Tunable Antimicrobial Polypropylene Surfaces: Simultaneous Attachment of Penicillin (*Gram* +) and Gentamicin (*Gram* −)

Nattharika Aumsuwan, Matthew S. McConnell, and Marek W. Urban*

School of Polymers and High Performance Materials, Shelby F. Thames Polymer Science Research Center, The University of Southern Mississippi, Hattiesburg, Mississippi 39406

Received November 21, 2008; Revised Manuscript Received January 6, 2009

Surface reactions were performed on polypropylene (PP) surfaces to retard the simultaneous growth of *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas putida* (*P. putida*) bacteria. Microwave plasma reactions in the presence of maleic anhydride (MA) resulted in the formation of acid groups on the surface of PP. Such surfaces were further modified by conducting two parallel reactions: (1) poly(ethylene glycol) (PEG) was attached to COOH groups of the PP surface, followed by penicillin V (PEN) reactions to target *S. aureus* destruction and (2) diglycidyl PEG was attached, followed by gentamicin (GEN) reactions, to create antimicrobial surfaces targeted at *P. putida*. Simultaneous gram "+" and gram "−" resistance was obtained by varying the PEN/GEN ratios on such modified PP surfaces, thus providing the controllable degree of gram "+" and gram "−" antimicrobial strength. While spectroscopic analyses revealed chemical attachments of PEN and GEN, the effectiveness against proliferation of *S. aureus* (*Gram* +) and *P. putida* (*Gram* −) bacteria was determined using liquid culture tests. These studies show for the first time the formation of tunable antimicrobial polypropylene surfaces with controllable strength.

Introduction

Numerous studies have shown that surface modifications of polymers is beneficial in achieving or altering polymeric materials compatibility, surface energies, surface reactivities, and other properties while maintaining useful bulk properties.[1−5] Among numerous surface modifications, ranging from grafting to,[6−11] grafting through,[12] and grafting from,[13−17] microwave plasma reactions in the presence of maleic anhydride (MA) have several useful attributes that resulted in a new platform of surface functionalization leading to carboxylic acid group formation on surfaces of poly(tetrafluoroethylene) (PTFE),[18,19] poly(dimethylsiloxane) (PDMS),[20−24] and poly(vinylidene fluoride) (PVDF).[25] While these solventless reactions provide convenient means for generating surface reactive groups, the lack of accessibility due to steric constraints[26] may inhibit the effectiveness of surface modifications. One way to enhance accessibility of surface reactive groups is to provide adequate surface molecular weight distribution (or "molecular roughness") by attaching one end of a variable length molecular spacer to functionalized surface, while leaving the other end unreacted for further functions. This approach provides mobility to the unreacted end of the molecule, thus providing accessibility for further reactions as well as enhancing antimicrobial activity.[19,27] As previous studies have shown,[19,27] a 50/50 molar mixture of linear 200 and 600 g/mol polyethylene glycol (PEG) facilitated molecular roughness and subsequently enhanced antimicrobial activity of PEN-terminated PEG-MA-ePTFE surfaces.

Utilizing this approach, we have attached ampicillin[15] that inhibits transpeptidase enzyme reactions by intercepting cross-linking of the bacteria cell walls, thus causing the bacterial cell to succumb to osmotic forces.[28,29] Although ampicillin provides antimicrobial effectiveness against gram "+" and "−" bacteria, there are situations where the simultaneous presence of two independent mechanisms of destruction governed either by osmotic forces (+) or transition though ribosome (−) may be beneficial for microbial film distruction. Thus, having a controllable balanced combination of gram "+" and "−" antibiotics attached to polymeric surfaces may be beneficial, with the gram "−" aminoglycosides family of antibiotics represented by gentamicin, amikacin, or streptomycin and gram "+" β-lactum ring containing molecules, such as penicillin and its derivatives. These studies focus on chemical modifications of PP surfaces that contain controllable amounts of covalently bonded gram "+" (PEN) and gram "−" (GEN) antibiotics, attached through a molecular spacer to a PP substrate. By controlling their molar ratios this approach provides an opportunity to simultaneously

