Antibacterial Surfaces on Expanded Polytetrafluoroethylene; Penicillin Attachment

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Expanded polytetrafluoroethylene (ePTFE) was chemically modified to retard the growth of S*taphylococcus aureus* bacteria. This was accomplished by microwave plasma reactions in the presence of maleic anhydride (MA) to create acid functional groups on ePTFE surfaces, followed by esterification reactions with 200 and 600 molecular weight linear polyethylene glycol (PEG). Such surfaces were utilized for further reactions with penicillin (PEN) through etherification reactions to create anti-microbial surfaces. These reactions resulted in surface morphological changes, and spectroscopic analysis using attenuated total reflectance Fourier transform infrared spectroscopy (ATR FT-IR) revealed the formation of ester linkages resulting from reactions between PEN and PEG functionalities. Antibacterial activities were evaluated by a series of experiments where PEN-modified ePTFE specimens were immersed in a liquid *aureus* culture, and the bacteria growth was quantified by measuring % absorbance of the suspension at 600 nm wavelength. The lowest absorbance was observed for the solution containing PEN-PEG-MA-ePTFE specimens, thus showing highly effective anti-bacterial activity toward gram-positive *Staphylococcus aureus* bacteria. To our best knowledge, this is the first study that shows PEN-ePTFE surface modifications that are effective against gram-positive *aureus* bacteria.

Introduction

Ongoing search for materials that could be either utilized as implants or other devices in contact with a human body continues to be a major challenge. Although various polymers have found diversified biomedical applications, it is apparent that there is virtually no polymeric material that requires no surface modifications. By modifying surfaces, one may achieve a number of desirable properties, which range from blood clotting prevention to controllable drug release, and other applications, while maintaining useful bulk polymer properties. A variety of polymers are being utilized in these applications; for example, poly(vinyl chloride) (PVC) serves in cardiac catheters, surgical tapes, artificial hearts, blood pumps, and artificial limbs,[1−5] and requires different surface modifications than poly(methyl methacrylate) (PMMA) utilized for contact lenses, bone cement, artificial teeth, and dental fillings.[1,6−8] Along the same lines, poly(dimethyl siloxane) (PDMS) is used for contact lenses, artificial skin, oxygenators, and drug delivery systems,[1,9−14] thus necessitating different surface modifications than expanded poly(tetrafluoroethylene) (ePTFE) utilized for vascular graft prostheses, heart patches, or stapes prosthesis.[1,15−20]

Regardless of specific surface modifications, all biomaterials are susceptible to bacterial attacks, which may have detrimental effects. Thus, many efforts have been made to generate polymeric surfaces with desirable bio-properties that exhibit anti-microbial activity, and recent studies showed PDMS-amoxicillin surface modifications.[14] Because ePTFE is a nonreactive and nontoxic flouro-containing polymer, multiple medical devices ranging from vascular grafts to mitral valve tendon replacements, or orthopedic surgeries and soft tissue in plastic and reconstructive surgeries,[21−23] have been developed. However, its surfaces, just like other polymeric materials implanted into biological environments, are not exempt from the bacterial attacks.[24,25] *Staphylococcus aureus* bacteria is particularly important because it causes suppurative infections and toxinoses in humans as well as superficial skin lesions such as styes, boils, and furunculosis.[26]

In view of these considerations, one possible solution is to attach antibiotic molecules to polymeric surfaces to inhibit the growth of bacteria. This study focuses on surface modifications of ePTFE that lead to the attachment of penicillin, which was selected because of its known ability to inhibit gram-positive bacteria growth.[27] This paper describes for the first time simple surface modifications using microwave plasma and chemical reactions that effectively prevent the growth of S*taphylococcus aureus* bacteria on ePTFE surfaces, which is schematically illustrated in Figure 1, where the employment of surface microwave plasma reactions in the presence of maleic anhydride, followed by surface hydrolysis, provides the platform for generating acid groups, followed by esterification reactions employing polyethylene glycol (PEG), and reactions with penicillin (PEN).

