Developing an In Vitro Dynamic Test System for Thrombogenicity Evaluation of Medical Devices and Biomaterials

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Plain Language Synopsis

This project involves developing a reliable benchtop test system to assess the potential for biomaterials and medical devices to induce blood clot formation under flow conditions. This effort is meant to improve the design, development, and safety evaluation of blood-contacting medical devices, while reducing the need for acute animal studies.

Introduction

A reliable in vitro dynamic thrombogenicity test system to assess the potential for biomaterials and medical devices to induce thrombus formation is needed for improving the design, development, and safety evaluation of blood-contacting medical devices, while reducing the need for acute animal studies. In order to establish a robust in vitro thrombogenicity test, the key test conditions impacting thrombosis need to be understood and optimized to improve test reliability and sensitivity. In this study, we developed an in vitro blood flow loop test system and investigated the effects of blood species and blood temperature on thrombogenicity test results.

Materials and Methods

Blood Preparation

- Four types of animal blood were utilized in this study: abattoir porcine, donor porcine, donor ovine, and donor bovine.
- Fresh human blood was obtained from the NIH's Blood Donor Research Program.
- A static pre-test using latex tubes was used to determine the donor-specific heparin concentrations for the flow loops.

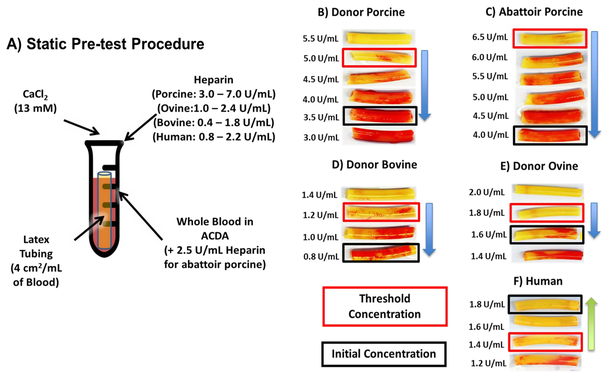

Figure 1. Static latex pre-test performed to predict donor-specific heparin concentrations. A) Uniform latex tubes were incubated in re-calcified blood with a series of heparin concentrations for 15 to 30 minutes at room temperature or 37°C. Example images of the pre-test results: B) Donor porcine blood, C) Abattoir porcine blood, D) Donor bovine blood, E) Donor ovine blood, and F) Fresh human blood. **Threshold Concentration** is defined as the minimum heparin concentration that resulted in a thrombus surface coverage ≤ 10% on latex tubes. **Initial Concentration** is the initial heparin concentration that was used in the pilot dynamic flow loop testing of the negative and positive controls to determine the final donor-specific concentration used for the rest of dynamic testing. The **Initial Concentration** was selected based on empirical data. The differences between the Initial Concentration and the Threshold Concentration is blood species dependent, as indicated by the arrows .

Dynamic Flow Loop

- The anticoagulated whole blood was recirculated at 200 mL/min through a polyvinyl chloride (PVC) tubing loop containing a test sample for 1 hour at room temperature (**Figures 2B and 2C**) or for 1 or 2 hours at 37°C (**Figure 2D**)
- One test material (12 cm length, introduced through the sidewall of the PVC tubing) was evaluated per loop (Figure 1A)
- Four test materials were evaluated:
  - Thrombo-resistant negative control: polytetrafluoroethylene (PTFE)
  - Thrombogenic positive control: latex
  - Silicone
  - High-density polyethylene (HDPE)
- For the pilot dynamic flow loop testing, if the **Initial Concentration** produced a thrombus surface area coverage of < 10% on the PTFE and > 50% on the Latex, then it was used as for the rest of dynamic testing. Otherwise, the heparin concentration was adjusted accordingly and the test repeated until the above thrombus criteria were met.

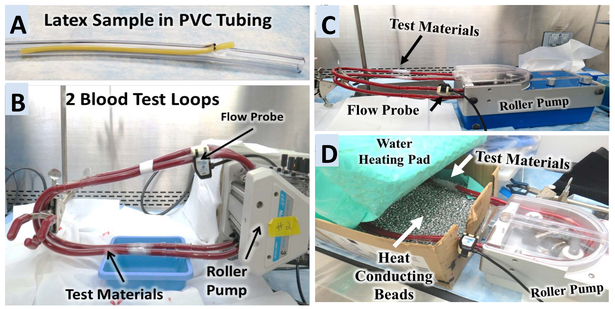

Figure 2. A) Representative image of how a test material is introduced into the flow loop. B) Experimental set-up of the dynamic flow loop system for the effects of blood species study. Dynamic flow loop set-up at C) room temperature and D) 37°C for the effects of temperature study.

Blood source	Anticoagulant at blood drawing	Blood storage time before use (hr)	Final heparin concentration for dynamic test (U/ml)
Human	ACDA	2 - 4	1.4 - 2.8
Donor ovine	ACDA	24 – 36	1.4 - 1.8
Donor bovine			0.6 - 1.4
Donor porcine			3.5 – 7.0
Abattoir porcine	ACDA plus heparin	2 – 12	3.0 - 4.5

Table 1. Final concentrations of heparin used in the dynamic flow loop system for each blood species. Final concentration was within ± 0.4 U/mL (Ovine, Bovine, and Human) or ± 1.0 U/mL (Porcine) of the initial concentration indicated by the pre-test.

