Fibrin Gelation During Blood Clotting

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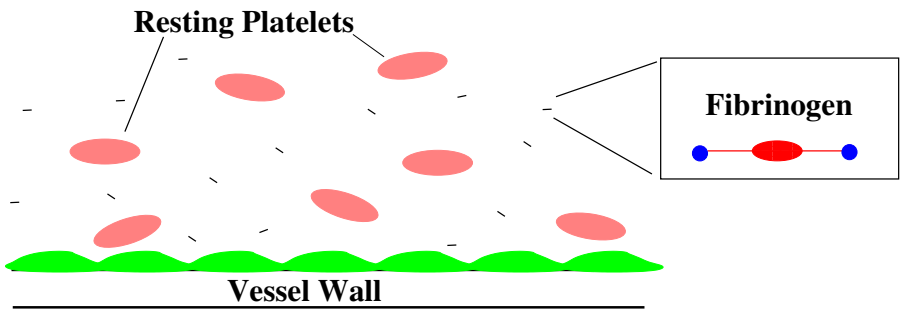
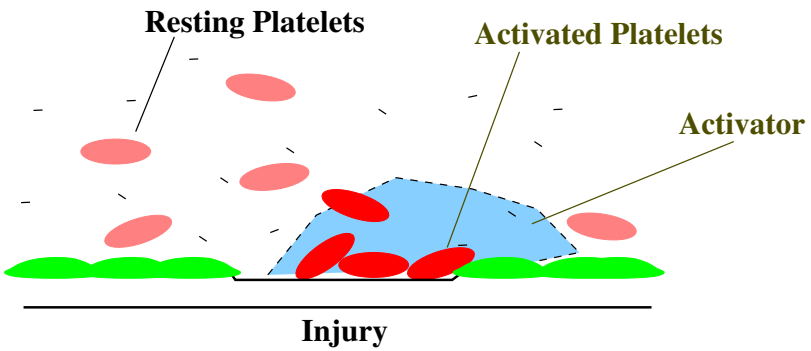
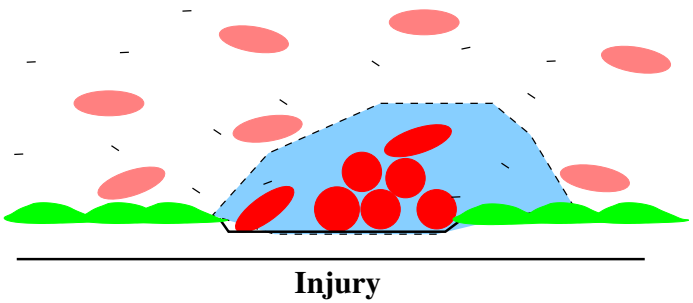
Acknowledgments

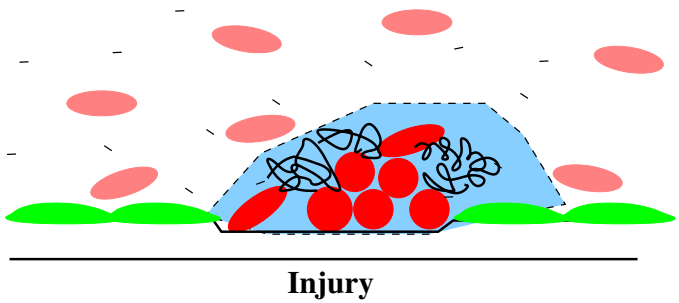
- Joint work with Jim Keener and Cheryl Zapata-Allegro, drawing on earlier work with Bob Guy
- Funding from NSF and NHLBI.

Blood Clotting Overview



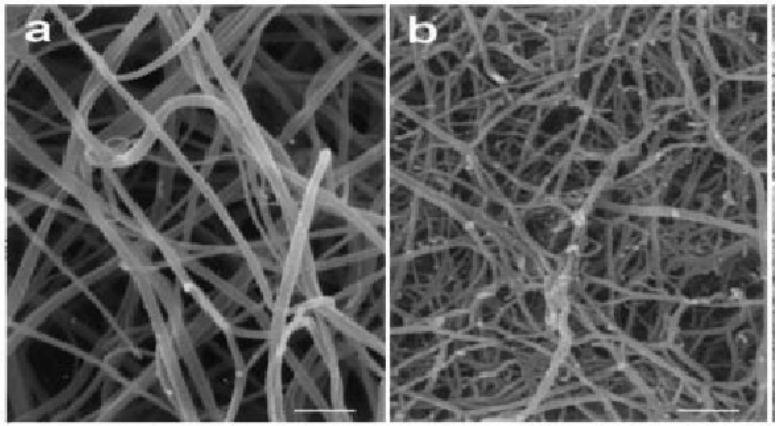






- Platelet aggregation produces platelet plug
- Coagulation enzyme reactions produce enzyme thrombin
- Thrombin cleaves fibrinogen to produce fibrin monomers
- Fibrin polymerizes and gels

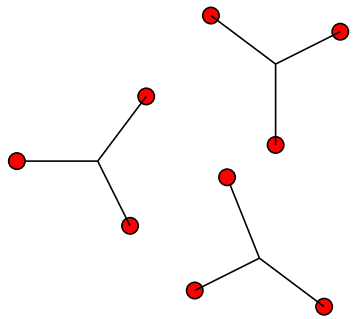
Fibrin Polymerization



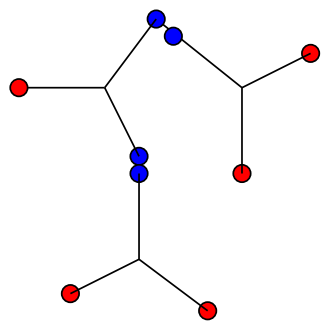
- Low thrombin concentrations induce "coarse" fibrin clots.
- High thrombin concentrations induce "fine" fibrin clots.
- Coarse/fine clots differ mechanically and degrade differently.
- How does thrombin concentration affect clot structure?
- Goal: develop model of fibrin gelation that gives information about the clot structure to
  1. Explain thrombin-concentration-dependent gel structure.
  2. Study fibrin gelation during clotting under flow.

Kinetic Gelation - The Ziff Model

Assume



- Monomers have f 'reactive' sites



- Reactive sites combine, forming a polymer chain
- There are no closed loops
- C_k – species consisting of k joined monomers – "k-mers"
- Chemical reactions: $C_i + C_j \xrightarrow{k_{i,j}} C_{i+j}$

cf. Ziff and Stell, J. Chem. Phys, 1980.

The Chemical Reaction Equations

Let c_k be the concentration of k-mers

$$\frac{dc_k}{dt} = \frac{1}{2}\sum_{i+j=k} r_i r_j c_i c_j - r_k c_k R,$$

$$\frac{dR}{dt} = -R^2,$$

where $r_j = (f-2)j + 2$ is the number of reactive sites on each j-mer, and R is the total concentration of reactive sites.

Remark: Before a gel forms, $R(t) = R_s(t) \equiv \sum_{k=1}^{\infty} r_k c_k$. Then, the ODEs for c_k imply

$$\frac{dR_s}{dt} = -R_s^2.$$

One form of Ziff's model postulates that R satisfies such an ODE for all time even after gelation. We will revisit this later.

Approach 1: Moments

Define moments

$$M_1 = \sum_k k c_k, \qquad M_2 = \sum_k k^2 c_k, \qquad A = \frac{M_2}{M_1} = \sum_k k \left(\frac{k c_k}{M_1} \right),$$

$$Y = (f-2)M_2 + 2M_1.$$

Find that

$$\frac{dM_1}{dt} = 0, \qquad \frac{dY}{dt} = (f-2)Y^2.$$

Average oligomer size $A \to \infty$ iff $Y \to \infty$. We regard the event that the average cluster size becomes infinite as gelation.

For $f > 2$, $Y \to \infty$ at finite time t_{gel}. For IVP starting with monomers only,

$$t_{gel} = \frac{1}{f(f-2)c_1(0)}.$$

Question: What happens after gel time t_{gel}?

Approach 2: Moment Generating Function

Set $g(z,t) = \sum_k z^{r_k} c_k(t)$ and calculate that

$$g_t = \frac{1}{2} g_z^2 - z g_z R.$$

Introduce the change of variables $W = zR - g_z$.