Experimental Section

ePTFE specimens were purchased from Philips Sci Inc. (Rock Hill, SC), cut to 7 × 7 mm squares, followed by washing with acetone in an ultrasonic washer, and dried at room temperature under vacuum conditions before use. Plasma reactions were conducted using open reactor conditions, as described elsewhere.[28] The ePTFE substrate and 100 mg of solid maleic anhydride (MA) (Aldrich Chemical Co.) were placed into the microwave reactor chamber and spaced 8.5 cm apart of each other. In a typical experiment, the reactor was evacuated to

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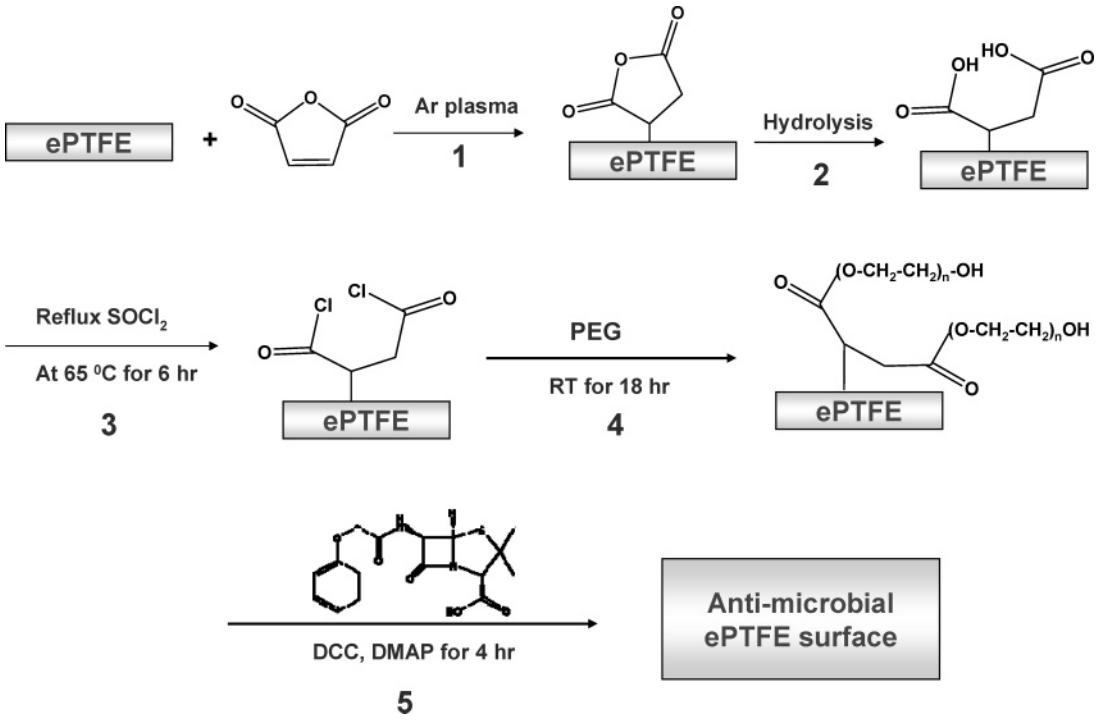


Figure 1. Schematic diagram of surface reactions on ePTFE: step 1, Ar microwave plasma reaction; step 2, hydrolysis of MA-ePTFE; step 3, conversion of acid groups; step 4, PEG reaction; step 5, PEN reaction.

150 mTorr, followed by purging it with Ar gas to reach a steady-state pressure of 250 mTorr at a flow rate of 2.96 mL/min. At this point, microwave radiation at 600 W of power with an output frequency of 2.45 GHz was turned on to induce plasma formation (Figure 1, step 1). Under these conditions, the reaction chamber pressure increases continuously during the microwave plasma discharge. In an effort to maintain plasma environment during longer exposure times, a vacuum was applied continuously to maintain pressure conditions during the experiment. Because monomeric and polymeric forms of MA are water soluble, in an effort to determine stability of the surface treatments and to ensure that the newly formed species are not physisorbed on the surface, the samples were boiled in water for 30 min (Figure 1, step 2). After being dried, specimens were stored in a desiccator under ambient conditions.