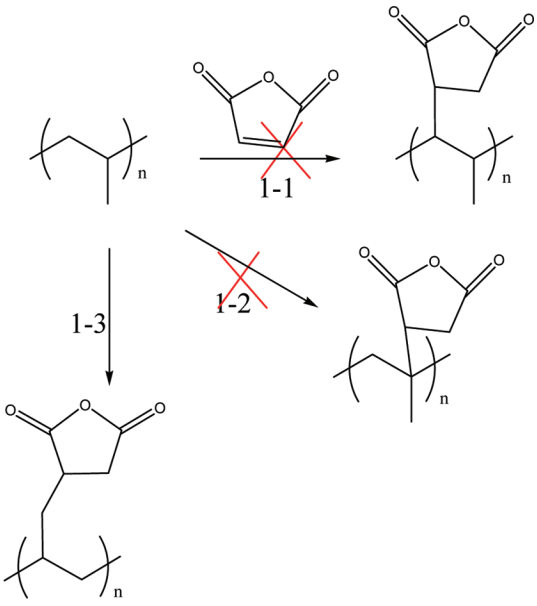

Figure 1. Possible reaction sites along polypropylene (PP) backbone.

* To whom correspondence should be addressed. E-mail: marek.urban@usm.edu.

10.1021/bm8013473 CCC: $40.75
Published on Web 01/27/2009

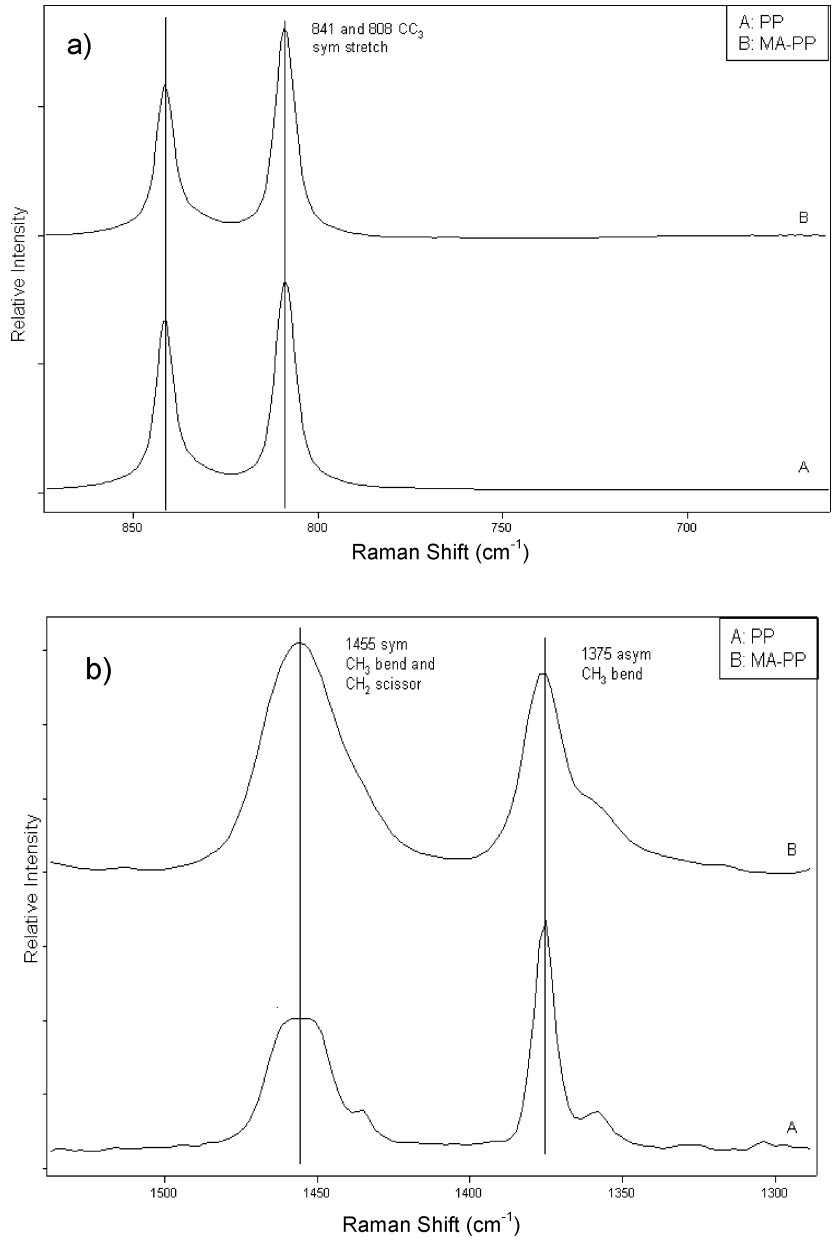

Figure 2. (a) ATR-FTIR spectra of (A) PP and (B) MA-PP in the 1300−1500 cm^{-1} region. (b) Raman spectra of (A) PP and (B) MA-PP the 700−850 cm^{-1} region.

