Ex-Vivo Human Clot Analog Fabrication for Mechanical Thrombectomy Device Validation

ENGINEERING HONORS PROGRAM
UNIVERSITY OF MICHIGAN

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Introduction and Background

This project aims to help address the wider problem of acute ischemic stroke treatment. In the U.S. roughly 795,000 patients suffer from ischemic stroke every year [1]. Proper vessel recanalization is essential for effective treatment and is often done using mechanical thrombectomy devices to remove obstructing thrombi. To improve recanalization rates and achieve better patient outcomes, proper device validation is required. Such validation can be more easily, and cost effectively, completed using ex-vivo thrombus or clot analogs. As such in this project novel human clot analogs are fabricated, and various coagulation conditions are explored to form clot analogs that mimic real human thrombi properties so the ex-vivo analogs can be used for device validation.

Project Objectives

1. Fabricate repeatable human clot analogs with commercial or donated blood products and added chemical agents to induce coagulation.
2. Explore the effects of ingredients and coagulation conditions such as time, motion, pressure, and temperature on analog properties.
3. Quantify the mechanical and histological properties of fabricated clot analogs via dynamic mechanical analysis (DMA) testing and histology testing, respectively.
4. Compare clot analog properties to real human thrombi properties to ensure similarities and that the analogs are suitable for validation use.

Materials and Methods

Over the course of project work various blood products and biochemical ingredients were collected and combined in different ratios for different analog fabrication trials [2].

- Human red blood cells (RBCs) were obtained from the Michigan Medicine blood bank, human platelet rich plasma (PRP) and porcine (pig) whole blood were obtained from online suppliers.
- Solutions of $CaCl_2$, type I collagen, and thrombin were prepared to initiate clot formation and increase coagulation in certain trials.
- Analogs were fabricated within 15 mL conical tubes and later SLA 3D printed cylinder molds for improved standardization.

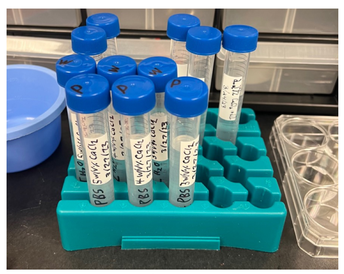

Figure 1. Solutions of $CaCl_2$ acting as the primary clotting initiator across all analog fabrication trials.

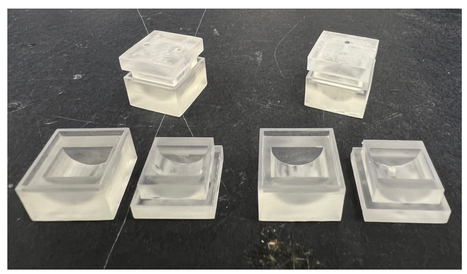

Figure 2. SLA 3D printed molds used for later analog fabrication, ingredients would be mixed and injected into the mold yielding cylindrical clots.

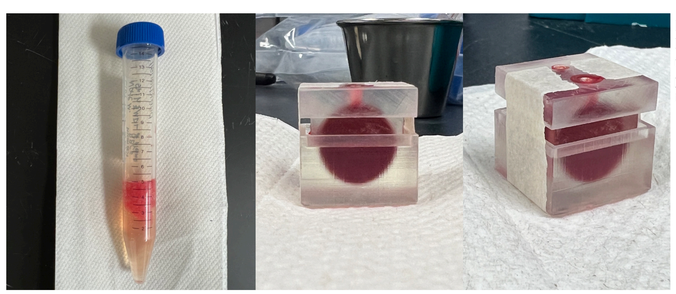

Figure 3. On the left, a mixed human RBCs and PRP clot analog in a conical tube, and on the right, a pair of mixed porcine whole blood clot analogs set to form within the new clot molds.

Results and Discussion

Human blood clot analogs were successfully fabricated. Multiple trials of analog fabrication were completed and differed by their ingredient ratios and coagulation conditions as different cases were explored to produce progressively better analogs.

- Analogs made with human RBCs and PRP were comprised of 70% to 90% PRP and 30% to 10% RBCs.
- New analogs were later made with 100% porcine whole blood to explore the effect of different blood sources and reduce costs.
- In all fabrication trials $CaCl_2$ ranging from 2.27 w/v% to 5 w/v% was added in a 1:10 ratio of $CaCl_2$ solution to blood product volume to increase $[Ca^{2+}]$ and initiate clot formation.
- Solutions of thrombin and type 1 collagen were added to improve coagulation and clot matrix formation.
- Coagulation typically occurred at room temperature, with other analogs being incubated at 37° C human body temperature.
- Static coagulation and coagulation with orbital shaking was done.
- Observed coagulation time required for solid clot formation ranged from just 10 seconds with thrombin present, to 1-2 hours for most other cases.

