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Supplemental information

Effects of clot contraction on clot degradation: A mathematical and experimental approach

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Supplementary Information

A. Modeling methods

i. Model details

In this manuscript, the macroscale model from Bannish et al. (Bannish et al., 2017) was significantly expanded to include red blood cells (RBCs). Briefly, the macroscale model tracks tPA molecules as they diffuse through the clot, bind to fibrin, and unbind from fibrin. When a tPA molecule binds to a fibrin fiber, we access empirical distribution functions obtained from the microscale model (specific microscale model details are provided in Bannish et al. 2014 and Bannish et al. 2017 (Bannish et al., 2017; Bannish et al., 2014)) — first, we sample the cumulative distribution function of tPA leaving times to determine when tPA unbinds from the fiber; second, we sample the cumulative distribution function of single fiber lysis times to determine if/when that particular fiber will degrade. When a fiber degrades in the macroscale model, any tPA still bound to that fiber is assumed to be on a large fibrin degradation product (FDP). These FDPs can diffuse along and away from the clot but are too big to diffuse farther into the clot. Eventually, after an average time of 1/ (kinetic unbinding rate), the tPA kinetically unbinds from the FDP and is able to freely diffuse anywhere in the domain. The model is a reaction-diffusion master equation for $P(\vec{m}, t)$, the probability of a tPA molecule being at a given position in a given state (bound or unbound) at a given time:

$$\frac{dP(\vec{m},t)}{dt} = \text{diffusion of tPA} + \text{binding/unbinding of tPA}$$

and it is implemented algorithmically. A flow chart depicting the main steps of the algorithm used to simulate the master equation is shown in Supplementary Figure 1.

The macroscale model is 3-dimensional (3D). The model domain (which includes a fibrin-free region in which tPA is initially distributed, a peripheral clot region that only contains fibrin, and a core region that includes RBCs and fibrin (main text Figure 1A)) is approximately 100 μm wide, 30.4 μm (uncontracted clot) or 8.2 μm (contracted clot) tall, and 1 pore size deep (1.014 μm for an uncontracted clot and 0.22 μm for a contracted clot). tPA and fibrin are the only proteins explicitly included in the macroscale model; plasminogen and plasmin - as well as tPA and fibrin - are included in the microscale model, results from which are read into the macroscale model. A single RBC is included in the modified macroscale model by defining a 7.09 μm x 7.09 μm x pore size μm, as this corresponds to the average RBC diameter, volume of the fibrin clot to be undegradable fibrin through which tPA cannot diffuse. In this way, the model RBC acts as a physical barrier to tPA diffusion. The size of the model RBC was chosen to best approximate the size of an actual RBC, which has diameter of about 8 μm. Assuming that the RBC is circular when viewed in the appropriate orientation, the area an RBC should take up in our clot is 50 μm^2 = (7.09 $\mu m)^2$. Since the depth of the model clot is small (0.22 - 1.0135 μm), we assume that the RBC fills that entire dimension. Hence, a single model RBC is a (7.09 μm x 7.09 μm x pore size μm) volume of clot. For every simulation, we randomly distribute 50 of these RBCs throughout the core region of the clot.

To model external fibrinolysis, 9,350 tPA molecules (shown as green stars in main text Figure 1) were randomly initialized in the fibrin-free region (white region in Figure 1). As tPA diffuses through the clot (diffusion coefficient equal to 5×10^{-7} cm^2/second) and binds to fibers, it initiates the lytic process, and results in a front-like degradation of fibrin, as seen experimentally in previous studies (Tutwiler et al., 2019). Most model clot scenarios contain a peripheral fibrin-only region (without RBCs), which models the fibrin that is typically redistributed during the contraction process, and a core region that contains both fibrin and RBCs. The fibrin fibers in all scenarios have a diameter of 0.0727 μm. Contracted clots (90RCT, 50RCT, 90RCL, and 0RC) have a clot volume of 883 μm^3 with a pore size of 0.22 μm. Uncontracted clots (50RUL, 90RUL, and 0RU) have a clot volume of 5620.0 μm^3 with a pore size of 1.014 μm; both the volume and the pore size are significantly larger in the uncontracted clots. Clot parameters for all scenarios are listed in Supplemental Table 1.

Scenario	Length of Each Fibrin Fiber in Periphery (μm)	Length of Each Fibrin Fiber in Core (μm)	Volume of Periphery (μm^3)	Volume of Core (μm^3)	Volume of Entire Clot (μm^3)	Number of Fibrin Fibers in Periphery	Number of Fibrin Fibers in Core	Number of Fibrin Fibers in Entire Clot
90RCT	0.22	0.22	79.2	800.8	882.8	13,325	1,481	14,806
50RCT	0.22	0.22	39.6	838.2	882.8	7,175	7,175	14,350
90RCL	1.0135	0.22	259.6	620.4	882.8	2,827	1,466	4,293
50RUL	1.0135	1.0135	334.5	5,179	5,622	1,112	1,112	2,224
90RUL	1.0135	1.0135	780.4	4,743.2	5,662	2,224	247	2,471
0RU	N/A	1.0135	N/A	5662	5,662	N/A	2,224	2,224
0RC	N/A	0.22	N/A	882.8	882.8	N/A	14,350	14,350

Supplementary Table 1. Values of parameters for the scenarios discussed in the main text (with RBC-rich cores). Exact values are included for reproducibility.

