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Contact angle studies of the surface properties of covalently bonded poly-L-lysine to surfaces treated by glow-discharge

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Abstract: Contact angle data, measured by using a sessile drop arrangement in conjunction with Axisymmetric Drop Shape Analysis-contact Diameter (ADSA-CD), were used to quantify the effects of ammonia gas plasma treatment on the surface properties of previously untreated polystyrene surfaces. The surface tension of treated polystyrene samples is considerably higher than that of untreated samples. The increase in surface tension following plasma treatment is attributed to the addition of amine groups to the surface.

Next, conformational changes following the attachment of poly-L-lysine to the untreated samples by simple adsorption and plasma treated samples by covalent bonding were investigated. Surface tension values obtained from contact angle data indicate that conformational changes to poly-L-lysine occur in both cases, because these values are lower than the surface tension of poly-L-lysine in solution. However, contact angle data show that covalently bonded poly-L-lysine undergoes less conformational changes than simply adsorbed poly-L-lysine.

Key words: Contact angles on poly-L-lysine – plasma treatment of polystyrene surface – adsorption of poly-L-lysine – axisymmetric drop shape analysis of poly-L-lysine surfaces

Introduction

Protein adsorption to vascular biomaterials plays an important role in determining the biocompatibility of these implants. Most biological interactions with materials occur after protein adsorption has taken place so that blood cells do not interact directly with the polymer implant or tissue material, rather, they interact with the adsorbed material-protein interface [1, 2]. In addition, clogging of vascular prostheses, blood oxygenators, intravascular catheters, and hemodialysis equipment is a consequence of the adsorption of plasma proteins and adhesion of blood components to the synthetic surfaces. Studies have shown that on natural surfaces, plasma proteins remain in their native state and are reversibly adsorbed [3], while on artificial surfaces, on the other hand, the proteins are irreversibly adsorbed and undergo substantial changes in conformation [4]. This then leads to subsequent thrombogenic events of platelet and erythrocyte adhesion and surface induced coagulation [1, 2]. It is apparent then that thrombus formation on artificial surfaces is strongly influenced by the nature of the adsorbed protein layer. Thus, the possibility arises of minimizing the number of thrombogenic events by adsorbing a protein layer, which does not undergo substantial conformational changes, on the artificial surface.

Many approaches have been developed to recreate, as closely as possible, a surface which mimics the real system. Some researchers have attempted to graft endothelial cells onto artificial surfaces thereby completely recreating the natural surface [5]. The major difficulty of this approach is that after five generations of cells have been cultured, the phenotype of the cells begins to change from endothelial cells to non-differentiated cells, causing them to lose their non-thrombogenic qualities.

A more promising approach would be to attach irreversibly a closely packed layer of protein to the artificial surface without altering significantly the conformation of the protein molecules. In order not to change the conformation of the protein, the number of bonds between the protein and the surface should be kept to a minimum. This requires bonds which are stronger than van der Waals type interactions: covalent bonding. It can be assumed that any conformational changes resulting from covalent bonds will be local and very close to the synthetic surface so that the remainder of the protein molecule will be unaffected. This approach has been applied to enzymes which were covalently bonded to surfaces but still kept their reactivity [6], indicating that the bonding process did not greatly affect the properties of the enzymes.

In this paper the feasibility of using a gas plasma to add functional groups, which are then subsequently used to bond covalently the protein, onto artificial surfaces, is investigated. Gas plasma is an attractive method which can be used to modify the properties of surfaces because it is a simple one-step process. On the other hand, other currently used methods involve multi-step reactions to attach functional groups onto solid surface and elaborated rinsing procedure to ensure that all the by-products of multi-step reaction and unused reagents between each step are removed [7, 8]. In addition, by its very nature, it is a very clean process because a surface is in contact only with a flowing gas. The flowing gas continuously removes from the system any unused reactants and unwanted by-products thereby reducing the need to use stringent rinsing procedures of the conventional techniques.