eliminate proliferation of various strengths gram "+" and gram "−" microbial growth.

Experimental Section

Medical grade PP specimens were purchased from San Diego Plastics (San Diego, CA), cut to 1 × 1 cm squares, followed by washing with acetone in an ultrasonic washer, and dried at room temperature. Plasma reactions were conducted using open reactor conditions, as described elsewhere.[23,24] PP substrate and solid powder 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 150 mTorr, followed by purging it with Ar gas to reach a steady-state pressure of 250 mTorr at a flow rate of 4.0 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 for 5 s To ensure that the newly formed species are not physisorbed on the surface and all MA surface groups are converted to COOH groups the specimens were washed in water for 30 min and stored in a desiccator under ambient conditions.

COOH groups on PP surfaces were converted to acid chloride using oxalyl chloride ($C_2O_2Cl_2$) at room temperature for 3.5 h. Upon completion the specimen was removed from the flask and washed with chloroform to eliminate excess of oxalyl chloride. The acid chloride PP surfaces were then placed into a chloroform solution of PEG (Aldrich) (50% by volume) containing a 1:1 volume ratio of linear PEG 200 and 600 g/mol 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 tripropylamine was added into the reaction flask at the onset of the reaction to neutralize hydrochloric acid generated during the reaction. Each specimen was washed with chloroform several times to remove unreacted PEG, followed by a final wash with distilled water for 1 h.

In an effort to attach penicillin (PEN) (Sigma Inc.) esterification reactions using 4-(dimethylamino)-pyridine (DMAP) catalyst and dicyclohexyl-carbodiimide (DCC) coupling agent were conducted. The K salt of PEN (1.5 mmol) was dissolved in a 20 mL 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. PEG-MA-PP specimens and DMAP (0.25 mmol) were placed into a 100 mL flask

