X COATING™: A NEW BIOPASSIVE POLYMER COATING

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Evidence of functional mechanisms at the blood molecular level when in contact with X Coating is demonstrated. X Coating creates a hemocompatible surface on a broad range of materials used during cardiopulmonary bypass (CPB). This paper explains the X Coating mechanism and summarizes the various tests performed in order to demonstrate the benefits of X Coating compared to non-coated and other types of commercially available coatings. It is concluded that X Coating reduces the contact activation stresses that occurs during CPB.

IMPACT OF MATERIALS ON BLOOD

Cardiopulmonary bypass has been linked to significant pathophysiological morbidity. Poor patient outcomes have been shown to be caused by: perfusion and hemodynamic alterations[1-3], gas exchange[4-5], hypothermia, acid-base balance[6-8] and blood contact activation[9-11]. Contact activation is caused when the formed elements of the blood (red blood cells, white blood cells, and platelets) and plasma are exposed to nonendothelial surfaces. This contact activation results in a tendency to bleed following CPB, as well as leads to pulmonary, renal, cerebrovascular and other organ dysfunctions.

Platelets and Factor XII are directly activated by contact with synthetic materials. All of the subsequent reactions in blood during CPB are consequences of this direct activation. As blood first contacts the synthetic material, plasma proteins are immediately adsorbed onto the surface. Fibrinogen, albumin, immunoglobulin and hemoglobin are all adsorbed, but the amount of each protein adsorbed varies with the surface material[12-13]. Hydrophilic surfaces adsorb less protein than hydrophobic surfaces. Adsorbed fibrinogen is rapidly displaced by an activated form of high molecular weight kininogen (HK) and to a lesser extent by Factor XII. The initial activation of platelets is not completely understood. In the presence of fibrinogen, activated platelets adhere to the fibrinogen as well as to each other[14]. Some of the platelets partially or completely release granular contents. Activated platelets release thromboxane A2, a potent vasoconstrictor and platelet agonist. Aggregated platelets form emboli that can block arterioles and capillaries. Even though filters and membrane oxygenators reduce the number and size of these platelet fibrin emboli, some emboli have been observed in retinal vessels during and after CPB[15].

Factor XII is one of the primary plasma proteins of the contact system. In the presence of kallikrein, surface bound Factor XII is cleaved into two active serine proteases, Factor XIIa and XIIf. This cleavage produces bradykinin, and also initiates the coagulation cascade, activates complement and neutrophils. Factor XIIa with kallikrein activates the fourth primary contact protein, Factor XI. Activated Factor XI initiates the intrinsic pathway of coagulation and the generation of thrombin, which converts fibrinogen to fibrin. Factor XIIa activates the first component of complement and generates the anaphylactoxins C3a and C5a, via the classical pathway.

Activation of platelets, complement, neutrophils, and endothelial cells produces a number of vasodilator substances. These substances all play a role in the whole body inflammatory response to CPB. These vasoactive agents have been shown to increase vascular permeability, cause hypotension or vasoconstriction. These vasoactive substances mediate the inflammatory response associated with CPB and contribute to the morbidity and mortality of cardiac surgery patients[16-17].

Contact activation is one of the key deleterious effects occurring during CPB. As explained, blood contacting a foreign surface will trigger a variety of activation complexes involving numerous blood components. In light of these findings, manufacturers of CPB equipment and disposable

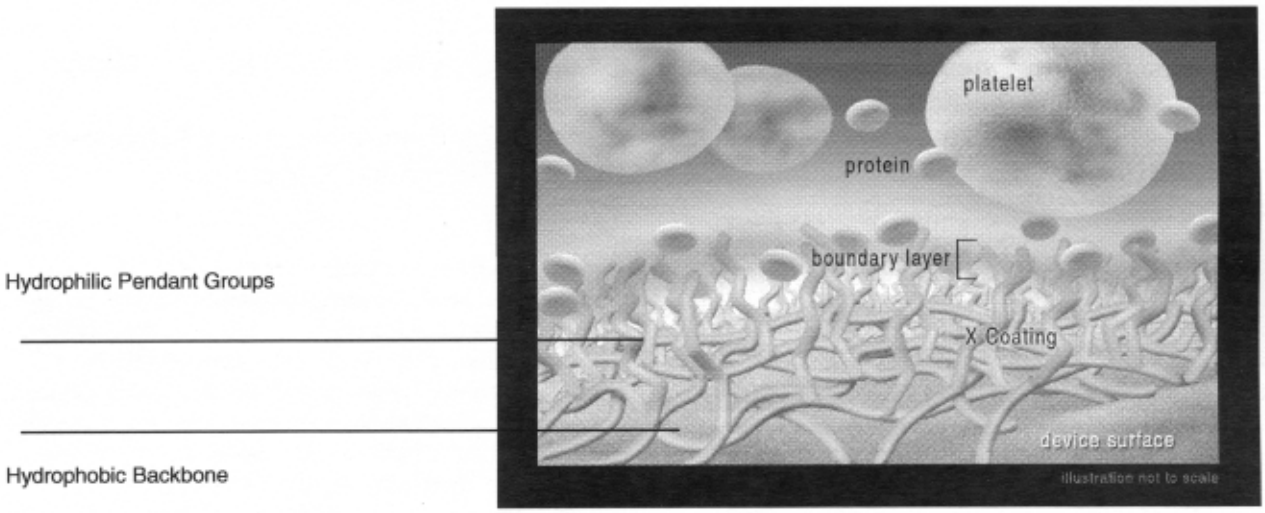

Figure 1: *X Coating mechanism.*

products have invested significant amounts of resources in developing surface coatings in hopes of improving the overall bypass experience.

