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Visualization and analysis of biomaterial-centered thrombus formation within a defined crevice under flow

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ABSTRACT

The blood flow pathway within a device, together with the biomaterial surfaces and status of the patient's blood, are well-recognized factors in the development of thrombotic deposition and subsequent embolization. Blood flow patterns are of particular concern for devices such as blood pumps (i.e. ventricular assist devices, VADs) where shearing forces can be high, volumes are relatively large, and the flow fields can be complex. However, few studies have examined the effect of geometric irregularities on thrombus formation on clinically relevant opaque materials under flow. The objective of this study was to quantify human platelet deposition onto Ti6Al4V alloys, as well as positive and negative control surfaces, in the region of defined crevices (~50–150 μm in width) that might be encountered in many VADs or other cardiovascular devices. To achieve this, reconstituted fresh human blood with hemoglobin-depleted red blood cells (to achieve optical clarity while maintaining relevant rheology), long working optics, and a custom designed parallel plate flow chamber were employed. The results showed that the least amount of platelet deposition occurred in the largest crevice size examined, which was counter-intuitive. The greatest levels of deposition occurred in the 90 μm and 53 μm crevices at the lower wall shear rate. The results suggest that while crevices may be unavoidable in device manufacturing, the crevice size might be tailored, depending on the flow conditions, to reduce the risk of thromboembolic events. Further, these data might be used to improve the accuracy of predictive models of thrombotic deposition in cardiovascular devices to help optimize the blood flow path and reduce device thrombogenicity.

1. Introduction

Many blood-contacting medical devices remain associated with thrombotic and thromboembolic events that result in morbidity, mortality, and ultimately, a deterrent to the broader adoption of these technologies [1–5]. The blood flow pathway within a device, together with the biomaterial surface and status of the patient's blood, is a well-recognized factor in the development of thrombotic deposition and subsequent embolization [6]. For instance, regions of flow separation, recirculation, and stagnation are known to lead to increased platelet deposition [7–10], and such flow conditions may occur near cardiac valves, arterial stents, and vascular grafts due to the disturbances introduced by the specific device architecture [10–14]. Blood flow patterns are of particular concern for devices such as blood pumps (i.e. ventricular assist devices, VADs) where shearing forces can be high, volumes are relatively large, and the flow fields can be complex.

Geometric irregularities, such as steps and crevices, within the flow path of continuous flow VADs serve as a nidus for thrombus formation [7]. Manufacturers make a great effort to reduce the presence of such features within their devices prior to implantation through precise machining and polishing. However, VADs are comprised of multiple parts that serve to create the blood contacting surfaces, so the introduction of internal steps and crevices is

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unavoidable at some level. There is also the possibility that surface imperfections can be introduced during the operation of some devices, for instance when a non-fixed VAD impeller collides with a housing surface and creates a scratch [15].

Understanding how surface irregularities affect the development of thrombotic deposition on clinically relevant biomaterials would provide a useful tool for improving the blood flow path design and materials selection process for VADs and other blood-contacting medical devices. Such data could be used directly or in the development of mathematical models that seek to predict thrombotic deposition risk for a given biomaterial/geometry combination. To date, the use of flow visualization technologies such as particle image velocimetry (PIV) have become routine in blood pump design, as has the early evaluation of candidate biomaterials by acute blood contact under well-defined flow conditions or simple mixing [6].There have been a limited number of *in vitro* studies that have examined real-time platelet deposition onto biomaterials in regions of disturbed flow [16,17]. These reports did not evaluate geometries associated with device joints or surface imperfections and generally evaluated disturbances on a much larger scale. Further, these reports did not evaluate the opaque metallic materials used in the rotary blood pumps in common clinical use today. The objective of this report was to quantify human platelet deposition onto Ti6Al4V alloys, as well as positive and negative control surfaces, in the region of crevices (~50–150 μm in width) that might be encountered in many VADs or other cardiovascular devices. To achieve this, reconstituted fresh human blood with hemoglobin-depleted red blood cells (RBC ghosts, to achieve optical clarity while maintaining relevant rheology), long working optics, and a custom designed parallel plate flow chamber were employed.

2. Materials and methods

2.1. Blood analog preparation

Fresh whole blood was collected after informed consent from 12 healthy donors (7 male, 5 female), who had refrained from taking any platelet altering medications 14 days prior to collection, with a mean age of 27 ± 6 years in accordance with Institutional Review Board guidelines. Platelet rich plasma (PRP) was collected by centrifuging citrated blood (collected into 5 mL Vacutainer tubes, [0.105 M] citrate, BD, Franklin Lakes, NJ, USA) at 250×g for 15 min. Platelets were fluorescently labeled by the addition of quinacrine dihydrochloride (0.5 μM final concentration, Sigma-Aldrich, St. Louis, MO, USA) to the PRP. Since the PRP was taken from healthy donors it was assumed to have normal blood protein levels. Packed RBCs (type O-, Valley Biomedical Products & Services, Inc., Winchester, VA, USA) were utilized to create RBC ghosts through an established protocol [18]. RBC ghosts were used to provide optical clarity. In order to investigate real-time platelet deposition onto opaque surfaces the objective must be focused through the perfusate in the flow channel. However due to the opaque nature of native RBCs, this visualization technique is not possible with the utilization of whole blood. The blood analog used as the perfusate was made by mixing the fluorescently labeled PRP with the RBC ghosts to produce a final hematocrit of 25% (25% of the total volume consisted of RBC ghosts and 75% consisted of PRP) and a final platelet concentration of $2.7 \pm 0.30 \times 10^8$ per mL.