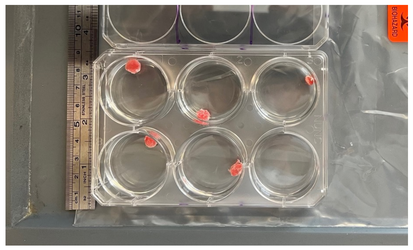

Figure 4. Samples of coagulated/solid human RBCs and PRP clot analogs suspended in 1x PBS and cut to size for later DMA testing.

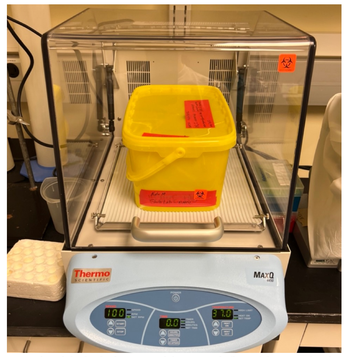

Figure 5. Incubation and orbital shaker machine used for either or both incubating clots at human body temperature and mechanically agitating clots during coagulation.

Fabricated analogs from different conditions/trials were able to be successfully characterized mechanically and histologically.

- Mechanical characterization via DMA testing was done over a 0% to 40%, 60%, or 80% strain range with a typical 1% strain rate.
- Stress versus strain data was collected for various analog trials and allowed analog stiffness/elastic modulus to be quantified.
- Histology data was collected by fixing analog samples in 10% formalin, cutting and staining sections with trichrome, allowing observation under a microscope for histological analysis.

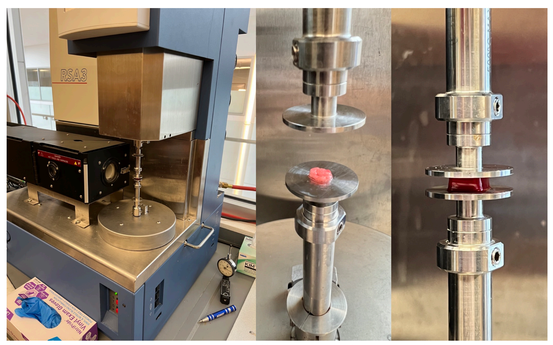

Figure 6. On the left, DMA machine used for clot analog stiffness testing via compression, in the middle a human RBCs and PRP analog about to undergo testing, and on the right a porcine whole blood analog partially compressed during a test cycle.

Conclusions and Future Work

Project results indicated that while clot analogs based on human and porcine derived blood ingredients could be successfully fabricated, their mechanical and histological properties still have significant room for improvement to better mimic the stiffness and histology of real human thrombi and be suitable for thrombectomy device validation.

- The impacts of time, motion, and temperature on clot analog coagulation were explored though further testing and characterization is needed to determine the exact nature of their impacts on stiffness and histology.
- Analogs made from human RBCs and PRP with added $CaCl_2$, type I collagen, and thrombin were found to be stiffer than human RBCs and PRP analogs with added $CaCl_2$ and type I collagen only based on DMA results, but not nearly as stiff as real human thrombi.
- Analogs made from human RBCs and PRP with added $CaCl_2$, type I collagen, and thrombin failed to form real thrombi-like collagen networks based on histology results.
- Analogs made from porcine blood coagulated faster with higher w/v% solutions of $CaCl_2$ added in the 1:10 volume ratio.

Future work on this project includes further exploring the effects of stronger motion/vibration and pressure on clot coagulation to achieve stiffer clots and better mimic real blood vessel environments. Future work also includes repeating DMA and histology testing to obtain better baseline analog results and adding new chemical/coagulation agents to gauge their impacts and obtain stiffer and more histologically accurate clots. This project is ongoing and current work includes conducting more fabrication trials with porcine whole blood analogs. DMA and histology data has yet to be analyzed for porcine whole blood-based analogs.