X COATING™

Extensive research has been put forth in formulating X Coating (also referred in this paper as Poly(2-methoxyethylacrylate) (PMEA)) a non-heparin, biocompatible polymer coating. X Coating has an intricate action mechanism that allows reduction in protein denaturation and platelet adhesion when in contact with blood. X Coating is composed of an amphiphilic polymer, which means that it has both hydrophobic and hydrophilic properties. It is the dual properties of X Coating, working in tandem, that allow it to adhere to a variety of materials in the extracorporeal circuit and form a new surface that reduces protein denaturation and platelet adhesion. While X Coating is hydrophobic where it contacts the device surface, its blood contact surface is hydrophilic (Fig.1). Water in the blood collects at the coating's hydrophilic interface, causing the coating to swell and create a water layer structure referred to as the boundary layer. Protein molecules associate freely within this boundary layer, maintaining their native conformation as they move between the boundary layer and the blood stream, just as they would in normal circulation. Because proteins in contact with X Coating do not denature, platelets will not adhere to the surface.

X Coating enhances the hemocompatibility properties of various materials used during cardiopulmonary bypass procedures. Furthermore, PMEA demonstrates the following characteristics: it is insoluble in water; it is a flexible material and has adhesive properties making this coating stable and durable. Over that last few years, several studies and rigorous technical analysis of this new polymer have been undertaken to better understand the complex nature of X Coating. The following are the results of investigations performed on this polymer to confirm the hemocompatibility properties and the mechanism of PMEA.

PROTEIN ADSORPTION WITH X COATING

A study published in Biomaterials Journal[18] demonstrated the blood compatible aspects of X Coating in relationship to protein adsorption and platelet adhesion on its surface. The author evaluated protein adsorption and platelet adhesion. The number of platelet adhered to the surface of adhesion on poly(meth)acrylates is shown in Fig.2. It was demonstrated in this study that the number of platelets adhered to the PMEA surface was the least.

In this study, PMEA was closely compared to PHEMA (Poly(2-hydroxyethylmethacrylate)), which has been widely used in various biomedical fields including soft contact lenses. Washed platelet adhesion was examined. In the absence of fibrinogen (FNG), the adhesion of platelets to the surface of PMEA and PHEMA was almost inhibited. On the other hand, in case of surfaces precoated with FNG,

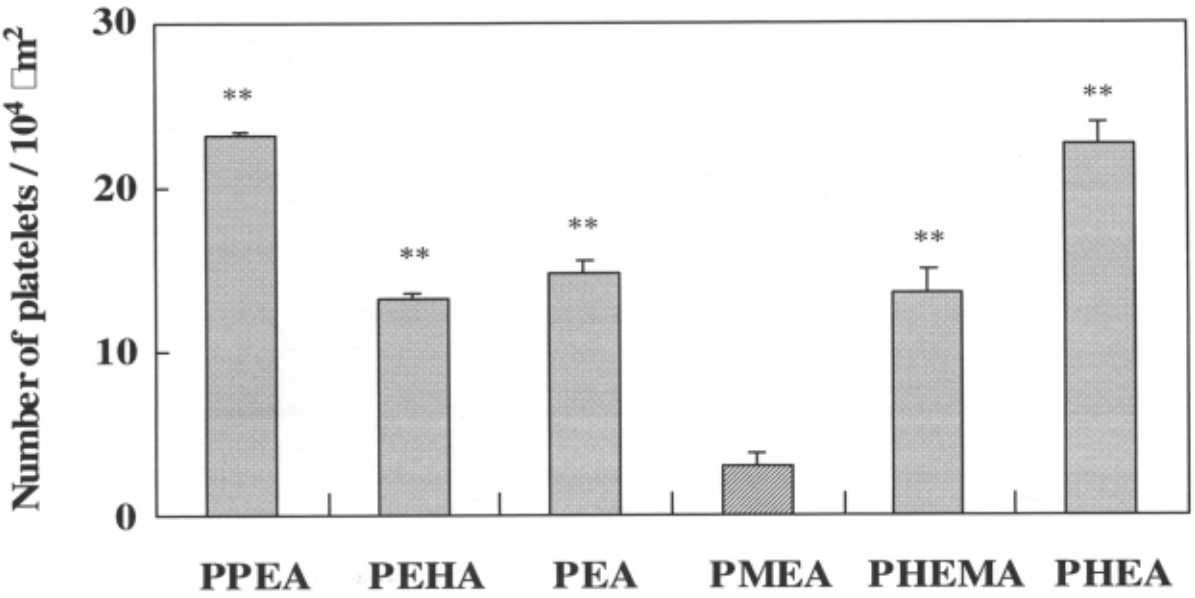

Figure 2: Number of platelets adhered to the surface of poly(meth)acrylates (**P<0.01 vs PMEA, mean ± SD, n=5)

much platelet adhesion and spreading were observed on PHEMA, but little platelet adhesion was recognized on PMEA. The platelets adhered to PMEA maintain their original round shape compared with those adhered to PHEMA (Fig.3).