Using PDE for g and

$$\frac{dR}{dt} = -R^2,$$

find that

$$W_t + W W_z = 0.$$

Quantities of Interest

$$W = zR - g_z$$

- Concentration of reactive sites in solution,

$$R_s = \sum_k r_k c_k = g_z|_{z=1^-}$$

- Concentration of reactive sites *not* in solution, i.e., in gel

$$R_g = R - R_s = W|_{z=1^-}. \qquad (R_g = W|_{z=1^-} \text{ and } R_s = R - R_g).$$

- A gel forms at the time t_{gel} for which $\lim_{t \to t_{gel}} W_z|_{z=1^-} = \infty$.
- Sol mass density

$$\theta_s \equiv \sum_k k c_k(t) = \frac{1}{f-2}\left(2\int_0^1 W(z)dz - W\Big|_{z=1^-}\right)$$

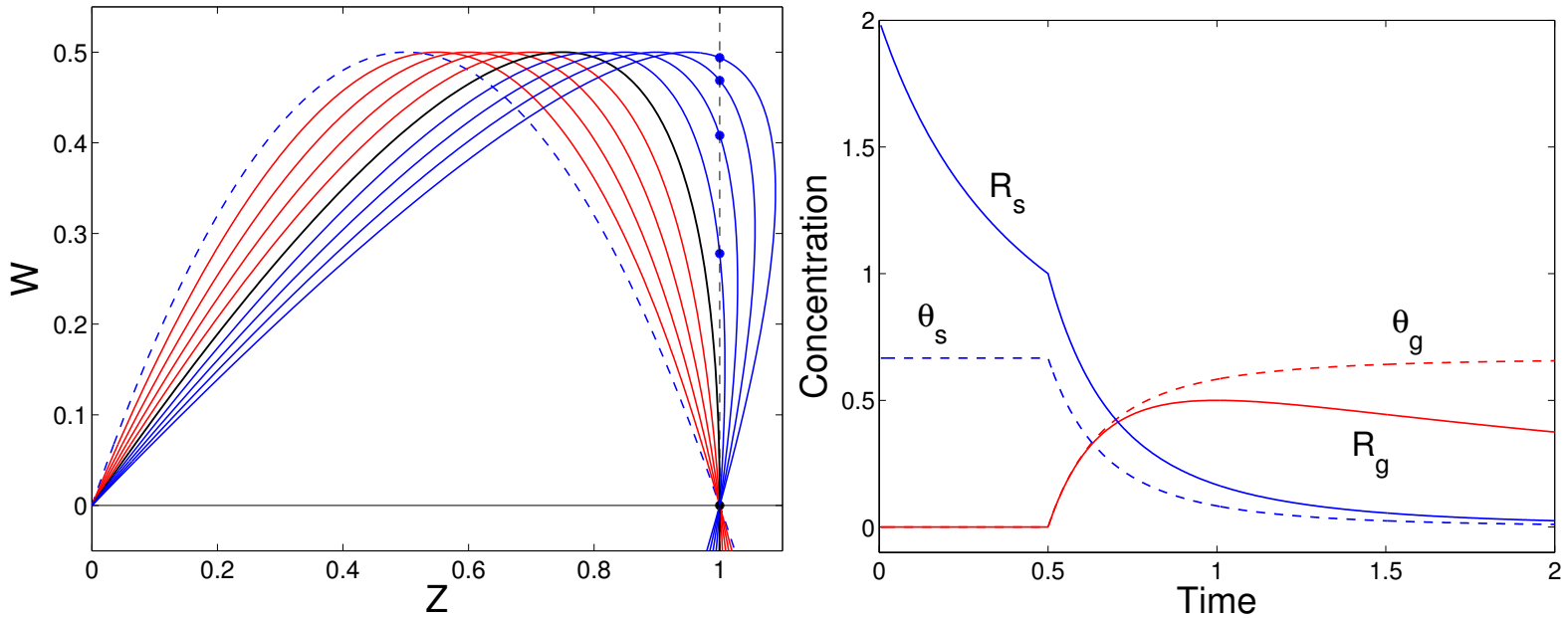
- Only values $W(z,t)$ for $0 \le z < 1$ matter for our purposes.

Determine W

Initial data: Monomer only $\Rightarrow c_j(t=0) = m_0\delta_{j1}$

$\Rightarrow W(z,t=0) = m_0\ f\ (z - z^{f-1})$.

Solve for $W(z,t)$ using the method of characteristics:



$W(1,t) = 0$ and $W_z(1,t) < \infty$ until time $t = t_{gel}$.

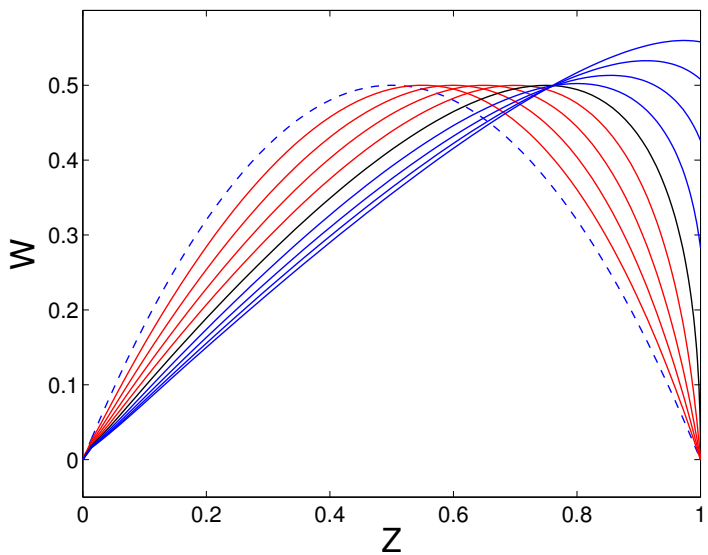
Eliminate Gel-Gel Reactions

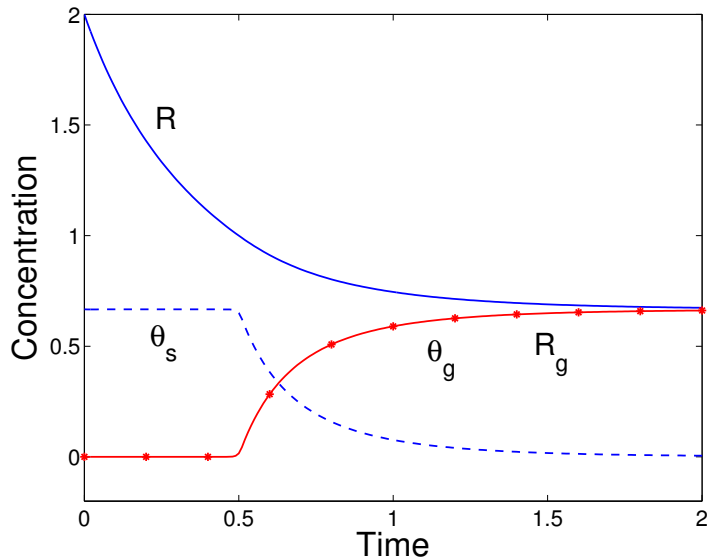
Eliminate gel-gel reactions so gel forms no cycles:

$$\frac{dc_k}{dt} = \frac{1}{2}\sum_{i+j=k} r_i r_j c_i c_j - r_k c_k R,$$

$$R_t = -(R^2 - R_g^2), \qquad R_g = W|_{z=1},$$

$$W_t + WW_z = zR_g^2 \qquad \text{(Non-local)}$$





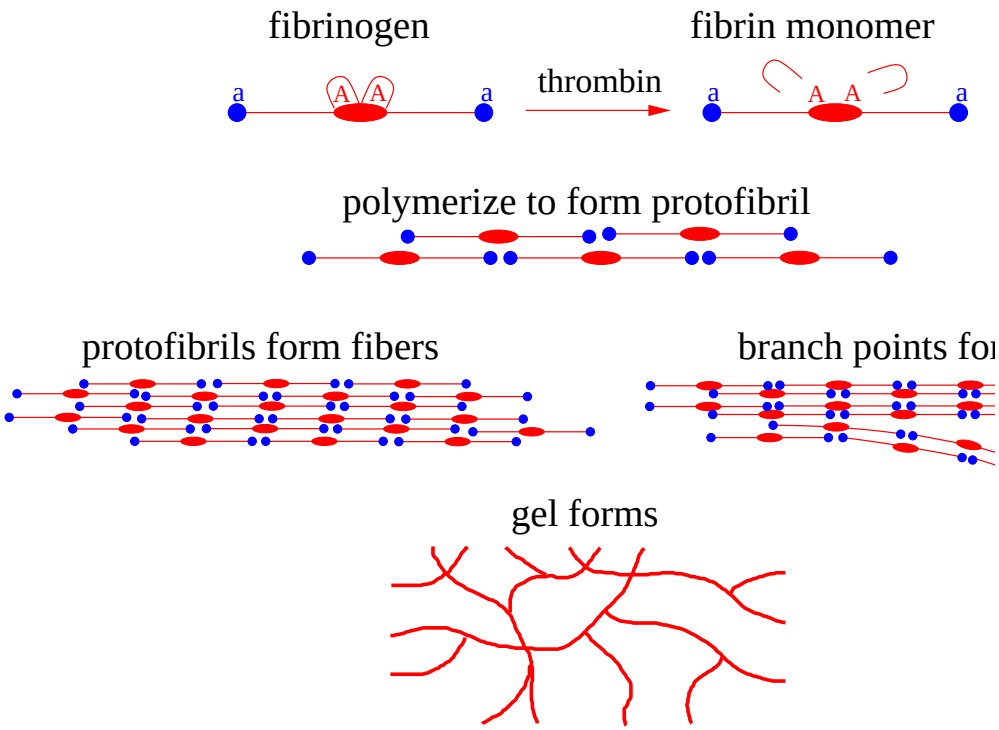
Diffusion of Polymer, Time-dependent Source

Allow polymer (but not gel) to diffuse with diffusion coefficient D and add time-dependent source of monomer $S_1(t)$.

$$\begin{aligned}
\frac{dc_k}{dt} &= \frac{1}{2}\sum_{i+j=k} r_i r_j c_i c_j - r_k c_k R + \delta_{k,1} S_1(t) + D\Delta c_k, \\
W_t + WW_z &= zR_g^2 + f(z - z^{f-1})S_1(t) + D\Delta(W - zR_g), \\
R_t &= -(R^2 - R_g^2) + f\ S_1(t) + D\Delta(R - R_g), \\
R_g &= W|_{z=1}
\end{aligned}$$

With diffusion in x, MoC in z won't work.
Numerical scheme uses centered differencing in x and 2nd-order upwind differencing in z exploiting smoothness of W for $z < 1$.