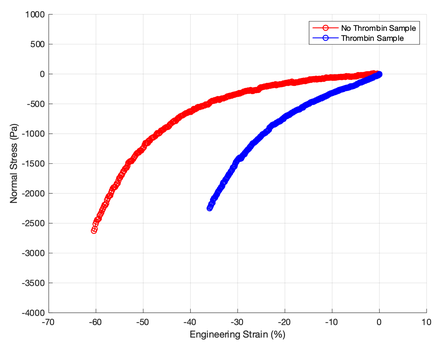

Figure 7. Stress-strain curve of selected human RBCs and PRP analog samples with added $CaCl_2$, type I collagen, and thrombin or no thrombin, from the plotted data the analog with thrombin added (blue) is significantly stiffer than the analog without thrombin added (red).

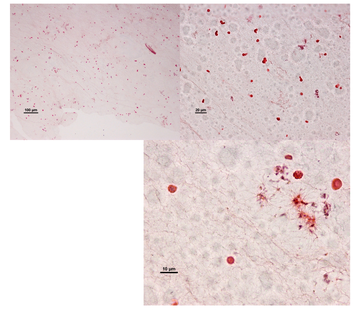

Figure 8. Histology image results with trichrome stain for selected human RBCs and PRP analog samples with added $CaCl_2$, type I collagen, and thrombin, with 100x zoom top left, 400x zoom top right, and 1000x zoom below, all showing a lack of strong and real clot-like networking.

Acknowledgements

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References

[1] Boehme, A. K., Esenwa, C., & Elkind, M. S. (2017). Stroke Risk Factors, Genetics, and Prevention. *Circulation research*, 120(3), 472–495. https://doi.org/10.1161/CIRCRESAHA.116.308398

[2] Liu, Y., Reddy, A. S., Cockrum, J., Ajulufoh, M. C., Zheng, Y., Shih, A. J., Pandey, A. S., & Savastano, L. E. (2020). Standardized Fabrication Method of Human-Derived Emboli with Histologic and Mechanical Quantification for Stroke Research. *Journal of stroke and cerebrovascular diseases*, 29(11), 105205. https://doi.org/10.1016/j.jstrokecerebrovasdis.2020.105205