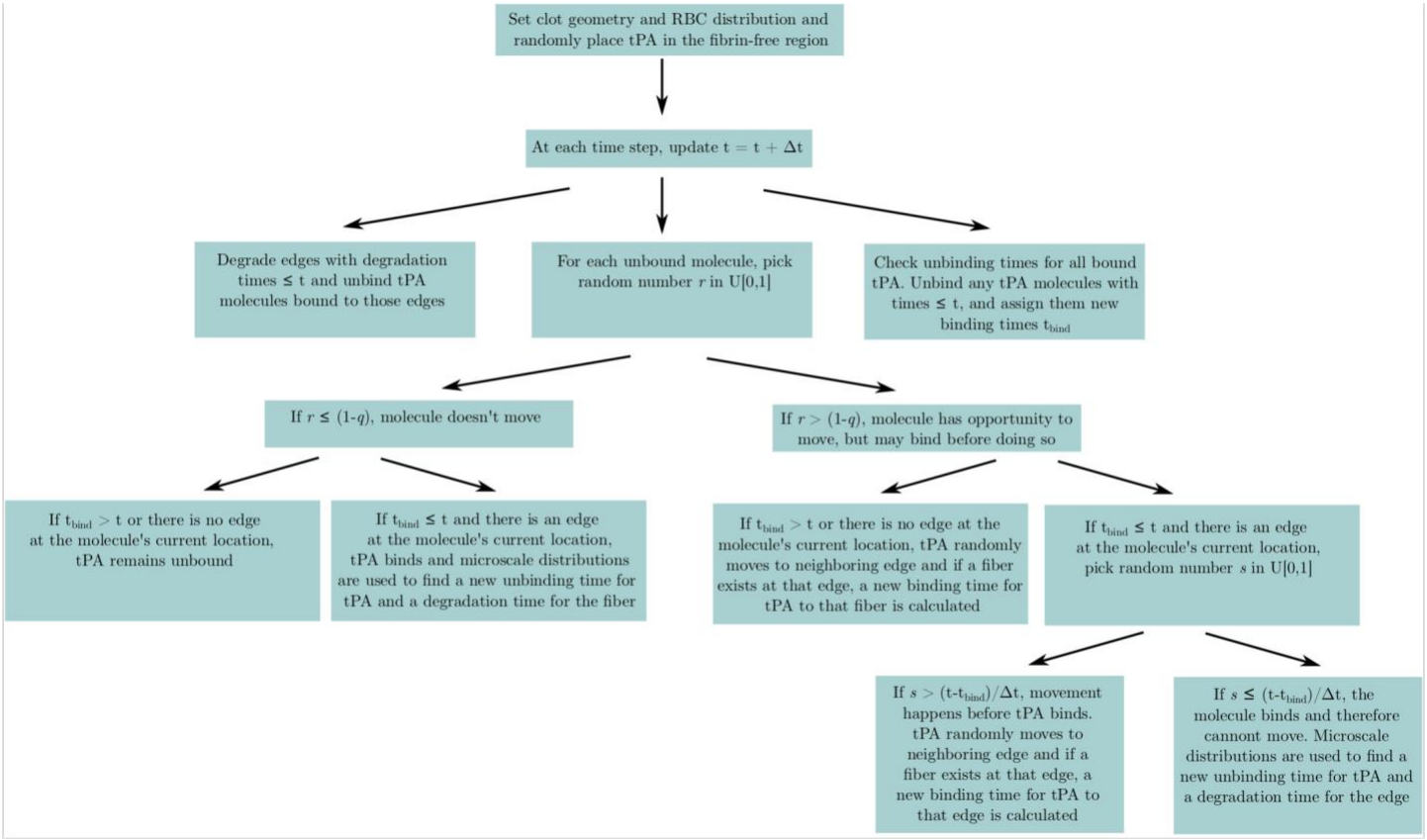
ii. Computational details

The model is simulated using custom MATLAB and Fortran codes. Three independent simulations are run for each model scenario tested. Data files containing information about the distribution of RBCs, the distribution of fibrin fibers, and the distributions from the microscale model are read into the Fortran code. The microscale model of a single fiber cross-section, which is exactly as described in (Bannish et al., 2017), uses a 2 μM plasminogen concentration, no initial plasmin, and 1 randomly placed tPA molecule on the outer edge of the fiber cross-section. Many biochemical and physical reactions (tPA conversion of plasminogen to plasmin, plasmin degradation of fibrin, etc.) are modeled, and the single fiber lysis time and the time at which tPA left the cross-section are recorded. Distributions of these lysis times and tPA leaving times are

generated from 50,000 independent simulations of the microscale model, and these data are what is used by the macroscale model. The macroscale model is run for 15 minutes of simulated time, after which data files with the spatiotemporal location of tPA and of undegraded fibrin are created. These files are post-processed in MATLAB and Prism 9.0 to create the figures and data displayed in this manuscript.

To calculate average degradation rate for a specific scenario, we average the individual degradation rates of the three independent runs for that scenario. The individual degradation rates are obtained as follows. We use Prism 9.0 to plot the fraction of fibrin remaining versus time for each clot region. For example, for scenario 90RCT, the fraction of fibrin remaining in the periphery, core, and full clot are each plotted as a function of time (Supplemental Figure 4). Then, the slopes of these curves are calculated by fitting a line to the most linear part of each plot. The slope of this line is the degradation rate (measured in fraction of fibrin per second). The slopes from the three trials are averaged together to get an average degradation rate for each region in each scenario.

