

Original Article

Quantification of Hydrophobicity of Heparin-Treated Polyvinylchloride Tubing

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Keywords: cardiopulmonary bypass, heparin-coating, biocompatibility, hydrophobicity

Presented at the AmSECT 37th International Conference, April 8–11, 1999, New Orleans, Louisiana

ABSTRACT

It is understood that the biocompatibility of a material depends upon the proteins that bind to its surface. It is this protein layer that interacts directly with components of the coagulation and inflammatory systems. Fibrinogen, capable of platelet binding and activation, is a particularly important protein in determining a material's biocompatibility. Hydrophobic materials tend to have a greater affinity for fibrinogen, making them less biocompatible than hydrophilic materials. We compared the hydrophobicity of three different heparin-coated polyvinylchloride (PVC) tubing preparations with uncoated PVC tubing. We also determined if there would be a difference in the applied solution; water, an ionic solution (saline), and a protein solution (fresh frozen plasma). Hydrophobicity was quantified with five separate measurements of contact angles of water, saline, and plasma, and droplet spread diameter of water and saline. We found that Durafluo II displayed hydrophobicity similar to that of uncoated PVC tubing (initial contact angles of water were 78.0 ± 1.1 and 79.6 ± 0.6 degrees, respectively). Carmeda and 3M heparin-coated tubings displayed significantly ($p < .01$) less hydrophobicity (initial contact angles of water were 59.8 ± 2.1 and 39.6 ± 1.9 , respectively). Three minutes after initial contact, the 3M heparin coating was the only preparation that remained significantly ($p < .01$) less hydrophobic than the uncoated PVC. These data suggest that 3M heparin-coated PVC is the most biocompatible of the tubings we examined, followed by Carmeda, Durafluo II, and the untreated PVC tubing.

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INTRODUCTION

Occasionally, patients undergoing open cardiac operations experience an adverse reaction to cardiopulmonary bypass (CPB) known as "postperfusion syndrome." The symptoms can include prolonged pulmonary insufficiency, accumulation of extravascular fluid, renal dysfunction, fever, vasoconstriction, and coagulopathy. These symptoms can occur after an apparently successful operation with good hemodynamic performance. These deleterious effects of CPB are directly related to the contact of the patient's blood with the synthetic surfaces of the CPB circuit. This contact initiates a "whole body inflammatory response" involving elements that, under normal circumstances, act locally at sites of injury, such as the Kinin-kallikrein system, the coagulation cascade, the fibrinolytic system, the complement cascade, and platelets (1).

The absorption of protein is the first thing that happens when blood comes into contact with a synthetic material, occurring within seconds of exposure (2). The biocompatibility of a material depends primarily on the proteins that bind to its surface, because it is this layer of proteins that interacts directly with all the other components of the biological system. This layer of adhered proteins mediates any effects that a material may have on a biological system. Understanding the dynamics of protein adhesion to the surface of synthetic materials at the blood-material interface, and how synthetic surfaces dictate the behavior of bound proteins is key in understanding biocompatibility. Hydrophobicity is a particularly important surface characteristic in terms of protein adsorption. Protein adsorption, in general, is greater on hydrophobic than on hydrophilic surfaces, making hydrophobicity highly predictive of a material's ability to bind proteins at the blood-material interface (3). Several studies have demonstrated that plasma protein adsorption is less readily reversible on hydrophobic surfaces than on hydrophilic ones (4, 5). This indicates that hydrophobic materials not only tend to bind plasma proteins in higher quantities, but also with stronger interactions.

It is not only the quantity of proteins that adheres to a synthetic surface at the blood material interface that determines a material's biocompatibility. Different types of synthetic surfaces will bind different proteins preferentially upon exposure to blood. Every different type of synthetic material will have a characteristically different layer of adsorbed proteins at the blood-material interface. Studies have shown a clear correlation between the hydrophobicity of synthetic surfaces and their ability to bind and retain fibrinogen (6). Numerous studies have shown that fibrinogen is a primary component of the adsorbed protein layer (7-9). During thrombus formation, fibrinogen acts as a bridge for aggregating platelets, interacting with GP IIb-IIIa receptors on two different platelets (10). Plasma-bound fibrinogen is unable to interact with platelets that have not been activated. However, several studies have demonstrated that upon binding to a synthetic surface, fibrinogen is able to adapt a conformation that allows it to interact