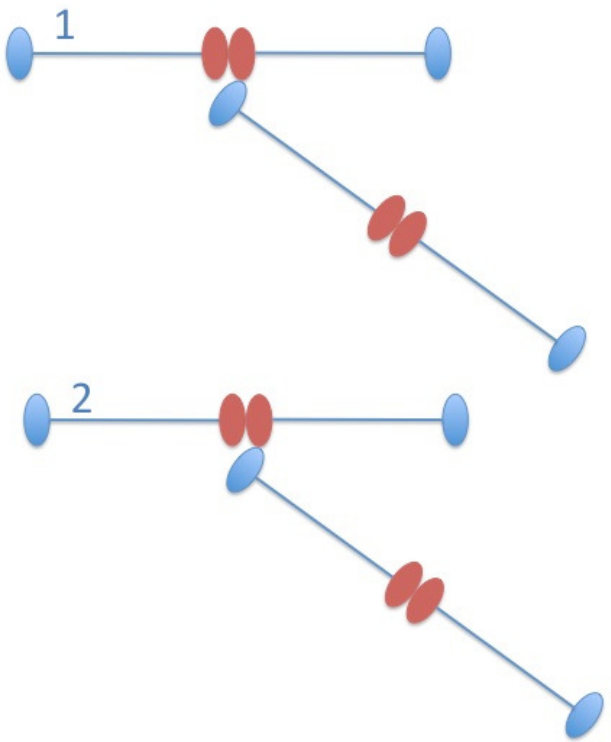
Fibrin Polymerization Process



What mechanism of branch formation would make the final branch point density sensitive to the thrombin concentration?

Monomer Binding Reactions

- Fibrin monomers bind center to end and end to center in separate steps.



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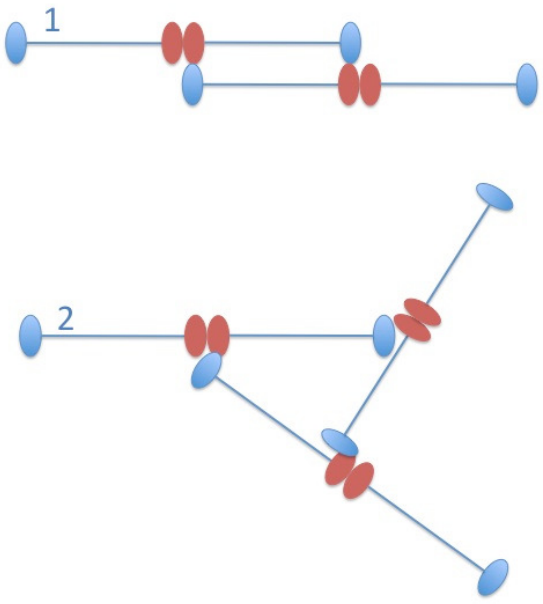


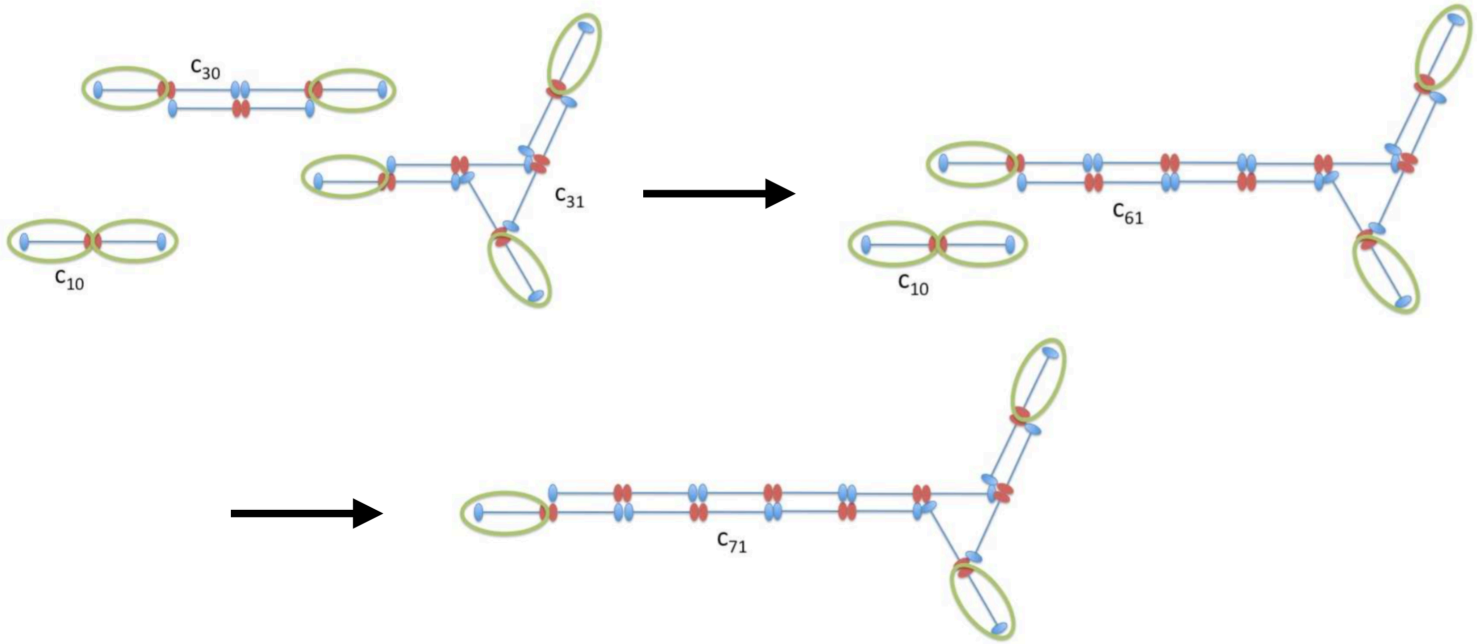
- Model first path as bimolecular reaction
- Model second path as trimolecular reaction

Fibrin Reactions – Elongation

$C_{m,b}$ denotes oligomer with b branches and $m + 2b$ monomers.

Two oligomers bind to create larger oligomer:

$$C_{m_1,b_1} + C_{m_2,b_2} \xrightarrow{k_l} C_{m_1+m_2,b_1+b_2}$$

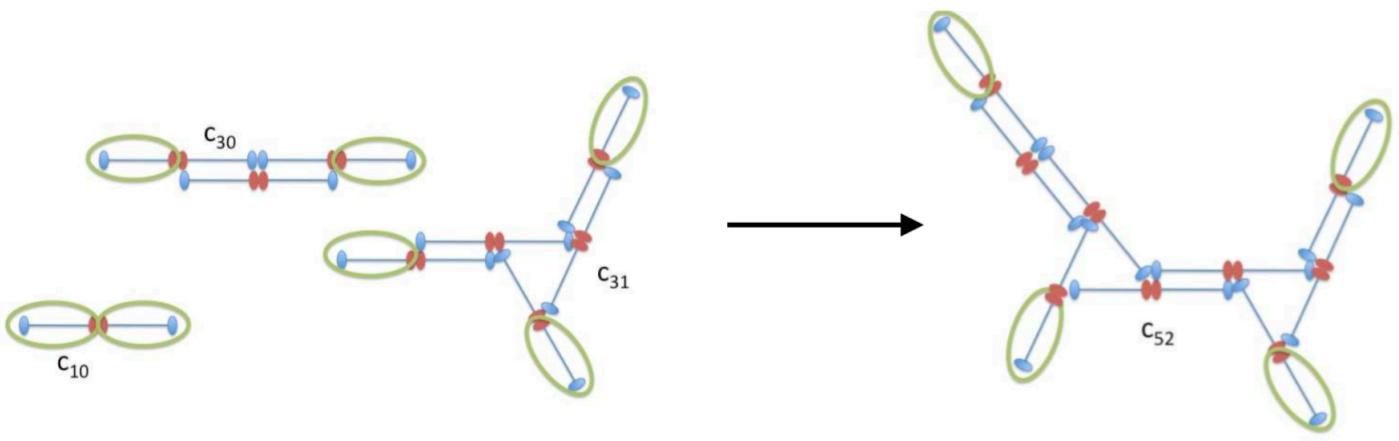


Lose two reaction sites per elongation reaction.

Fibrin Reactions - Branching

Three oligomers bind to create larger oligomer with new branch:

$$C_{m_1,b_1} + C_{m_2,b_2} + C_{m_3,b_3} \xrightarrow{k_b} C_{m_1+m_2+m_3-2,b_1+b_2+b_3+1}$$



Lose three reaction sites per branching reaction.

Fibrin Reaction Kinetics

Two types of reactions lead to elongation or branch formation

$$C_{m_1,b_1} + C_{m_2,b_2} \xrightarrow{k_l} C_{m_1+m_2,b_1+b_2}$$

$$C_{m_1,b_1} + C_{m_2,b_2} + C_{m_3,b_3} \xrightarrow{k_b} C_{m_1+m_2+m_3-2,b_1+b_2+b_3+1}$$

$$\frac{dc_{mb}}{dt} = S_{10}\delta_{m1}\delta_{b0} + \frac{k_l}{2} \sum_{\sum b_j=b} \sum_{\sum m_j=m} r_{b_1} r_{b_2} c_{m_1 b_1} c_{m_2 b_2} - k_l r_b c_{mb} R$$
$$+ \frac{k_b}{6} \sum_{\sum b_j=b} \sum_{\sum m_j=m} r_{b_1} r_{b_2} r_{b_3} c_{m_1 b_1} c_{m_2 b_2} c_{m_3 b_3} - \frac{k_b}{2} r_b c_{mb} R^2,$$

$$R_t = 2S_{10} - k_l R^2 - \frac{k_b}{2} R^3,$$

Number of reactive sites on C_{mb} oligomer is $r_b = b + 2$.