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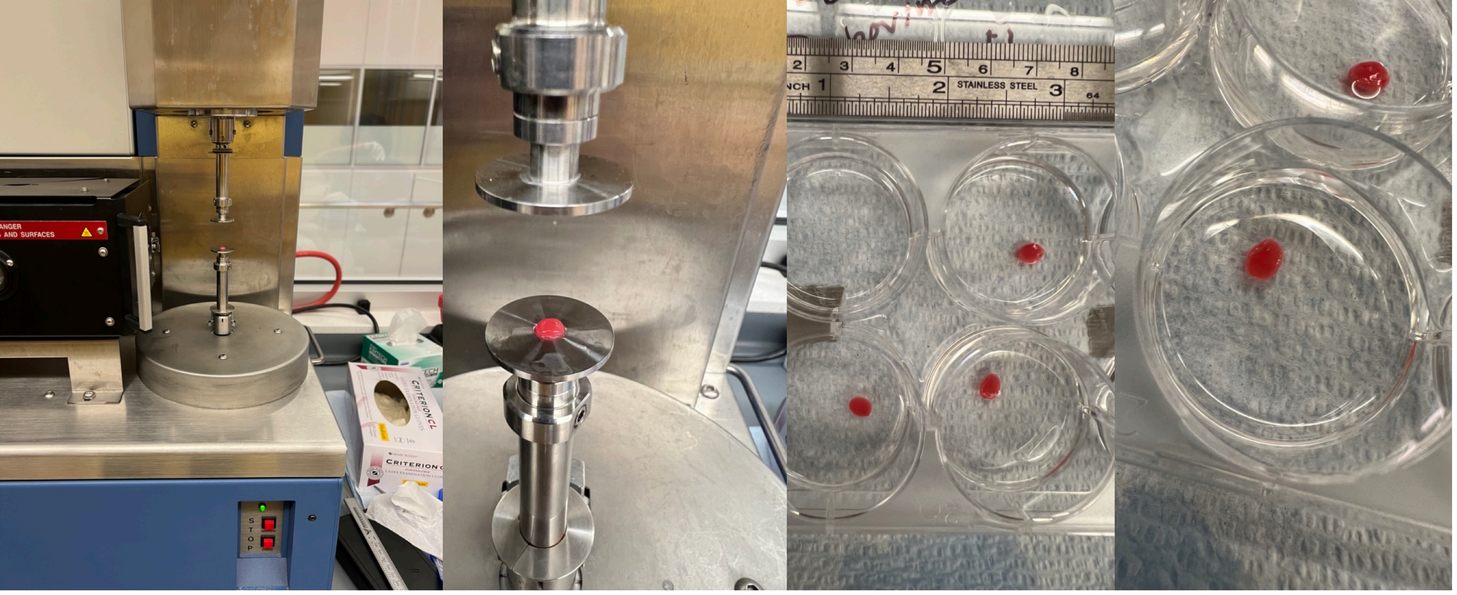
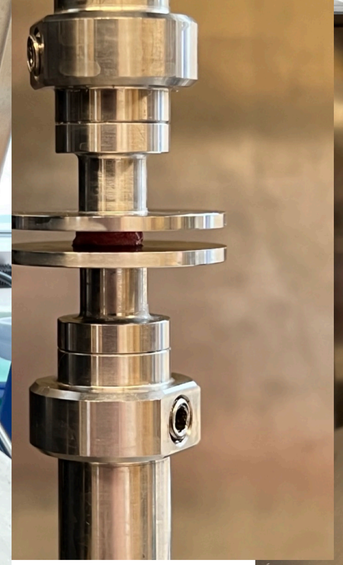
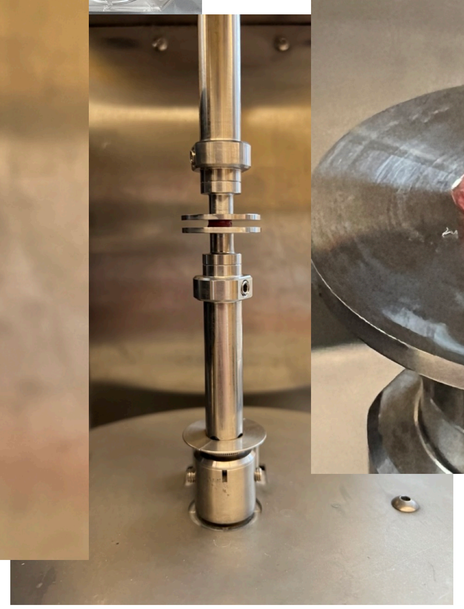
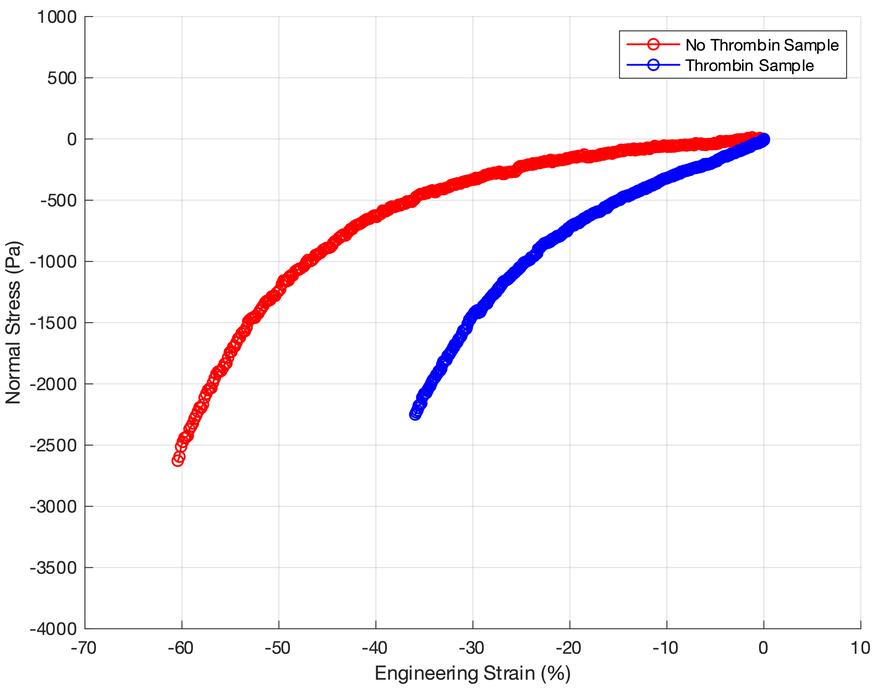