Results and Discussion

Effects of Blood Species

- For many *in vitro* assays it is not feasible to use human donor blood due to the limited blood availability. Therefore, identifying appropriate and reliable substitutes for human blood (e.g., animal blood) is essential for developing robust test methods for device thrombogenicity assessments.
- For all blood types, latex had significantly greater thrombus surface coverage, thrombus weight, and platelet count reduction than all other biomaterials ($P < 0.01$).
- Silicone exhibited intermediate thrombogenicity only in donor ovine and bovine blood tests, with more thrombus surface coverage and platelet reduction than PTFE and HDPE ($P < 0.01$).

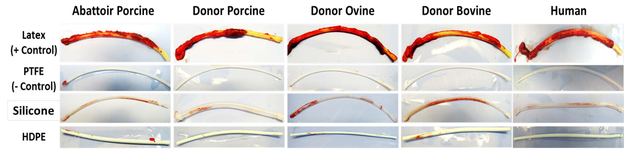

Figure 3. Representative images of thrombus formation on the test materials after 1 hour of circulation with different blood species. Blood flow direction was from right to left.

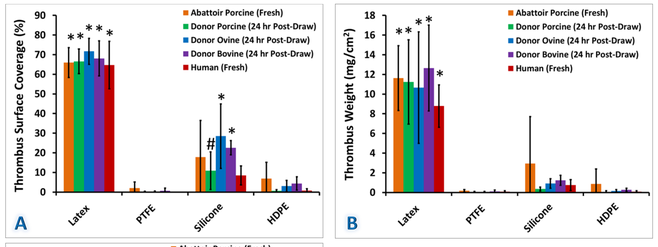

Figure 4. Comparison of human and animal blood sources. The effects on: A) thrombus surface coverage, B) normalized thrombus weight, and C) platelet count reduction.
(Bars show mean ± SD;
* P<0.01 or # P<0.05 vs PTFE and HDPE,
α P<0.05 vs PTFE,
n=5 for animal blood and n=6 for human blood)

Effects of Blood Species

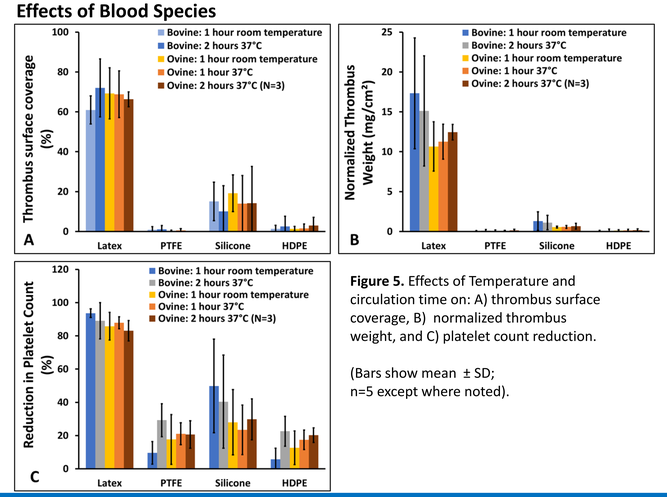

Figure 5. Effects of Temperature and circulation time on: A) thrombus surface coverage, B) normalized thrombus weight, and C) platelet count reduction.

(Bars show mean ± SD;
n=5 except where noted).

- Testing at room temperature eliminates the need for cumbersome heating equipment and simplifies the test system (**Figure 2C** vs **Figure 2D**). However, to establish clinical relevancy, test results need to be compared with those obtained at a physiological temperature of 37°C.
- Only ovine and bovine blood were used for this study.
- For ovine blood:
  - There was no significant difference in the relative thrombogenicity results between any of the test conditions ($p > 0.05$).
  - For three out of the five ovine donors, the heparin concentration had to be decreased by approximately 0.2 U/ml at 37°C to produce acceptable amounts of thrombus deposition on the positive and negative controls when compared to the room temperature tests.
- For Bovine blood:
  - There was no significant difference in the relative thrombogenicity results between the 1 hour blood circulation at room temperature and 2 hour blood circulation at 37°C ($p > 0.05$).
  - At 37°C, the circulation time had to be increased from one to two hours and the heparin concentration decreased by approximately 0.2 U/mL to produce the acceptable amount of thrombus deposition on the positive control when compared to the room temperature tests.

Conclusion

- All animal blood sources tested in this study (from 2 to 36 hours post-draw) can effectively differentiate between thrombogenic and thrombo-resistant materials and may be suitable alternatives to fresh human blood for dynamic thrombogenicity testing.
- Donor-specific anticoagulation levels and thrombosis marker sensitivity were dependent on the animal blood source.
- The utilization of donor ovine and donor bovine blood in this system may provide better differentiation between thromboresistant materials and materials with intermediate thrombogenicity. Additional testing is needed to validate these results and determine their clinical relevance.
- Testing at 37°C for 1 or 2 hours does not appear to significantly increase the sensitivity of the *in vitro* flow loop thrombogenicity assay.
- Further research, including interlaboratory studies, are needed to validate the test methodology prior to the establishment of a consensus standard.

Disclosure: The mention of commercial products and/or manufacturers does not imply endorsement by the FDA or the U.S. Department of Health and Human Services.

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