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 thrombus formation, with the goal of improving the safety of blood-contacting medical devices, while reducing the need for acute animal studies.

Introduction

A reliable in vitro thrombogenicity test system is necessary to assess the potential for biomaterials and medical devices to induce thrombus formation, as this is a key factor in the development of many medical devices. Currently, the gold standard for testing thrombogenicity involves the use of acute animal models, which can be both time-consuming and costly. Developing an in vitro test system could significantly reduce the need for acute animal studies.

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.

Blood 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 (Figure 2B and 2C) or for 1 or 2 hours at 37°C (Figure 2D).
- One test material (1 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 coverage of < 10% on the PTFE and > 50% on the Latex, then it was used as the test system. Otherwise, the heparin concentration was adjusted accordingly and the test was repeated until a thrombus surface coverage of 50% was achieved.

Uniform latex tubes were incubated in re-calcified blood with a series of heparin concentrations (0.6 - 1.4 U/mL) 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.

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.

Dynamic Flow Loop

- 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).

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 relevance, test results need to be validated 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 controls while maintaining 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 while compared to the room temperature tests.

Conclusion

- All animal blood sources tested in this study (from 2 to 3 hours post-draw) can effectively differentiate between thrombogenic and thrombo-resistent 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 thrombogenic 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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