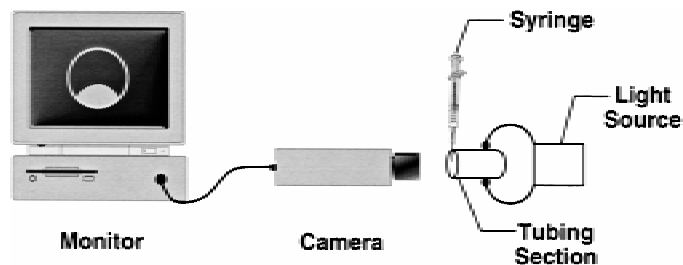


Figure 1: Contact angle measurement device: this device, consisting of a digital camera with micro lens, monitor, and light source, was assembled to measure contact angles

with GP IIb-IIIa receptors causing platelet activation and aggregation (11, 12). Because of its high affinity for synthetic surfaces, reactivity toward platelets, and integral involvement in clotting and thrombosis, fibrinogen adsorption is particularly important in determining a material's biocompatibility. Because hydrophobicity and fibrinogen binding have been demonstrated to be highly correlative, the hydrophobicity of a material's surface is clearly indicative of its biocompatibility.

We compared the hydrophobicity of the luminal surfaces of several different commercially available heparin-coated polyvinylchloride (PVC) perfusion circuit tubings to uncoated PVC tubing. To do this, we used contact angle measurement and droplet spread diameter. The heparin-coated tubings tested include 3M,^a Carmeda,^b and Duraflo II.^c

MATERIALS AND METHODS

Contact angle measurements were performed by administering a 10.0 μ L droplet of distilled water, phosphate buffered saline, or fresh frozen plasma to a 1-cm section of tubing using a Hamilton syringe. A MTI CCD 72^{TMd} camera with a NIKKOR AF Micro^{TMc} 60-mm lens was positioned horizontally adjacent to the tubing section. The tubing was illuminated with a Fibrinoleptic Specialties LS 86/110^f light source, and the image was fed to a Sony HR Trinitron^{TMg} monitor. (Figure 1) The contact angle was measured directly from this image. (Figures 2-5) A horizontal line was drawn intersecting the two contact points on the outer edge of the droplet. Using a ruler, a line was drawn tangential to the surface of the droplet at the point of contact with the luminal surface of the tubing. The angle between the line intersecting the contact points and the line tan-

- a 3M Corp., St. Paul, MN
- b Medtronic, Minneapolis, MN
- c Baxter, Deerfield, IL
- d MTI International, Menomonee Falls, WI
- e Nikon, Tokyo, Japan
- f Fibrinoleptic Specialties Inc., Peabody, PA
- g Sony Electronics Inc., Park Ridge, NJ

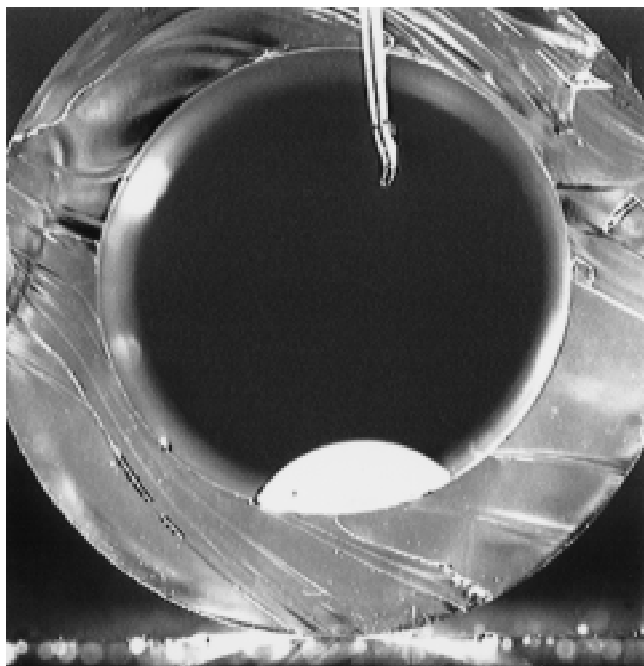


Figure 2: 10 µl water droplet on 3 M heparin-coated PVC tubing for contact angle measurement; this image was obtained using the contact angle measurement device; contact angles were measured directly from images like this one