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$$C_{m_1,b_1} + C_{m_2,b_2} + C_{m_3,b_3} \xrightarrow{k_b} C_{m_1+m_2+m_3-2,b_1+b_2+b_3+1}$$

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$$+ \frac{k_b}{6} \sum_{\sum b_j = b} \sum_{\sum m_j = m} r_{b_1} r_{b_2} r_{b_3} c_{m_1 b_1} c_{m_2 b_2} c_{m_3 b_3} - \frac{k_b}{2} r_b c_{mb} R^2,$$

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Number of reactive sites on C_{mb} polymer is $r_b = b + 2$.

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$$+ \frac{k_b}{6} \sum_{\sum b_j=b} \sum_{\sum m_j=m} r_{b_1} r_{b_2} r_{b_3} c_{m_1 b_1} c_{m_2 b_2} c_{m_3 b_3} - \frac{k_b}{2} r_b c_{mb} R^2,$$

$$R_t = 2S_{10} - k_l R^2 - \frac{k_b}{2} R^3,$$

Number of reactive sites on C_{mb} polymer is $r_b = b + 2$.

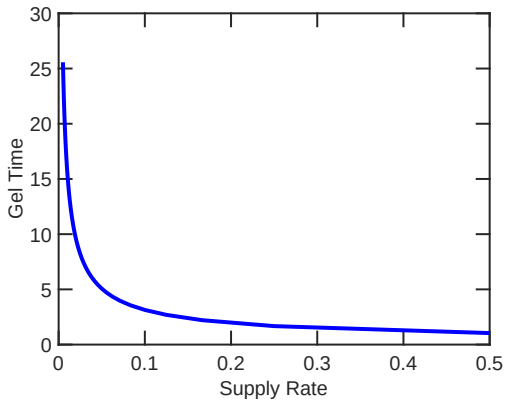
Branching Structure vs Supply Rate

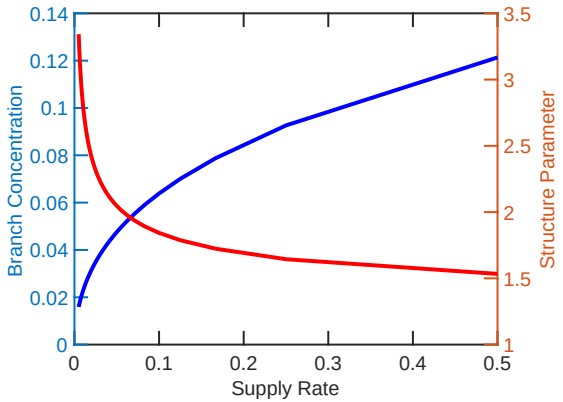
$$M = \sum_{m,b}(m+2b)c_{mb}, \qquad A = \frac{1}{M}\sum_{m,b}(m+2b)^2 c_{mb}, \qquad A \to \infty \text{ as } t \to t_{gel}.$$

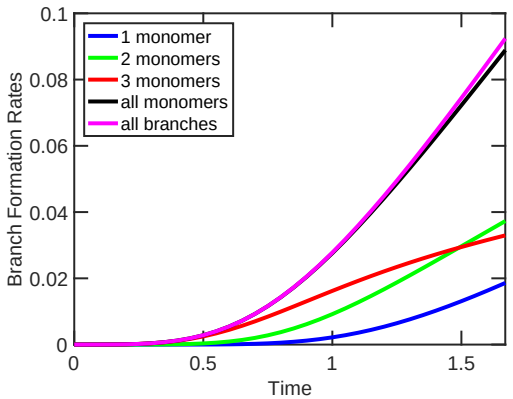
Derive and solve ODEs up to t_{gel} for quantities of interest.

$$R = \sum_{m,b}(b+2)c_{mb}, \qquad B = \sum_{m,b} bc_{mb}, \qquad O = M - c_{10}.$$

Experiments with different constant monomer supply rates S_{10}.







- Gel time decreases with S_{10}.
- B increases and structure parameter O/B decreases with S_{10}.
- Almost all branch formation involves at least one monomer.

PDE Form of Fibrin Equations

Generating function $g(x,t,y,z) = \sum_{m,b} y^m \; z^{b+2} \; c_{mb}(x,t)$.

$$W = zR - g_z|_{y=1}, \qquad R_g = W|_{z=1}, \qquad R_s = R - R_g, \qquad V = -g_{zy}|_{y=1}.$$

With diffusion, source of monomer, and no gel-gel reactions,

$$\begin{aligned}
W_t &= -\left\{ \frac{k_l}{2} W^2 + \frac{k_b}{6}(zR - W)^3 - \frac{k_b}{2}(R^2 - R_g^2)(z^2 R - zW) \right\}_z \\
&\quad + k_l z R_g^2 - \frac{k_b}{2} z \Big(R^3 - (3R_s R_g^2 + R_g^3) \Big) + D\big(W - zR_g\big)_{xx} \\
R_t &= -k_l(R^2 - R_g^2) - \frac{k_b}{2} \left\{ R^3 - (3R_s R_g^2 + R_g^3) \right\} + 2S_{10} + D(R_s)_{xx} \\
V_t &= -\left\{ \left(k_l W - \frac{k_b}{2}(zR - W)^2 + \frac{k_b}{2} z (R^2 - R_g^2) \right) V \right\}_z \\
&\quad + k_b\big((zR - W)^2 (R - W_z)\big) - 2S_{10} z + DV_{xx}.
\end{aligned}$$

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\end{aligned}$$

Auxiliary Quantities

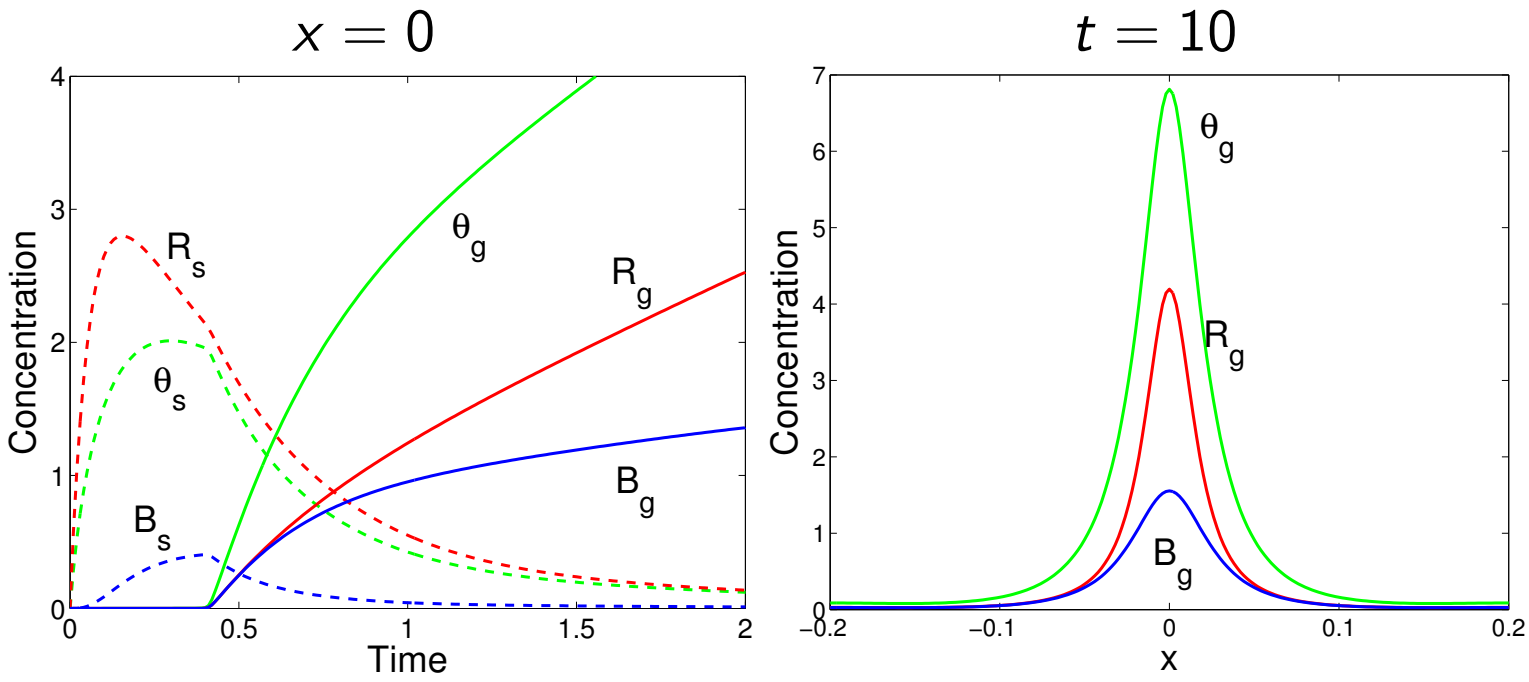
- Polymer branch concentration $B_s = \sum_{mb} bc_{mb}$
- Polymer mass density $\theta_s = \sum_{mb}(b + 2m)c_{mb}$
- Total branch concentration B, total mass density θ
- Gel branch concentration $B_g = B - B_s$

$$\begin{aligned} \theta_t &= D(\theta_s)_{xx} + S_{10} \\ B_t &= D(B_s)_{xx} + \frac{k_b}{6}(R^3 - (3R_sR_g^2 + R_g^3)). \end{aligned}$$

$$\begin{aligned} \theta_s(x,t) &= 4\int_0^1 W(x,t,z'),dz' - \int_0^1 V(x,t,z')dz' - 2W(x,t,1) \\ B_s(x,t) &= 2\int_0^1 W(x,t,z')dz' - W(x,t,1) \end{aligned}$$

