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TITLE SURFACE MODIFICATION OF POLYMERIC MATERIALS AND ITS
EFFECT ON BLOOD COMPATIBILITY

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SURFACE MODIFICATION OF POLYMERIC MATERIALS AND ITS EFFECT ON BLOOD COMPATIBILITY

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ABSTRACT

The surfaces of commercially available polymeric materials have been modified through two techniques, the chemical infusion process and physical vapor deposition. The surfaces of poly(methylmethacrylate) (PMMA) have been modified through a chemical infusion process by treatment of the sample with a solution containing varying amounts of titanium(IV)isopropoxide and polyvinylpyrrolidone (PVP). The surfaces of silicone rubber samples have been coated with a thin coating of titanium dioxide with an ion beam sputtering technique. The treated samples were characterized by scanning electron microscopy, optical microscopy, and neutron activation analysis. The infused samples were evaluated for blood compatibility using two biological assays: 1) an adherence assay in which the adherence of human polymorphonuclear leukocytes to the samples was determined, and 2) a hemolysis assay using rat blood erythrocytes to determine the hemolytic activity of the samples. Based on the results of these assays, the PMMA samples treated with PVP alone resulted in an improvement in reactivity with the blood cells.

INTRODUCTION

Polymeric materials are used in a wide range of medical applications because of their unique physical properties such as strength, elasticity, and pliability. A potential application could include small diameter vascular implants if one of the major problems of polymeric biomaterials in contact with blood, thrombus formation[1], could be overcome. Although the underlying processes are not completely understood, an initial step in the thrombogenic sequence is the adsorption of plasma proteins to the surface of a material followed by adhesion of platelets and/or leukocytes[2]. The extent of thrombosis, in turn, is related to the surface properties of the polymer exposed to blood such as surface charge and polarity, surface energy, hydrophilicity, chemical composition, and surface roughness[3]. Due to the detrimental consequences of one or more of these surface properties, even the best commercially available polymers continue to show limited blood compatibility[4].

One of our approaches to surface modification uses a technique originally intended to smooth polymer surfaces, the chemical infusion process[5,6]. This technique can introduce normally insoluble materials into the outermost layer of a polymeric material, thereby, altering the surface without changing its bulk physical properties. With a second approach, the ion beam deposition technique can deposit very thin coatings of materials on polymeric surfaces with minimal heat generation. In our attempts to improve its blood compatibility, these techniques were used to impregnate a polymeric surface with biocompatible materials.

Polyvinylpyrrolidone (PVP) and titanium dioxide (TiO₂)

materials to be incorporated into polymeric surfaces because they could potentially provide a non-thrombogenic surface. PVP was chosen because of its intrinsic blood compatibility. PVP was used extensively as a blood plasma substitute or blood volume expander during World War II by the German army and is responsible for saving thousands of lives[7, 8]. In fact, a follow-up study of people who had received transfusions of PVP solution showed no permanent kidney or liver damage[9]. Therefore, because of its benign nature, PVP showed promise as a material for further investigation. Titanium dioxide was considered as a surface coating material for our experiments because of the unusually low bioreactivity and non-toxic nature of titanium in soft tissue[10-12] which is attributed to the high thermodynamic stability of the protective surface oxide. Moreover, in a study of the dielectric properties of various cardiovascular implant materials, strong correlations were found between materials that demonstrate antithrombogenicity and their dielectric constants[13]. Exposure of these material surfaces to blood proteins produced pronounced electrokinetic and dielectric charge. Therefore, titanium dioxide with its high thermodynamic stability, low reactivity, and high dielectric constant of the order of 110 and the blood compatible PVP may provide a stable, low thrombogenic surface on polymeric surfaces. Our evaluations of the blood compatibility of the infusion treated samples have been conducted in terms of how such modifications of the PMMA affect blood cell interactions with the surfaces.

EXPERIMENTAL

PMMA, Plexiglas VM^R, was purchased from Rohm & Haas (Philadelphia, PA) and injection molded into a disk shape. The silicone rubber disks were prepared using Dow Corning^R (Midland, MI) MDX-4-4210 Clean Grade Elastomer. Titanium(IV)isopropoxide and polyvinylpyrrolidone (PVP, molecular weight 10,000) were purchased from Aldrich Chemical, Co. The former was used as received while the PVP was dried as previously reported[14]. Baker reagent grade isopropanol (IPA) and acetone were dried over Drierite^R, distilled and handled under argon.