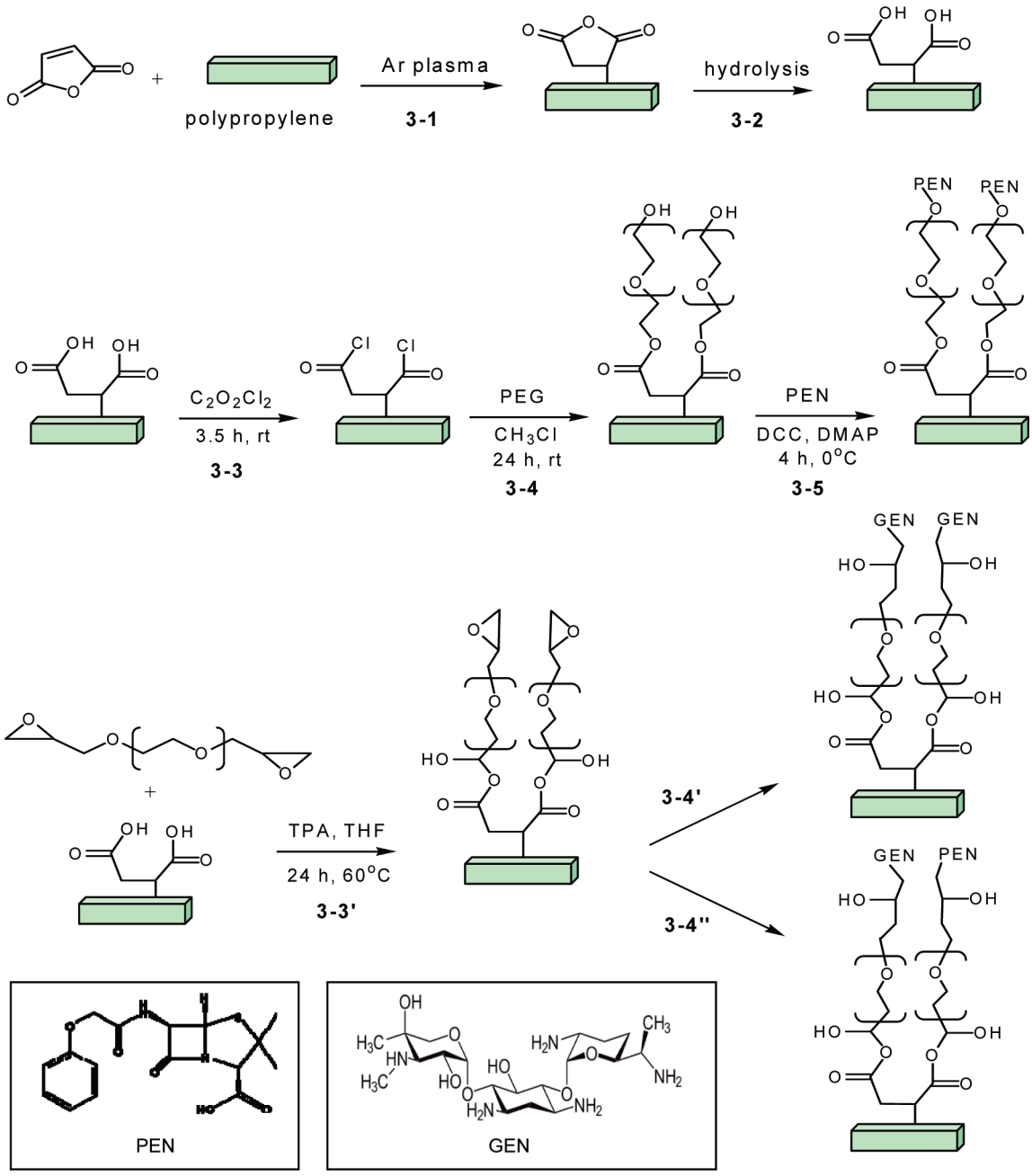

Figure 3. Schematic diagram illustrating reactions leading to the formation of GEN-PEG-MA-PP and PEN-PEG-MA-PP surfaces.

with 20 mL of methylene chloride. In the next step, dried PEN V was added to the mixture, 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.

In an effort to attach GEN to PP surfaces, MA-PP specimen was refluxed with 20 mL of diglycidyl PEG (Aldrich Chemical Co.; 526 g/mol), 5 mL of tripropylamine (Aldrich Chemical Co.), and 50 mL of tetrahydrofuran (THF) for 24 h at 60 °C, washed in THF for 1 h, and allowed to dry in a desiccator under ambient conditions (Figure 3, step 3-3′). GEN (Sigma Inc.) was purchased as a sulfate salt at 660 μg base/mg. A total of 0.15 g GEN was dissolved in 20 mL of distilled water, 10 mL of 1 M KOH solution was added to neutralize the GEN salt, and the solution was saturated with NaCl. The solution was filtered using a separatory funnel, where extraction with 100 mL of chloroform took place. The bottom layers of the extractions were collected into a round-bottom flask and the chloroform was evaporated under reduced pressures. The resulting white solid was dissolved in 150 mL of DMF and refluxed at 100 °C with glycidyl-PEG-MA-PP for 24 h. The sample was washed in DMF for 1 h, followed by washing it in chloroform for 1 h. The simultaneous attachment of PEN and GEN on the surface on previously modified PP surfaces was pursued by simultaneous PEN and GEN reactions to (glycidyl) PEG-MA-PP for 24 h, followed by subsequently washing in DMF and chloroform (1 h each), dried, and kept in the refrigerator.

Antimicrobial activity of PEN-PEG-MA-PP and GEN-PEG-MA-PP surfaces against *S. aureus* (RN 6390) and *P. putida* (ATCC, Rockville, MD) was determined by overnight growth in LB broth and King's medium, respectively. A series of specimens (PP, MA-PP, PEG-MA-PP, PEN-PEG-MA-PP, and GEN-PEG-MA-PP) were immersed into freshly incubated cultures of each bacteria and incubated at 37 °C for 5 h. Antimicrobial activity was determined by measuring the absorbance at 600 nm using a UV−vis spectrometer (Beckman DU-600).