Another aspect of proteins that was discussed in this paper is the determination of conformational changes of adsorbed proteins. The amounts of adsorbed BSA (bovine serum albumin) and FNG from their mono-component solutions onto the surface of polymers were investigated (Fig.4). The amounts of BSA and FNG adsorbed onto PMEA were lower than those adsorbed onto PEHA (Poly(2-ethylhexylacrylate)), but they were very similar to those adsorbed onto PHEMA).

Finally, the last aspect of proteins investigated in this study was the α-helical contents of native BSA and FNG in Phosphate Buffered Solution (PBS). The α-helix content of BSA was about twice that of FNG. It was found that the degree of conformational change of the adsorbed protein depended on the property of the polymer surface. The percentages of α-helix content in BSA adsorbed onto the surface of polymers and in PBS are summarized in Fig.5.

In conclusion, the PMEA surface suppressed platelet adhesion and spreading as compared to other poly(meth)acrylates. These results indicated that the PMEA surface minimized its interactions with blood.

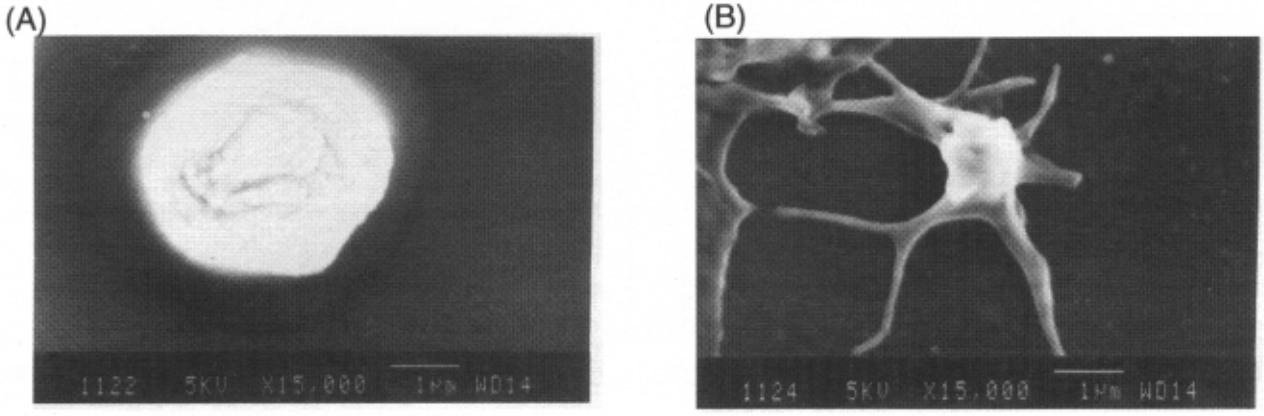

Figure 3: Scanning electron micrographs of washed platelets adhered to polymer pre-coated with FNG. (A) PMEA (B) PHEMA (magnification X 15 000).

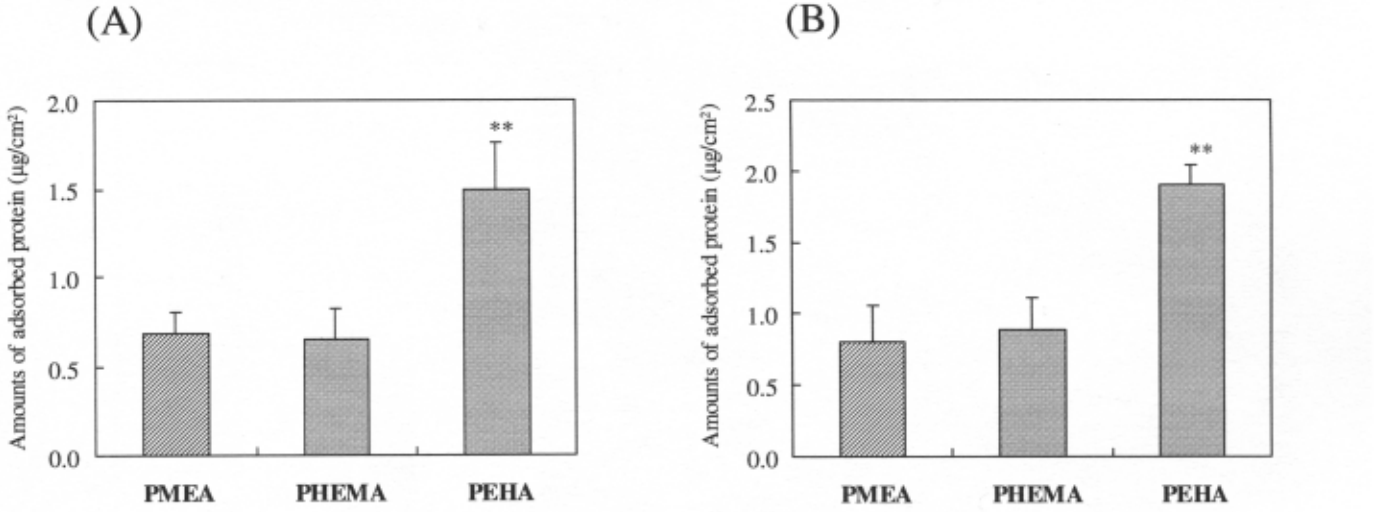

Figure 4: Total amount of protein adsorbed onto the surface of polymers. (A) BSA (B) FNG (**P<0.01 vs PMEA, mean ± SD, n=5)