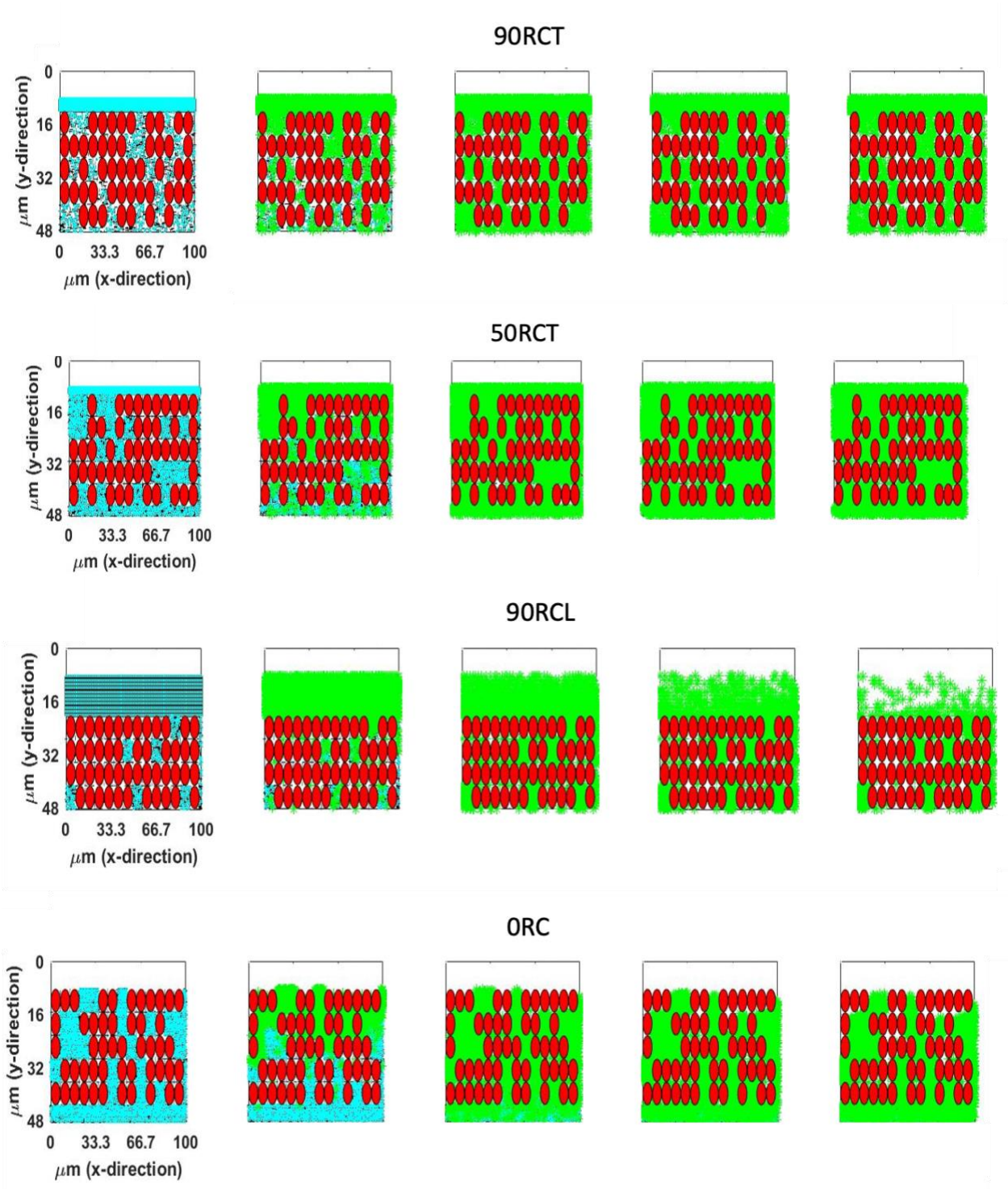
To obtain the times to the 50% and 90% degradation, we plot the fraction of fibrin remaining versus time in MATLAB. We locate the respective time points which correspond to 0.5 of the total initial fibrin and 0.1 of the total initial fibrin remaining (because when 0.1 of the initial amount remains, 90% has been degraded). We average these times over the three trials for each clot region in each scenario.