To modify MA-ePTFE surfaces with penicillin (PEN) (Sigma Inc.), we utilized an esterification reaction using 4-(dimethylamino)-pyridine (DMAP) catalyst and dicyclohexyl-carbodiimide (DCC) coupling agent.[29,30] Polyethyleneglycol (PEG) (Aldrich) was used as a spacer between modified ePTFE surfaces and PEN. Acid groups on ePTFE surfaces were first converted into acid chloride using thionyl chloride under reflux conditions at 65 °C for 6 h (Figure 1, step 3). The sample was removed from the flask and washed with chloroform to eliminate excess thionyl chloride. The acid chloride ePTFE surfaces were then placed into a chloroform solution of PEG containing a 1:1 molar ratio of linear PEG 200 and 600 molecular weight. The esterification reaction was carried out in a sealed flask at room temperature for 18 h. A small amount (1−2 drops) of triethylamine was added into the reaction flask at the onset of the reaction to neutralize hydrochloric acid that was generated during the reaction (Figure 1, step 4). The sample was washed with chloroform several times to remove unreacted PEG, followed by the final wash with distilled water for 2 h.[31]

Reactions of PEG-MA-ePTFE with PEN were conducted using the esterification process (Figure 1, step 5). The K salt of penicillin V (PEN V) (1.5 mmol) was dissolved in a small volume of water, cooled, and acidified with 0.1 N HCl. Precipitated PEN V was filtered and dried in a vacuum oven at room temperature for 1 h.[22] PEG-MA-ePTFE specimens and DMAP (0.25 mmol) were placed into a 100 mL flask with 20 mL of methylene chloride. In the next step, dried PEN V was added to the mixture, then stirred and cooled in an ice−water bath. DCC (1.3 mmol) was added, and the mixture was continuously stirred for 4 h. Upon removal, all specimens were washed in methylene chloride sequentially for 2 h, dried for 24 h, and analyzed.

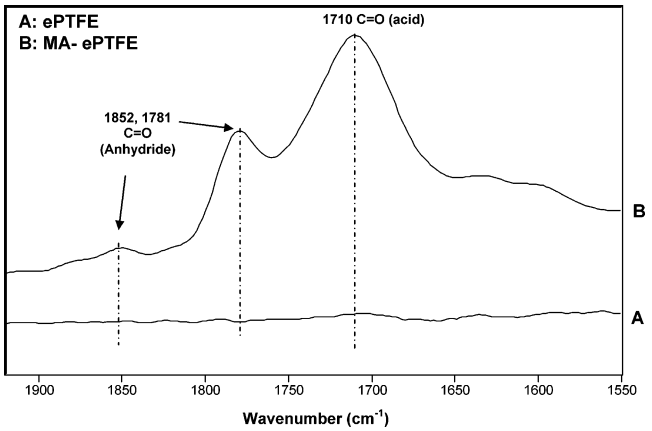


Figure 2. ATR-FTIR spectra of (A) ePTFE, and (B) maleic anhydride (MA)/ePTFE.

To determine anti-microbial activity of PEN-PEG-MA-ePTFE surfaces, *Staphylococcus aureus* (RN 6390) and *Pseudomonas aeruginosa* (ATCC, Rockville, MD) were allowed to grow overnight in LB broth and King's medium, respectively. A series of specimens (ePTFE, MA-ePTFE, PEG-MA-ePTFE, PEN-PEG-MA-ePTFE, and PEN-ePTFE) were immersed into freshly incubated cultures of each bacteria and incubated at 37 °C for 3−4 h. Anti-microbial activity was determined by measuring the absorbance at 600 nm using a UV−vis spectrometer (Beckman DU-600).