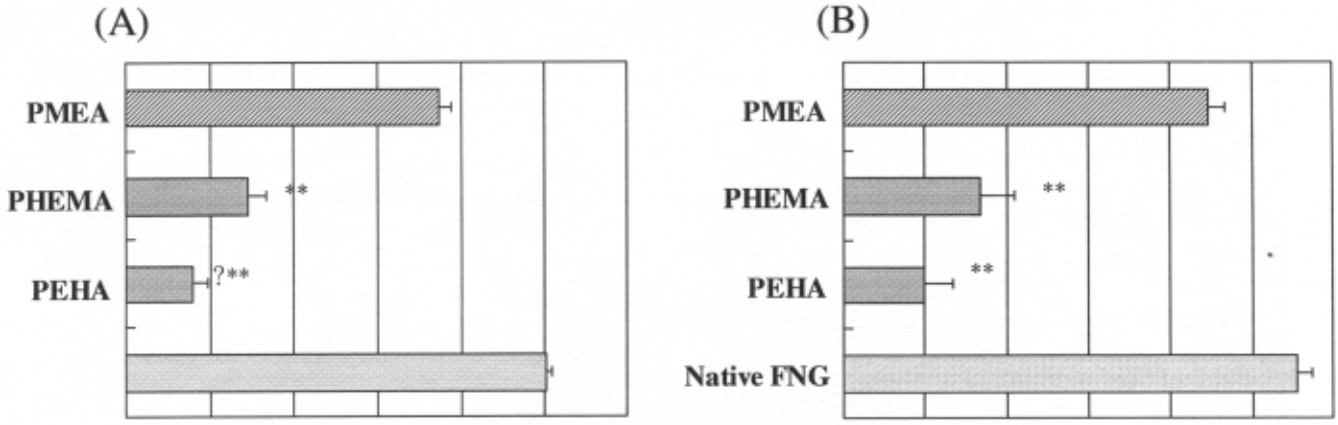

Figure 5: Percentage of α-helix content of (A) BSA (B) FNG adsorbed onto the surface of polymers and in PBS (**P<0.01 vs PMEA, mean ± SD, n=5)

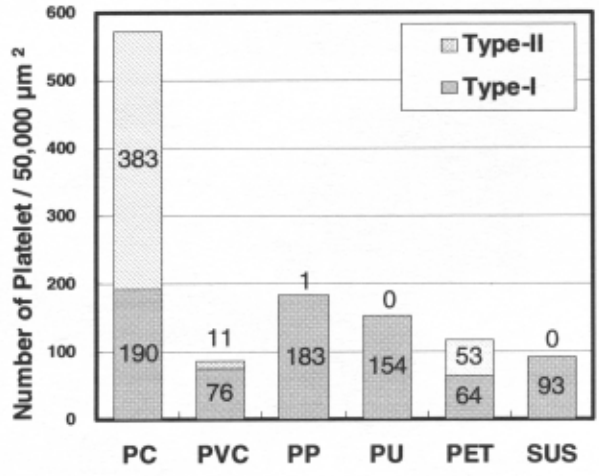

Figure 6: Results of platelet adhesion test on non-coated surfaces

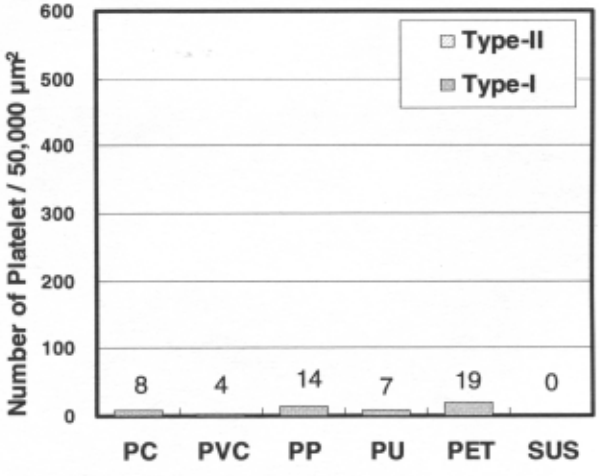

Figure 7: Results of platelet adhesion test on coated surfaces

PC (polycarbonate), PVC (polyvinyl chloride), PP (polypropylene), PU (polyurethane), PET (polyethyterephtalate), SUS (stainless steel)