In the present studies the attachment of a polypeptide, poly-L-lysine, to treated and untreated polymer surfaces was investigated. The changes in surface properties of poly-L-lysine following attachment to the different surfaces were quantified by means of contact angle data interpretation. Contact angle measurements were obtained from the contact diameter of sessile drops by means of the Axisymmetric Drop Shape Analysis-Contact Diameter technique (ADSA-CD) [9, 10]. The advantage of using contact angle interpretation to study the surface attachment of poly-L-lysine is that contact angles are the most direct manifestation of surface energetics.

Materials and methods

Materials

The polymer that was used as the surface to be plasma treated was polystyrene (Central Research Laboratory, Dow Chemical); it is readily available in a thin film which is very smooth and uniform as confirmed by the high reproducibility of contact angle results.

The plasma gas used to add functional groups to the surface was anhydrous ammonia, NH_3 with minimum purity of 99.99% (Canada Liquid Air). Argon, with purity in excess of 99.999% (Canada Liquid Air) was used to flush the harmful ammonia gas from the plasma chamber once the surface treatment was completed.

Once surface treatment was completed a polypeptide, poly-L-lysine (Sigma Chemical, St. Louis, Missouri), with a molecular weight between 1000 and 4000, was bonded on the surface.

Methods

A Harrick plasma chamber (Fig. 1) was used to chemically treat the polystyrene surfaces. The power input to the plasma is by means of an inductive coil. The maximum power of the apparatus is 30 W at a frequency of 13.56 MHz (radio frequency).

Surface treatment proceeded as follows: The polystyrene strips were placed in the chamber near the rear of the chamber where the plasma is most uniform [11]. The chamber was then evacuated to approximately 100 μm Hg and the ammonia gas was introduced. The pressure in the chamber was allowed to equilibrate at the desired value (typically between 100 and 500 μm Hg) before the full power of 30 W at 13.56 MHz was applied. The power was applied for the desired length of time after which the power was turned off and the ammonia gas supply was shut off. Argon gas was then introduced into the chamber to flush out the remaining ammonia gas. After flushing for approximately 5 min with argon gas, the vacuum pump was shut off, allowing the chamber to fill with argon to atmospheric pressure. Once the front cover of the chamber was removed the treated polystyrene surfaces were carefully removed and were either immediately placed in a 6% glutaraldehyde solution (Grade I,

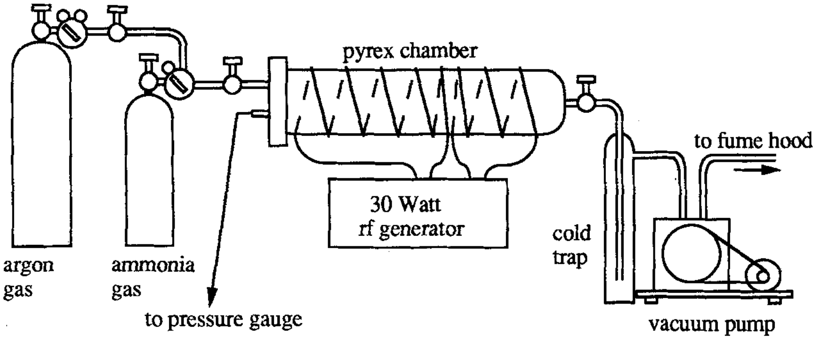

Fig. 1. Schematic of Harrick Plasma System used for chemical modification of surfaces.

Sigma Chemical, St. Louis, Missouri), as discussed in the next section, or mounted on clean glass slides for contact angle measurements.

Argon treated surfaces were also investigated. The reason for studying argon treated surfaces is twofold: 1) argon, being an inert gas, allows the determination of the effects of the plasma treatment itself on the surface properties of the material; 2) because no amine groups are introduced on the surface during argon plasma treatment, the differences in the contact angle obtained following the two different gas plasma surface treatments can be directly attributed to the amine groups.