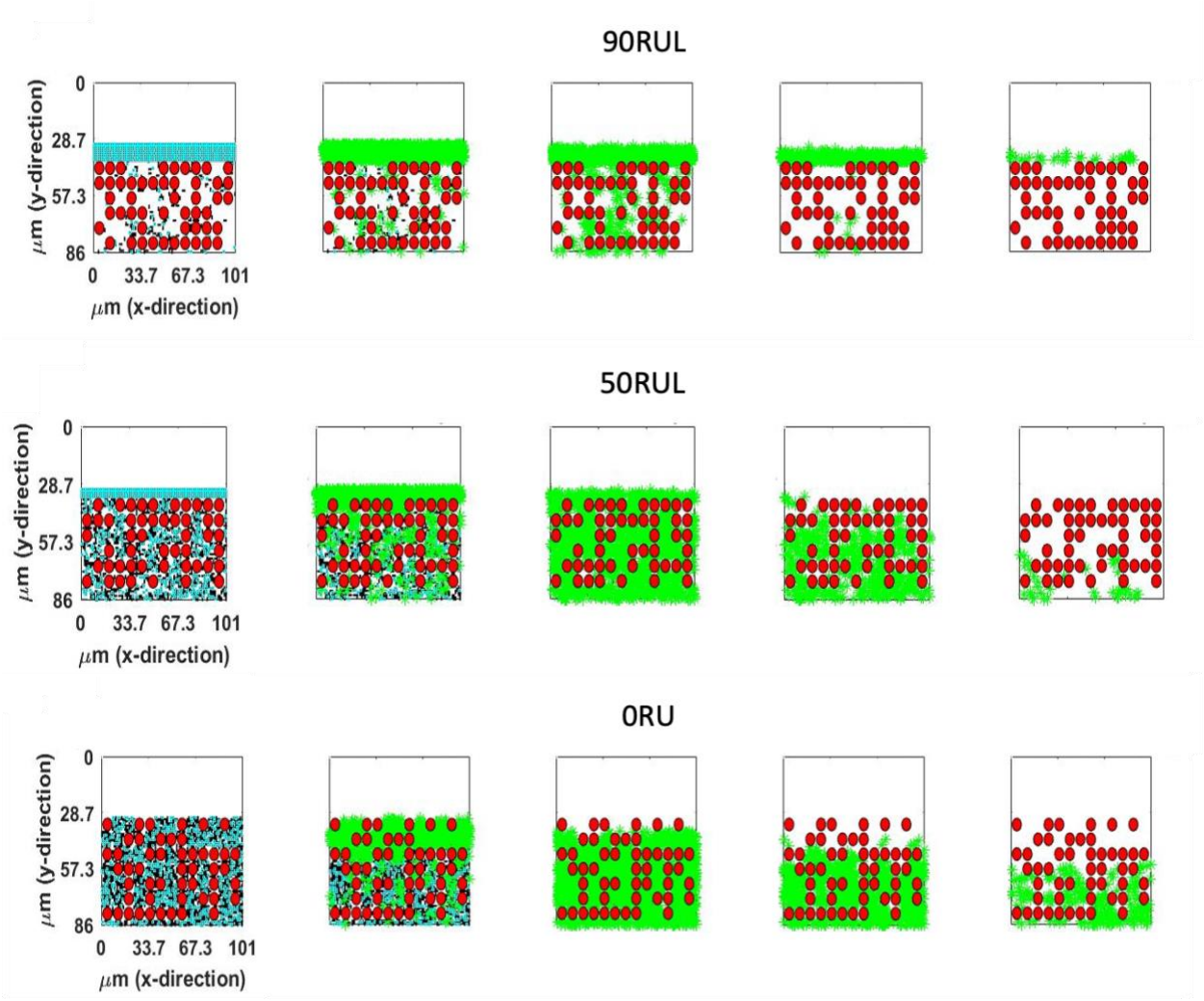
Supplementary Figure 1. Model flow chart.

B. Supplemental Results

i. RBC-rich core



Supplemental Figure 2. Contracted blood clot scenarios (90RCT, 50RCT, 90RCL, 0RC) time snapshots from 0 seconds (first snapshot) to 180 seconds (last snapshot) in 60 second intervals. RBCs are represented as red ovals, fibrin fibers in the plane of the paper are in black, fibrin fibers perpendicular to the plane of the paper are in cyan, and bound tPA molecules are represented as green asterisks.



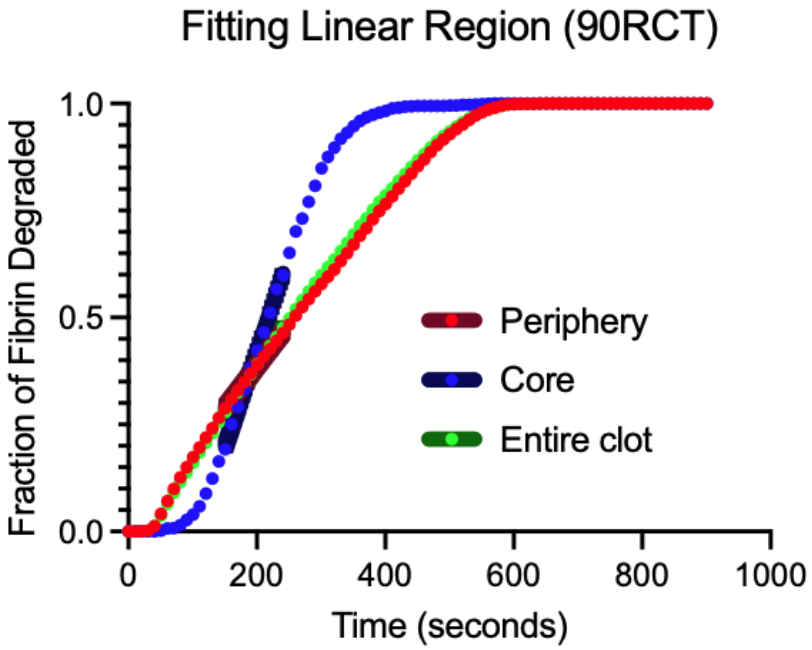
Supplemental Figure 3. Uncontracted blood clot scenarios (50RUL, 90RUL, 0RU) time snapshots from 0 seconds (first snapshot) to 180 seconds (last snapshot) in 60 second intervals. RBCs are represented as red ovals, fibrin fibers in the plane of the paper are in black, fibrin fibers perpendicular to the plane of the paper are in cyan, and bound tPA molecules are represented as green asterisks.