TEST PARAMETERS			*RESULTS* N=5 *(mean / sd)*				
BLOOD FLOW L/min	GAS FLOW ml/min	V/Q	CONTROL	XCOATED	*p* value	JUDGEMENT	
0.5	250	**0.5**	34.8 / 1.3	34.5 / 1.1	0.722	Not significant	O_2 Transfer (ml/min)
0.5	500	**1**	34.9 / 0.8	35.3 / 0.8	0.449	Not significant	
0.5	1000	**2**	34.6 / 1.3	34.9 / .5	0.675	Not significant	
7.0	3500	**0.5**	381.0 / 11.5	380.8 / 7.3	0.977	Not significant	
7.0	7000	**1**	382.1 / 14.3	386.1 / 7.8	0.597	Not significant	
7.0	14000	**2**	389.3 / 7.1	386.9 / 9.9	0.671	Not significant	
0.5	250	**0.5**	13.4 / 0.6	13.3 / 0.8	0.965	Not significant	CO_2 Transfer (ml/min)
0.5	500	**1**	23.8 / 1.1	25.1 / 2.9	0.362	Not significant	
0.5	1000	**2**	39.7 / 1.5	40.6 / 1.8	0.416	Not significant	
7.0	3500	**0.5**	207.5 / 6.0	210.7 / 7.2	0.472	Not significant	
7.0	7000	**1**	331.9 / 14.3	332.9 / 12.3	0.910	Not significant	
7.0	14000	**2**	460.0 / 21.2	466.2 / 15.2	0.612	Not significant	

Figure 8: *In-vitro* Gas Transfer of X Coated vs. Uncoated CAPIOX SX18 Oxygenators

The reason why PMEA showed excellent compatibility with platelet lies on the fact that the adsorbed plasma proteins found on the surfaces were not altered. Therefore, PMEA would serve to modify the surface of artificial materials to attain higher blood compatibility.

PLATELET ADHESION WITH X COATING™

A platelet adhesion test was performed on various materials to observe the direct impact that X Coating has on platelets. Different materials will generate a varying degree of platelet adhesion (type I) and activation (type II). As seen in Fig. 6, polycarbonate (PC) can be classified as one of the harshest materials in relations to platelet adhesion and activation.

However, a marked difference is noted when platelets encounter a surface coated with X Coating (Fig. 7). In fact, when comparing results between both polycarbonate samples, the non-coated sample had a combination of 573 platelets/50,000μm^2 either adhered on the surface and/or activated. On the surface with X Coating, only 8 platelets/50,000μm^2 could be found adhered and in their original round shape. X Coating exhibits strong hemocompatible properties and as demonstrated, its mechanism allows for protein to maintain their native conformation and for platelets to remain in circulation.

CAPIOX® SX OXYGENATOR PERFORMANCE WITH X COATING™

Providing a true hemocompatible environment during cardiopulmonary bypass is the ultimate goal to be achieved when using a coated surface. However, the twin challenge facing designers of a biocompatible blood oxygenation system is to have the benefits of an improved blood/material interface without changing the basic physical parameters of the material itself. Coating the surfaces does not change the inherent flexibility, hardness, brittleness and tensile strength of the plastics and metals used to construct the devices while improving their interactions with blood. The greatest area of concern with any coated circuit is the potential to change the gas flux of microporous fibers used in membrane oxygenators.

Terumo has developed a proprietary chemical formulation controlled by a unique automated manufacturing process to apply X Coating in a way that does not obstruct the micropores of the polypropylene fibers used to build the CAPIOX oxygenators. The result is that oxygenators with X Coating perform identically to equivalent models without X Coating with respect to gas transfer. This has been demonstrated experimentally *in-vitro* using bovine blood. The experiment tested populations of 5 uncoated and 5 coated SX18 oxygenators. Fig. 8 shows the data analyzed and summarized. For all samples at 0.5 L/min and 7 L/min blood flow and gas flow rates ranging from 250 ml/min to 14,000 ml/min no statistical differences were detected (Ho = not equivalent, 90% confidence) in oxygen or carbon dioxide transfer. No increase in variability was noted across the population of test values. Other experimental data has shown no substantial changes in performance for six hours of *in-vitro* bovine blood