A scanning electron microscope (SEM) Quanta FEI series 200 FEG was used to evaluate surface morphologies. All specimens were sputter coated with gold and analyzed at a 45° angle with a scanning electron beam. Attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectra were collected using a Bio-Rad FTS-6000 FT-IR single-beam spectrometer set at a 4 cm^{-1} resolution equipped with a deuterated

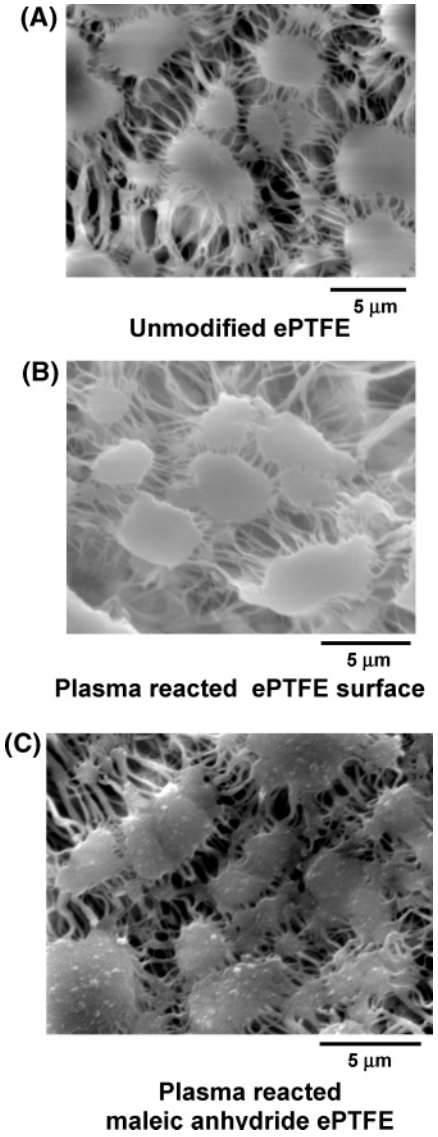


Figure 3. SEM images of (A) ePTFE, (B) plasma reacted ePTFE, and (C) MA plasma reacted ePTFE.

triglycine sulfate (DTGS) detector and a 45° face angle Ge crystal. Each spectrum represents 400 co-added scans ratioed against a reference spectrum obtained by recording 400 co-added scans of an empty ATR cell. All spectra were corrected spectral distortions using Q-ATR software.[32]

Results and Discussion

Figure 1 illustrates a schematic diagram of surface reactions employed to obtain anti-microbial ePTFE surfaces. As indicated in the Introduction, the effective method of generating acid groups on polymeric surfaces is the employment of surface microwave plasma reactions in the presence of maleic anhydride, followed by surface hydrolysis.[28] These reactions are illustrated in Figure 1, steps 1 and 2. In an effort to confirm that indeed these reactions have occurred on the ePTFE surface, we analyzed the surfaces.

Figure 2, traces A and B, illustrate ATR-FTIR spectra recorded from the surface of ePTFE before and after reactions, respectively. As expected, there are no bands in the 1900−1500 cm^{-1} region for ePTFE (trace A). In contrast, as illustrated in trace B, the bands at 1781, 1852, and 1710 cm^{-1} are detected as a result of microwave plasma reactions (Figure 1, steps 1 and 2). These bands are attributed to anhydride C═O and acid C═O stretching vibrations,[33−35] and their presence indicates that indeed ePTFE surfaces were chemically modified through a C═C bond opening of the maleic anhydride ring and its hydrolysis.[28]

One of the significant differences between PTFE and ePTFE polymers is surface morphology. As shown in Figure 3A, SEM images of ePTFE show mesh-like network morphology, which is functional in bio-environments as it exhibits the ability for body tissues to network with and grow into it. As shown in Figure 3B and C, as a result of microwave plasma surface reactions without and in the presence of maleic anhydride, respectively, the morphology remains virtually the same, as manifested by SEM images, and the only difference is the formation of whitish particle-like sparkles. However, ATR-FITR measurements illustrated in Figure 2 clearly show that COOH modifications have occurred. It should be also noted that these reactions result in water contact angle changes from 125° for ePTFE to 95° for COOH-modified ePTFE.

