Rate-Dependent Mechanical Properties of Fibrin Biomaterial Scaffolds

E. Freeman, B. Tawil,[1] and M.C. Shaw
Bioengineering Department, California Lutheran University, 60 W. Olsen Rd. #3750, Thousand Oaks, CA 91360
[1]Baxter Healthcare Corporation, Westlake Village, CA 91360

Statement of Purpose: Many biomaterial scaffold materials, including fibrin, exhibit a strong viscoelastic response. The viscoelastic behavior results from the inherent viscoelasticity of the fibrin fibrils themselves, as well as from viscous fluid flow within the hydrated fibril structure. At present, the role of each mechanism has not yet been completely elucidated. In part, this has been the result of the lack of established experimental methods for measuring the viscoelastic response *in vitro*. Furthermore, there is a lack of predictive models accounting for the underlying mechanisms at the microscopic level of strain-rate dependent deformation. Here, a recently established punch indentation technique was used to explore the basic strain-rate dependence of the mechanical stiffness and stress relaxation behavior of fibrin biomaterial scaffolds of varying biochemical compositions. The data obtained from these experiments are then analyzed using both elastic and viscoelastic models.

Methods: Scaffold synthesis – Fibrin and thrombin solutions were diluted to the appropriate concentrations in TBS and 30mM $CaCl_2$ in TBS, respectively. Two concentrations were used: 17.3 mg/ml of fibrinogen and 1U/ml of Thrombin (Formulation A) and 5 mg/ml of fibrinogen and 1U/ml of Thrombin (Formulation B).
Clot Construct Preparation – 5 ml of diluted fibrinogen and 5 ml of diluted thrombin were added simultaneously to individual wells of a standard 6-well plate. Following 1 hour at 25˚C, 2ml of TBS were added to cover the clots and then they were placed in a 5% CO_2 incubator at 37ºC for 24 hours prior to testing.
Rate-Dependent Indentation Assay - The fibrin constructs were then tested following a recently developed, two-part compressive indentation protocol. An Instron 3365 Universal Testing Machine equipped with a 2.5N load cell was used to perform the experiments. In the first part of the experiment, a circular, flat-ended glass punch (diameter of 3 mm) was used to indent the constructs to a displacement of up to 5 mm while measuring the force required. Three loading rates were used: 1mm/min, 10mm/min and 100mm/min. Two clots for both formulations were subjected to four indentations at each load rate, such that n = 8 for each condition. Data acquisition rates ranged from 4ms to 400ms. In the second part of the experiment, the indenter was held at a fixed displacement for 60 s after completing the first part of the indentation experiment.

Results / Discussion: Initial Load/Displacement Response - Representative data for Formulation A are shown in Fig. 2 for the first part of the experiment.
Stiffness and Young's modulus-Consistent with earlier studies [1], increasing the concentration of fibrinogen led to a linear increase in the Young's modulus. Furthermore, a higher load rate resulted in a significantly higher Young's Modulus (Fig. 2).

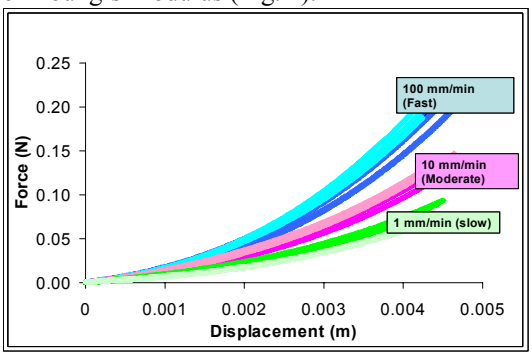

Figure 1. Typical force vs. displacement data from formulation A at three different load rates: 1, 10 and 100 mm/min. A nominal Young's modulus was extracted from the linear portion of the data between 0 and 1.0 mm

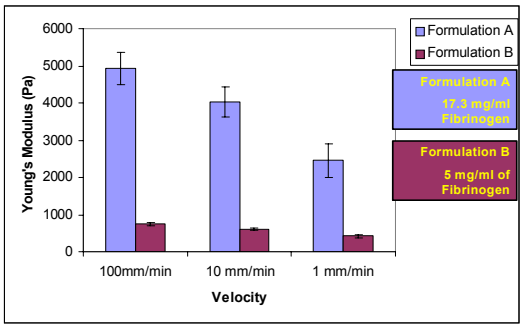

Figure 2. Summary of Young's modulus for both Formulation A and B at the three loading rates.

Stress Relaxation and Viscoelastic Modeling - A lumped element Maxwell (spring-dashpot) model was developed and fitted to the stress relaxation data obtained from the second part of the experiment. Specifically, the viscoelastic time constants were extracted from the relationship:

$$\text{Force} = \boldsymbol{B} + \boldsymbol{C}^{*}\ \text{EXP}^{(-\text{time}/\boldsymbol{A})} + \boldsymbol{E}^{*}\ \text{EXP}^{(-\text{time}/\text{D})}$$

where the viscoelastic time constants are A and D; B, C and E are fitting parameters. Significant differences were observed between both formulations and between the three loading rates in each formulation.

Conclusions: The strain-rate dependence of fibrin was examined both experimentally and through a lumped element model. The nominal stiffness of fibrin was found to scale with the concentration of fibrinogen, and to depend upon the initial loading rate. The subsequent stress relaxation of the fibrin under constant displacement also exhibited a strong dependence on initial loading rate. These results are interpreted through a viscoelastic model with varying time constants.

References: 1) Costales C.A., Mooney R.G., Curtin J., Tawil B., and Shaw M.C. 2005. *MRS Conference Proceedings*, San Francisco, CA.