	90RCT			50RCT			90RCL			90RUL			50RUL			0RU		
Subregion	E	C	P	E	C	P	E	C	P	E	C	P	E	C	P	E	C	P
Degradation Rate	0.0022	0.0043	0.002	0.0021	0.0025	0.0026	0.0069	0.0052	0.0079	0.0066	0.0157	0.0056	0.0066	0.0081	0.0126	0.005		0
Time to 50% Lysis	241	221	258	241	318	171	91	211	88	101	121	111	101	154	71	134	141	0
Time to 90% Lysis	464	341	484	448	488	391	204	288	171	181	161	191	181	211	121	218	228	0

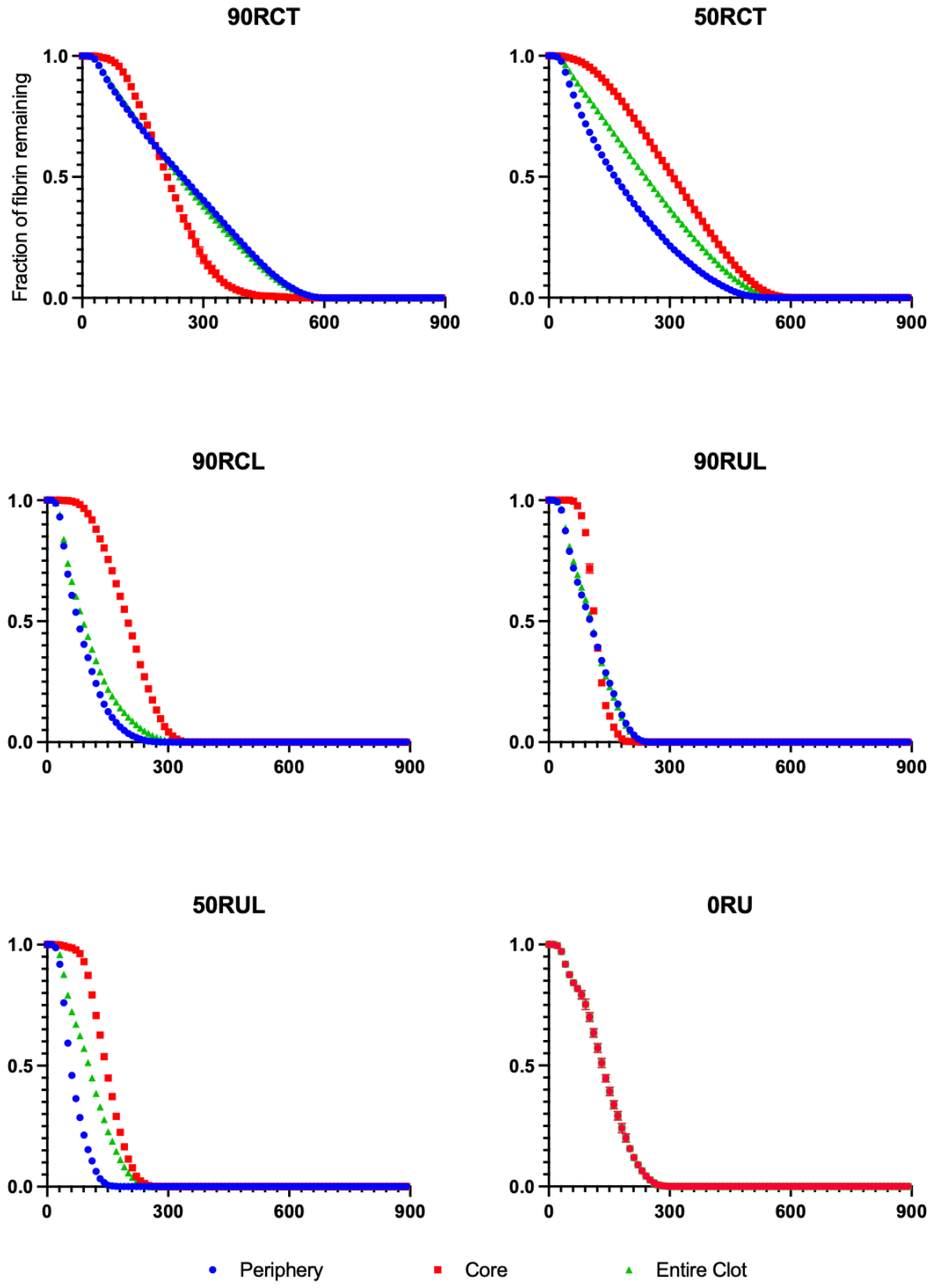
Supplemental Table 2. Summary table of degradation rates (fraction of fiber remaining per second), time to 50% lysis (seconds), and time to 90% lysis (seconds) for each scenario. The subregions are broken up into the entire clot (E), the core (C), and the periphery (P).

