Partial Thromboplastin Time and Prothrombin Time for the Hemocompatibility Evaluation of Biomaterials

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Statement of Purpose: Currently, there are no widely accepted in vitro test methods to assess thrombogenicity of biomaterials for applications in blood-contacting medical devices. Partial thromboplastin time (PTT) and prothrombin time (PT) assays are commonly used in clinical settings to monitor anticoagulation therapy or to screen for coagulation-factor deficiencies in patients, but these tests have also been adapted by the medical device industry to evaluate blood compatibility of biomaterials using animal and human blood with normal coagulation factors. In the hemocompatibility testing standard ISO10993-4:2009, PTT and PT are among the in vitro coagulation test methods listed for assessing implant devices. However, these tests lack sufficient validation or analysis of test parameters that may affect the results, and their usability for device/biomaterial thrombogenicity characterization has not been well-established. The goals of this project are to evaluate whether PTT and PT assays are capable of differentiating materials with different thrombogenic potentials, and to determine the effects of the test parameters on the results.

Methods: The test parameters and conditions investigated are summarized in Table 1. Fresh human blood was obtained from the National Institutes of Health Blood Donor Research Program, and pig blood was obtained from a local slaughterhouse. PTT reagent (rabbit brain cephalin without additional activators) and PT reagent (tissue factor and Ca^{2+} ions) were purchased from BioData (Horsham, PA). Whole blood was centrifuged at 3000g for 10 min to obtain platelet poor plasma, which was used fresh or frozen at -80 ˚C for later use. To prepare test samples for both assays, test materials (4 cm^2) were incubated in 1 ml of plasma at 37 ˚C for various times (15, 30 and 60 min), and then the samples were chilled and kept on ice. PTT and PT clotting times were measured using a 4-channel photo-optical anticoagulation analyzer (ThromboScreen 400C from Pacific Hemostasis) following the typical test procedures provided by the reagent and instrument manufacturers.

Table 1: Test parameters and conditions

Parameters	Conditions
Blood species	Human, Porcine
Anticoagulant	Sodium Citrate, Acid Citrate Dextrose Solution A (ACDA)
Plasma storage	Fresh, Frozen
Test materials	Nylon, HDPE, 316L Stainless Steel, Buna-N rubber, Glass, Latex
Incubation time	15, 30, 60 minutes

Results and Discussion: PTT and PT test results (normalized to the clotting times from their corresponding plasma controls) with sodium citrate anticoagulation are shown in Figure 1 below.

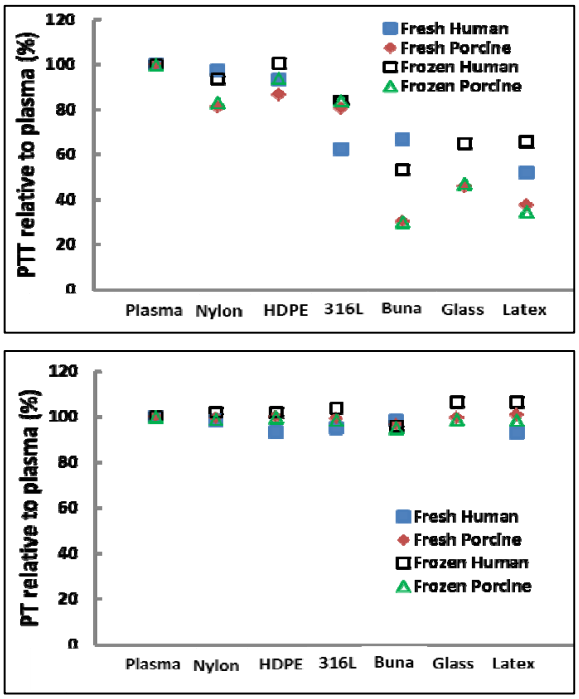

Figure 1 Normalized PTT (top) and PT (bottom) results. 30 min incubation, n=2 to 5 for each material.

Generally, absolute PTT clotting times using human plasma were almost twice as long compared to porcine plasma. When exposed to the positive control materials (Buna-N, Glass, Latex), porcine plasma also clotted faster compared to human plasma based on the normalized PTT values. Regardless of the anticoagulant used, plasma storage method, or incubation time (Table 1), PTT values exhibited the same trend: Plasma = Nylon = HDPE > 316L Stainless Steel > Buna, Glass, Latex, indicating that the PTT assay may be useful in differentiating biomaterials with different thrombogenic tendencies. In contrast, PT values for all test materials were almost the same (around 100% relative to their corresponding plasma controls) for all the test conditions (Table 1). As suggested by the much shorter clotting times for the PT test compared to the PTT test (e.g. PT 10-15 sec vs. PTT 100-200 sec for plasma controls), it is possible that the strong coagulation activator (tissue factor plus Ca^{2+} ions) in the PT reagent might have masked any activation effects of the test materials on the plasma coagulation.

Conclusions: Our preliminary results demonstrate that the PTT assay has the potential to differentiate between materials with different thrombogenic tendencies, while the PT assay did not show any difference between any of the test materials. Further research is needed to fully validate the PTT assay for in vitro material thrombogenicity testing and to establish a pass/fail criterion that has clinical relevance.