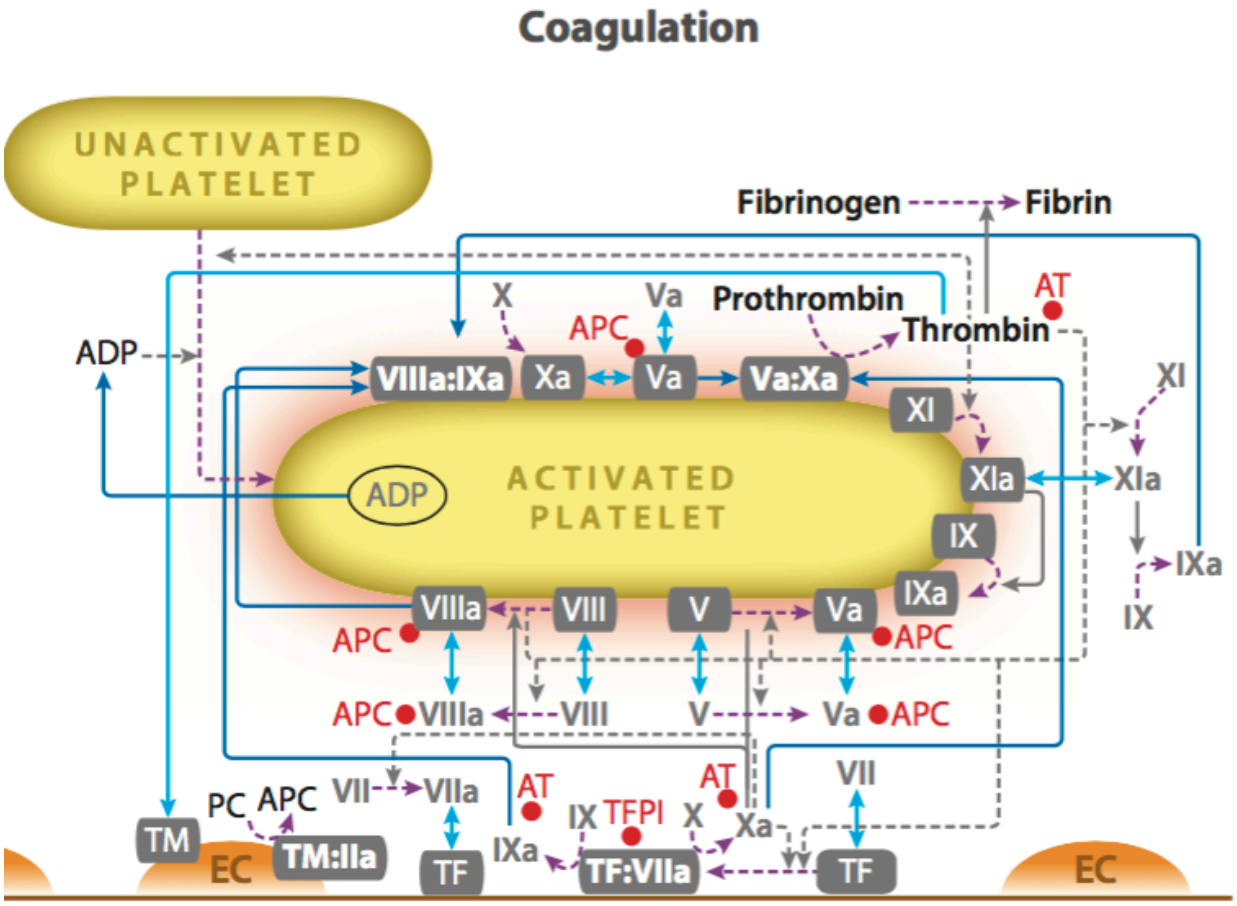
Fibrin Model Results

- Monomer supplied near $x = 0$ at exponentially decreasing rate

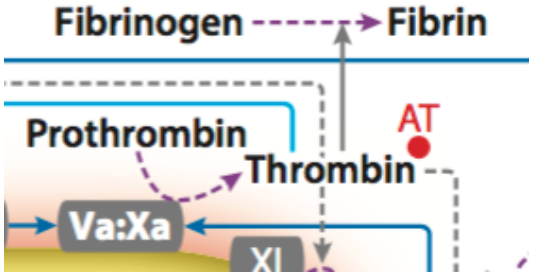


Coagulation Reaction System

Fibrin monomers are produced by coagulation system.



Vascular Injury Model



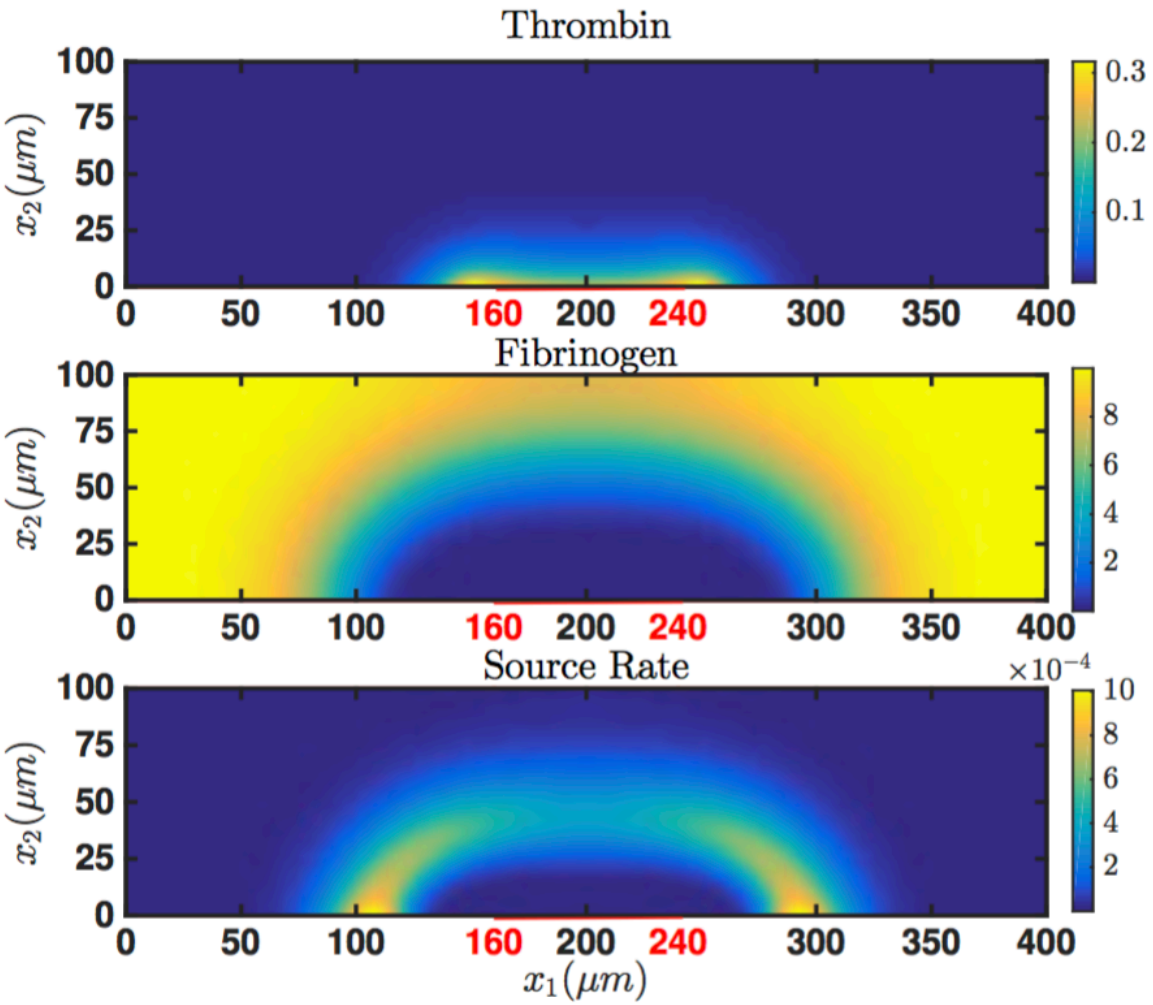
Model Assumptions

- Prothrombin P and fibrinogen F enter upstream and move by advection and diffusion.
- P is converted to thrombin E on injury at rate $\frac{\alpha\ P}{K_m+P}$.
- E moves by advection and diffusion, is degraded, and converts F into fibrin monomers $C_{1,0}$ at rate $S_{10} = \frac{k^{cat}EF}{K_m+F}$.
- Fibrin polymerizes as before and fibrin oligomers move by advection and diffusion.
- Gel does not move.