	90RCT	50RCT	90RCL	90RUL	50RUL	0RU
90RCT	X	0.9948	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
50RCT		X	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
90RCL			X	**0.0196**	**0.0106**	**<0.0001**
90RUL				X	>0.9999	**<0.0001**
50RUL					X	**<0.0001**
0RU						X

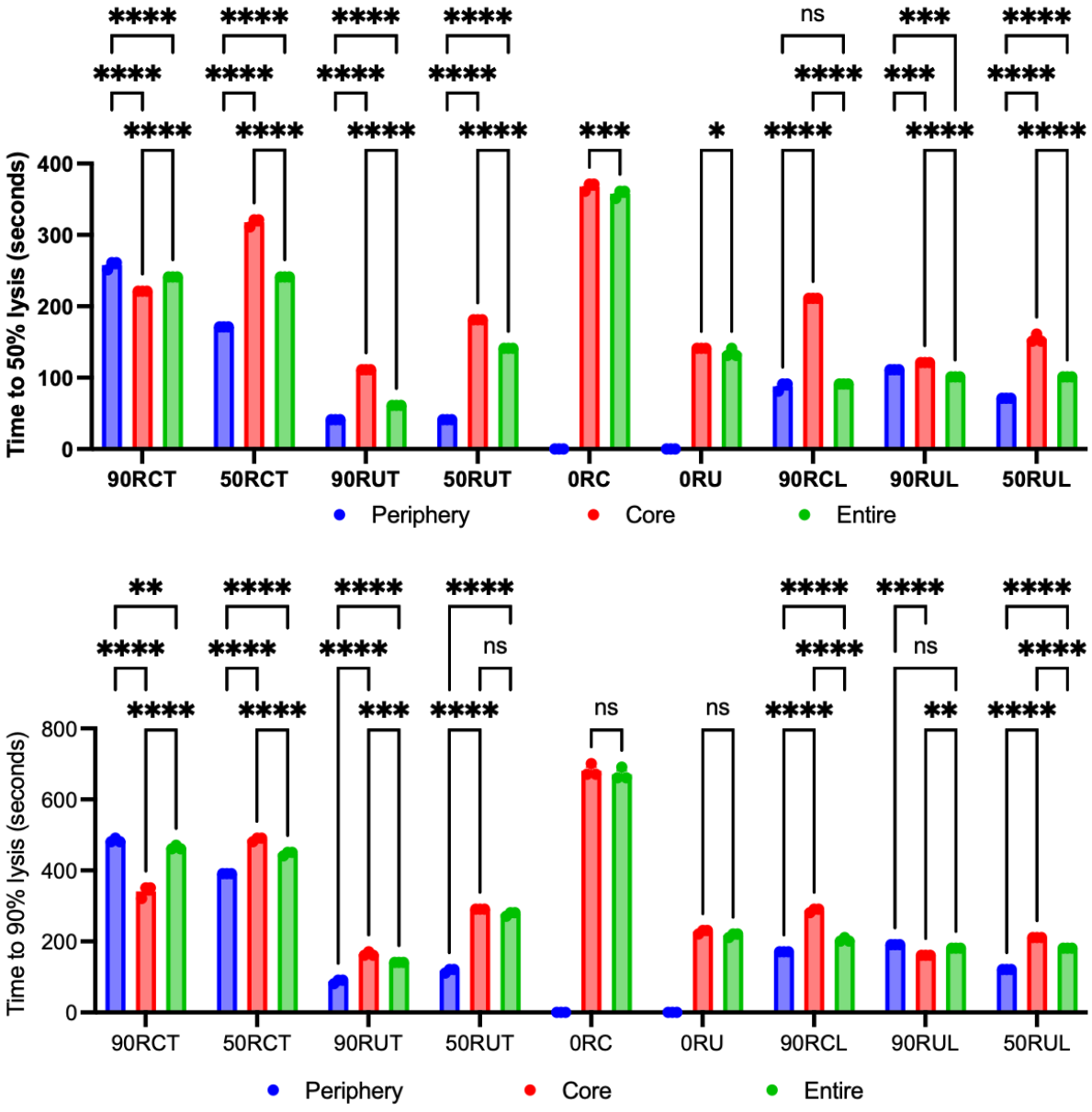
Supplementary Table 3: Statistical significance of entire clot degradation rates. Significant values are bolded.



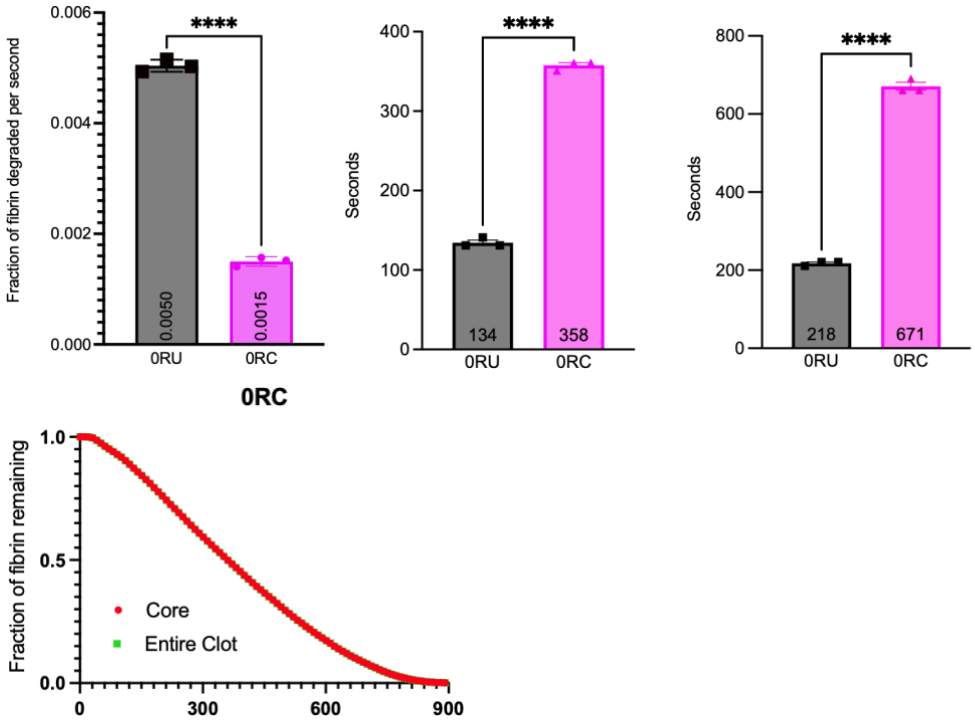
Supplementary Figure 4. Representative example for how degradation rate was calculated. A linear regression was approximated within the linear region of degradation.