The chemical infusion process requires preparation of two solutions. The diluent solution was prepared by addition of titanium(IV)isopropoxide to a 35% solution PVP (w/w) in IPA. The amount of titanium(IV)isopropoxide was varied from 0 to 50% by weight. The starting solution was prepared by adding 35% acetone by weight to the diluent solution. The infusion chamber (Figure 1) is initially charged with this starting solution. The apparatus consists of two glass tubes connected by silicone tubing. The solution in one tube is mechanically stirred in order to ensure complete mixing while the other tube holds the samples. One peristaltic pump circulates the solution in the infusion chamber while a second pump adds the diluent solution in small increments. In this manner, the solution in the apparatus gradually becomes richer in diluent. The total volume of 325 ml for the system requires about 7 hours dilution time at a rate of 2 ml/min for complete replacement of the starting solution with the diluent. The final solution contains greater than 95% diluent. Upon removal from the final solution, the samples were rinsed with IPA or deionized water for samples with or without titanium in the diluent solution, respectively.

The physical vapor deposition of titanium was performed in a 1 meter diameter, cryopumped chamber (pressure 5×10^{-5} torr, Figure 2). The titanium target was placed 9 cm from a 1500 EV hollow cathode ion source. A second similar source was aimed at the silicone rubber samples in a rotating fixture 15 cm from the target to clean the samples with an argon ion beam at a density ~ 20 ma/cm² or ~ 1.5 KEV for 2 min. After cleaning, the second source was turned off and the deposition of titanium was accomplished using

Figure 1. Schematic of the chemical infusion apparatus used for infusion of moisture sensitive additives such as titanium(IV)isopropoxide.

Figure 2. Schematic of apparatus for ion beam sputter deposition of titanium on silicone rubber substrates. Pictured is 1) the narrow focus 10 KVV ion source, 2) wide focus 10 KVV ion source, 3) titanium target, 4) rotating substrate upon which the samples are mounted within the 5) 1 meter vacuum chamber.

the first source at about the same power settings. A deposition rate of 5 Å per sec. was measured by a piezo electric crystal monitor, while a glass witness slide was included on the rotator by the samples. The 500Å titanium coating thickness was measured with a stylus step gauge. Type K thermocouple measurements showed the ambient sample temperature to be 45°C.

The blood compatibility of infusion treated samples was evaluated through two biological assays: an adherence assay and a hemolysis assay. The adherence assay determined the adherence of human polymorphonuclear (PMN) leukocytes to the polymeric samples. The PMN suspension was prepared as previously described[14]. The cell adherence assay procedure was a modified version[14] of that previously described by Lehnert and Ferin[15]. Adherent cells were calculated from the total number of cells initially added to each sample well in the blood testing chamber minus the nonadherent cell numbers following the oscillation phase of the assay. The hemolysis assay used rat blood erythrocytes to determine the hemolytic activity of the samples. The hemolysis of erythrocytes was quantitated spectrophotometrically using a modification [14] of the hemolysis assay described by Harrington and colleagues [16] and red blood cells obtained from adult, male rats. This assay allows us to determine what impact modifications of the polymer surface have on the rupture of cell membranes.

RESULTS AND DISCUSSION

Because surface roughness is one of the surface properties believed to affect the thrombogenicity of the substrate, the chemical infusion process and ion beam deposition were chosen to modify the surfaces of polymers. Both of these techniques can potentially provide smooth surfaces or coatings on polymers in addition to being useful to incorporate biocompatible materials. Silicone rubber substrates have been coated with titanium by the vapor deposition technique. Typical surface characteristics of an oxygen cleaned silicone rubber substrate with a 500Å titanium coating are shown in Figure 3. This sample shows macroscopic cracks in the coating, visible as straight dark lines along with a surface "wavyness". This "wavyness" feature is observed for all samples regardless of variation in deposition conditions such as sputter cleaning with argon or oxygen gas and argon sputter deposition with or without oxygen assist. Because of the difference in thermal expansion of the substrate and the titanium coating, one cause of the cracks and surface features could be the heat generated during the deposition process. By reducing the rate of deposition by reduction of the ion beam density, the substrate temperature should be lowered giving smoother surfaces.

