Reduction of Bacterial Adhesion to Biocompatible Polymer Surfaces Via Plasma Processing

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ABSTRACT: Plasma processing of the surfaces of biomaterials is interesting because it enables modification of the characteristics of a surface without affecting bulk properties. In addition, the results are strongly influenced by the conditions of the treatment. Therefore, by adjusting the plasma parameters it is possible to tailor the surface properties to best fulfill the requirements of a given application. In this work, polyurethane substrates have been subjected to sulfur hexafluoride glow discharge plasmas. The influences of different SF6 plasma exposure times and pressures on the adhesion of Staphylococcus aureus and Pseudomonas aeruginosa to the polymer have been investigated. The wettability and surface free energy have been evaluated via contact angle measurements. At low pressure (6.7 Pa) the contact angle decreases with increasing exposure time in the 180 s to 540 s interval, but at higher pressure (13.3 Pa) it increases as a function of the same variable. Bacterial adhesion has been quantified from in vitro experiments by determining the growth of colonies on Petri dishes treated with agar nutrient. It has been observed that the surface properties play an important role in microbe adhesion. For instance, the density of adhered P. aeruginosa decreased as the surface contact angle increased. S. aureus preferred to adhere to hydrophobic surfaces.

KEY WORDS: biocompatibility, polymer surfaces, plasma treatment, Staphylococcus aureus, Pseudomonas aeruginosa

I. INTRODUCTION

The technological development of medicine and materials engineering has made possible the use of many artificial materials in devices designed to operate in contact with body fluids or within the human body. In this context, polymers are the most frequently employed materials. They can be found, for instance, in artificial hearts, catheters, insulators of pacemaker wires, and heart valves. Bacterial contamination, however, is one of the most serious problems associated with the use of polymers in medical devices. A classical example of such a problem occurs in intubated and mechanically ventilated patients. The endotracheal plastic tube provides all the favorable conditions for bacterial colonization, mainly by Pseudomonas aeruginosa and Staphylococcus aureus, which are responsible for more than 30% of nosocomial infections.
Normally, bacterial colonization can be eliminated by surface disinfection or via antibiotic therapy. Bacteria, however, can adhere to material surfaces in an irreversible way. Such adhered microorganisms can reproduce, forming complex cultures and producing substances that can act as protective coatings. Frequently, the eradication of this complex structure, referred to as a biofilm, is a difficult clinical procedure since substances produced by the microorganisms can act as physical and chemical barriers, protecting them from external agents.

Biofilm formation has been observed, for instance, in voice prostheses, dental implants, and urinary and intravenous catheters. In most cases, the presence of biofilms requires the removal and replacement of the implant. In voice prostheses, the frequency of such replacement is, on average, four months.

It has been observed that material surface properties have important effects on bacterial adhesion and proliferation. Thus a possible solution to biofilm formation depends on the interactions between the bacteria and the biomaterial surface. Plasma-based material treatment techniques are therefore of potential interest because they allow intensive modifications of surfaces of virtually any kind of material without affecting the bulk properties. In this work, polyurethane substrates have been exposed to sulfur hexafluoride plasmas and the influence of different treatments on the adhesion of Pseudomonas aeruginosa and Staphylococcus aureus to the modified samples has been investigated. P. aeruginosa is frequently responsible for the contamination of catheters and prostheses; S. aureus presents similar threats.

II. EXPERIMENTAL

Figure 1 shows the experimental system used for the plasma treatments. This consists of a cylindrical, six-liter stainless-steel vacuum chamber, which, during the plasma treatments, was evacuated continuously by a rotary-vane pump rated at 18 m$^3$h$^{-1}$ (Edwards, UK). The system pressure was monitored using a capacitance manometer (Datametrics Barocel, Edwards, Wilmington, MA, USA). Sulfur hexafluoride (at least 99.9% pure, White Martins, Sorocaba, SP, Brazil) was fed to the system from a cylinder via a regulator and a needle valve. The partial pressure of SF$\textsubscript{6}$ measured in the absence of the discharge was used as a control parameter. Discharges were generated by the application of radiofrequency power (13.56 MHz supply, maximum power 300 W, Tokyo HyPower, Japan) to the upper of two horizontal, circular, stainless-steel parallel-plate electrodes, mounted inside the chamber. An RF matching circuit (Tokyo HyPower, Japan) allowed the minimization of the reflected power, typically to less than 5 W. The lower electrode, which also served as substrate holder, was grounded.

Prior to the treatments, 1.5-cm-diameter discs of commercially pure polyurethane (PU) were sonicated in detergent solution and rinsed in distilled deionized water. To ensure that all the surfaces were exposed to the plasmas, thumbtacks were inserted in the discs to keep their faces vertical and, therefore, uniformly enveloped by the plasma. After positioning the samples on the substrate holder, the chamber was pumped down
to 1 Pa to remove atmospheric contaminants, the desired pressure was set, and then the discharges were generated for the predetermined time.