Diffusion But No Flow

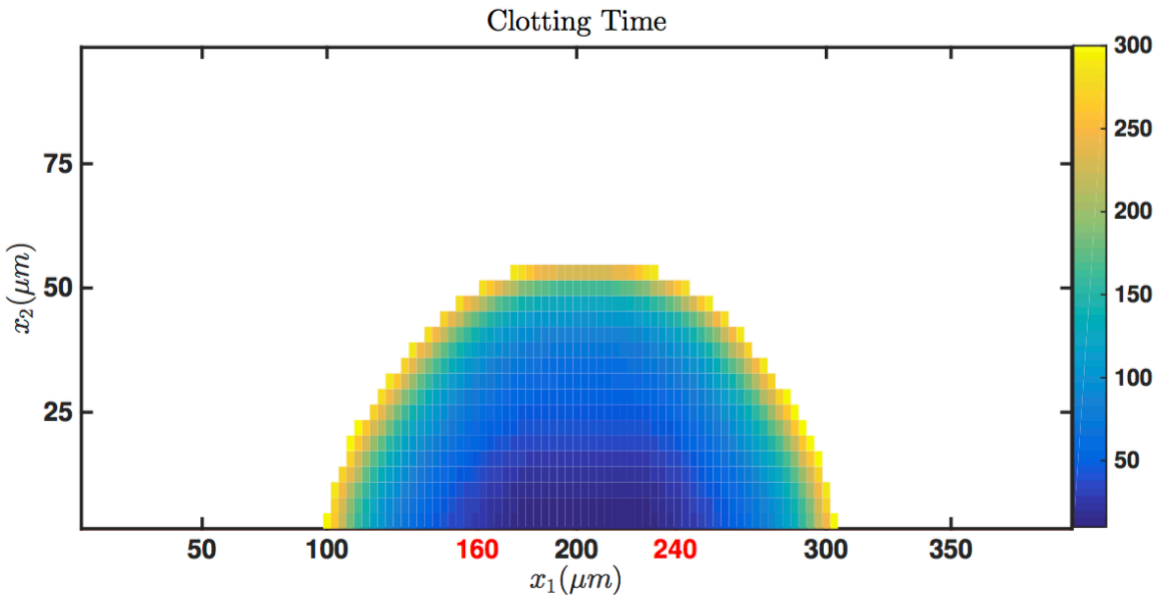
(Top) Thrombin (Middle) Fibrinogen

(Bottom) Monomer Source Rate $S_{10} = \frac{k^{cat} EF}{K_m + F}$



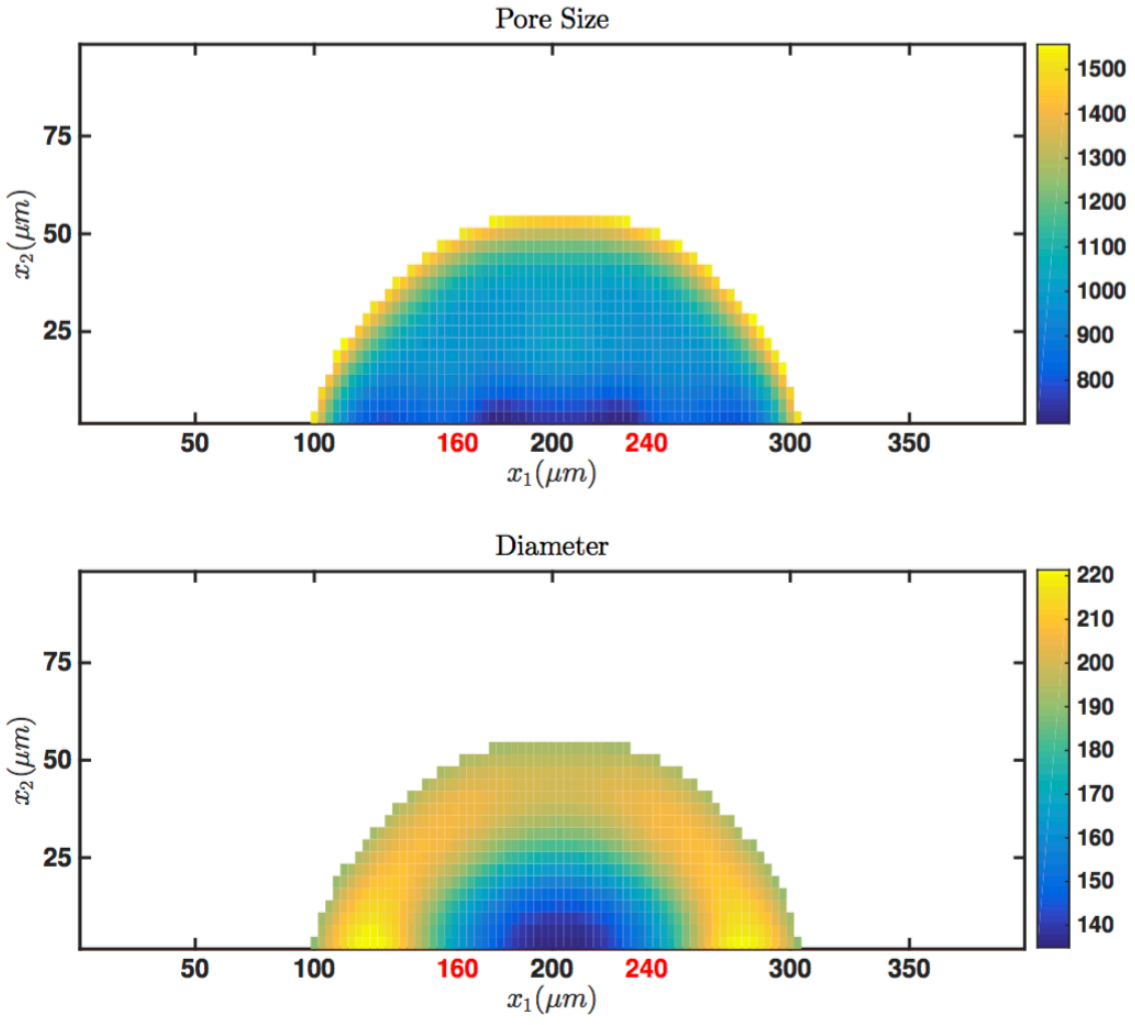
Diffusion But No Flow

Gel Time



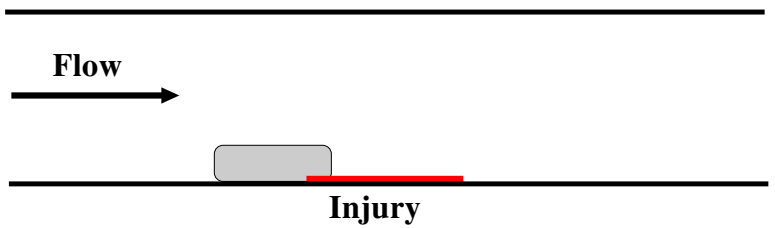
Diffusion But No Flow

(Top) Pore Size (Bottom) Fiber Diameter



Fibrin Gelation with Flow

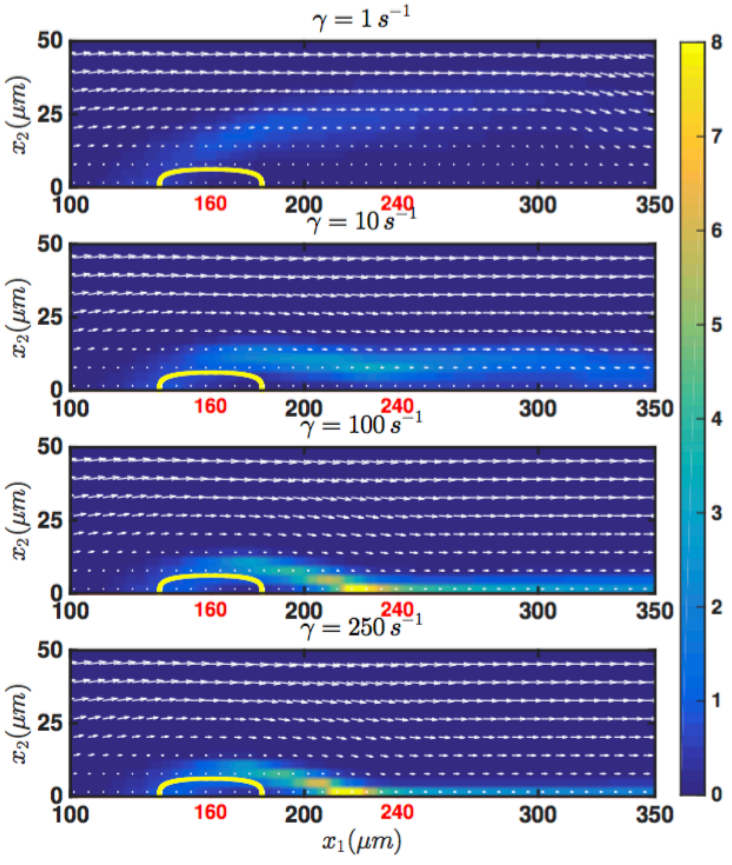
With flow, gel forms far downstream of injury.
Need flow obstacle for fibrin formation over injury.



