Designing an interlaboratory study to develop standardized best practices for performing in vitro dynamic thrombogenicity testing of medical devices and materials

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Plain Language Synopsis

We designed an interlaboratory study to develop standardized best practices for performing benchtop testing to assess the potentials of medical devices and materials to induce blood clot formation under clinically relevant flow conditions. This study will evaluate the key test parameters on the reliability and reproducibility of dynamic test systems.

Introduction

Thrombosis (blood clot formation) is a common issue for blood-contacting medical devices. Currently, there are no standardized or widely accepted in vitro test methods to evaluate device thrombogenicity under clinically-relevant flow conditions. We aim to develop best practices for performing in vitro flow loop tests that can be used to assess thrombogenic potentials to improve the design and safety evaluation of various blood-contacting medical devices and materials, while reducing the need for animal studies.

Materials and Methods

- In order to design an appropriate interlaboratory study, the FDA performed several preliminary studies to aid in the development of the test protocol.

Blood Preparation

- Four types of animal blood were utilized in this study: porcine, ovine, and bovine blood from live animal donors and abattoir porcine blood.
- Fresh Human blood was obtained from the National Institutes of Health (NIH)/ Research Blood Donor Program.
- The donor specific heparin concentration was determined by a static latex pre-test.

Static pre-test for donor-specific heparin concentration

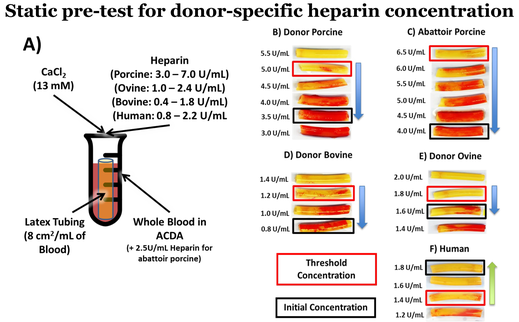

Figure 1. Static latex pre-test performed to predict donor-specific heparin concentrations. A) Uniform latex tubes were incubated in re-calcified blood with a series of heparin concentrations for 15 to 30 minutes at room temperature or 37°C. Example images of the pre-test results: B) Donor porcine blood, C) Abattoir porcine blood, D) Donor bovine blood, E) Donor ovine blood, and F) Fresh human blood.

- Threshold Concentration: The minimum heparin concentration that resulted in a thrombus surface coverage ≤ 10% was selected as the threshold concentration.
- Initial Concentration: The initial heparin concentration utilized in the dynamic flow loop below.

Materials and Methods (Cont.)

Dynamic Flow Loop

- Whole blood heparinized to a donor-specific concentration was circulated through a 6.4 mm inner diameter (ID) polyvinyl chloride tubing loop containing a test sample (3.2 mm diameter, 12 cm length,) at 200 ml/min (**Figure 2A**, **2B**, and **2C**).
- Materials investigated included a positive control latex, a negative control polytetrafluoroethylene (PTFE), silicone, High-density polyethylene (HDPE), natural rubber (BUNA) and 3D printed nylon.
- The effects of several key factors including blood temperature, incubation time, and blood species (human, pig, cow, and sheep) were investigated.
- To accommodate more device sizes, a larger flow loop system using 9.5 mm ID tubing was also developed and evaluated. (**Figure 2D**)
- The percent of thrombus surface coverage and platelet count reduction were measured to characterize the thrombogenicity of the test samples.

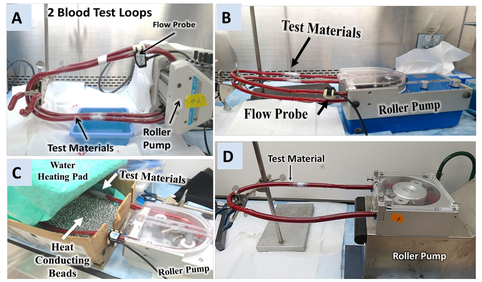

Figure 2. Experimental setup of the 6.4 mm ID dynamic flow loop system for: A) the effect of blood species study, and the effect of temperature study at B) room temperature (RT) and C) 37°C. D) Experimental setup of the 9.5 mm ID tubing dynamic flow loop system.

Results and Discussion

Effect of Blood Species (6.4 mm ID Tubing Test System):

- For many in vitro test labs, it is not feasible to use human donor blood due to its limited availability. Therefore, identifying appropriate and reliable substitutes (e.g. animal blood) for human blood is essential for developing robust assessments of device thrombogenicity.
- The similarity in test results (**Figure 3**) between different blood species suggests that multiple animal blood sources (particularly donor ovine and bovine blood) may be suitable alternatives to fresh human blood for dynamic thrombogenicity testing.

Effect of Blood Temperature (6.4 mm ID Test System):

- Testing at room temperature (RT) eliminates the need for cumbersome heating equipment and simplifies the test system (**Figure 2B** vs **Figure 2C**).
- However, to establish clinical relevancy, test results need to be compared with those obtained at a physiological temperature of 37°C .
- Test results with ovine and bovine blood demonstrate that the test system can differentiate the relative thrombogenicity of different materials at both 37 °C and RT (**Figure 4**).
- For bovine blood, testing at RT may accelerate in vitro thrombus formation and shorten the test time from 2 hours to 1 hour.