The surface contact angle, $\theta$, defined as the angle at the intersection of a plane tangent to the drop and a plane containing the surface, was measured using a Ramé-Hart 100-00 goniometer, in which a syringe dispenses a sessile drop of the test liquid onto the film surface. Distilled deionized water and methylene iodide were employed. Three drops placed at different surface sites on each film were measured. Ten measurements per site were taken.

The effects on $\theta$ of different SF$_6$ plasma exposure times between 180 s and 540 s were investigated at two pressures: 6.7 Pa and 13.3 Pa. Similarly, the dependence of $\theta$ on the SF$_6$ pressure in the 6.67 to 13.3 Pa range was investigated.

The concentrations of $P. aeruginosa$ and $S. aureus$ obtained from samples treated with SF$_6$ plasmas (70 W, 13.3 Pa) for times of up to 540 s were measured. Bacterial adhesion assays were performed using controlled bacterial strains. Selected samples in triplicate and a control (a pristine sample) were placed in a 480–mL tube containing BHI (brain heart infusion) broth inoculated with $10^6$ colony forming units (CFU) of $P.$

**FIGURE 1.** Experimental apparatus used for the plasma treatments.
aeruginosa (ATCC 27853) or S. aureus (ATCC 6538). After 48 h of incubation at 37°C, the samples were removed from the tube, rinsed, and transferred to an Erlenmeyer containing sterile saline. The bacterial cells were then harvested by stirring at 50 rpm for 1 h in a refrigerated incubator. After centrifugation, solutions derived from each sample were diluted and deep plated, as well as the polymer discs themselves, which were placed in dishes with agar and incubated for 24 h at 35°C. The quantification of adhered bacteria was made via the pour plate method.\(^{11}\)

### III. RESULTS AND DISCUSSION

It has been observed that the surface characteristics are strongly influenced by the plasma conditions. As an illustration, Fig. 2 shows the contact angle of PU surfaces as a function of the exposure time, \(t\), to discharges excited at 70 W and at two different pressures. As can be observed, there are two opposite tendencies in \(\theta\) as \(t\) is increased. In the lower pressure regime (6.7 Pa), the contact angle decreases as \(t\) is increased. On the other hand, \(\theta\) increases with the exposure time when the pressure is 13.3 Pa. As observed previously,\(^{12}\) the modification of the contact angle following the exposure to SF\(_6\) plasmas is due to the incorporation of fluorine-containing groups by the surface. Thus, a possible explanation for these trends is the different reaction rates associated with the quantity of energy deposited in the material by collisions with species from the discharge. As the pressure is increased, the mean free path of plasma species is decreased, as well as the average energy of the colliding particles. Owing to these factors, the rate of reactions involving surface and plasma species is reduced, requiring longer exposures. Inversely,

![Figure 2](https://example.com/figure2.png)

**FIGURE 2.** Contact angle of the polyurethane surfaces as a function of the exposure time to discharges excited at 70 W and at two different SF\(_6\) pressures. The contact angle of the as-received material is indicated by the dotted line.
the bond breakage caused by the impingement of energetic species can result in the formation of free radicals on the surface. Such radicals can remain active in the polymer structure, capturing atmospheric hydrophilic groups (hydroxyl from water vapor, for instance) when the sample is removed from the vacuum chamber. Therefore, the longer the sample is exposed to the plasma, the higher is the density of active sites produced on the polymer, resulting in a decrease in $\theta$ due to the post-plasma reactions. In both cases it was possible to produce highly hydrophobic surfaces with an ultimate contact angle near 127°. It is worth noting, however, that the gas pressure determines the time necessary to reach the desired hydrophobicity. Whereas contact angles around 127° could be attained with exposures of 180 s when the pressure was 6.7 Pa, such hydrophobicity was obtained only after 540 s when the pressure was raised to 13.3 Pa.

The dependence of surface properties on the plasma conditions can be explored by considering Fig. 3, which shows the contact angle as a function of SF$_6$ pressure, $P$, in the gas feed. As can be seen, $\theta$ remains nearly constant over a range of values of $P$, and when $P$ is further increased to 13.3 Pa, the contact angle abruptly decreases. A possible explanation for this tendency is, again, the reduction of the reaction rates involving surface and plasma species caused by the decrease in the mean free path as $P$ increases. When the pressure is higher than about 12.0 Pa, in this case, the energy of the reacting species reaches a threshold below which surface modification is insufficient to cause strong modifications of $\theta$.

