Bioengineering a 3D Fibrin-Based Thrombus Model to Study Osteosarcoma Under Shear Stress

Haley Neumiller,[1] Sriya Sagi MS,[2] Gisele Charpentier BS,[2] Nikolaos Pipis PhD,[2] Avery Nguyen,[1] Rameshwar (Ram) R. Rao MD PhD [2, 3]

[1]Department of Bioengineering, University of Washington, Seattle, WA; [2] Ben Towne Center for Childhood Cancer and Blood Disorders Research, Seattle Children's Research Institute, Seattle, WA; [3] Division of Pediatric Hematology, Oncology, Bone Marrow Transplant, and Cellular Therapies, Department of Pediatrics, University of Washington School of Medicine, Seattle, WA

Motivation

Metastatic osteosarcoma (OS) carries a poor prognosis, with an estimated 5-year survival rate of 20%.

The OS metastatic cascade is a multi-step process which involves malignant cells traveling through the bloodstream to distant sites in **fibrin (FIB)-rich microthrombi.**

The influence of fluid flow within FIB microthrombi during the circulation phase is poorly understood.

Goal

Develop a 3D FIB hydrogel model under flow to investigate the state in which OS cells travel through circulation in FIB-rich thrombi.

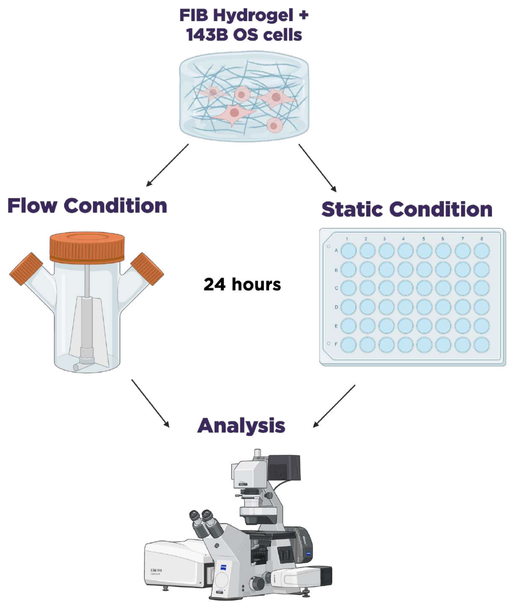

Figure 1: General schematic of flow vs. static experiment workflow.

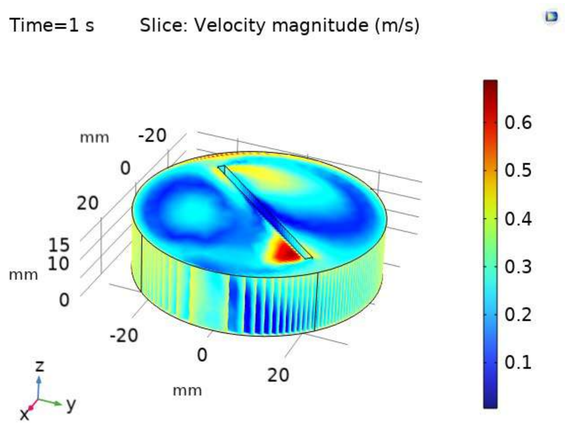

Figure 2: COMSOL Multiphysics computational model of flow velocity magnitude within spinner flask at 1 s. This model demonstrates heterogeneous flow throughout the spinner flask with maximum velocity near the spinning impeller.

RPM	~128	Interpretation
R_e	3396	Some areas of turbulent flow
V_{max}	26.7 $\frac{cm}{s}$	Large artery: 10-40 $^{cm}/_{s}$
τ_{avg}	0.213 $\frac{dynes}{cm^2}$	Shear stress will not kill cells
η	89 µm	> 20 µm will not kill cells

Methods

FIB hydrogels were formulated at 10 mg/mL to model thrombi. Material characterization (DMA, viscoelastic rheology, and confocal reflectance) were performed at day 0 prior to flow.
For flow vs. static experiments, hydrogels were allowed to compact for 24 h in a 24-well plate; flow samples were then transferred to a spinner flask for 24 h to model thrombi in circulation. 143B OS cells were seeded at 500k/mL density in cellular formulations.

Figure 3: Image of EZBlue stained FIB hydrogels under flow conditions modeled above.