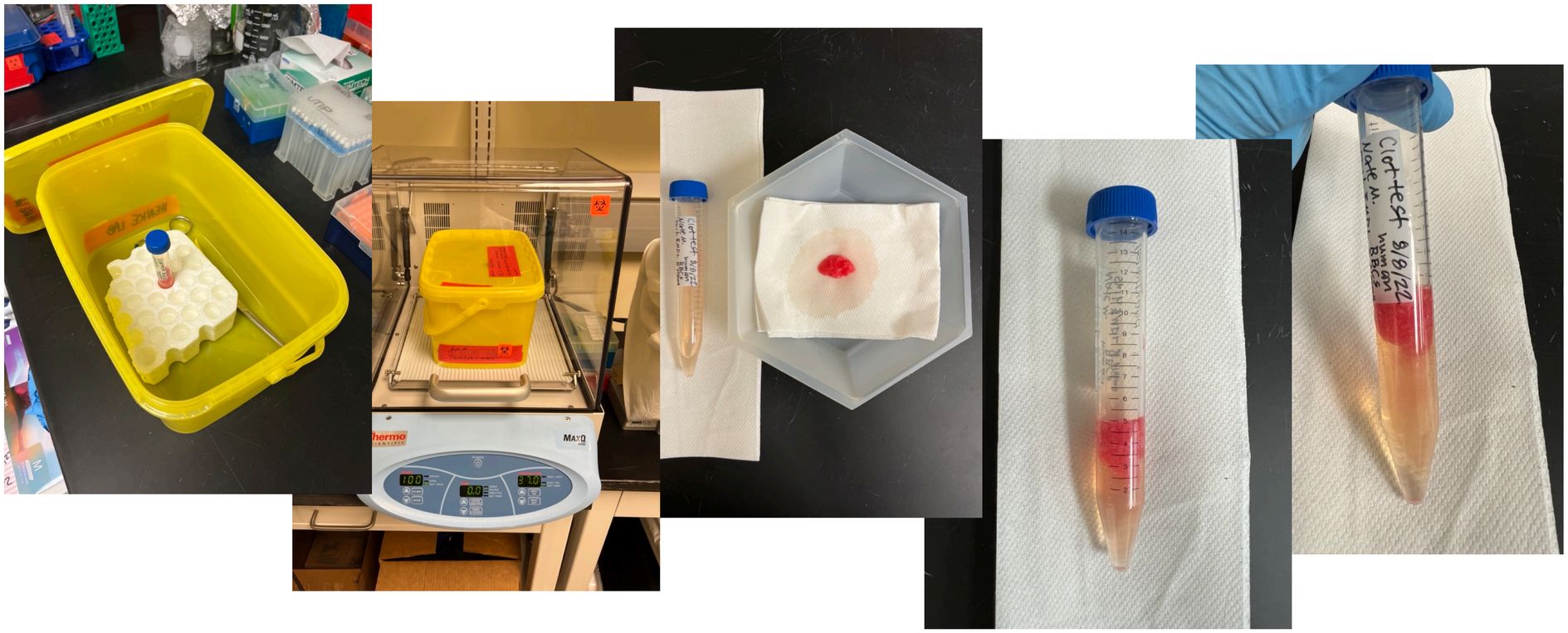
Clot DMA 8/3/22

Clot DMA 8/10/22

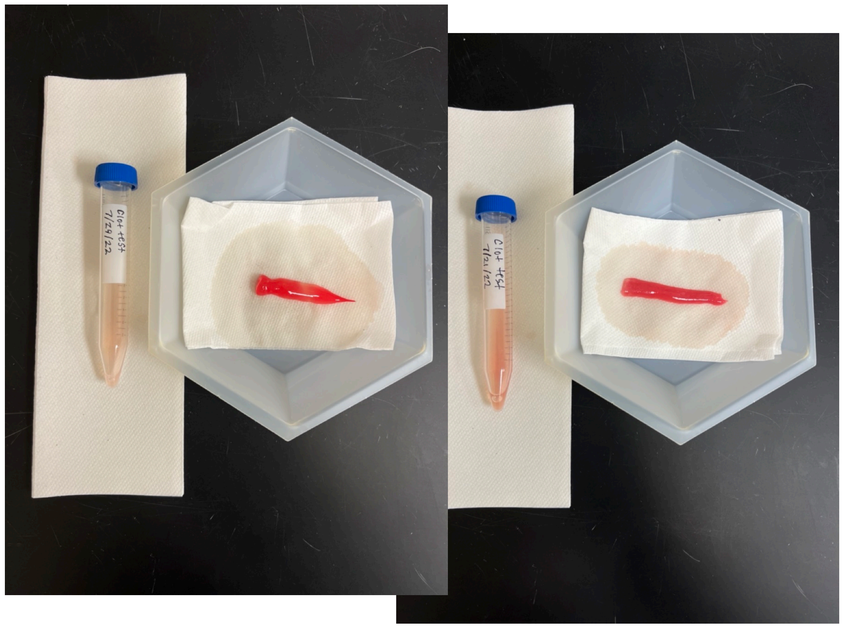






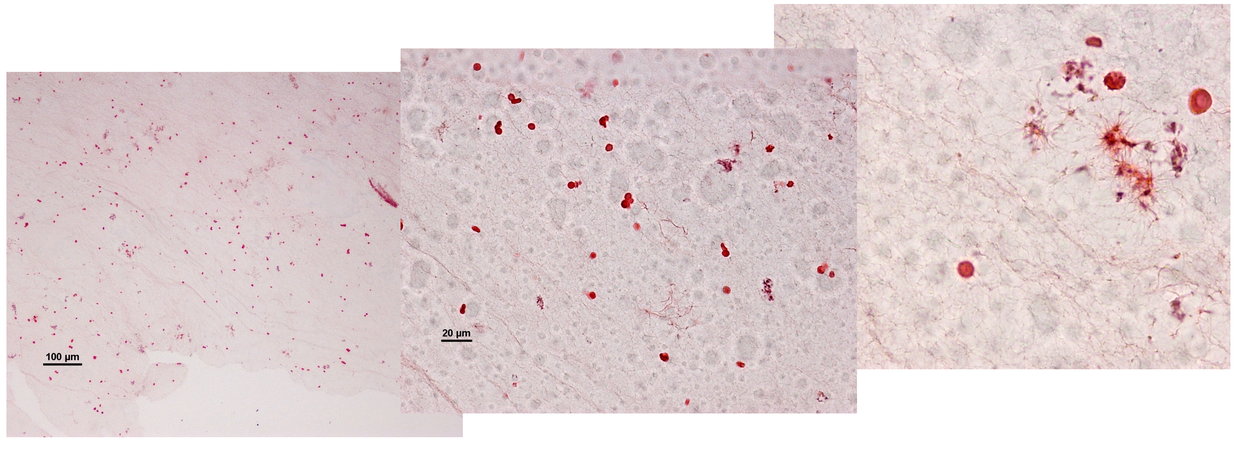