As is evident from Fig. 4, which shows the concentration of $P.$ aeruginosa colony forming units in solutions obtained from samples treated for different exposure times, differences in surface properties influence the amount of adhered bacteria. Figure 4

![FIGURE 3](image-url)

**FIGURE 3.** Contact angle of polyurethane surfaces as a function of SF$_6$ pressure, $P$, in the plasma gas feed. In all the treatments the exposure time and the RF power were kept constant at 180 s and 70 W, respectively. The dotted line indicates the contact angle of the pristine polymer.
shows that the CFU concentration increased as \( t \) was increased. Since the microorganisms in the solution originated from the sample, higher CFU concentrations indicate weaker adhesion and therefore a smaller number of bacteria adhered to the surface. Consequently, longer treatments produce surfaces less attractive to \( P. \ aeruginosa \). One should note, however, that the concentration at \( t = 540 \) s is higher than \( 10^6 \) CFU/mL, the inoculated density, suggesting that problems in the initial inoculation procedure or bacterial reproduction may have occurred. Since these possibilities may occur together, it is difficult to affirm what has really happened. In spite of this, as the contamination process is well controlled, most probably the bacteria have found an environment more suitable to proliferation than to adhesion.

Another possibility for the increase in the CFU concentration may be the modification of the microorganism-surface interaction caused by the enhancement in \( \theta \) as the exposure time was increased. Figure 5 shows that slight modifications in the contact angle, when \( \theta \) is larger than \( \sim 120^\circ \), resulted in large variations in the CFU concentration. As can be observed, beyond that point the adherence is reduced as the contact angle increases. Although not completely understood, biofilm formation involves attachment and maturation steps. It is generally believed that the modification of contact angle directly affects the first step. As \( \theta \) increases, the free surface energy decreases, leading to a less receptive material.\(^{13}\) Such a supposition is reinforced, for instance, by the findings of Pereni et al.,\(^{14}\) who observed a good correlation between \( P. \ aeruginosa \) retention and total surface free energy of silicon and stainless steel.

The results presented in Fig. 5 are in opposition to those obtained by Triandafillu et al.,\(^{18}\) who observed a reduction in the amount of adhered \( P. \ aeruginosa \) on PVC as the
hydrophilicity of the polymer surface was increased. It should be pointed out, however, that different mechanisms are involved in these two situations. As previously observed, the hydrophilization of PVC is mainly due to the loss of chlorine-containing species, which reduces electrostatic repulsion between water molecules and the surface. Owing to this, the bacteria can be easily washed from the surface. On the other hand, the incorporation of fluorine in polyurethane surfaces promotes both electrostatic repellence and bactericidal effects, which contribute to a decrease in the density of adhered microbes.

The results for adherence of *S. aureus* were contrary to those observed with *P. aeruginosa*. For instance, as observed in Fig. 6, which shows the CFU concentration of *S. aureus* as a function of the exposure time to SF₆ plasmas, the bacterial concentration decreased as *t* was increased. Once more, as the concentration indicated in the figure refers to organisms in the solution, and since the increment in *t* causes the contact angle to decrease, this result indicates that *Staphylococcus aureus* prefers more hydrophobic surfaces. It has been stated that microbial adhesion to surfaces is an interplay between the hydrophobicity and charge properties of the interacting surfaces. In this sense, it is interesting to observe Fig. 7, which shows the CFU concentration of *S. aureus* as a function of the dispersive (nonpolar) component of the surface free energy, *E*<sub>D</sub>. The concentration of microorganisms in suspension decreases as *E*<sub>D</sub> increases. This result may be explained by the electrostatic repulsion between charges in the bacterial membranes and negative charges due to the presence of highly electronegative fluorine-containing groups on the surface.

**IV. CONCLUSIONS**

The exposure of polyurethane samples to SF₆ plasmas is observed to be an effective procedure to modify their surface hydrophobicity. Furthermore, the properties of the treated
surfaces are strongly influenced by the conditions of the treatment. For instance, the effect of the exposure time on the contact angle depends on the gas pressure. When the plasmas were generated from an atmosphere at 6.7 Pa, the contact angle decreased with increasing $t$. On the other hand, $\theta$ increased with the exposure time when the pressure was 13.3 Pa.

Differences in surface characteristics resulted in different affinities between the microbes and the modified surfaces. Each microorganism interacted in a different way with the surface. While *Stapylococcus aureus* preferred to adhere to more hydrophobic surfaces, *Pseudomonas aeruginosa* preferred to adhere to more hydrophilic ones.

**FIGURE 6.** Concentration of *S. aureus* colony forming units in solutions obtained from samples treated in SF$_6$ plasmas (70 W, 13.3 Pa) for different exposure times.

**FIGURE 7.** Concentration of *S. aureus* colony forming units as a function of the dispersive component of the surface free energy.
Finally, the present study has shown that it is possible to reduce bacterial adhesion to polymers via plasma surface modification. Further studies are under development to investigate the influence of other parameters, such as surface chemical composition and roughness, on biofilm formation.

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