneous microstructure comprising random macrobundles of fibrin fibrils observed via confocal fluorescence microscopy. An analytical model was developed to rationalize the micromechanics of the fibrin based on these observations.

Methods: Fibrin Constituent Preparation: Fibrinogen and thrombin solutions (Baxter BioScience) were diluted by adding TBS to the fibrinogen and 30mM $CaCl_2$ in TBS to the thrombin. The fibrinogen was diluted to concentrations ranging from 5-34 mg/ml. The thrombin was kept constant at 1 U/ml.
Fluorescently Labeled Construct Formation: AlexaFluor 488 (Molecular Probes) fluorescently labeled fibrinogen was prepared into a 1mg/ml stock solution and added to the fibrinogen dilutions at a concentration of 1:50. 150µl of the resulting solution was then used to prepare 300µl constructs on glass coverslips with 150µl of diluted thrombin. After 24 hours, the constructs were examined using confocal fluorescent microscopy and analyzed for fibrin fibril network pore size and microstructure.
Indentation Protocol: To obtain the nominal Young's Modulus of the fibrin biomaterials, a novel compressive indentation protocol was used [1]. With a 2.5N load cell and a 3mm circular, flat-ended glass punch attached to an Instron 3365 Universal Testing machine, 4 ml fibrin constructs, prepared as above, were tested subject to a displacement rate of 10mm/min to a final displacement, u, of 5mm. The force, F, in Newtons, and displacement, u, data were recorded and analyzed to determine the nominal Young's modulus [1].
Micromechanics Modeling: The load-displacement relationships were modeled using simple beam theory. Together with the Bousinessq solution for the macroscopic indentation response [1] the following expression is obtained:

$$F = \left[\frac{38.4\pi a E^{*} N}{1-\upsilon^{2}}\left(\frac{R}{L}\right)^{4}\right]u \quad \text{(Eq. 1)}$$

where E* is the intrinsic Young's modulus of the fibrin, a is the indenter radius, ν is the Poisson's ratio, R is the radius of the fibrils, L is the network pore size and N is the number of fibrils per area.

Results / Discussion:
Stiffness vs. Concentration of Fibrinogen: There was a linear relationship observed between the nominal Young's Modulus and the concentration of fibrinogen (Fig. 1). According to Eq. 1, these data are consistent with the model predictions that the number of fibrils, N, is increasing, rather than a fixed number of fibrils that are increasing in diameter (Solid lines, Fig. 1).

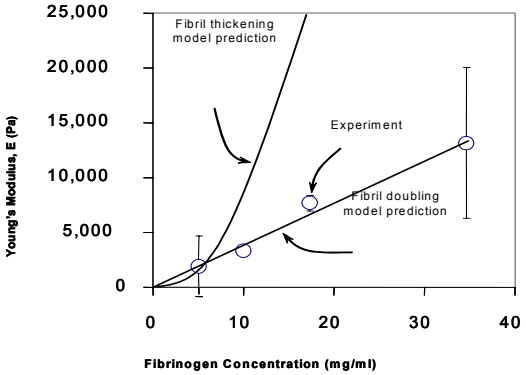
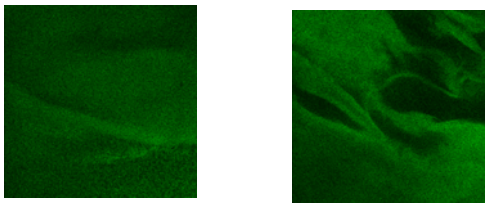


Fig. 1. Effect of fibrinogen concentration on Young's modulus, E for fibrin [1]. These results indicate that the Young's modulus is linearly dependent upon fibrinogen concentration (R^2 = 0.983). The two solid lines are the predictions of Eq. 1 for varying N or R..

Fluorescence Microscopy: Confocal fluorescence micrographs of fluorescently labeled fibrin constructs of different concentrations of fibrinogen showed that the constructs with higher levels of fibrinogen exhibited larger macrobundles of fibrils which were not observed in lower concentrations of fibrin (Fig. 2). These macrobundles of fibrin are thus concluded to play the dominant role in contributing to the linear increase in fibrin stiffness with increasing fibrinogen concentration.



(a) *(b)*

Fig. 2. Confocal fluorescent micrographs (100X original magnification) of fibrin: a) 5mg/mL; b) 34 mg/mL fibrinogen.

Conclusions: Structure-function relationships in fibrin have been explored using coupled experimental and micromechanical approaches. Differences between the stiffness of different formulations have been rationalized in the context of a new micromechanical model and are correlated using confocal fluorescence microscopy.

References: [1] Costales C.A., et. al MRS Conference, San Francisco, CA 2005; [2] Cox S., Cole M., and Tawil B. 2004. Tissue Engineering. Vol 10 (5/6): 942; [3] Shigley, J.E., "Mechanical Engineering Design," McGraw-Hill, 1983.