Polypeptide bonding

A simple chemical reaction was used to bond poly-L-lysine covalently to the chemically modified surface. First, glutaraldehyde was bonded to the amine groups on the surface and then the poly-L-lysine was bonded to the free ends on the glutaraldehyde molecules [12]. A schematic of the reaction is given in Fig. 2 and the details of the procedure were as follows:

After removal from the plasma chamber the surfaces were placed in a 6% glutaraldehyde solution at 4 °C for 3 h. Then, the surfaces were rinsed thoroughly in running distilled water. The surfaces were then placed in a 0.1 g/L poly-L-lysine solution in a 0.01 M phosphate buffer solution (pH 8.0) at 4 °C and incubated overnight. After incubation, the surfaces were again rinsed in distilled water and washed with 0.01 M TRIS buffer at pH 7.5 and 0.1 M NaCl. The buffer was poured off and replaced every 15 min for about 2 h. Finally, the surfaces were once again rinsed thoroughly with distilled water. After this procedure the surfaces were mounted on clean glass slides and contact angles on these surfaces were measured.

For comparison purposes, poly-L-lysine adsorption onto untreated polystyrene strips was also attempted. The procedure followed was the same as that described for plasma treated surfaces with the exception that the untreated surfaces were not placed in the glutaraldehyde solution. Contact angle measurements were also performed on these surfaces.

Contact angle and liquid surface tension measurements

Contact angle measurements with water were used to quantify the effects of plasma treatment on the polystyrene surfaces. It is expected that the chemical treatment of the surfaces will affect the surface tension of the solid surfaces which will be reflected in the contact angle value. An equation of state will be used to estimate the surface tension of the chemically treated surfaces [13, 14].

The addition of amine groups to the polystyrene surface will increase the surface tension of the substrate thereby decreasing the contact angle formed by a water drop. It has been our experience that small contact angles cannot be accurately measured from the profile of the drop using a goniometer. Thus, in order to improve the accuracy of the results, a drop shape analysis called ADSA-CD was used. [9, 10]. In essence, it is a technique where the contact angle is determined by numerically minimizing the difference between the volume of a drop predicted by the Laplace equation of capillarity and that prescribed experimentally. The required input parameters are: drop volume, liquid surface tension and contact diameter of the sessile drop. The last parameter,

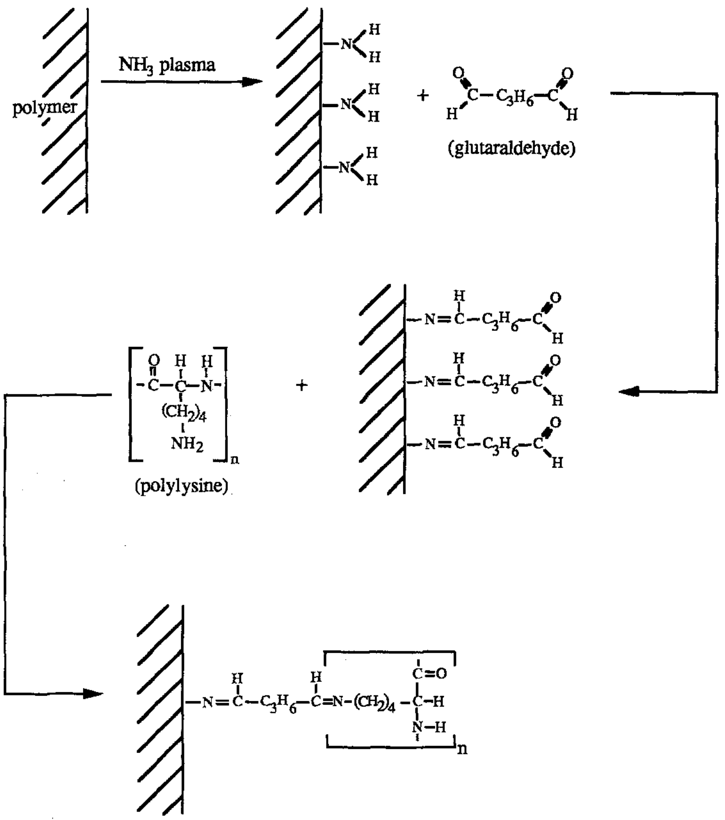

Fig. 2. Schematic diagram of chemical reaction used to bond poly-L-lysine to a polymer surface.