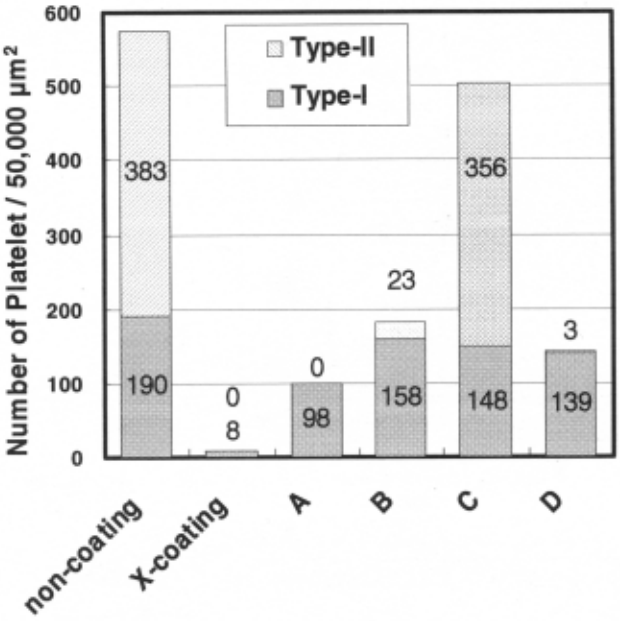

Figure 9: Platelet Adhesion test on PC

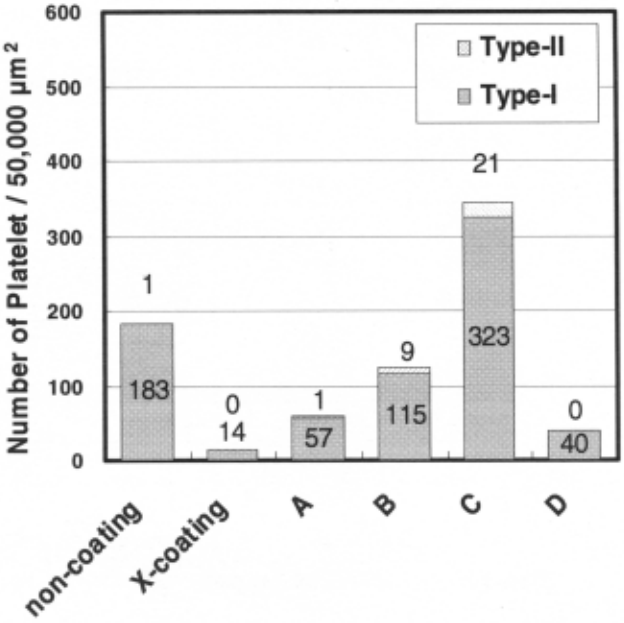

Figure 10: Platelet Adhesion test on PP

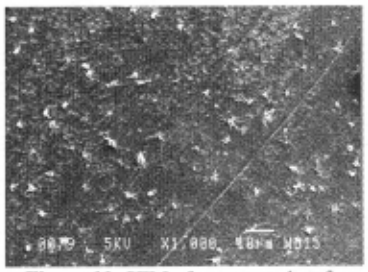

Figure 11: SEM of non-coated surface

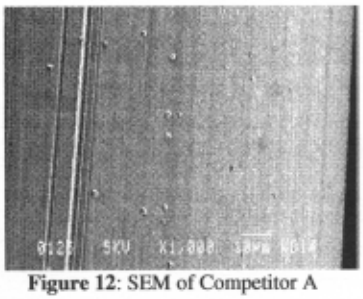

Figure 12: SEM of Competitor A

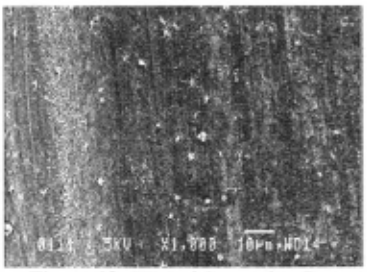

Figure 13: SEM of Competitor B

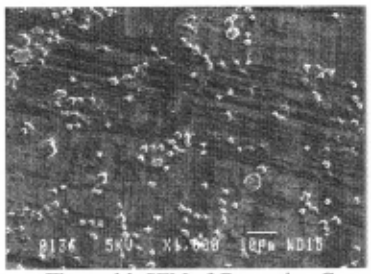

Figure 14: SEM of Competitor C

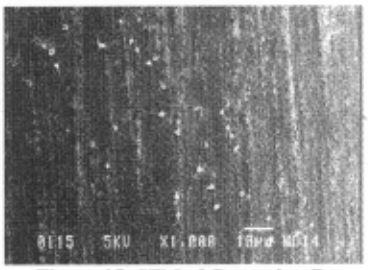

Figure 15: SEM of Competitor D

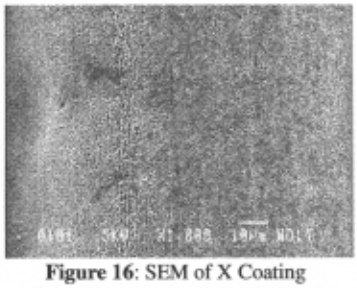

Figure 16: SEM of X Coating

runs and for six continuous hours of clinical use using a porcine model.

The conclusion of these experiments is that X Coating does not interfere with or change the performance of CAPIOX oxygenators to exchange gases with blood across a wide range of conditions encompassing those found during extracorporeal cardiovascular procedures.

COMPARATVE STUDY WITH OTHER SURFACE COATINGS

PLATELET ADHESION TEST

Products with X Coating offer improved hemocompatibility and reliable performance. However, a crucial question remains: how does X coating compare against other commercially available coatings? In the following report, X Coating is compared to other commercially available

coatings for both platelet adhesion (type I) and platelet activation (type II).

Three material samples of polycarbonate (PC) and polypropylene (PP) are obtained from one oxygenator for all product samples to be evaluated i.e. non-coated, with X Coating™, and four other available coated oxygenators. Of the four competitive coatings, three are heparin based and one is a co-polymer. Each material sample is dipped for 30 minutes in platelet controlled plasma (PCP). PCP is composed of platelet rich plasma (PRP) and platelet poor plasma (PPP). This mixture ensures a defined number of platelets that can be accounted for during the analysis phase of the study. All material samples were cleaned prior to testing and the surfaces were fixed with glutaraldehyde after exposure to PCP.

Scanning Electron Micrograph (SEM) photographs were taken on the polypropylene material for all products tested. A visual examination of the polypropylene surfaces (Fig.11-15) clearly demonstrates that X Coating (Fig. 16) is superior compared to the non-coated and other coated surfaces related to the amount of platelets adhering to its surface. The inspection denotes significant amounts of platelet adhesion (type I) and platelet activation (type II) on the non-coated surface and some of the competitive coatings tested.

THROMBO-RESISTANT PERFORMANCE TEST

Thrombo-resistance performance testing was done comparing X Coating™ and heparin coating.