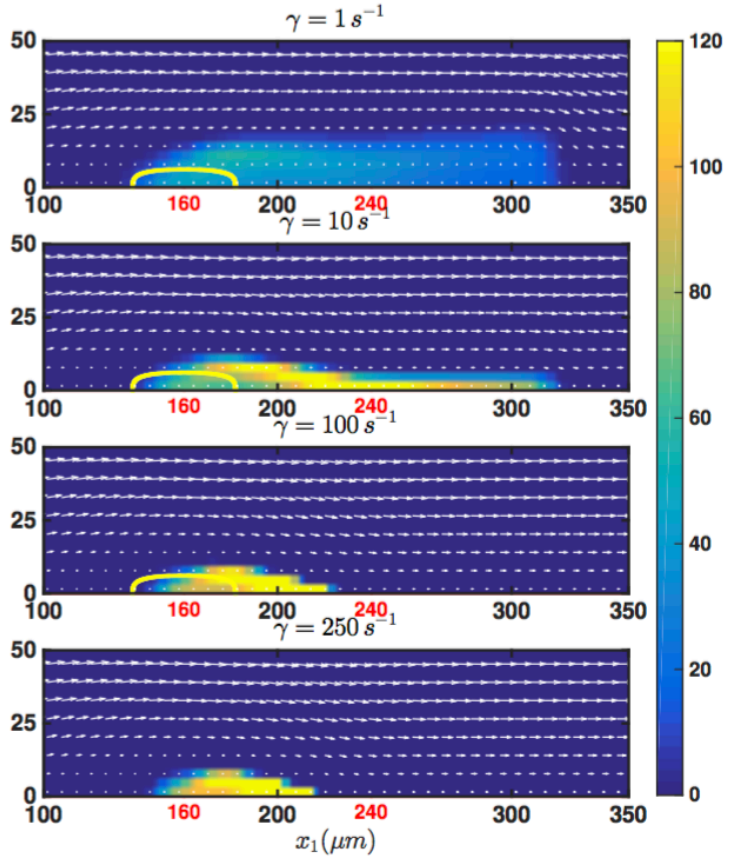
Insert porous 'bead' representing platelet aggregate.

Fibrin Gelation with Flow

Monomer Source Rate



Gel Mass Density



Final Words

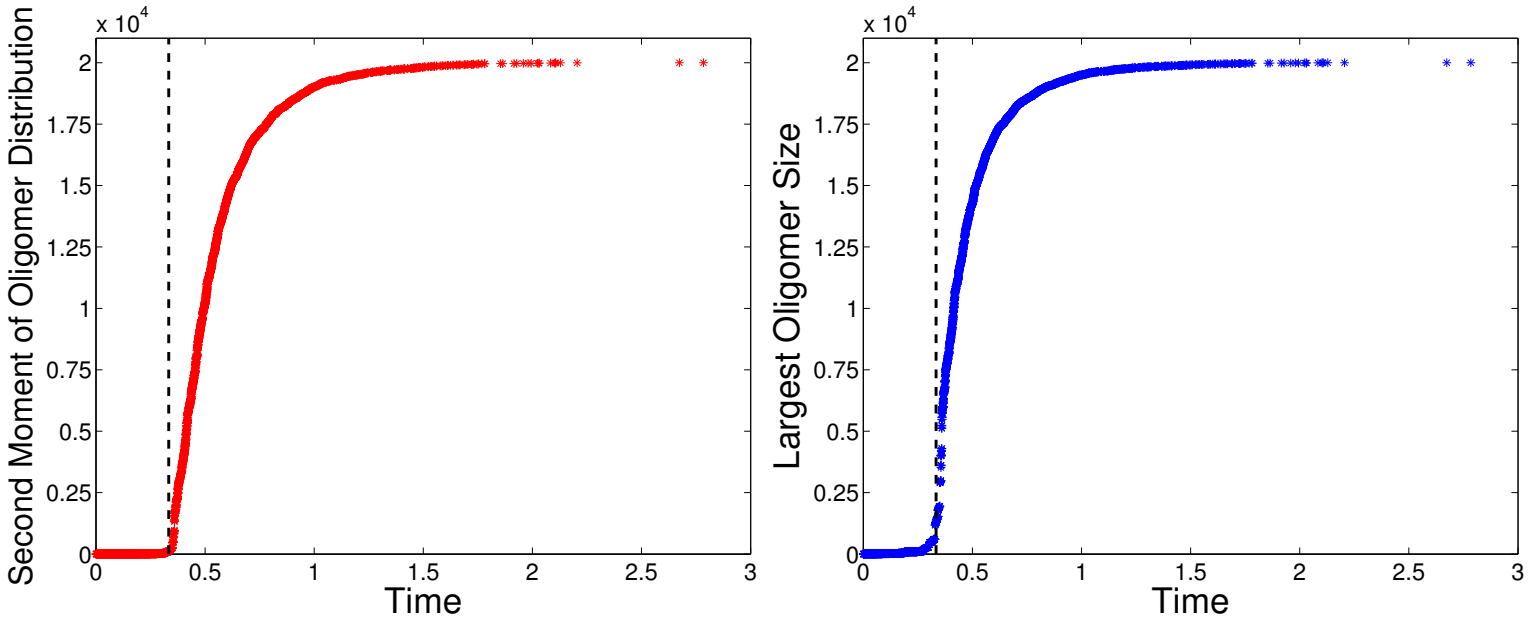
- Have developed model of fibrin polymerization in which the resulting gel structure is a function of the thrombin concentration.
- Have coupled this model to reduced coagulation model to begin to study fibrin gelation under flow during blood clotting.
- Model gives reasonable times for gel formation, pore size, fiber diameters, and predicts need for shelter to allow fibrin gelation at injury.
- Model can be combined with our more comprehensive models of coagulation and platelet deposition to study the clotting response to vascular injury.

References

Ziff and Stell, J. Chem. Phys, 1980.
Guy, Fogelson and Keener, MMB, 2007.
Fogelson and Keener, PRE, 2010.
Fogelson and Keener, SIAP, 2015.
Zapata-Allegro, U of U PhD thesis, 2016.

How valid are mean-field gel models?

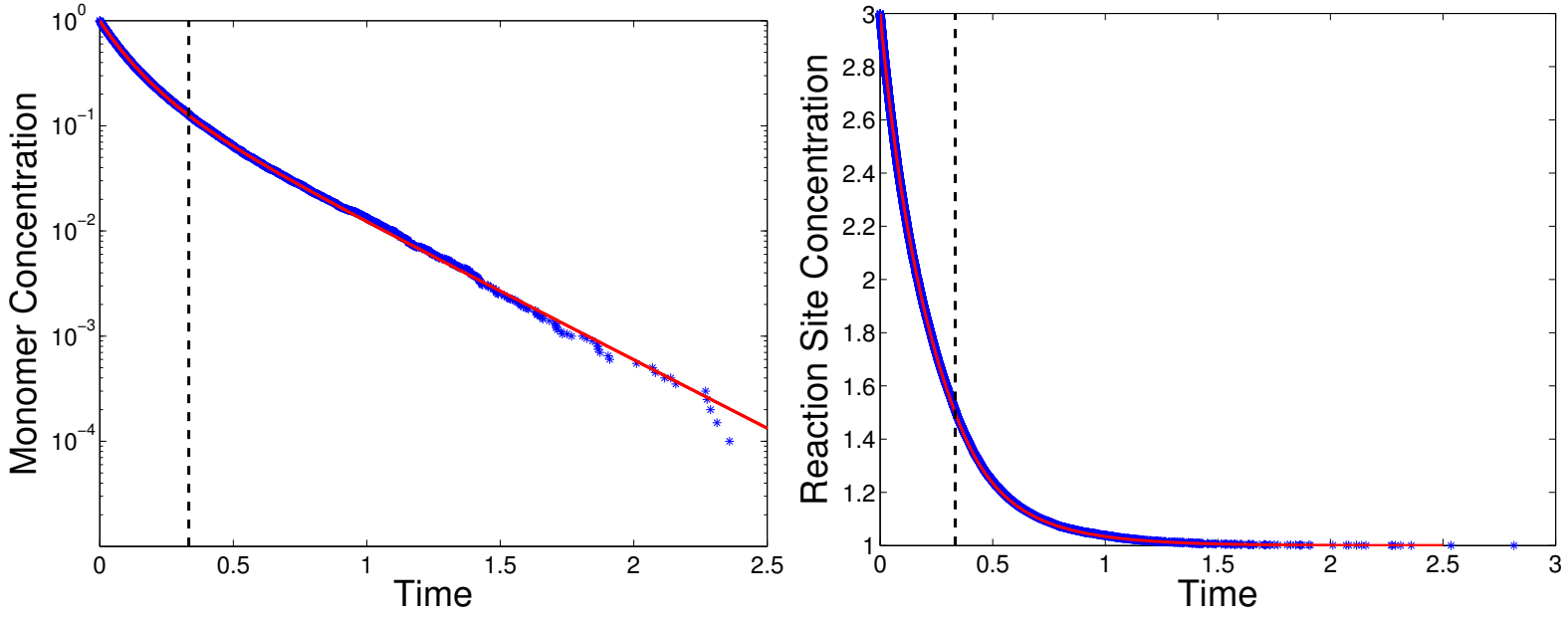
- The chemical reactions $C_i + C_j \xrightarrow{k_{i,j}} C_{i+j}$
- Use Gillespie algorithm to simulate dynamics with large number of monomers.