Results

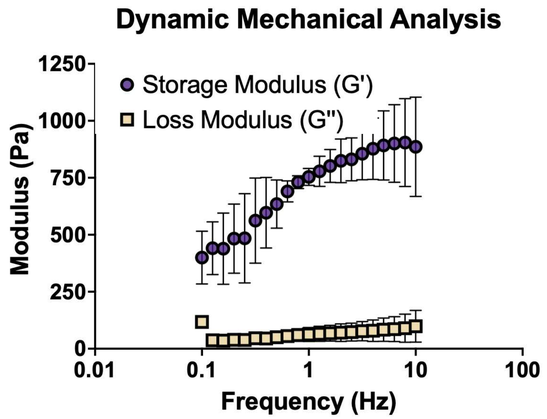

Figure 4: DMA frequency sweep demonstrates an average storage modulus of approximately 750 Pa at 1 Hz (n=4 technical hydrogel technical replicates, mean +/- SD).

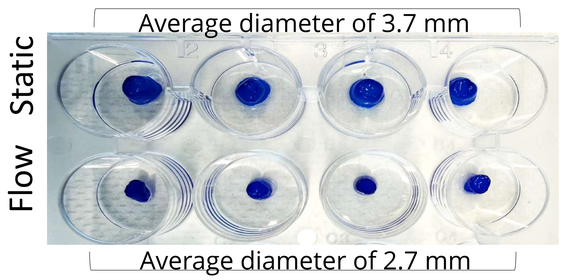

Figure 5: Compaction of acellular FIB hydrogels under static vs. flow conditions after 24 hours.

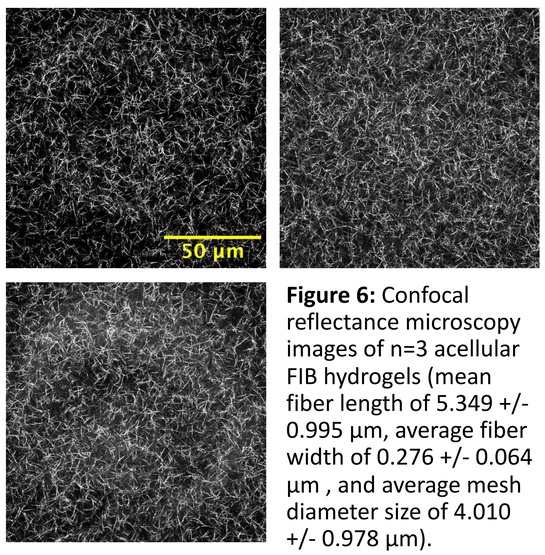

Figure 6: Confocal reflectance microscopy images of n=3 acellular FIB hydrogels (mean fiber length of 5.349 +/- 0.995 µm, average fiber width of 0.276 +/- 0.064 µm , and average mesh diameter size of 4.010 +/- 0.978 µm).

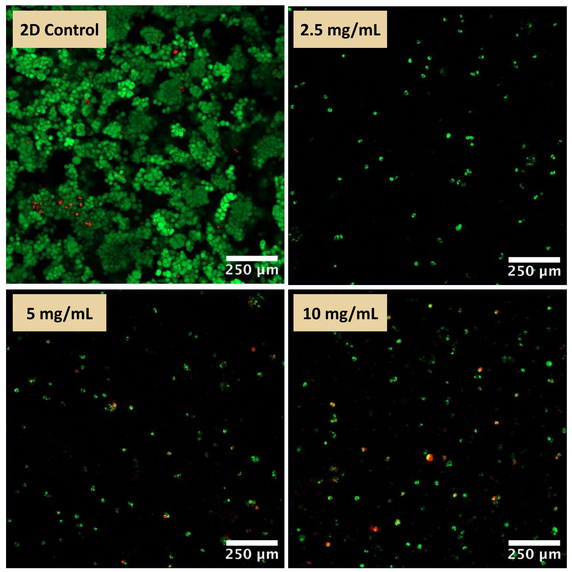

Figure 7: 143B OS cell viability imaging at 1 day of culture. Viability was high in all FIB hydrogel concentrations. The cells dispersed throughout the matrix.

Conclusions

We developed a stable 3D FIB hydrogel model to study metastatic OS thrombi under physiologically relevant flow conditions. Flow was found to induce significant hydrogel compaction, altering thrombus FIB architecture. Our spinner flask system mimics arterial circulation with calculated shear stresses and flow velocities. 143B OS cells were found to live in a range of FIB hydrogel concentrations, making this an ideal model for future experiments.

Current Work

In flow vs. static conditions, OS cells evaluated using live/dead and immunocytochemistry imaging. To evaluate metabolic activity, quantitative assays including AlamarBlue and CellTiter-Glo are performed.