Multiple tubing loops are constructed, each containing three replicates of uncoated connectors and seven replicates each of X Coating and heparin coating connectors. Heparinized bovine blood is circulated through each loop at 37°C for six hours. Each loop is then drained of blood and rinsed with normal saline. Unheparinized bovine blood collected in sodium citrate is then recalcified, and placed in each loop for approximately 25 minutes. Blood is not circulated during this incubation period. The blood is then drained and the loops are rinsed again with a normal saline solution.

Connectors are shown in fig. 17. X coated connectors developed substantially less thrombus than did uncoated connectors, and slightly more thrombus than did heparin coating connectors. This experiment may not have relevance in a clinical model, other than demonstrating that X Coating remains on the device surface for six hours of continuous recirculation. However, it is interesting that X Coating shows significant thrombo- resistance performance in this test, considering that X coating does not have any anti-coagulation function similar to heparin coating.

WATER STRUCTURE

The exact reason why PMEA prevents denaturation and adsorption of proteins onto material surfaces still remains a mystery. Other polymers exhibiting hydrophilic, sometimes even amphiphilic properties similar to the ones found in X Coating do not always achieve the same level of

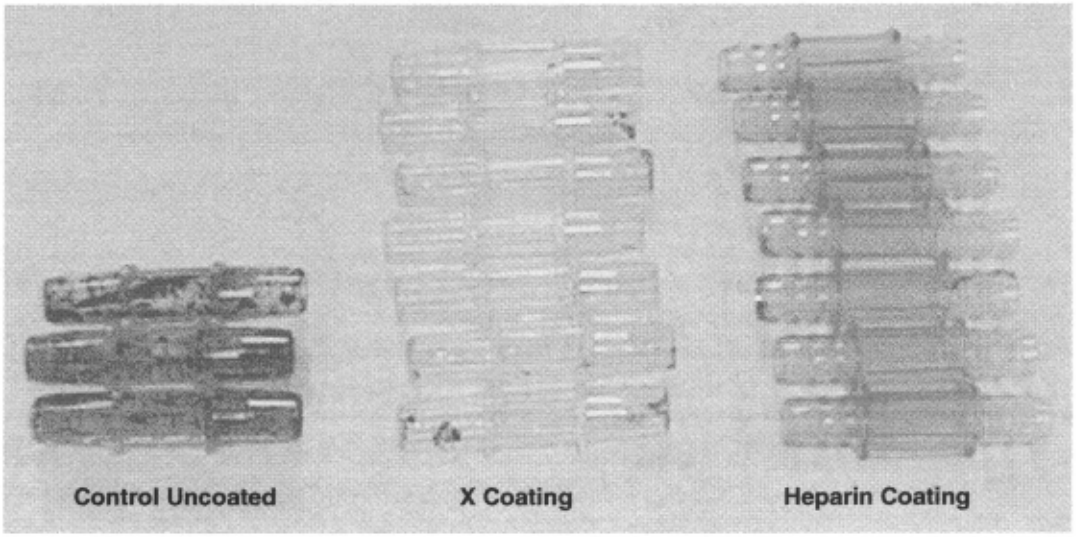

Figure 17: Thrombo-resistance Performance Test

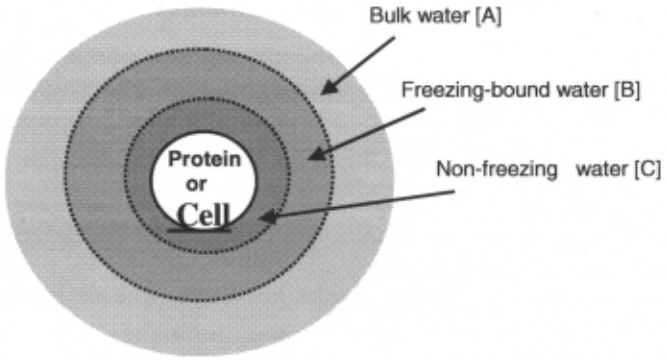

Figure 18:. Water arrangement around native protein. [A] Bulk water, [B] Freezing-bound water, [C] Non-freezing water.

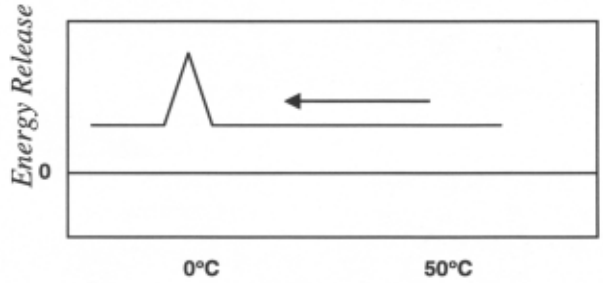

Figure 19: Energy curve of water molecules transitioning from amorphous form to crystal form.

hemocompatibility. A study investigated in details the different water structures in polymer. There is a peculiar phenomenon observed on the surface of protein, cell and some biocompatible materials. The phenomenon is called Cold Crystallization. The freezing-bound water structure is responsible for this phenomenon.

It is well known that in a hydrated polymer, there are two kinds of water: water which shows first-order phase transition, such as crystallization and melting, called freezing water (bulk water) and water which never crystallizes even at -100°C called non-freezing water. Non-freezing water is strongly bound to polymers. Proteins or cells have typically three layers of water surrounding them (Fig. 18). The layer of water directly in contact with the protein or cell is called non-freezing water, the second layer of water in contact with the non-freezing water is referred to as freezing-bound water, while the remaining water touching the cell is called free or bulk water[19].