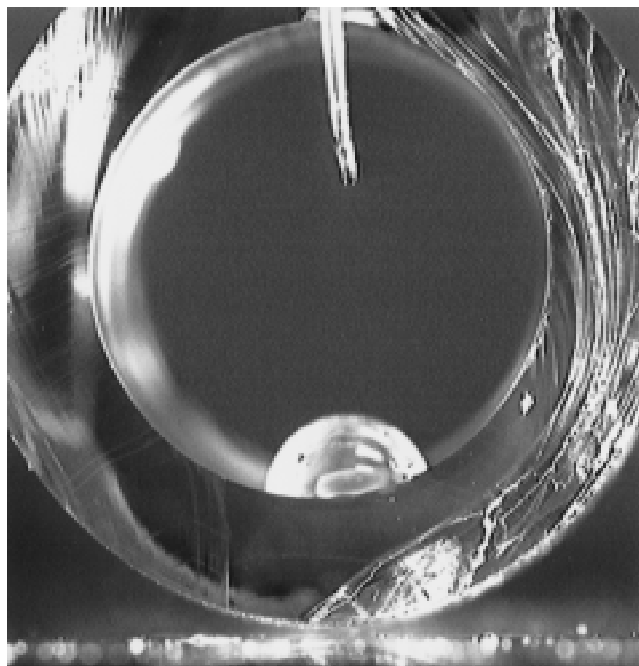


Figure 4: 10 µl water droplet on Duraflo II heparin-coated PVC tubing for contact angle measurement; this image was obtained using the contact angle measurement device

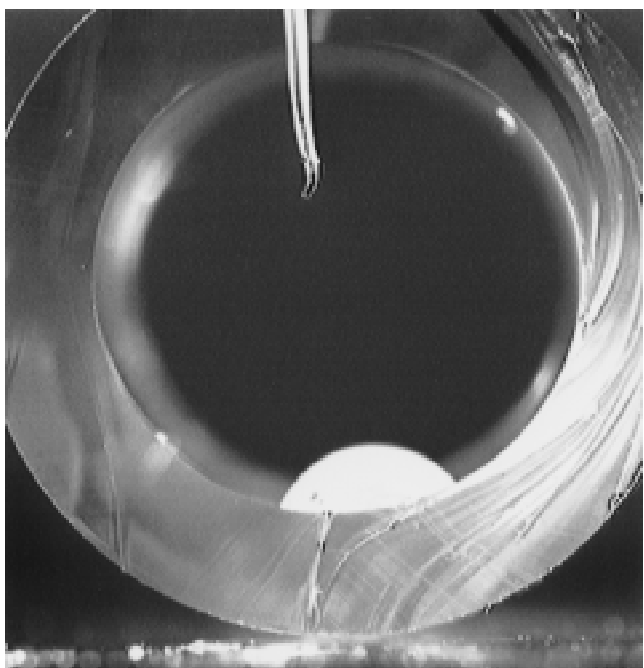


Figure 3: 10 µl water droplet on Carmeda heparin-coated PVC tubing for contact angle measurement; this image was obtained using the contact angle measurement device

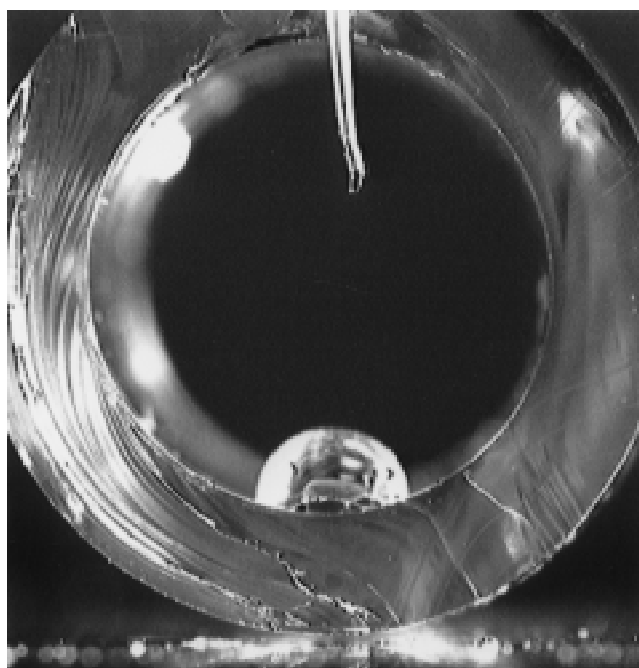


Figure 5: 10 µl water droplet on uncoated PVC tubing for contact angle measurement; this image was obtained using the contact angle measurement device.

