

Examination of Platelet Adhesion and Activation on Gamma-sterilized Medical Polymers

Introduction

The blood compatibility of polymers used for medical devices which facilitate the movement or storage of blood is critical, particularly in devices whose applications where device-contacting blood will be re-introduced into a patient (dialyzer housings, storage vessels, IV components, etc.). Standard biocompatibility testing conducted in accordance with the ISO 10993 series of standards for these materials includes various tests for hemocompatibility¹, including platelet interactions, but direct microscopic examination of platelet adhesion and activation is less common. This white paper presents the results of a scanning electron microscopic (SEM) examination of human platelet adhesion on polymeric materials, which shows that CYROLITE® materials may be less likely to aggregate and activate platelets than some other commonly-used blood-contacting materials.

Background

Platelet activation after adhesion to blood contacting materials occurs with various steps, starting with the initial attachment of cells, the spreading of the cell, and the final release of granule contents². Although as many as 5 distinct forms, corresponding with increasing degrees of platelet activation, have been described³, this paper will focus on the three morphologies. The first morphology, the non-activated state (Figure 1), describes platelets in which the platelet retains its disk-like or round-like state. The second morphology, occurring subsequently is the partially-activated (dendritic) state (Figure 2), where one or more pseudopodia have become visible. The final morphology is the fully activated (spread) state, where the pseudopodia have largely disappeared and formed a characteristic fried egg shape (Figure 3).



Figure 1: Unactivated Platelet

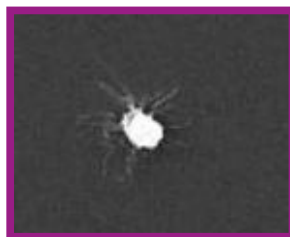


Figure 2: Partially Activated Platelet

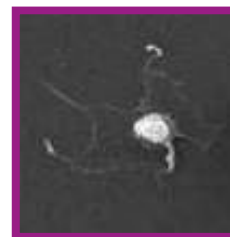


Figure 3: Fully Activated Platelet

Sample description

Samples of 6 polymeric materials (CYROLITE® Med 2, CYROLITE G-20 HIFLO®, co-polyester, Impact modified SMMA, transparent ABS, and medical grade polycarbonate) were molded into 4.00" diameter x 0.062" plaques by Lansen Mold Company of Berkshire, MA. Samples plaques were then sent to Steris Isomedix Services of Spartanburg, SC, for gamma irradiation at 50-60kGy. Once received from sterilization, polymer samples of approximately 0.2"x0.4" dimension were cut from the sterilized plaques for use as microscopic samples.

Blood exposure and sample preparation

Prior to microscopic examination, samples were exposed to human blood and prepared for microscopic examination using established procedures. Unspun single-donor citrated human whole blood (Biological Specialty Corporation, Colmar, PA) was supplied along with the polymer samples to the North Carolina State University Center for Electron Microscopy (Raleigh, NC). Blood was warmed to 35°C in a water bath, and samples submerged in blood for a period of 5 minutes. After exposure, samples were progressively washed and cells fixated with 4% paraformaldehyde/1%glutaraldehyde.

After a final, wash cycle using citrated saline, the samples were dehydrated in graded ethanol, and then critically point dried in liquid CO₂. Samples were sputter coated with 25Å gold-palladium, and stored in a vacuum desiccator until viewing.

Sample Examination: Platelet enumeration and morphology

Sample images were generated using a 10kV JEOL JSM-5900LV (JEOL U.S.A. Peabody, MA) SEM at 1000x magnification. Each polymer sample was photographed in five random areas on the mold-formed (smooth) side of the sample, representative of the fluid-flow surface in device components. Images were digitally acquired as 1280x960 TIFF images.

Sample images were examined and platelets were enumerated by categorizing as non-activated, partially-activated, and fully-activated depending upon their morphological state. Individual platelet counts for the five regions of each sample were combined and averaged over the image area to yield platelet counts per square millimeter.

Results

Figure 3 presents the observed platelet adhesion and activation by material type and platelet morphology.