As stated earlier, it is believed that most polymers do not contain the freezing-bound water molecular layer. One theory, currently under further investigation, holds the premise that proteins that come in contact with a polymer surface containing only freezing water and bulk water will denature.

A study published in Polymer International[20] demonstrated that X Coating™ does contain the freezing-bound water layer. Each water molecular structure owns defined freezing characteristics. In this study, the author's objective is to assess freezing-bound water in PMEA. The cold crystallization of water mentioned earlier will be interpreted as the phase transition from the amorphous ice to crystalline ice, which belongs to the freezing-bound water in the polymer. Therefore, the study will evaluate the enthalpy (chemical energy or heat content from a molecule) released at various temperature end-points. The hydrated PMEA sample was first cooled to -100°C at the rate of 2.5°Cmin^{-1}, held at -100°C for 10 min, and then heated to room temperature at the same rate.

To better understand the results from the Cold Crystallization study, one needs to understand the enthalpy dynamics of water molecules when either being cooled below freezing temperatures or being reheated to transition back to its original aqueous form. Fig. 19 illustrates a normal energy curve of bulk water transitioning to ice. As bulk water loses heat or energy, the temperature gradually decreases

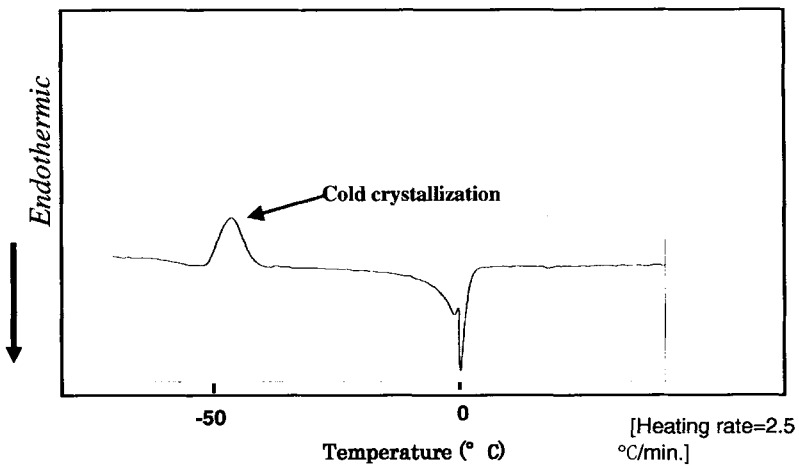

Figure 20: Shows DSC heating curves of PMEA-water system

from 50 degrees to zero degree. There is a release of energy when water molecules transition from liquid to crystal formation. As a result, a peak of positive energy (exothermic peak) is observed when the water molecules release energy to transition into ice at 0°C. The same phenomenon holds true when water molecules transition from ice back to a liquid form. However, the energy curve observed will show a negative peak of energy (endothermic peak) as energy from the surroundings is drawn by the water molecules to decrease the affinity between each other, thus releasing themselves from a crystal formation into a liquid formation.

Figure 20 is the energy curve of water molecules when in contact with PMEA. There are three energy peaks indicating that there are three transition temperature points (one for bulk water and two for freezing-bound water). As previously discussed, non-freezing water will under no circumstances achieve crystallization. At -100°C, bulk water is crystallized, while non-freezing and freezing-bound water remain in an amorphous state. The positive energy peak (exothermic peak) observed at -42°C indicates the release of energy and the transition phase for freezing-bound water to change from an amorphous arrangement to a crystallized form. Secondly, two endothermic peaks are present at -3°C and -1°C. Analysis of these peaks revealed an increase in molecular motion indicating a higher consumption of energy from the water molecules, which indicates that the freezing-bound water's melting point is at -3°C while the bulk water's melting point is reached at -1°C.

The Differential Scanning Calorimetric (DSC) method provides a scientific methodology to identify quantitatively the ratios of all three types of water contained in PMEA. These ratios can be determined from the enthalpies of melting and crystallization of the water structures. The total water content (i.e. equilibrium water content) of PMEA includes 32% non-freezing water, 48% freezing-bound water and 20% of bulk water. This means that the major part of the hydrated water in the equilibrium state is the most crucial type involved in the protein denaturation process, the freezing-bound water. In some earlier publications on cold crystallization of water in hydrated polymers, such as polysaccharide-water, poly (ethylene oxide) (PEO), cold crystallization is observed. In general, cold crystallization is observed in a very narrow water content range. For PMEA, cold crystallization is observed over a wide water content range and is very reproducible.

In conclusion, this investigation did confirm that when hydrated, X Coating™ contains a significant amount of freezing-bound water. This particular molecular structure of water plays a key role in the hemocompatibility of surfaces in contact with blood during cardiopulmonary bypass procedures. Consequently, proteins in contact with an X coated surface will maintain their native conformation, therefore X Coating will reduce protein denaturation.

SUMMARY

X Coating has been shown to reduce protein denaturation and platelet adhesion when applied to the various materials found in cardiopulmonary bypass circuits. Early clinical data reported[21] demonstrates that X Coating improves patient outcomes. Further studies are currently in progress to evaluate the benefits of this new polymer coating.

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