INTRODUCTION

Ventricular Assist Devices (VADs) have become a means to both preserve and prolong the lives of patients with heart failure. By providing circulatory support, VADs lessen the load of the heart. Compared to traditional medical management of heart failure, VADs have a higher patient survival rate [1]. VADs are used as both a bridge to transport as well as form of destination therapy for patients requiring long-term or permanent circulatory support.

While VADs do increase survival rate for heart failure patients, survival percentage of patients using this type of mechanical circulatory support for permanent or long-term support decreases significantly the longer the patient is reliant upon the device. In a study by Lietz K. et al., it was seen that the percent of patients still alive one-month post VAD implantation was 86.1%, but only 30.9% of patients with VADs were still alive twenty four months after implantation [2].

VADs do pose risks to patients since this implanted medical device can be thrombogenic. Blood cells in contact with the VAD can become activated, forming cell aggregates, which can lead to thrombosis, as well as other complications. To improve patient survival rates, many studies focus on improving the biocompatibility of the VAD material in contact with the blood.

Platelet activation, since it is the initial step to thrombus formation is often used as a way to quantify material biocompatibility. A good indicator as to whether or not a material will cause the blood to clot, low platelet activation in blood samples corresponds to a biocompatible material. Material coatings or surface modifications have shown an increase in the biocompatibility of materials, as the coated materials display lower levels of platelet activation. A previous study was done by Ye et al. to assess the biocompatibility of a siloxane functionalized phosphorylcholine polymer coating. The study revealed the incorporation of the coating resulted in decreased platelet deposition on the coated titanium alloy surface as well as lower platelet activation levels than the unmodified titanium alloy. The results of this study suggests that an incorporation of a siloxane functionalized phosphorylcholine polymer coating onto a titanium alloy can improve the material’s biocompatibility [3].

OBJECTIVE

The goal of this study is to evaluate whether the incorporation of a siloxane functionalized phosphorylcholine polymer (MPCMPSi) coating onto a titanium alloy (Ti₆Al₄V) will improve the biocompatibility of the titanium alloy. Using flow cytometry, the study will compare platelet activation levels of blood samples exposed to a coated titanium alloy, as well as a non-coated titanium to test and see if the titanium surface modification will result in lower levels of platelet activation.

SUCCESS CRITERIA

The MPCMPSi coating can be considered successful in increasing the biocompatibility of the titanium alloy if the coated titanium shows platelet activation levels in the 8 – 10% range.

METHOD

The study evaluated three different materials: titanium alloy (Ti₆Al₄V), titanium alloy coated with a siloxane functionalized phosphorylcholine polymer (MPCMPSi), and alumina (Al₂O₃). Alumina was chosen as a positive control. Samples were polished and cut to 1 x 2.5cm dimensions. Titanium samples were coated with MPCMPSi using a simple silanization technique after the surfaces were passivated with a 35% nitric acid for 1 hr [3]. All materials were sterilized by alternating washing between unadulterated acetone and ethanol. Following cleansing, samples were stored in 70% ethanol.

Whole ovine blood was collected by jugular venipuncture using an 18 gauge 1 ½” needle directly into a syringe after discarding the first 3 ml. The blood was withdrawn into a 60 ml syringe which contained 6 ml of 0.1 M sodium citrate. Three sterilized samples (uncoated titanium, coated titanium, and alumina) were fixed in three separate tubes, and each tube was subsequently filled with 5.2 ml of citrated whole ovine blood. A tube with no material was also included to provide a baseline of how activated the blood sample was. After submersion, the samples were incubated at 37°C and rocked for 45 min. Once incubation time was complete, blood samples were prepared for flow cytometry using a platelet activation assay.

Blood (5 µl) was transferred from the incubation tubes into 5ml round bottom polystyrene tubes with 35 µl of Tyrode’s buffer with BSA and citrate, 5 µl CAP2A (Serotec, USA) at a concentration of 60 µg/ml. The samples were incubated for 20 minutes, and then washed with 1 ml of Tyrode’s buffer with BSA and citrate. After the first wash, 5 µl of MCA 2418 (Serotec, USA) at a concentration of 25 µg/ml was added to each sample. The samples were incubated for a second time, again, for 20 minutes and washed with a final time with 1 ml of Tyrode’s buffer with BSA and citrate. Samples were then fixed with 500 ml of 1% Paraformaldehyde.

After fixation, samples were run through a flow cytometer to count the number of fluorescently labeled platelets in each sample.

RESULTS

After three trials, blood exposed to no material averaged a 17.34% activation. Uncoated titanium displayed a 14.48% activation level, while the coated titanium displayed an average platelet activation level of 16.88% activation. Alumina showed a 15.91% activation level.
Figure 1. Percent Gated for each of the three completed trials. In trials 1 and 2, the blank sample displayed the lowest levels of platelet activation compared to any of the blood samples exposed to any of the other materials, as expected. For trial 3, all samples displayed unusually high levels of platelet activation, all in at least the mildly activated range. The blank sample displayed platelet activation levels in the highly activated range (>30%).

DISCUSSION

While past studies suggest that surface modifications have been successful in increasing the biocompatibility of materials, the data from the flow cytometry analysis did not show any significant decrease in platelet activation levels. The averages for each of the samples actually showed the coated titanium sample displaying slightly higher levels of platelet activation. In only one trial (trial 3) did the coated titanium display lower levels of platelet activation; however, all samples in this trial were at least moderately activated, including the blank sample with no material exposure.

Since only three completed trials were accomplished with the finalized protocol, not enough data has currently been collected to comment on whether or not the incorporation of the siloxane functionalized phosphorylcholine polymer coating onto a titanium alloy improved the biocompatibility of the titanium alloy. More trials need to be completed before commenting on the ability of the MPCMPSi coating to improve the biocompatibility of a titanium alloy.

Along with more trials, coating characteristics need to be evaluated as well, specifically looking into how well the coating adheres to the surface and whether of the coating is evenly distributed.

Previous studies suggest that the incorporation of a siloxane functionalized phosphorylcholine polymer coating onto a titanium alloy showed promising results for improving the biocompatibility of a titanium alloy. Continuing in-vitro evaluation of this coating could lead to the establishment of a siloxane functionalized phosphorylcholine polymer coating as a viable option for improving the biocompatibility of VAD materials, and therefore improving patient survival.

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REFERENCES