Spectroscopic analysis of PP surfaces was conducted using attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectroscopy. In a typical experiment, ATR FT-IR spectra were collected using a Bio-Rad FTS-6000 FT-IR single-beam spectrometer set at a 4 cm^{-1}

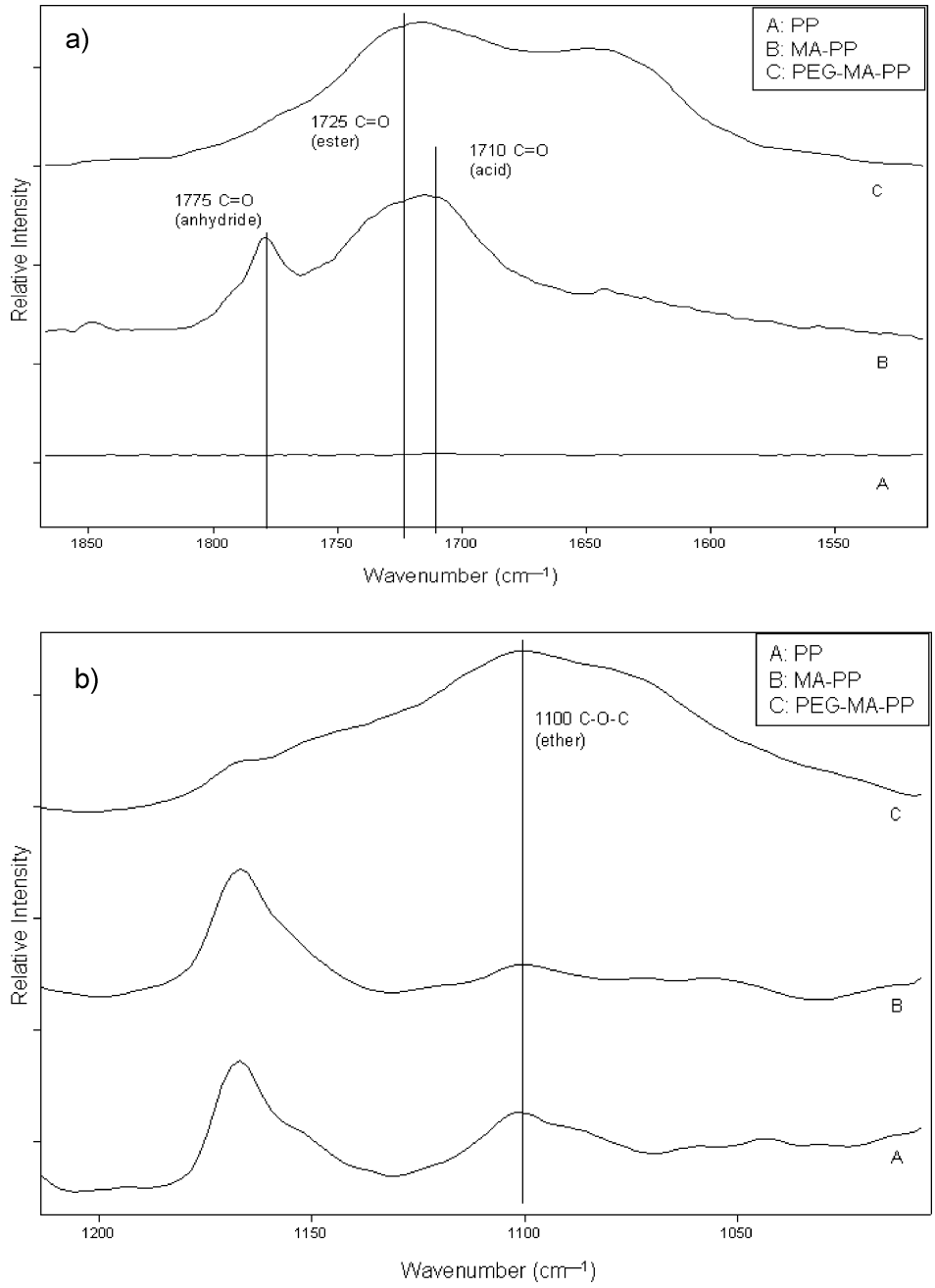

Figure 4. ATR-FTIR spectra of (A) PP, (B) MA-PP, and (C) PEG-MA-PP in the (a) 1850−1550 cm^{-1} region and the (b) 1200−1050 cm^{-1} region.

