The purpose of this test method is to predict the thrombogenic potential of materials used in the manufacture of blood-contacting medical devices. By evaluating surface-induced platelet activation and subsequent adherence to a material, as measured by depletion of platelets and leukocytes from blood, a material’s potential for thrombus formation can be assessed. If a significant decrease in platelets and/or leukocytes is observed in whole blood when compared to a appropriate control, the tested device may be considered to be at risk of inducing an in-vivo thrombogenic response.

The present standard for the testing of platelet and leukocyte response to cardiovascular materials, ASTM F2888-13 (Standard Test Method for Platelet Leukocyte Count—An In-Vitro Measure for Hemocompatibility Assessment of Cardiovascular Materials), mandates the use of several reference materials in the presence of blood anticoagulated with sodium citrate. This study was designed to assess a potential improvement in the assay design using an alternate anticoagulation method. Current studies on changing this methodology are under investigation at the FDA also by using blood anticoagulated with 2-3 IU/mL of heparin®. For this study, the effects of several biomaterials were evaluated when exposed to blood anticoagulated with sodium citrate and, concurrently, an alternatively less potent anticoagulant protocol, heparin at 1 IU/mL. We believe this alternative test method allows for a more sensitive test that can predict potential thrombogenicity outcomes of medical device materials with a better overall assay performance.

Materials and Methods

The positive controls recommended in the ASTM F2888-13 method utilized black rubber, natural rubber latex, and crushed borosilicate glass which were prepared in triplicate for each assay (4 replicate assays, n=3 per samples per anticoagulant type). The blank consisted of an empty polypropylene tube and the negative control consisted of medical-grade high density polyethylene (HDPE).

Fresh human blood was drawn into blood collection tubes with either 3.2% sodium citrate or sodium heparin at a final concentration of 1 IU/mL. A complete blood count (CBC) was obtained to qualify the blood samples and ensure that the platelet and leukocyte counts fell within a normal range (platelets 116 – 329 k/μL and leukocytes 3.4 – 8.4 k/μL) and then the blood samples were stored on ice until use. Each control was exposed to blood at ratios of 12 cm²/mL (HDPE, latex) or 1 gram/mL (black rubber, glass) and incubated at 37.2°C for 1 hour5 minutes with continuous agitation. A minimum of 1 mL of blood was used for each sample.

Following exposure, the tubes were removed from incubation and immediately placed on ice. Ethylenediaminetetraacetic acid (EDTA) was added to each tube to stop further clotting activity, and the blood was then decanted from each article and placed into a new, pre-chilled polypropylene tube. All biomaterials were removed from the tubes and articles with a visible clot were placed in weigh boats. The samples were again subjected to a CBC (Hemavet HV950) to determine platelet and leukocyte counts. The blood counts from each of the biomaterials were compared per anticoagulant and the mean percentage of blank results were compared by use of one-way ANOVA with a post-hoc Tukey analysis of means.

Introduction

The selection of the potent anticoagulant sodium citrate used in ISO 10993-4 biological assessment of medical devices has a critical impact on the performance of this ASTM test. Evaluation of the feasibility of using a less effective anticoagulant at a reduced concentration, heparin (1 IU/mL), as performed in this study demonstrates that such a protocol has the potential to significantly improve the predictive power of the ASTM Platelet and Leukocyte Assay. Devices or materials that have been screened in the standard ASTM assay using sodium citrate as an anticoagulant may frequently show false negatives (appear non-thrombogenic) than would be seen if they were re-screened in blood anticoagulated with low level heparin.

This study confirms the potential improvement in sensitivity of the assay when the anticoagulant conditions used for blood preparation were modified to a lower threshold for platelet activation and subsequent clot formation. Early, rapid, and cost-effective screening of materials in this assay may allow for reduction in later-stage development product failure due to material-mediated thrombogenicity. The capability of black rubber to produce consistent positive results in the heparin anticoagulated assay (and not in a citrated anticoagulated assay) supports the use of heparin at levels of 2-3 IU/mL and even at levels as low as 1 IU/mL (possibly even less) as the anticoagulant of choice for this assay. We recommend that further investigation be performed on test devices and materials with known clinical histories using the heparin anticoagulated blood. Presently a study incorporating the Abbott Response Electrophysiology Catheter is underway at APS and has produced responses similar to those seen in both the in vitro blood loop and in vivo NAVI model.

Summary

References: