Rheological and Turbidimetric Characterization of Early-Phase Wound Gels

Jonathan Garvin, Dennis Piercy, Stephanie Jorgensen, and Robby Sanders
Tennessee Tech University, Department of Chemical Engineering

Introduction

- Fibrinogen is a 340 kDa protein found in the blood that is essential to wound healing.
- Normal physiological concentration is 2-4 mg/mL.
- During the early stages of wound healing, fibrinogen is enzymatically cleaved by thrombin to generate a fibrin clot.
- Rheology, the study of material deformation, can be used to characterize fibrin structure and stiffness. As indicated in Figure 1 and the listed equations, the stress-strain response of a material can be used to extract values for mechanical properties.

$$G(t) = \frac{\tau}{\gamma}$$

$$G'(\omega) = G\frac{\omega^2\lambda^2}{1+\omega^2\lambda^2}$$

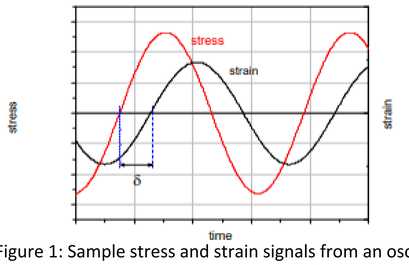

Figure 1: Sample stress and strain signals from an oscillatory rheology experiment [1]

Where **(G)** is the shear modulus, **t** is time, **τ** is the strain, **γ** is the stress, **ω** is the angular frequency, and **λ** is the relaxation time

- Storage modulus (G'), which indicates a gel's ability to elastically respond to deformation, can give insight on the physical characteristics of the wound gel.
  - These properties are important since wounds are constantly subjected to constant strain and deformation in the body.
- Turbidity is a measure of how cloudy or opaque a material is at a chosen wavelength. As a fibrin clot forms and becomes more structured its turbidity increases.

Research Questions

- How does the concentration of fibrinogen affect the physiochemical properties of an early-phase wound during gelation?
- What effect does temperature have on fibrin gelation and final gel structure?

Methods

- Aliquots of three components were prepared to produce final mixed concentrations of fibrinogen at 1, 3, 6 and 12 mg/mL, thrombin at 1 U/mL, and $CaCl_2$ at 5 mM in 1x Tris-buffered saline.
- Fibrinogen, thrombin, and $CaCl_2$ solutions were rapidly mixed with a micropipette and analyzed using a TA Instruments rheometer and a Tecan spectrophotometer.
- Evolution of fibrin storage modulus at 37°C was recorded for 2 hours using a 2° cone at a 0.005 strain rate and 1 rad/s angular frequency (see Figure 2).
- Fibrin turbidity was measured at 450 nm for 1 hour at 23, 30 and 37°C (see Figure 3).

Results

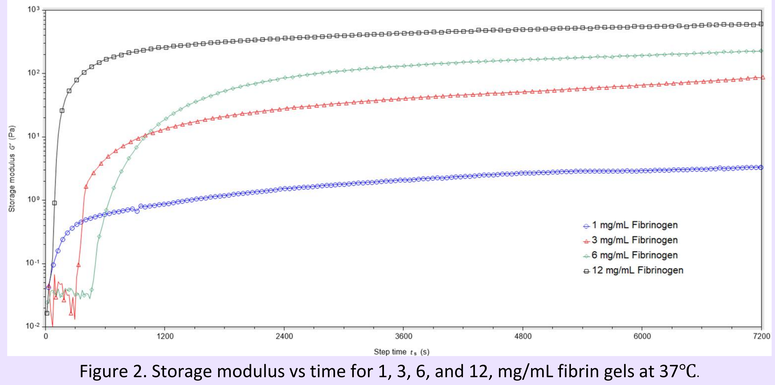

Figure 2. Storage modulus vs time for 1, 3, 6, and 12, mg/mL fibrin gels at 37°C.

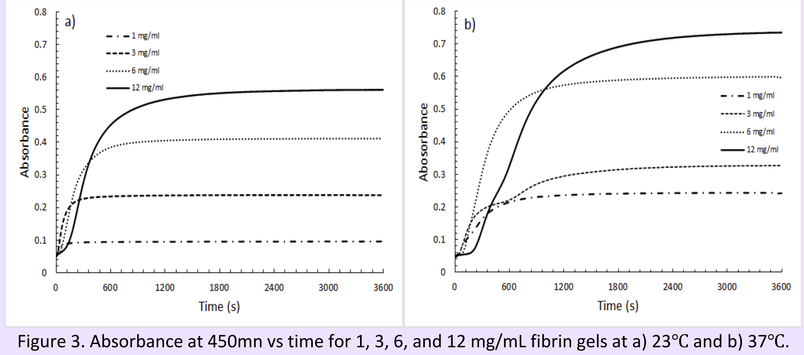

Figure 3. Absorbance at 450mn vs time for 1, 3, 6, and 12 mg/mL fibrin gels at a) 23°C and b) 37°C.

Discussion

- Rheology data (n=2) indicates that higher fibrinogen concentrations yield gels with higher storage modulus (representative of stiffer structure) with structured gels forming sooner than with lower concentration gels.
- Turbidity results (n=3) indicate formation of more dense fibrin networks with increasing concentration.
- Increasing temperature from 23°C to 37°C resulted in more turbid samples (see Figure 3).
- Increase in network density may be attributed to an increase in fiber count, thickness, and length[2]

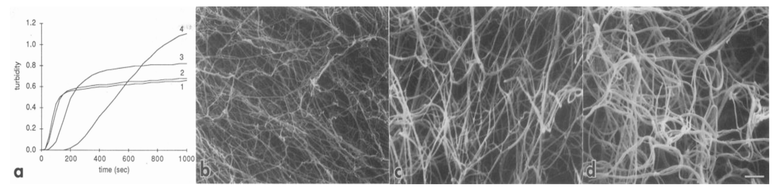

Figure 4. (a)Turbidity measurements for varying concentrations of thrombin: (1) 1 U/ml, (2) 0.1 U/mL, (3) 0.01 U/mL, (4) 0.001 U/mL. Scanning electron micrographs for fibrin gels using thrombin concentrations of: (b) 1 U/mL, (c) 0.01 U/mL, (d) 0.001 U/mL. [2]

- Individuals with fibrinogen-related disorders are more susceptible to bleeding.
- Tunability of gel structure and mechanical properties has lead to interest in fibrin scaffold implants.
- Fibrin implants must closely mimic structure and function of target tissue to promote cell viability and subsequent healing [3,4].

Future Work

- Utilize confocal and scanning electron microscopy imaging techniques to determine the impact of fibrinogen concentration on the microstructure of fibrin gels.
- Given the relative simplicity of turbidity measurements, determine if rheological and turbidimetric results can be used interchangeably to describe microstructural changes.
- Further explore temperature effects on fibrin gel rheology.

References

[1] Franck A. *TA instruments* "Viscoelasticity and Dynamic Mechanical Testing
[2] Weisel, J.W. et al. *Biophys. J.* 63:111-128 (1992).
[3] Thomson, K.S. et al. *Tissue Eng. Part A* **19**, 967-977 (2013).
[4] Bensaid, W. et al. *Biomaterials* **24**, 2497-2502 (2003).