The presence of COOH groups on the ePTFE surfaces is useful because such entities may serve for further reactions. Because our interest is the attachment of penicillin (PEN) that

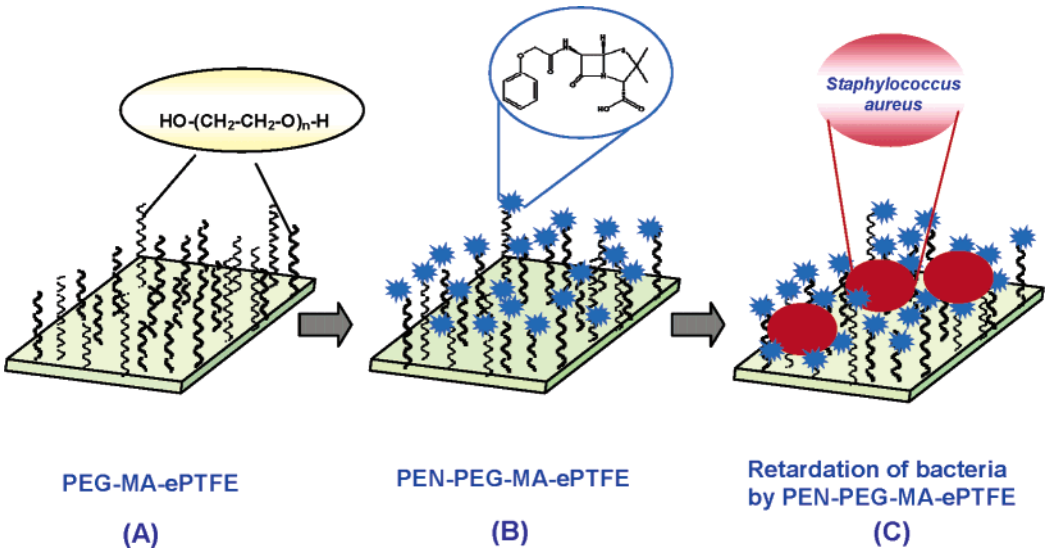


Figure 4. Representation of ePTFE surface modifications: (A) PEG-MA-ePTFE, (B) PEN-PEG-MA-ePTFE, and (C) retardation of bacteria by PEN-PEG-MA-ePTFE.

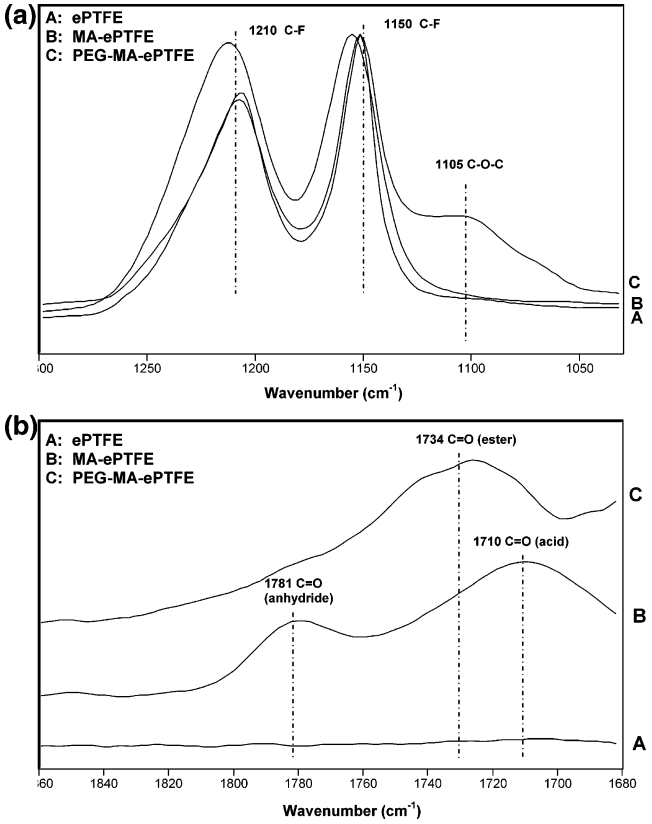


Figure 5. ATR-FTIR spectra of PEG-MA-ePTFE in (a) 1300−1000 cm^{-1} and (b) 1900−1600 cm^{-1}: (A) ePTFE, (B) MA-ePTFE, and (C) PEG-MA-ePTFE.