Results and Discussion (Cont.)

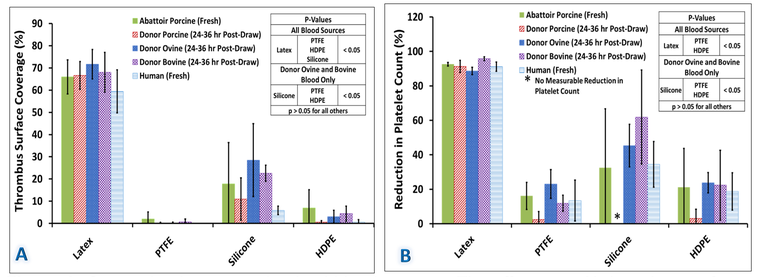

Figure 3. Comparing human and animal blood sources. The effects on: A) thrombus surface coverage and B) platelet count reduction. (mean ± SD; n=5 for animal blood and n=6 for human blood)

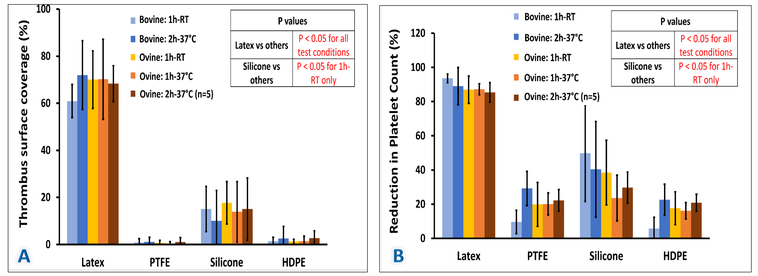

Figure 4. Effects of temperature and circulation time on: A) thrombus surface coverage and B) platelet count reduction. n = 6 except where noted.

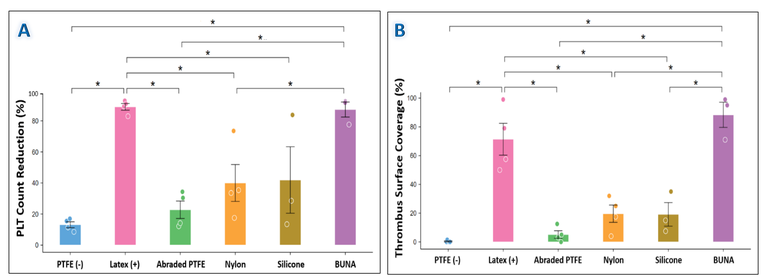

Figure 5. Comparison of different materials in the large diameter (9.5mm ID) test system: A) Platelet (PLT) count reduction and **B)** Thrombus surface coverage on test material after 1 hour blood recirculation at room temperature. Data are shown as a mean ± standard error. n=4 for all materials, except BUNA and silicone (n=3). *p<0.05.

Large Diameter (9.5 mm ID) In Vitro Flow Loop Test System

- Cardiopulmonary bypass roller pumps were utilized to accommodate the larger flow loop tubing with a flow rate of 527 ± 2 mL/min (**Figure 2D**).
- The study was performed at room temperature with bovine blood.
- The system was able to effectively differentiate the known thrombogenic materials (latex, BUNA) from the historically thromboresistant PTFE ($p<0.05$, **Figure 5**).
- The 3D printed nylon and silicone test samples were shown to have an intermediate thrombogenicity level with significantly less thrombus surface coverage and platelet count reduction than latex and BUNA, but significantly more than the unabraded PTFE ($p<0.05$).

Interlaboratory Study Design

- For all test conditions investigated in our laboratory, the flow loop systems were able to effectively differentiate between thrombogenic and thrombo-resistant materials when appropriate control materials and donor-specific anticoagulation levels were used.
- However, these results need to be validated in an interlaboratory study prior to standardization.
- Participating labs will perform a matrix of thrombogenicity tests on a set of test samples under a range of pre-described test conditions (**Table 1**) to determine which test conditions will allow the test system to better differentiate test samples with different thrombogenic potentials.
- There are currently 9 labs (including the FDA) participating in this study.

Table 1. Draft Round-robin Test Matrix

Parameters	Options
Test Materials	Total of 6-8 test articles different in material/geometry/surface roughness
Blood species	Bovine or ovine
Blood storage	Fresh or shipped overnight
Flow loop tubing ID	6.4 mm or 9.5 mm
Temperature	Room temperature or 37 °C
Test duration	1 hr, 2 hr, or 4 hr
Flow rate	200 ml/min for 6.4mm ID tubing loop, 500 ml/min for 9.5 mm ID loop
Donor-specific heparin concentration estimation	Static pre-test with latex tube, activated clotting time (ACT), or other coagulation measurements

*n=5 replicates preferred (at least n=3) using different blood pools for each of the above test conditions

Next Steps

A new ASTM standard work item was established by FDA to provide a platform for conducting the collaborative interlaboratory study. A pilot study based on FDA's draft protocol was initiated in January 2023 with multiple participating laboratories. The formal interlaboratory study is expected to start in late Spring 2023 and the outcomes will help establish standardized best practices for performing dynamic in vitro thrombogenicity testing of blood-contacting devices and materials.

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