CLOTTING UNDER THE INFLUENCE: A COAGULATION ASSAY FOR CANNABINOID RESEARCH

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BACKGROUND

- **Increased exposure to synthetic cannabinoid compounds has been associated with Cannabinoid-Associated Coagulopathy (CAC), a severe bleeding disorder characterized by dysfunctional clotting mechanisms [1].**
- **Although cannabinoids have been implicated in anticoagulant pathway alteration [2], their direct effects on clot formation dynamics remain poorly defined.**
- **This study aims to develop and validate a point-of-care coagulation assay that can both measure quantify clot formation and identify functional changes associated with cannabinoid exposure.**

METHODS

- **Whole blood samples were obtained from healthy volunteers recruited through the Institute of Sports Sciences and Medicine at Florida State University. Participants' blood was drawn before and after exercising, which enabling comparison of clotting responses under varying physiological conditions.**
- **For the cassette strips (Fig. 1), 160 microliters (µL) of blood was mixed with 5 µL of saline solution, and 15 µL of different concentrations of Calcium Chloride (50–500 mM in 50 mM increments) for the assay, of which 100 µL was pipetted into the sampling paper.**
- **For the square strips (Fig. 4), an equal proportion of blood, saline, and calcium chloride was used , and 40 µL was pipetted directly in the center of the square. The cassette canister was a strngth of this model and helped provide more uniform results.**
- **Blood transport was recorded over 20-minute intervals and quantitatively analyzed using a custom written Python code (Fig. 3, 4).**

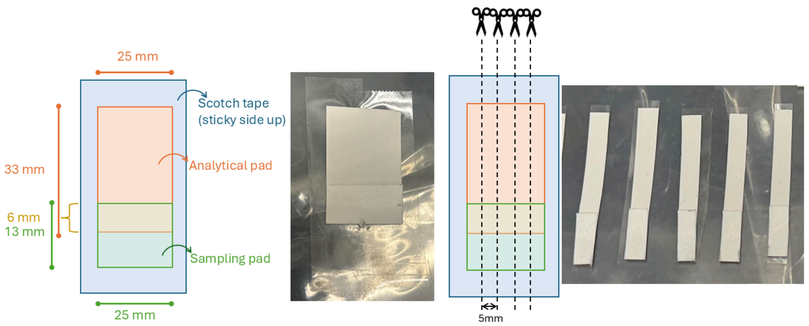

Fig. 1: Schematic and photographs of the lateral flow assay strip used to measure whole blood movement following calcium activation.

RESULTS

Fig. 2: Consistent coagulation behaviour and penetration length compared with previously published results [3] obtained using animal blood and across different LFA setups.

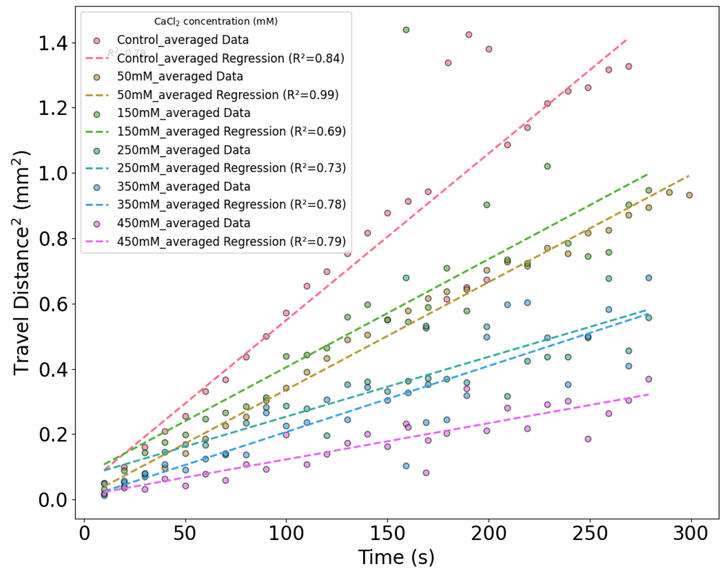

Fig. 3: Transport of whole blood sample in the developed lateral flow assay (LFA) microfluidic devices. Squared travel distance versus time for RBC components in whole blood samples measured across different assay configurations. Data points at each time point represent averages over all trials for each study condition, including the control group and varying $CaCl_2$ concentrations.

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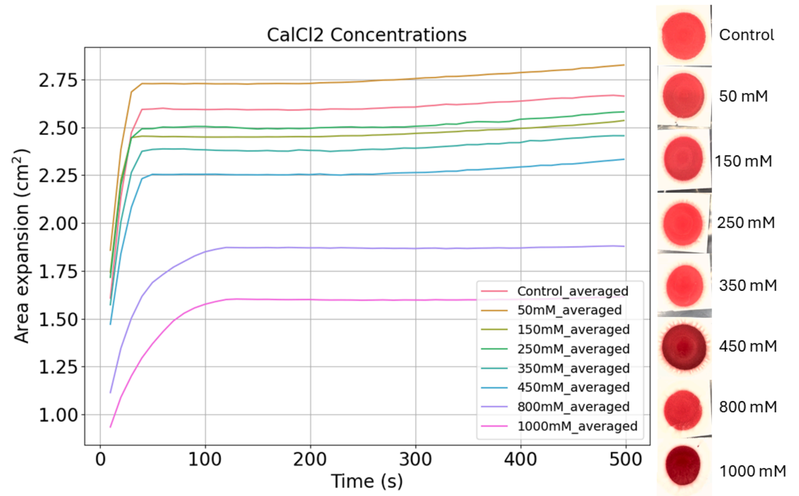

Fig. 4: Transport of whole blood sample in the developed novel circular dispersion assay (CDA). Plot shows the whole blood expansion area (cm2) as a function of time (s). Data points at each time point represent averages across all measured trials for each study condition (N=4), including the control group and varying $CaCl_2$ concentrations.

CONCLUSIONS

- **Our lateral flow and microfluidic assays have shown a concentration-dependent change in blood transport in response to increasing CaCl2 levels. Increasing $CaCl_2$ concentration resulted in a dose-dependent reduction in clot travel distance and expansion area. These findings are consistent with previously reported responses using animal models.**
- **The confirmation of the assay's sensitivity to calcium-dependent coagulation changes supports its utility as a clotting assessment tool, which is why we will continue our research for definitive results, while working to correct limitations such as measurement error created by uneven strips.**

FUTURE WORK

- **Our next step will introduce cannabinoids such as tetrahydrocannabinol (THC) and cannabidol (CBD) using this testing method to quanitfy the speed and strength of blood clot formation.**
- **Future studies will also incorporate cannabinoid and anticoagulant exposure to blood run through microfluidic chips simulating in-vitro arteries and hemodynamic conditions.**

REFERENCES

[1] G. M. Arepally and T. L. Ortel, "Bad weed: synthetic cannabinoid–associated coagulopathy," Blood, vol. 133, no. 9, pp. 902–905, Feb. 2019, doi: https://doi.org/10.1182/blood-2018-11-876839.
[2] M. A. Smythe, W. Wu, and C. L. Garwood, "Anticoagulant drug–drug interactions with cannabinoids: A systematic review," Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy, vol. 43, no. 12, pp. 1327–1338, Oct. 2023, doi: https://doi.org/10.1002/phar.2881.
[3] H. Li, D. Han, G. M. Pauletti, and A. J. Steckl, "Blood coagulation screening using a paper-based microfluidic lateral flow device," Lab Chip, vol. 14, no. 20, pp. 4035–4041, 2014, doi: https://doi.org/10.1039/c4lc00716f.