gential to the droplet surface at the contact point was measured using a protractor. Measurements were recorded at 0, 1, and 3

min. With each fluid, the experiment was repeated five times using five different sections of each type of tubing. The mean

Table 1: Measured contact angles

Distilled Water Contact Angles (degrees)				
	Uncoated	3M	Durafluo II	Carmeda
0 min	79.6 ± 0.6	39.6 ± 1.9*†	78.0 ± 1.1	59.8 ± 2.1*
1 min	55.4 ± 1.3	29.8 ± 1.6*†	63.8 ± 1.1*	51.8 ± 2.9
3 min	44.6 ± 1.2	23.0 ± 1.9*†	47.8 ± 2.2	48.4 ± 2.9
Phosphate Buffered Saline Contact Angles (degrees)				
	Uncoated	3M	Durafluo II	Carmeda
0 min	75.6 ± 0.4	45.8 ± 1.4*†	80.0 ± 0.5*	52.0 ± 2.1*
1 min	58.4 ± 0.5	40.4 ± 1.0*†	70.0 ± 1.2*	47.2 ± 2.0*
3 min	49.8 ± 1.5	33.4 ± 1.1*†	57.2 ± 0.9*	41.8 ± 1.2*
Fresh Frozen Plasma Contact Angles (degrees)				
	Uncoated	3M	Durafluo II	Carmeda
0 min	70.6 ± 1.3	46.8 ± 1.0*†	66.8 ± 1.4	48.8 ± 2.1*
1 min	51.4 ± 1.2	37.6 ± 1.3*†	54.6 ± 1.0	45.8 ± 1.0*
3 min	45.0 ± 0.3	30.2 ± 0.7*†	48.4 ± 0.7	38.8 ± 0.2*

* $p < .05$ compared to uncoated group.

† $p < .05$ compared to Durafluo II and Carmeda.

contact angles and standard errors for each time point were calculated using *Microsoft Excel*TM (Table 1).^h

To measure the droplet spread diameter, a section of each tubing type was cut lengthwise and spread open to lay flat. Five droplets, each consisting of 5.0 μL of distilled water were administered to each section of tubing. The diameter of each droplet was measured immediately upon contact using a ruler. The droplet spread diameter of PBS was calculated similarly, using 5.0 μL droplets of PBS. Mean droplet spread diameters and standard errors were calculated using *Microsoft Excel*TM (Table 2).^h

Statistical Methods

All data are expressed as mean \pm standard error of the mean. Data were analyzed with Student's *t*-test to compare the results between two groups using Minitab for Windows, release 9.ⁱ A *p*-value under .05 was considered significant.

RESULTS

Contact Angles

The Durafluo II heparin-treated PVC tubing displayed an initial contact angle of water similar to that of the uncoated tubing, with contact angles of 78 ± 1.1 , and 79.6 ± 0.6 degrees, respectively. The Durafluo II and uncoated tubings also displayed similar contact angles after 1 min and 3 min of exposure, indicating that Durafluo II heparin-coated and uncoated

PVC tubings display similar initial wettability and similar wettability over time. The 3M and Carmeda heparin-coated tubings displayed significantly less hydrophobicity than the uncoated and Durafluo II tubings, with initial contact angles of 39.6 ± 1.9 and 59.8 ± 2.1 , respectively. Over time, the Carmeda heparin-coated tubing displayed a contact angle of water that was more similar to that of the uncoated and Durafluo II tubings, but the 3M heparin-coated tubing continued to show a more acute contact angle. This indicates that the 3M heparin-coated PVC tubing is not only the most initially hydrophilic of the tubings that we examined, but also the most wettable over time. The same experiment was repeated with phosphate buffered saline (PBS) and fresh frozen plasma (FFP). Similar results were obtained. These data showed similar initial contact angles and time dependent wettability of Durafluo II and uncoated PVC tubing using both FFP and PBS. The Carmeda heparin-coated tubing displayed a more acute initial contact angle, and over time, displayed contact angles that were more similar to Durafluo II and untreated tubings. 3M heparin-coated tubing displayed the most acute initial contact angle with both PBS and FFP, and maintained more acute contact angles throughout the time course.

