Introduction

ISO 10993-4 in vivo thrombogenicity testing is required for regulatory approval of all blood-contacting medical devices and is often a key part of submission packages. Given the current state of in vivo thrombogenicity assays, the industry needs to create robust and reproducible assay design including in vivo models. Saint Jude Medical has partnered with American Preclinical Services (APS) to advance the science of the in vivo blood loop. This assay integrates freshly-harvested ovine blood containing minimal thrombin in a closed-pump loop. To confirm the reproducibility of this assay, control materials were identified that elicited either a positive or a negative thrombogenic response. For a direct comparison of the in vivo blood loop assay to the traditional in vivo nonanticoagulated venous implant (NAVI) assay, seven sheep were used as blood donors for the loop and then as subjects for a NAVI assay. In each assay - loop or NAVI - three study animals were used: the positive and negative control groups were run, approved cases. The resulting data show that this in vivo assay performs similarly to the in vivo NAVI assay. This in vivo blood loop model has the potential to predict a material's in vivo thrombogenicity, can substantially de-risk the materials or coating selection process and has been successfully used in lieu of in vivo models currently in use.

In-vitro Methods

Blood sourcing: A total of seventeen individual animals were used over the course of the project as repeat blood donors for the in vivo blood loop. Seven additional animals were used for the post-exposure to head comparison between in vivo and in vitro models and these animals were used only once. After the in vivo portion of the assay was completed, fresh ovine blood (<500 mL) was collected only less than two weeks every healthy donor sheep. During venipuncture and collection, porcine heparin was added to a final concentration of 1 UI/kg blood in the procedure for all sheep. After completion of all the NAVI results. For each study article, the median thrombogenicity scores were the same in two different assays, being 0, 1, and 5 for the negative control, the marketed, and the positive control, respectively. These data suggest that in vivo assay performs similarly to the in vivo NAVI assay. This in vivo blood loop model has the potential to predict a material's in vivo thrombogenicity, can substantially de-risk the materials or coating selection process and has been successfully used in lieu of in vivo models currently in use.

Results

Scoring and Observations: Neutral controls: Thrombus formation was evaluated at the end of the four hour indwelling period using similar techniques for the positive and negative assay as well as the seven blood loop assays in the head-to-head comparisons. Samples using standard positive and negative control conditions were collected for post-processing and scoring. The positive control was used as an uncoated polyurethane tubing of the same length as the negative control that was added to saline to increase the potential for thrombus formation. Both positive and negative control tube were purchased from The Joint Scientific. Each control was inserted -10 cm to the loop. The proximal portion of the tubing was then removed to prevent intragraft blood loss and the insert was sealed with PanSeal™ to prevent any leaking. The two remaining loops were used to assess intravascular thrombus formation from a wide range of client-generated materials, or in the case of the seven animals used for the head-to-head comparisons, the positive control. The test articles were St. Jude Medial Release™ Response™. Electrophysiology catheters. The blood was then introduced into each loop (total volume ~125 mL) without more than 26 minutes after the completion of the blood draw. Air was removed, the loop was placed into a peristaltic pump flowing at 0.8 mL/min with the loop partially submerged in a heated water bath (37 °C). After 4 hours, the pump was stopped, the loop was removed, opened and the blood sampled. The device sites for the test/control catheters in the loops were identified and cut on 3-4 cm after each one of the deployment loop. The tubing segments (15-20 cm) were cut longitudinally with a scalpel while care was taken to minimize the disturbance of any adherent thrombus (if any) with the tubing. The two segments were kept open and the study article was rinsed in situ with normal saline and then gently lifted out of the tubing. Any blood or thrombus inside the lumen of the catheter is rinsed with saline and the prepared study article was then placed on an absorbent pad for digital imaging and measurement of total surface thrombus.

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Discussion

We have developed an in vivo model which has proven suitable for the assessment of thrombogenicity for blood contacting medical devices. This in vitro blood loop model uses minimally heparinized ovine blood in a 125 mL loop that is circulated by a peristaltic pump at a flow rate comparable with large vein flow rate in adult sheep. The method was standardized using positive and negative control materials that simulate medical device catheters which were designed to have a permanent or maximal thrombus accumulation. Response for both the positive and negative controls have been tracked over an 13 month period. The thrombogenicity scoring used by this method was performed by direct observation of thrombus formation using a process identical to that used in the in vivo NAVI model currently accepted by ISO 10993-4 guidelines. The blood loop model has demonstrated reproducible and robustness for the qualitative assessment of thrombus formation and should be considered a replacement for the in vivo NAVI model. The value of any thrombogenicity assessment assay lies in its ability to accurately predict the apparent risk to the patient population which may be treated with the device being evaluated. There is widespread agreement within the medical device manufacturing and testing industry that the NAVI model is not ideal to accurately predict these risks whereby the performance of a test article is simply compared to a legally or commercially available control. The thrombogenicity scoring used by this method is performed by direct observation of thrombus formation using a process identical to that used in the in vivo NAVI model currently accepted by ISO 10993-4 guidelines. The blood loop model has demonstrated reproducible and robustness for the qualitative assessment of thrombus formation and should be considered a replacement for the in vivo NAVI model. The value of any thrombogenicity assessment assay lies in its ability to accurately predict the apparent risk to the patient population which may be treated with the device being evaluated. There is widespread agreement within the medical device manufacturing and testing industry that the NAVI model is not ideal to accurately predict these risks whereby the performance of a test article is simply compared to a legally or commercially available control. The thrombogenicity scoring used by this method is performed by direct observation of thrombus formation using a process identical to that used in the in vivo NAVI model currently accepted by ISO 10993-4 guidelines. The blood loop model has demonstrated reproducible and robustness for the qualitative assessment of thrombus formation and should be considered a replacement for the in vivo NAVI model.

The results of the full clinical trials with this in vivo blood loop method can be regarded as “salient” and provide insight into the scoring associated with a thrombogenic threshold of concern.

Adverse reactions were negligible for medical devices which have definitive clinical history and demonstrate suitable thrombogenic score in this in vitro model will be valuable in further defining the thrombogenic threshold of concern.