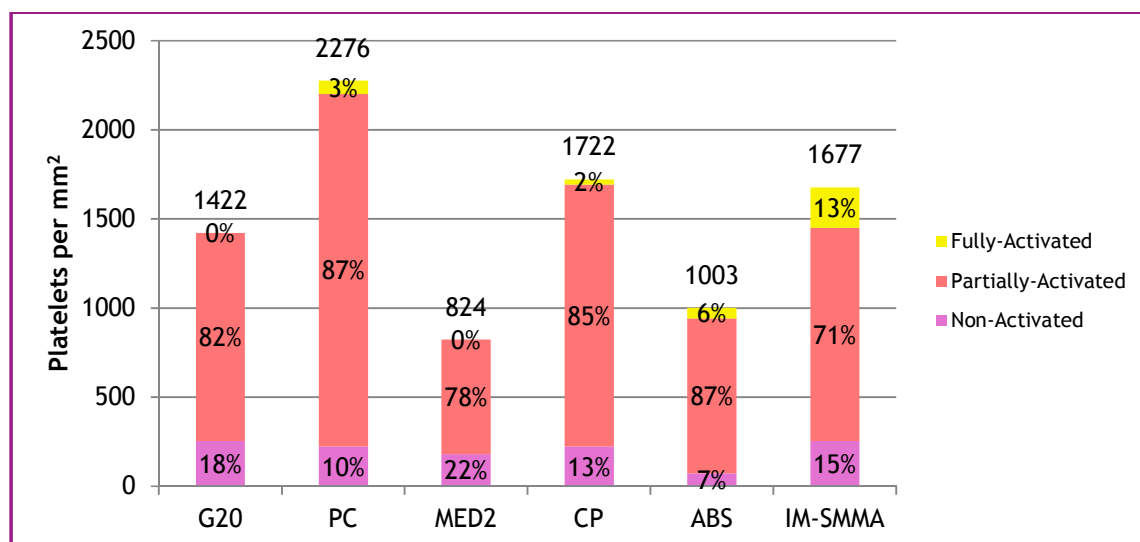


Figure 3: Platelet count and activation

Figure 4 presents the observed quantity of fully-activated platelets by material type

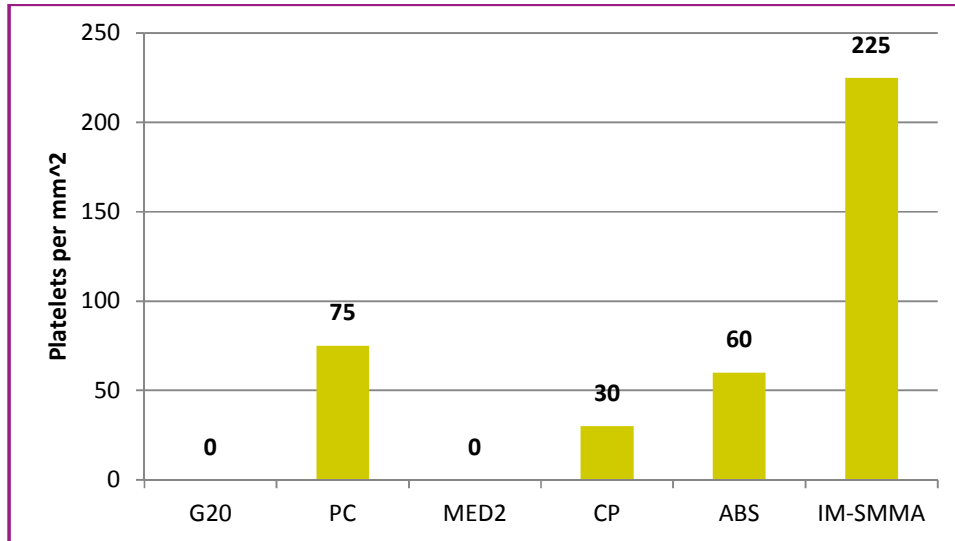


Figure 4: Fully-activated platelets by material

Discussion

The CYROLITE® Med 2 material showed the lowest number of adhered platelets among the six tested materials, with the ABS and CYROLITE® G-20 HIFLO® having the second and third lowest, respectively. The polycarbonate, co-polyester, and impact modified SMMA had the three highest numbers of adhered platelets.

Adhesion of platelets to a biomaterial is dependent upon many factors, including surface wettability, plasma protein absorption to the biomaterial surface, and the affinity of the surface to bind adhesion inhibiting proteins⁴. This initial data suggests that CYROLITE® Med 2 and G-20 HIFLO® materials may have qualities that reduce the adhesion and/or activation of human blood platelets when compared to certain other grades of polymers. Further research to verify results and determine mechanisms for this behavior, should be pursued.

References

1. ISO (2006). ISO 10993-4: 2002 Amd 1:2006 Biological evaluation of medical devices - Part 4: Selection of tests for interactions with blood. Geneva, Switzerland: ISO
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3. S. Goodman, "Sheep, pig, and human platelet-material interactions with model cardiovascular biomaterials," *J. Biomed. Mater. Res.*, **45**, 240-250 (1999)
4. J.H.Lee, H.B.Lee, "Platelet adhesion onto wettability gradient surfaces in the absence and presence of plasma proteins," *J.Biomed. Mater. Res.*, **41**, 304-311 (1998)

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