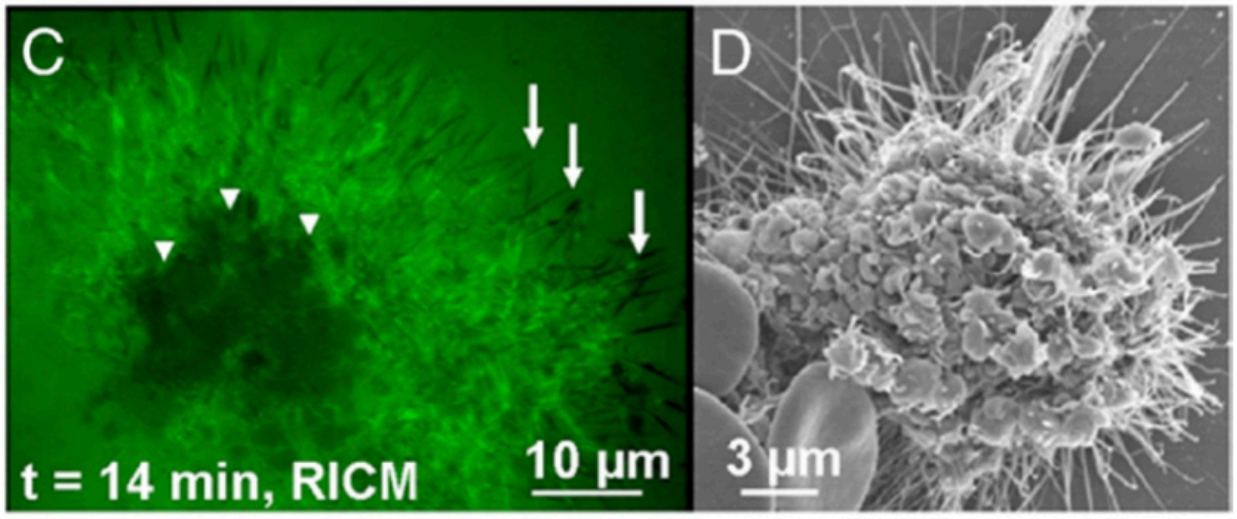
- Obvious transitions at gel time predicted by continuum model.

Which post-gel model is most reasonable?

- Model with no gel-gel reactions matches stochastic model results
- It like stochastic model does not allow cycles even in large oligomers (gel).



Fibrin at Base of Thrombus



Fibrin in Neeve's Experiments

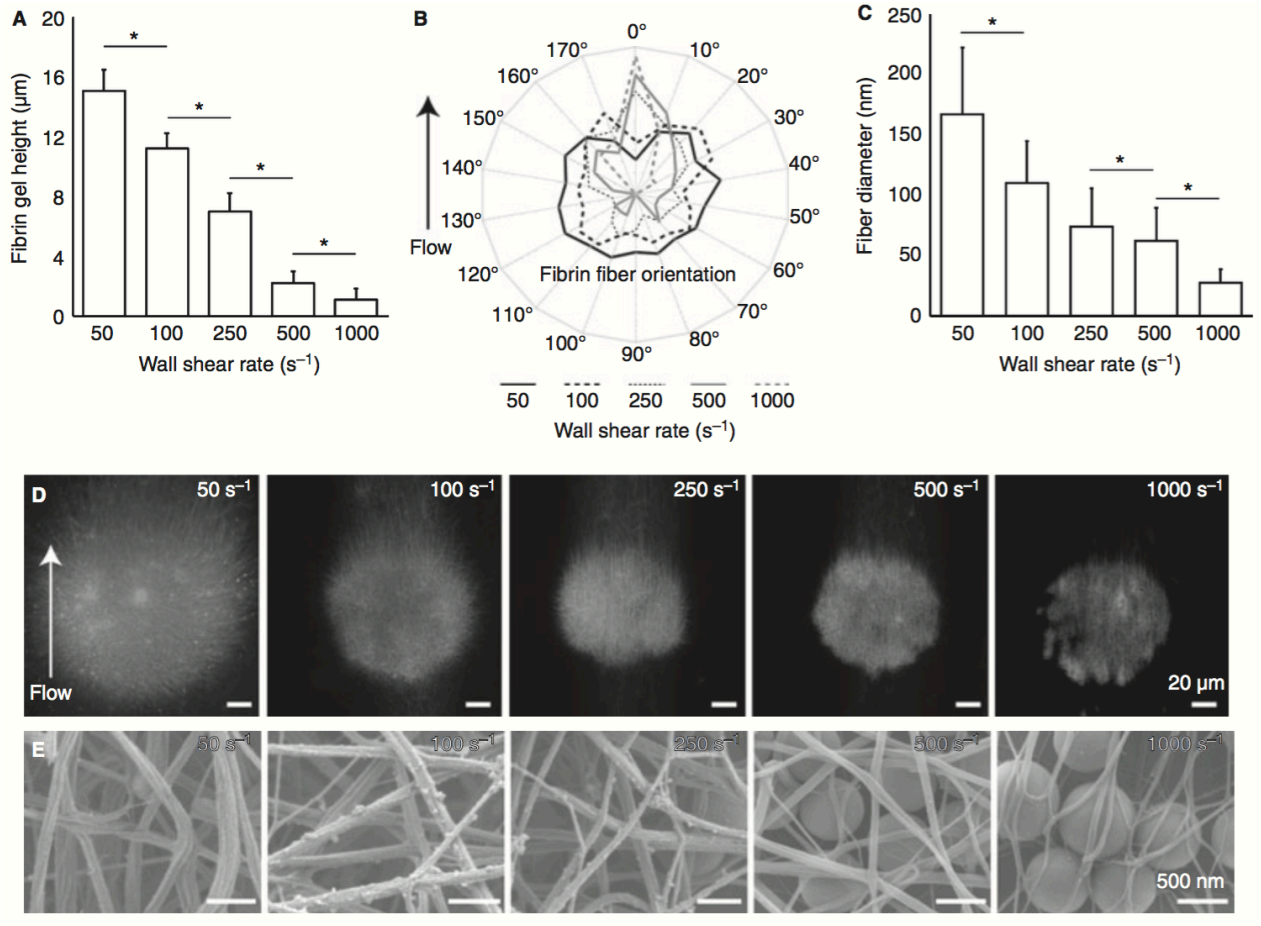


Fig. 5. The effect of shear rate on fibrin morphology. Normal pooled plasma (NPP) was perfused over a bead surface concentration of 8 molecules-TF μm^{-2} at 50 s^{-1} for 10 min. (A) Fibrin gel height was measured by confocal microscopy. (B) Orientation of fibrin fibers with the direction of flow. The peaks in the fiber orientation distribution for 500 and 1000 s^{-1} at 0° indicate alignment with flow. (C) Fibrin fiber diameter (n = 100). Epifluorescence (D) and SEM (E) images of fibrin deposition on bead spots. Differences within a group were determined by ANOVA and Bonferroni *post hoc* test to compare pairs. Significant differences ($P < 0.01$) are represented by an asterisk (*) and bars between pairs.

Fibrin in Wolberg's Experiments

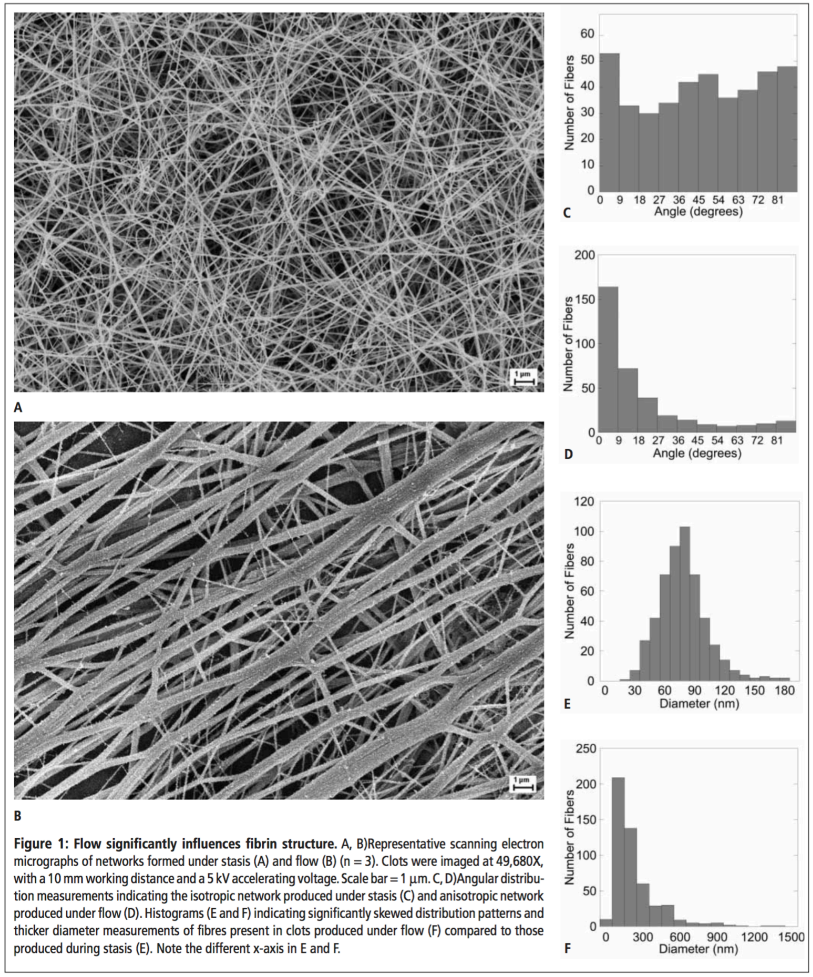


Figure 1: Flow significantly influences fibrin structure. A, B)Representative scanning electron micrographs of networks formed under stasis (A) and flow (B) (n = 3). Clots were imaged at 49,680X, with a 10 mm working distance and a 5 kV accelerating voltage. Scale bar = 1 µm. C, D)Angular distribution measurements indicating the isotropic network produced under stasis (C) and anisotropic network produced under flow (D). Histograms (E and F) indicating significantly skewed distribution patterns and thicker diameter measurements of fibres present in clots produced under flow (F) compared to those produced during stasis (E). Note the different x-axis in E and F.