the contact diameter, was obtained from a digital image of the drop taken from above [9, 10]. From this image coordinates of the three-phase line were digitized and the contact diameter calculated from a least squares fit of a circle to these points. The surface tension of water was measured by a pendant drop arrangement in conjunction with ADSA-P, which is another drop shape technique with an accuracy of 0.1 mJ/m^2 [15]. The volume of the drop deposited on the surface was controlled by a micrometer Gilmont screw syringe that was used to deliver the drop. The volume of the drop could be read with an accuracy of $\pm$ 0.15 μl, as claimed by the manufacturer. The ADSA-CD technique is capable of calculating contact angles, from the given input data, with an accuracy better than $\pm$ 1.0° [9]. It is a superior technique for measuring low contact angles on high energy solid surfaces. For small contact angles, the image of the drop appears fuzzy near the surface and it becomes exceedingly difficult to locate the point where the profile of the drop meets the surface. When techniques relying on the analysis of the drop profile are utilized, large errors may be produced [9]. On the other hand, ADSA-CD, requiring a top view of the drop and using the contact diameter instead of the profile of a liquid-vapour meniscus as the main experimental parameter, is well suited for determining small contact angles.

Results and Discussion

Contact angles with water, measured as a function of time of exposure to air after plasma treatment, are given in Fig. 3 for both ammonia and argon. In this figure and in all subsequent figures the errors correspond to 95% confidence limits. The contact angles increase substantially with time of exposure to air after a relatively stable initial period of about 20 min, as shown in Fig. 3. This same trend was observed for all the different plasma treatment conditions that were investigated. It was, therefore, decided to make all

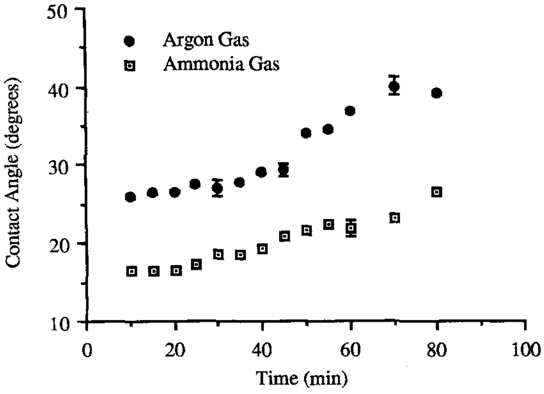

Fig. 3. Contact angles, measured by ADSA-CD, as a function of time of exposure to air after argon or ammonia plasma treatments. Plasma conditions: pressure = 300 μm Hg, length of treatment = 30 min.

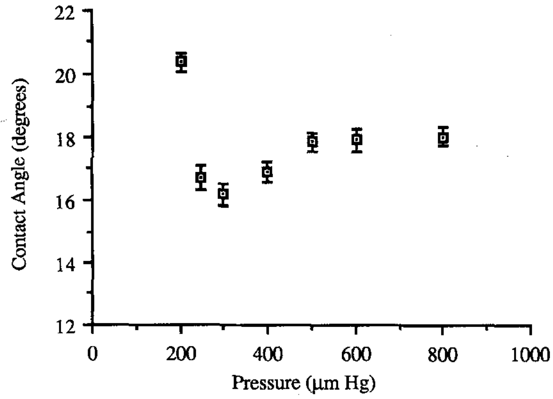

Fig. 4. Contact angles, by ADSA-CD, as a function of ammonia gas plasmas pressure for a constant length of treatment of 30 min.

subsequent contact angle measurements on plasma treated surfaces after 20 min exposure to air.