would effectively prevent the growth of bacteria, it is necessary to provide suitable surface functionality and morphology. Although acid groups are capable of reactions with PEN, to enhance anti-bacterial effectiveness of PEN, we introduced PEG flexible spacer between the COOH-functionalized surface and PEN molecules. The choice of PEG was dictated by its nontoxicity, biocompatibility, and the ability to swell in aqueous environments.[36,37] Furthermore, to enhance antimicrobial surface activity, nonuniform surface morphology was facilitated by varied lengths of PEG spacers. The premise behind this is that, by introducing random esterification reactions between acid chloride and hydroxyl groups of PEG with 200 and 600 MW, molecular roughness is introduced, which is capable of enhancing antimicrobial functionality. The latter is believed to be attributed to the ability of PEN-terminated PEG to inhibit the growth of the bacteria as they deposit on the surface. Thus, the enhanced surface roughness obtained by varying PEG chain lengths will increase the effective surface area in contact with the bacteria. This is illustrated in Figure 4A, which schematically shows the attachment of PEG, followed by reactions with PEN (B), and anticipated retardation of the bacteria in contact with the modified surface (C). However, for the surface groups to be more reactive, COOH groups were first converted to acid chloride functionalities using $SOCl_2$ solvent. This is schematically illustrated in Figure 1, step 3. This approach creates more reactive groups for further reactions with OH functionalities on PEG.

Similarly to the previous experiments, we utilized ATR-FTIR to determine the extent of surface reactions. Figure 5a and b, traces A−C, shows ATR-FTIR spectra of ePTFE (A), MA-ePTFE (B), and PEG-MA-ePTFE (C) in the 1300−1000 (a) and 1850−1680 (b) cm^{-1} spectral regions, respectively. While traces A and B serve as references, trace C illustrates

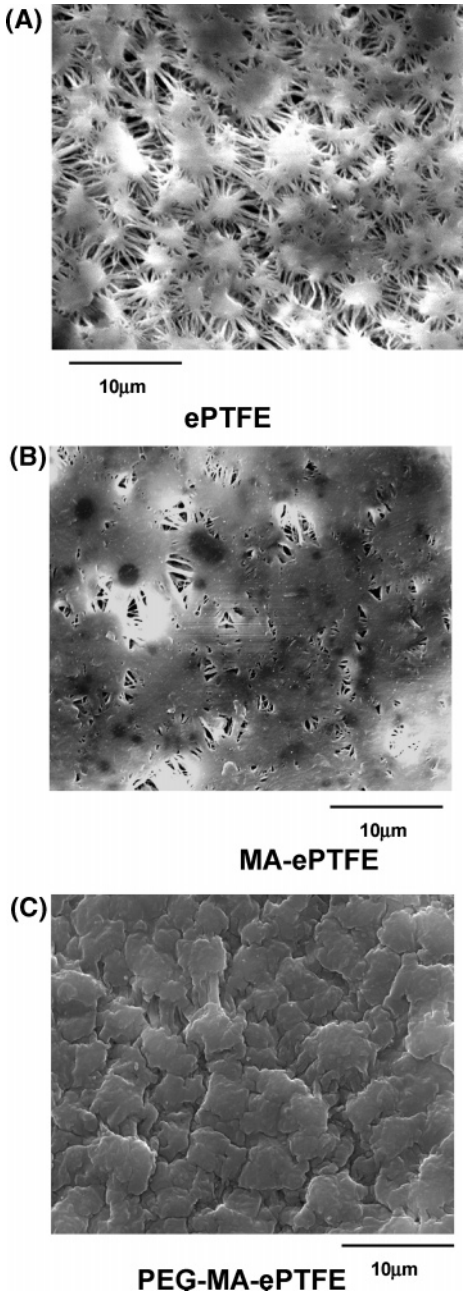


Figure 6. SEM images of (A) ePTFE, (B) MA-ePTFE, and (C) PEG-MA-ePTFE.

the presence of the 1105 and 1734 cm^{-1} bands due to C−O−C stretching and C═O ester vibrations[33−35] resulting from esterification reactions. These spectra were normalized to the C−C stretching vibrations at 1177 cm^{-1}. SEM images shown in Figure 6A−C illustrate surface morphologies of ePTFE, MA-ePTFE, and PEG-MA-ePTFE, respectively, which are significantly altered as a result of the surface reactions and exhibit fewer voids.