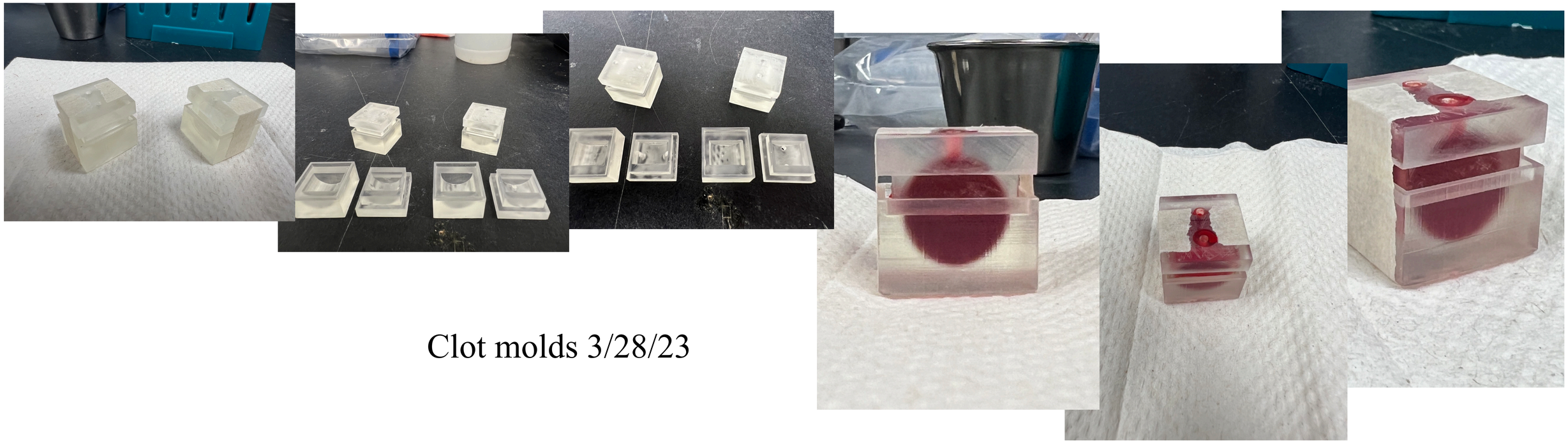
Clot setup 8/8/22



Clot tests 7/21/22 and 7/29/22

Clot histology 2/14/23





Clot molds 3/28/23

Clot testing setups 3/29/23