The increase in the contact angle value with time of exposure to air after treatment, as shown in Fig. 3, might be caused by surface relaxation, similar to polymorphic phase transitions. The increase in the contact angle value with time of exposure is very large suggesting a large change in surface tension value. However, contact angle changes associated with allotropic transitions of hydrocarbons are very small [16]. In fact, the changes in contact angle are so slight that only the capillary rise method of measuring contact angles (accuracy of ± 0.1°) is able to detect such transitions [16]. The more likely cause for the rise in the contact angle seems to be the slow reaction of surface and trapped radicals, formed by the exposure of ultraviolet radiation of the plasma, with atmospheric oxygen [17]. Specific reactions with the attached amine groups are unlikely to cause the rise in contact angles since a similar rise in contact angles is observed for samples treated with argon plasma, which does not add amine groups to the surface. It is apparent, however, that either argon or ammonia plasma treatments affect significantly the surface tension of polystyrene. Untreated polystyrene has water contact angle of approximately 90°, considerably higher than the contact angles, following plasma treatment, of 16° for ammonia and 25° for argon.

Next, experimental conditions for optimal chemical modification of the surfaces by ammonia gas plasma were investigated. Polystyrene surfaces were plasma treated for various pressures of the ammonia gas and for different lengths of treatment. The effects of the different treatments at various pressures were quantified using contact angle measurements. In Fig. 4 contact angles measured as a function of pressure of the ammonia plasma are plotted. The time of treatment for all cases was fixed at 30 min. From the results plotted in Fig. 4 it can be concluded that the maximum effect occurs at a pressure of 300 μm Hg since the lowest contact angle was observed at this pressure. It seems likely, that at this pressure, the maximum number of amine groups are introduced on the surface. These findings are in complete agreement with values reported in the literature [18].

In Fig. 5 contact angles measured as a function of length of treatment, for a constant pressure of 300 μm Hg, are given. The smallest contact angle was observed for a treatment time of 30 min. From the results presented in Figs. 4 and 5 the plasma treatment conditions for all subsequent surfaces were standardized to 30 min exposure at a gas pressure of 300 μm Hg.

It might have been expected that after a certain length of treatment the concentration of amine groups would reach a saturation value which would not change with length of treatment. One possible explanation for the observations is that ablation of the surface is occurring at the same time as the chemical modification of the surface takes place. At the longer times of exposure the gas temperature is higher, resulting in an

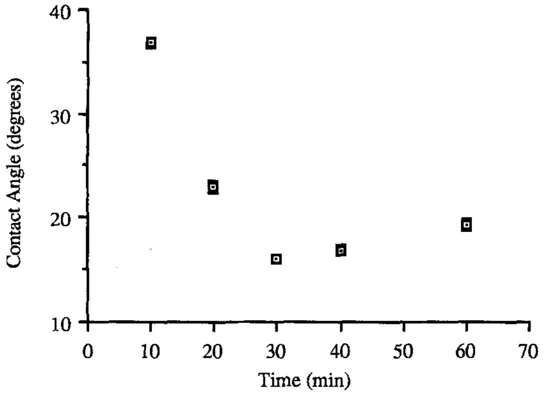

Fig. 5. Contact angles, by ADSA-CD, as a function of length of treatment for a constant ammonia gas plasma pressure of 300 μm Hg.

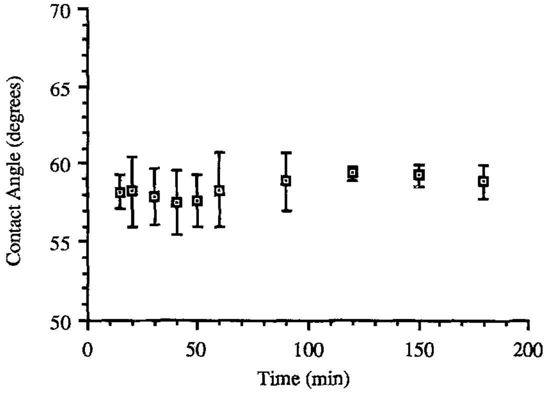

Fig. 6. Contact angles, by ADSA-CD, as a function of time of exposure to air following poly-L-lysine attachment to ammonia gas plasma treated surfaces.

increased kinetic energy of the particles bombarding the surface. The higher energy particles would have a higher probability of removing the attached amine groups from the surface.