As illustrated in Figure 1, step 5, the final step of the process involves reactions of PEG-MA-ePTFE with PEN. For that reason, we utilized esterification reactions with DCC as the catalyst and DMAP as the coupling reagent. These reactions were carried out in a one-step process and do not require prior activation of the reactants. As a result, PEN was attached to modified ePTFE surfaces via ester linkages. To illustrate that indeed this reaction occurred, ATR-FTIR analysis was performed, and Figure 7 shows the results. Again, for reference purposes, traces A and B represent the spectra of ePTFE and

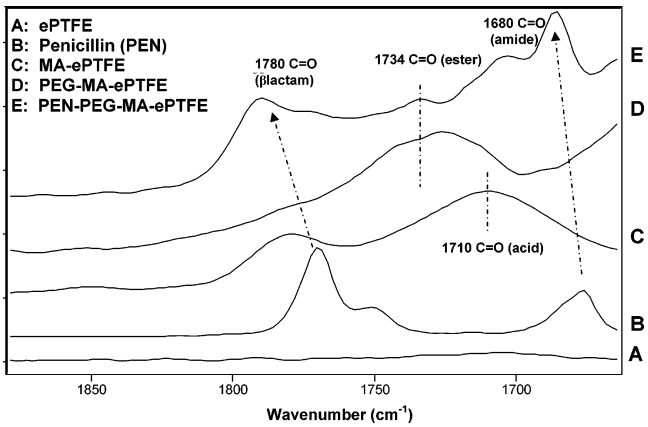
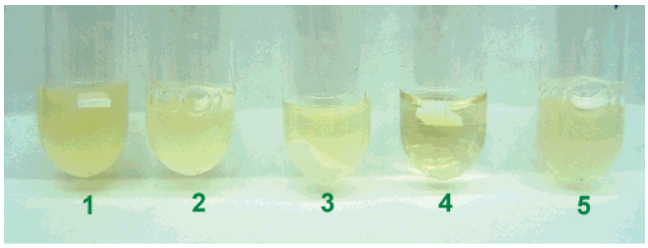


Figure 7. ATR-FTIR spectra in the 1900−1650 cm^{-1} region of (A) ePTFE, (B) PEN, (C) MA-ePTFE, (D) PEG-MA-ePTFE, and (E) PEN-PEG-MA-ePTFE.



Staphylococcus aureus

Figure 8. Photographs of test tubes containing the following specimens in *Staphylococcus aureus* cultures: ePTFE (1), MA-ePTFE (2), PEG-MA-ePTFE (3), PEN-PEG-MA-ePTFE (4), and PEN-ePTFE (5).

PEN, respectively, while trace E represents the spectrum of PEN-PEG-MA-ePTFE. As seen, the presence of the C═O vibrations at 1680, 1734, and 1780 cm^{-1}, which are attributed to amide, ester, and β-lactam C═O stretching vibrations,[33−35] respectively, is detected and indicates that the β-lactam ring, which is the bio-active group on PEN, remains intact during the coupling reactions. In summary, spectroscopic and morphological data illustrate that PEN was chemically attached to the ePTFE surface, and the next question is how effective this approach is in the presence of *Staphylococcus aureus* bacteria.

In an effort to demonstrate the effectiveness of these surface reactions, a series of experiments was conducted where modified and unmodified ePTFE specimens were placed into bacterial cultures. Figure 8 illustrates a photograph that depicts turbidity differences in *Staphylococcus aureus* cultures as a result of ePTFE exposure. While test tubes #1, 2, and 3 represent three controls of bacteria growth in the presence of neat ePTFE, MA-ePTFE, and PEG-MA-ePTFE, test tube #4 shows a bacterial cultures growth with a PEN-PEG-MA-ePTFE specimen. Only the PEN-PEG-MA-ePTFE specimen was able to retard bacterial growth, as seen by the lack of turbidity in the growth medium. In contrast, the cloudiness of the solutions containing ePTFE, MA-ePTFE, and PEG-MA-ePTFE (test tubes #1, 2, and 3) indicates bacterial growth. Another control that was utilized is exposure of a bacterial culture to the PEN-ePTFE specimen. For that purpose, direct ePTFE modification with PEN only (steps 1−4 in Figure 1 were skipped) was attempted. As seen in Figure 8, the solution in test tube #5 is turbid and supports bacterial growth, indicating that the reaction of PEN to unmodified ePTFE surfaces did not occur, and thus ePTFE surfaces were not capable of retarding the bacteria growth.