The chemical infusion process was first developed to smooth the surfaces of PMMA rods for use as mandrels for physical vapor deposition of thin metal foils[5]. This technique requires selection of an appropriate solvent for the polymer to act as the solvent component. A second solvent is chosen as a poor solvent for the polymer and will be referred to as the non-solvent component. The non-solvent component must be miscible with the solvent and any additives must be initially soluble in the solvent/non-solvent mixture. This starting solution causes the polymer matrix to swell and, thereby, facilitates the diffusion of the insoluble additives into the treated sector. As the infusion process proceeds, the initial solution is gradually replaced with the non-solvent mixture until the solution consists of greater than 95% diluent. During this latter phase of the process, the solvent diffuses out of the polymer, which results in the restoration of the polymer's surface rigidity. At the end of the process, the polymer samples are removed from the bath, rinsed with the non-solvent to remove additives adsorbed on the surface, and allowed to dry.

In order to smooth a polymer's surface, the proper combination of solvent, non-solvent, and polymeric additive must be selected. The first solvent system that gave a smooth surface was a solution containing poly(ethylene glycol) (PEG), water, and acetone. In addition to smoothing

Figure 3. Scanning electron micrographs of a 500Å argon ion deposited titanium coating on a oxygen sputter cleaned silicone rubber substrate.

the surface, it was noted that the additives (PEG and water) were incorporated in the sample. In this work, PVP and titanium dioxide were selected for incorporation in the polymer surface. Since titanium dioxide is insoluble in all common solvents, a soluble titanium alkoxide compound, titanium(IV)isopropoxide, was chosen as the additive. By preparing solutions with moisture free solvents, we were able to exploit the hydrolysis reaction indicated in Equation 1 to convert the soluble titanium alkoxide

compound into the insoluble titanium dioxide. Because this hydrolysis reaction is very favorable, the infusion apparatus must be rigorously kept free from moisture. Upon infusion of a PMMA sample with a solution containing titanium, the sample becomes opaque. The opaque nature of these samples can be attributed to the titanium alkoxide being converted into titanium dioxide. By looking at the cross-section of an infused sample, the infused sector can be easily seen; Figure 4 clearly shows that only the outermost layer is modified during the chemical infusion process.

The surfaces of the samples containing PVP, both with and without titanium, are much smoother than those without PVP. Deletion of PVP leads to a rough, uneven surface unsuitable for blood interaction studies. We examined cross-sections of samples treated with titanium solutions with and without PVP by scanning electron microscopy and energy dispersive X-ray analysis. The results of this study indicate that there is a significant decrease in titanium particle size between samples without PVP and samples containing PVP. In addition, the range of particle sizes is greater without PVP while more uniform particle size is evident with PVP. The PVP appears to aid in a more uniform distribution of the titanium. Most importantly, the overall surface is smoother with the PVP additive.

The amount of titanium in the infused samples was quantitated by neutron activation analysis as previously described[14]. By plotting the titanium concentration in the diluent solution versus the concentration of titanium in the infused sector, the concentration of titanium in the infused sector was estimated to be 38% of its concentration in the diluent. This implies that 38% of the infused layer and the surface is modified in the process. Thus, the process may alter a significant portion of the surface of the polymer.

Infusion of the PMMA samples with titanium (Ti) did not appear to importantly alter the adherence of human polymorphonuclear leukocytes (PMN) in a directional manner consistent with changing Ti concentration in the

Figure 4. Cross-section at 100X magnification of a PMMA disk treated with a diluent solution containing PVP and 40% titanium-(IV) isopropoxide in the diluent solution. The top of the sample is the boundry between the top (dark) and middle layer which corresponds to the infused sector of 280μm while the bottom layer is untreated polymer. Note the sharp interface between the infused region and the untreated PMMA region.

infused region. Greater than 70% of the PMN adhered to all the Ti-treated disks, the parent PMMA substrate, Teflon^R, the polystyrene disks, and to the commercially available polystyrene culture plates pre-treated by the manufacturer by electrostatic discharge. On the other hand, surface smoothing and infusion of the PMMA substrate with polyvinylpyrrolidone substantially reduced the adherence of the PMN. In results essentially identical to the Tecoflex^R and silicone rubber substrates, only ~50% of the PMN adhered to the PVP-treated disks. Infusion of the PMMA substrate with varying concentrations of Ti did not result in any consistent increases or decreases in the hemolytic activities of the disks as a function of Ti concentration in the infused region. All substrates tested lysed ~2-6% of the rat erythrocytes with the outstanding exception of polystyrene, which caused ~9% of the red blood cells to lyse. Thus, these preliminary biological studies suggest that the incorporation of PVP into a polymer's surface by the infusion process may favorably increase the polymer's compatibility with blood.

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