Thrombogenicity Evaluation of Medical Device Materials with an In Vitro Flow Loop System Under Varying Flow Conditions

Megan Jamiolkowski, Madelyn Golding, Zachary Goddard, Richard Malinauskas, Qijin Lu
US Food and Drug Administration

Statement of Purpose: Pre-clinical thrombogenicity evaluation is important to ensure the safety of blood-contacting devices, and is often necessary to support regulatory approval/clearance of new devices. To reduce animal use and overcome certain limitations inherent to in vivo studies, various in vitro flow loop systems have been developed for hemocompatibility testing of medical devices and biomaterials. However, due to the lack of standardized test protocols, and the inherent variability associated with donor blood, the results produced by these systems tend to differ greatly among studies. We aim to reduce these inconsistencies by using a static anticoagulation pre-test and a dynamic flow loop to develop a simple in vitro method to evaluate the thrombogenicity of medical devices and biomaterials at clinically relevant flow rates.

Methods: Ovine blood from live donors was drawn into containers with Anticoagulant Citrate Dextrose Solution A (ACDA) and used within 36 hrs. Immediately before starting each dynamic flow test, the blood was re-calcified (final $CaCl_2$ concentration of 13 mM) and heparinized to a donor-specific concentration. The heparin concentration used for each blood donor was determined using a static pre-test in which uniform latex tubes were incubated in re-calcified blood, under a series of heparin concentrations (1.0 to 2.4 U/mL in 0.2 U/mL increments), for 30 min at room temperature. The minimum heparin level that resulted in a thrombus surface coverage ≤ 10% on the latex was selected as the threshold concentration. The initial concentration used to start the flow loop testing was selected to be 0.2 U/mL less than the threshold concentration (e.g. threshold concentration = 1.8 U/mL, then initial concentration = 1.6 U/mL). Whole blood (26 mL) was recirculated at room temperature through 6.4 mm ID polyvinyl chloride tubing loops for 1 hr at three flow rates (100, 200, and 300 mL/min) using occlusion-controlled roller pumps. These flow rates correspond to venous shear rates between 60-200 s^{-1}. Each loop contained a single test article (length: 12 cm; diameters: 2.1 - 3.2 mm) that was introduced into the lumen through the tubing wall. Four materials were tested: a negative control polytetrafluoroethylene (PTFE), positive control latex, non-medical grade silicone (NM-Sil), and a medical grade silicone. For blood from each donor, the negative control PTFE and the positive latex were first tested in separate flow loops at 200 mL/min to verify that the selected pre-test heparin concentration would provide appropriate anticoagulation. If the thrombus surface coverage produced on the PTFE was ≤ 10% and on the latex was ≥ 60%, the initial concentration was used for the remainder of the experiments. If the deposition was > 10% on the PTFE or < 60% on the latex, the heparin concentration was increased or decreased by 0.2 U/mL, respectively. The % thrombus surface coverage, thrombus weight, platelet count reduction, and hemolysis were measured.

Results: Per the static pre-test, the final heparin concentrations selected for the blood flow loops ranged from 1.0 to 1.6 U/mL for blood from 4 donors. Preliminary results (Figure 1) indicate that a flow rate of 200 mL/min produced the least variance within the data. For this flow rate, latex had significantly more thrombus deposition and a greater platelet count reduction compared to the other test materials ($P < 0.01$). When the blood was circulated at 300 mL/min, the variance increased and the thrombogenicity endpoints were not statistically different for the materials tested ($P>0.05$). At a flow rate of 100 mL/min, latex had significantly more thrombus surface coverage only compared to PTFE ($P < 0.03$), but a greater thrombus weight than all of the other materials ($P < 0.01$). However, there was no significant difference in the platelet count reduction ($P > 0.05$) at 100 mL/min. The hemolysis generated by the system was greatest at 300 mL/min with significantly more plasma free hemoglobin produced than at 100 and 200 mL/min ($P <0.05$).

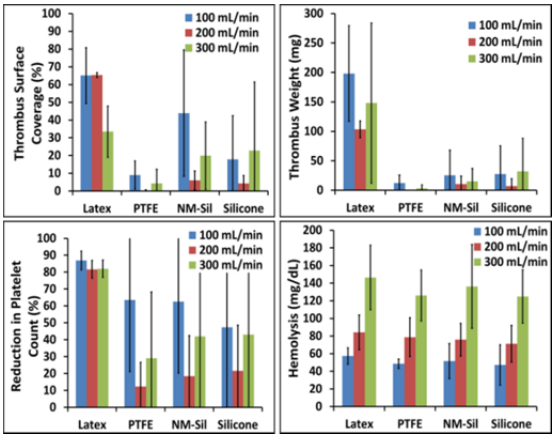

Figure 1. Effect of flow rate on thrombus formation, platelet reduction, and hemolysis. N=4 donors.

Conclusions: The data suggest that flow rate substantially impacts the thrombogenicity differentiation of biomaterials. For the current system, 200 mL/min provided the best distinction between the materials. The variability in thrombosis for in vitro blood flow loops may be decreased by using controlled donor-specific heparin concentrations and system-specific flow rates. The higher hemolysis levels produced at 300 mL/min may contribute to non-material mediated coagulation and therefore a flow rate at or above this level may not be appropriate for examining device thrombogenicity in this system.