2.2. Parallel plate flow chamber

A custom parallel plate chamber was designed for this study. Flow channels containing crevices of defined dimensions were prepared from polydimethylsiloxane (PDMS) using a photo-etched silicon mold. The perfusion apparatus is shown in Fig. 1. The main flow channel is 3 × 8 mm and includes one of three crevice dimensions $53 \pm 7 \times 122 \pm 4\ \mu m^2$ (n = 7), $90 \pm 12 \times 122 \pm 4\ \mu m^2$ (N = 7), or $137 \pm 10 \times 122 \pm 4\ \mu m^2$ (N = 80). The height of the flow channel is 100 μm. Silicon tubing (Silcon® Med-X Tubing; United States Plastic Corporation, Lima, Ohio, USA) was connected to the PDMS channel as inlet and outlet connectors. A titanium alloy sample (Ti6Al4V; LaunchPoint Technologies Inc., Goleta, CA, USA) acted as the bottom plate of the chamber. Ti6Al4V was selected for study since it is employed by several current and experimental VADs for blood-contacting surfaces [19]. Platelet deposition on rat tail, type I collagen coated glass coverslips (Neuvitro Corporation, Vancouver, WA, USA; N = 5) and a TiAl6V4 surface that had been modified with methacryloyloxyethyl phosphorylcholine polymer (MPC-Ti6Al4V, N = 5) were employed as positive and negative thrombogenic control surfaces respectively. The control surfaces were employed in perfusions utilizing the 90 μm wide crevice. MPC-TI6AL4V was prepared in the authors' laboratory using previously reported protocols [20]. The type I collagen coated cover slips were supplied by Neuvitro Inc. using their standard professional collagen coating procedure. Briefly, the coverslips were washed for 2 h and then rinsed 6 times with sterile water for 30 min each, and then treated with 100% ethanol for 2 h and 70% concentrated nitric acid for 24 h. After rinsing the coverslips 6 times for 1 h each, the G3 digital bio-coating system (Neuvitro) was utilized to coat them with 1 mg/mL rat tail type 1 collagen for 24 h at 4 °C. The coverslips were rinsed twice in sterile water for 10 min and allowed to dry in the fume hood for 12 h. A secondary coating was applied for 24 h at 4 °C and the coverslips were sterilized by radiation. A simple clamping mechanism held the plates together and acrylic shim stock was placed between the plates to provide a precisely defined channel height (Fig. 1B). The flow within this chamber was assumed to be one-dimensional laminar parallel plate flow (Reynolds numbers between 0.1 and 0.4) [21,22].

2.3. Blood analog perfusion and image acquisition

All non-test surfaces were passivated by incubation with 1% bovine serum albumin (BSA, microbiological grade powder; MP Biomedicals, LLC, Solon, OH, USA) in PBS for 20 min prior to perfusion. The whole blood analog was collected into a 20 mL polystyrene syringe (BD Biosciences) and pushed through the parallel plate flow chamber by syringe pump (Harvard Apparatus, Holliston, Massachusetts, USA) for 10 min at flow rates of 0.125 and 0.310 mL/min (wall shear rates of 400 s^{-1} and 1000 s^{-1} respectively at the center of the channel). These wall shear rates were selected because they are representative of regions within VADs that often contain undesired steps and crevices. A wide range of wall shear rates are produced within the pumps of continuous flow VADs, including supra-physiological wall shear rates of >25,000 s^{-1} (shear stress>100 N m^{-2}) [23–25]. However, these high levels of shear are localized almost exclusively to the tips of the impeller blades. The wall shear rates represented in this study are characteristic of those regions most susceptible to platelet deposition, including the inlet and outlet connectors, upstream and downstream of the stators, and along the outer wall of centrifugal VADs [23–25]. Platelet deposition was visualized within the crevices, in real time, using an inverted epifluorescence microscope (Olympus IX FLA, Olympus Corporation, Shinjuku, Tokyo, Japan) with a 40× super long working distance objective (PlanFL, phase contrast, working distance 6.5 mm – 8.3 mm, numerical aperture 0.55; Olympus Corporation) and a 103W HBO short arc mercury lamp light source (OSRAM GmbH, Munich, Germany). Images were acquired every 0.4 s beginning at 1 min after the start of perfusion