90RCT
50RCT
90RCL
90RUL
50RUL
0RU
Fraction of fibrin remaining
1.0
0.5
0.0
0
300
600
900
Periphery
Core
Entire Clot

Supplementary Figure 5. Plots of average (n=3) clot lysis for periphery region (blue), core region (red), and the entire clot (green) for scenarios 90RCT, 50RCT, 90RCL, 90RUL, 50RUL, and 0RU. The x-axis represents time (in seconds) and the y-axis represents the fraction of the clot degraded.

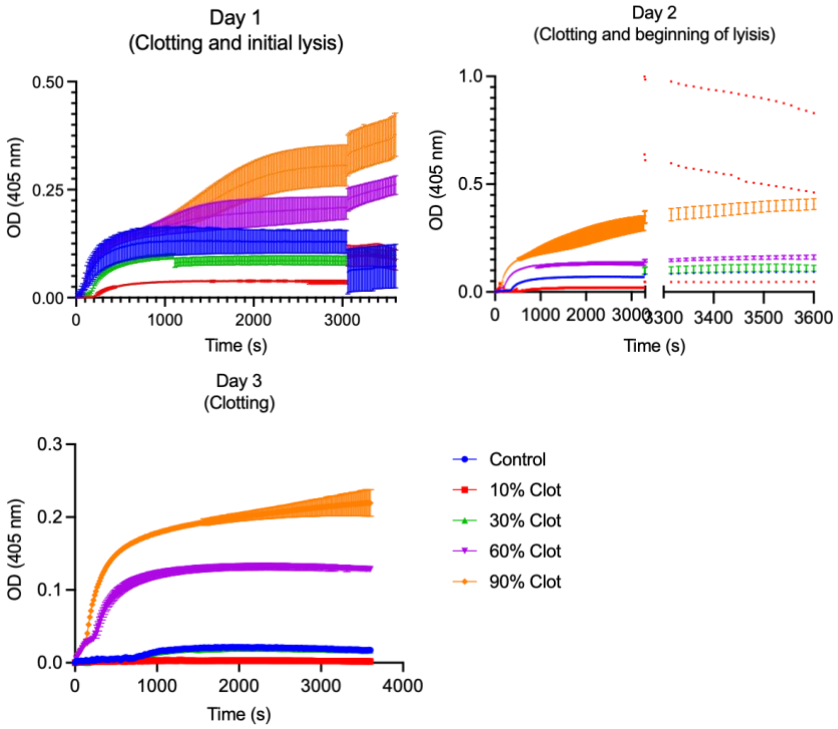


Supplementary Figure 6. Time to 50% and 90% lysis for the subregions and entire clot. (ns = not significant; * p>0.05; ** p<0.01; *** p<0.001; **** p<0.0001).



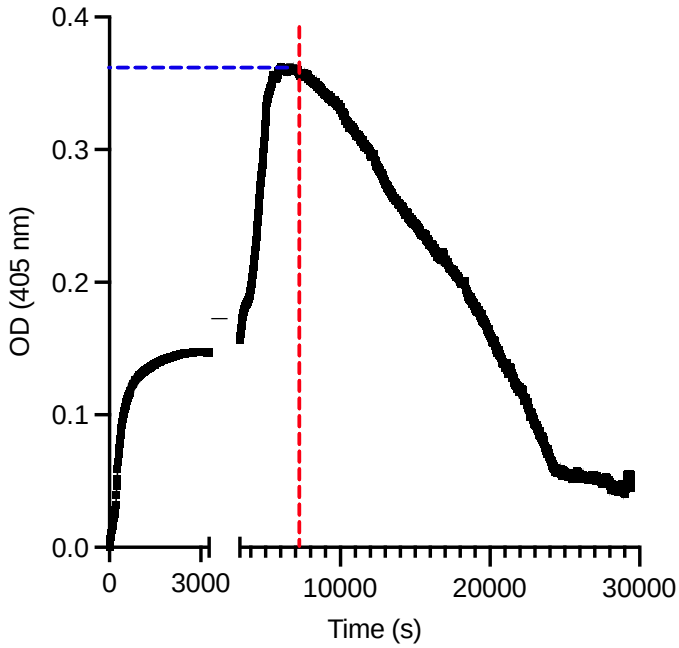
Supplemental Figure 7. Degradation rate, time to 50% lysis, time to 90% lysis, and time course of 0RC (**** p<0.0001).