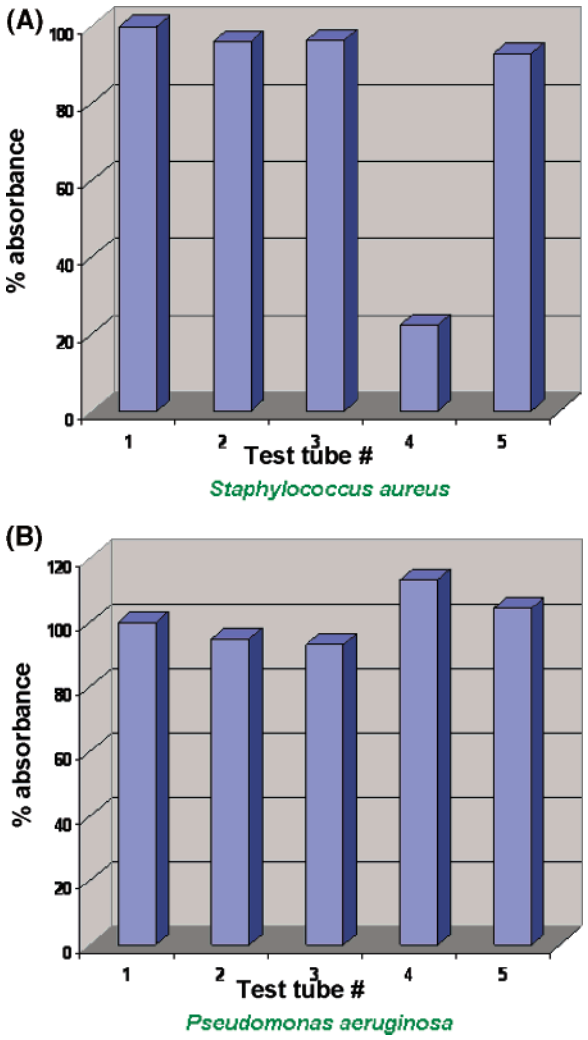


Figure 9. % absorbance plotted for liquid *Staphylococcus aereus* and *Pseudomonas aeruginosa* bacterial cultures growth in the presence of ePTFE (1), MA-ePTFE (2), PEG-MA-ePTFE (3), PEN-PEG-MA-ePTFE (4), and PEN-ePTFE (5).

To quantify the antimicrobial effectiveness of surface-attached PEN, the absorbance at 600 nm was measured for the solutions illustrated in Figure 8, 1−5. Figure 9A illustrates the results of these experiments and shows that the lowest relative absorbance is detected for the solution exposed to PEN-PEG-MA-ePTFE specimen, thus demonstrating the antibacterial activity of this specimen. It should also be noted the above experiments were conducted using *Staphylococcus aureus*, which is gram positive. The same series of experiments conducted using the gram-negative *Pseudomonas aeruginosa* showed that the solution containing PEN-PEG-MA-ePTFE is turbid and shows high relative absorbance values for all solutions including test tube #4 containing PEN-PEG-MA-ePTFE specimen. This is illustrated in Figure 9B. As anticipated, theses experiments showed that PEN attached to modified ePTFE surfaces is effective for gram-positive bacteria.

Conclusions

These studies show that maleic anhydride and carboxylic acid groups can be chemically bonded to ePTFE surfaces when microwave plasma radiation is utilized. Maleic anhydride reacts with ePTFE surfaces through a C═C bond opening of the maleic anhydride ring, and its hydrolysis results in chemically attached

carboxylic acid groups. Using esterification reactions in the presence of PEG spacer, PEN was reacted onto such ePTFE surfaces, which subsequently exhibits highly effective antimicrobial activity toward gram-positive *Staphylococcus aureus* bacteria. This approach may serve as a general surface modification process for the development of polymeric surfaces with anti-microbial properties.

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