Contact angles, as a function of time of exposure to air, were also measured on the plasma treated samples after removal from a 6% glutaraldehyde solution. The contact angles did not vary greatly with time of exposure to air. The average contact angle for ammonia treated surfaces was 60 ± 0.5° for a range of exposure times between 10 and 180 min. Similarly, the average contact angle for the argon treated surfaces was 32 ± 0.6°. The difference in the measured contact angles for the two different types of surfaces suggest that specific bonding of the glutaraldehyde molecules to the amine groups occurs on the ammonia plasma treated surfaces. If glutaraldehyde molecules simply adsorbed on the surface it would be expected that the contact angles for the ammonia treated surface would be similar to that of the argon treated surface.

Finally, following glutaraldehyde attachment to the ammonia treated samples, the same samples were placed in the poly-L-lysine solution. Contact angles were measured on these surfaces as a function of time after removal from solution and are given in Fig. 6. There is no significant variation in the contact angles with time after removal, indicating that poly-L-lysine attachment to the surfaces is quite stable. the average contact angle measured was 58.4 ± 0.4° corresponding to a solid surface tension, γ_{SV}, of 48.2 ± 0.2 mJ/m^2.

It is interesting to compare this value for poly-L-lysine covalently bonded to the surface to those from poly-L-lysine in an aqueous solution and from poly-L-lysine simply adsorbed onto a surface. Contact angles were measured for poly-L-lysine adsorbed onto untreated polystyrene from a 0.1 g/L aqueous solution. The measured contact angle was 90.1 ± 0.9°, corresponding to a γ_{SV} of 28.5 mJ/m^2 which is very close to the surface tension of bare polystyrene. These results might indicate that poly-L-lysine did not adsorb onto the surface at this concentration. Adsorption was attempted again but with a tenfold increase in the concentration of poly-L-lysine. The contact angle obtained for this concentration was 86.9 ± 0.9°, corresponding to a γ_{SV} value of 30.5 mJ/m^2.

The surface tension, γ_{LV}, at 20 °C for aqueous solutions of poly-L-lysine were measured with the ADSA-P technique. The surface tensions obtained were 72.3 and 72.9 mJ/m^2 for concentrations of 0.1 and 1 g/L, respectively. These values are very close to that of water, 72.8 mJ/m^2 at 20 °C. The obtained γ_{LV} values are in good agreement with literature values [19], for the same pH, which were obtained using the Wilhelmy plate method. Similar to the present work, in ref. [19] it was found that there was no significant dependence of γ_{LV} on the concentration of poly-L-lysine.

The results show that poly-L-lysine, at pH 8, is very hydrophilic in an aqueous state and that it becomes more hydrophobic when it is attached to a surface. It seems likely from the contact angle results and the corresponding surface tensions that covalently bonded poly-L-lysine undergoes less conformational change than a simply adsorbed poly-L-lysine layer. It also seems that covalent bonding of poly-L-lysine to the surface is a more efficient way of coating a surface than by simple adsorption from solution. While a 0.1 g/L concentration was sufficient to bond covalently poly-L-lysine to the surface, it was not sufficient to alter significantly the surface tension of polystyrene by simple adsorption.

The results presented indicate that conformational changes occur to poly-L-lysine when it is attached onto a solid substrate either by adsorption or by chemical bonding. While such changes cannot be avoided, it may be possible to reduce them greatly by the simple plasma treatment of the solid surface described in this work.

Conclusions

1) Contact angles can be used to investigate surface energetic changes following plasma treatment of surfaces. ADSA-CD is a highly accurate and sensitive technique for detecting differences in contact angles between different surface modification strategies used in this study.
2) Plasma treatment was used to modify the surface properties of polystyrene films. The surface tension of the film changed from 29 to 70 $\mathrm{mJ/m^2}$ following treatment with ammonia plasma.
3) Optimum plasma treatment conditions, for the present studies, were found to be at a gas pressure of 300 μm Hg and for a treatment period of 30 min.
4) Covalently bonding poly-L-lysine to a plasma treated surface produces a surface on which poly-L-lysine undergoes minimal conformational changes.

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