Droplet Spread Diameters

The droplet spread diameter data largely agreed with the results obtained from the contact angle studies. Durafluo II heparin-coated PVC tubing displayed water and PBS droplet spread diameters that were very similar to those displayed by uncoated PVC tubing. The Carmeda and 3M heparin-coated tubings displayed similar water and PBS droplet spread diameters that were approximately 30% larger than those displayed by Durafluo II heparin-coated and uncoated PVC tubings. (Figure 6 and Table 2)

DISCUSSION

The results for both the contact angle and the droplet spread diameter studies clearly demonstrated that the luminal surfaces

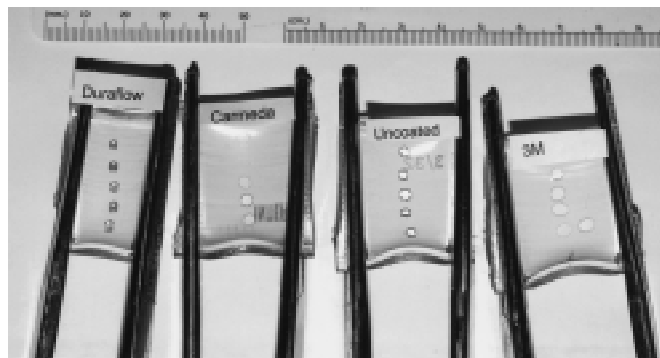


Figure 6: 5 μL phosphate buffered saline droplets on heparin-coated and uncoated PVC tubings for droplet spread diameter measurement; five droplets were administered to each of the tubings, and their diameters were measured

^h Microsoft Corp., Redmond, WA

ⁱ Minitab Inc., State College, PA

Table 2: Measured droplet spread diameters

Water Droplet Spread Diameter (millimeters)			
Uncoated	3M	Duraflor II	Carmeda
3.0 ± 0	4.0 ± 0.02*	3.1 ± 0.05	4.0 ± 0.04*
Phosphate Buffered Saline Droplet Spread Diameter (millimeters)			
Uncoated	3M	Duraflor II	Carmeda
3.0 ± 0.04	4.2 ± 0.07*†	3.1 ± 0.04	3.8 ± 0.07*

* $p < .05$ compared to uncoated group.

† $p < .05$ compared to Carmeda group.

of uncoated PVC tubing and Duraflor II heparin-coated PVC tubing were similarly hydrophobic. These studies also demonstrated that the luminal surfaces of Carmeda and 3M heparin-coated PVC tubings were less hydrophobic than those of Duraflor II and uncoated PVC tubings. Of the three heparin-coated tubing types examined, 3M had the most hydrophilic luminal surface.

The degree of hydrophobicity of a surface is a particularly important characteristic in determining its biocompatibility. Protein adsorption is generally greater and less reversible on hydrophobic than on hydrophilic surfaces, indicating stronger interactions between plasma proteins and hydrophobic surfaces. As previously mentioned, fibrinogen binding is particularly detrimental for a material's biocompatibility. Perez-Luna et al. (9) studied the correlation of several chemical and physical surface properties of commonly used polymeric materials with the amount of fibrinogen adsorbed from a complex mixture. The surfaces of 16 different polymers were characterized using static secondary ion mass spectroscopy, electron spectroscopy for chemical analysis, and contact angle measurements of several liquids. This study found a high degree of positive correlation between fibrinogen binding and a high dispersive component of surface energy was also observed to correlate with fibrinogen retention at the material surface, indicating stronger interactions (6). The results of our study suggest that 3M and Carmeda heparin-coated PVC tubings may offer the most biocompatible luminal surface with the least amount of fibrinogen binding and retention. The high degree of hydrophobicity displayed by the luminal surface of Duraflor II heparin-coated tubing clearly indicates that it may present the least biocompatible surface of the heparin-coated tubings we examined.

CONCLUSION

It is important to remember that synthetic surface biocompatibility involves many chemical and physical properties of the surface. Although it is highly correlative with biocompatibility, hydrophobicity is only one of these properties. The method devised to measure the contact angles allowed for the

measurements to be made without compromising the integrity of the heparin coating on the luminal surface of the PVC tubing. The tubing was not stretched, compressed, or deformed in any way that might effect the continuity or integrity of the heparin coating. To perform the droplet spread diameter measurements, the tubings were cut lengthwise and spread open to lay flat. This may have adversely affected the continuity or integrity of the heparin coating. However, the results of the droplet spread diameter and the contact angle measurement experiments correlated very well. This suggests that the manipulations of the tubings did not affect the integrity of the heparin coating.

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