ii. Supplemental Experimental results

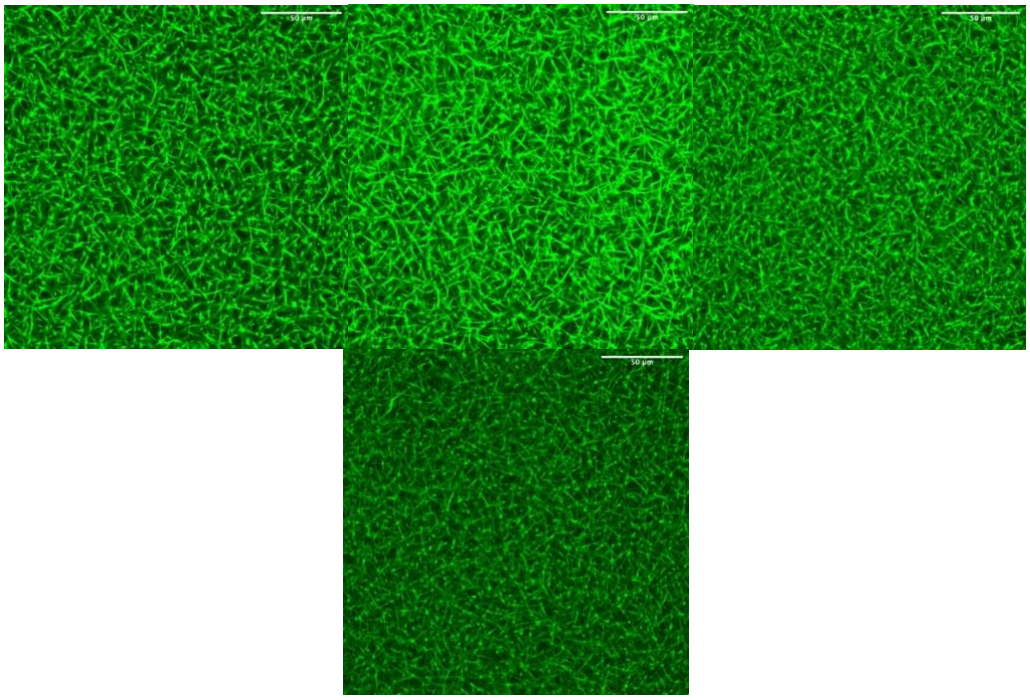


<u>Supplemental Figure 8</u>. The turbidity curves (normalized to the first point) for clotting and the beginning of lysis. Day 1 had successful clotting and lysis; all replicates were used in the

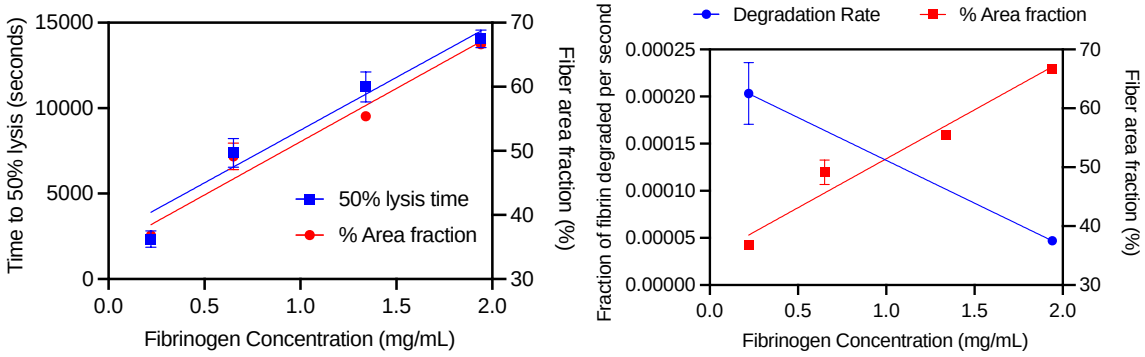
analysis. Day 2 had successful clotting, but some of the 10% clots had jumps in OD, which we speculate is due to a collapsed clot and thus irregular lysis. For this reason, those two replicates were removed from the final analysis; all other replicates were used in the analysis. Day 3 showed overall lower OD increases, possibly due to lower thrombin activity on that day. The lowest concentration appears to not have clotted at all, leading us to remove those three replicates from the final analysis. One replicate of the 30% sample from day 3 was removed from final analysis plots after being identified as an outlier for degradation rate and time to 50% lysis using the ROUT method on Prism.



Supplementary Figure 9. Turbidity clotting and lysis curves. tPA added after one hour of clot formation and turbidity plateau (arrow). Maximum OD (blue line, after an increase due to lateral aggregation during initial lysis) is used as the fully formed clot value to normalize clot curves, as described in the methods. Curves were shifted to 7200 seconds (red line) for plotting in main text.



Supplementary Figure 10. Confocal microscopy images of 0.22, 0.68, 1.33, and 1.94 mg/mL (left to right) fibrinogen.



Supplementary Figure 11. Relationship between time to time 50% lysis, fibrin network density, and fibrinogen concentration (left) and degradation rate, fibrin network density, and fibrinogen concentration (right).