resolution equipped with a deuterated triglycine sulfate (DTGS) detector and a 45° face angle Ge crystal. Each spectrum represents 400 coadded scans ratioed against a reference spectrum obtained by recording 400 coadded scans of an empty ATR cell. All spectra were corrected spectral distortions using Q-ATR software.[30]

Results and Discussion

As the first step in this study, it is important to establish which molecular entity along a PP backbone becomes accessible for further surface reactions with maleic anhydride in the presence of microwave plasmons. Figure 1 illustrates three possible surface reaction sites, paths 1-1, 1-2, and 1-3. To determine which of the reaction paths is responsible for further reactions to the PP surface, we recorded Raman (Figure 2a and b) spectra before (traces A) and after (traces B) microwave plasma reactions in the presence of MA. As seen in Figure 2b, the increase of the band at 1455 cm^{-1} and a simultaneous decrease of the band at 1375 cm^{-1} manifest lower concentration levels of -CH_3 groups. At the same time, the -CH_2- content increases. As shown in Figure 2a, traces A and B, the 841 cm^{-1} and 808 cm^{-1} band intensities remain unchanged, further indicating that the CC_3 groups remain inactive during the reactions and no new bands detected in the 750−650 cm^{-1} region signifies that the CC_4 groups are not formed. Thus, these data show that MA reacts to methyl groups on the PP backbone, such as shown in Figure 1, path 1-1. It should be also noted that the previous studies have shown that MA in microwave plasma environments result in the C═C bond saturation while maintaining the ring structure, unless further hydrolysis is conducted.[19] Consequently, free radicals resulting from the saturation are responsible for reactions with the PP surface, as illustrated in Figure 1, path 1-1.

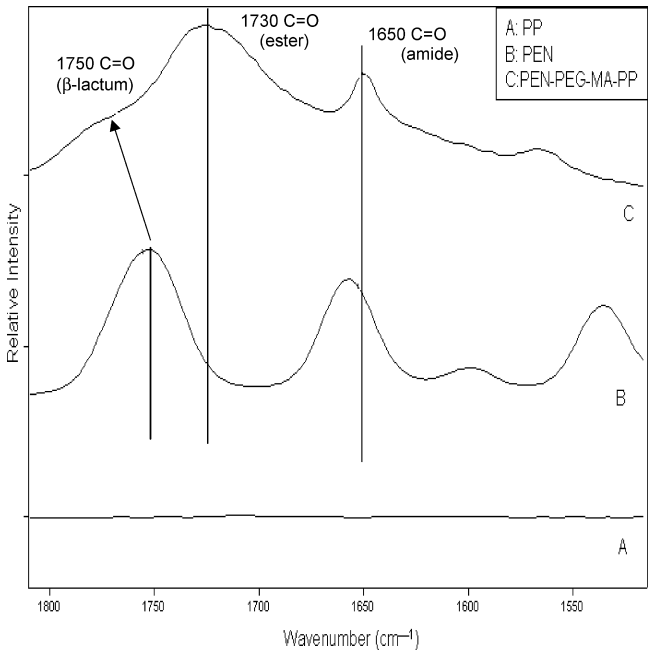

Figure 5. ATR-FTIR spectra of (A) PP, (B) PEN, and (C) PEN-PEG-MA-PP in the 1800−1550 cm^{-1} region.

The next step in these studies was to design and conduct reactions leading to the attachment of PEN and GEN that will be effective against gram "+" and gram "−" bacteria. Figure 3 illustrates a sequence of reactions leading to the attachment of PEN and GEN to MA modified PP surfaces. As stated above, formation of MA groups on the surface of PP through microwave plasma reactions in the presence of MA, followed by their hydrolysis into acid groups illustrated in steps 3-1 and 3-2 of Figure 3 represent a platform for surface modifications which are conveniently converted to COCl groups shown in step 3-3.

As indicated above, to achieve effective surface modifications the attachment of a molecular spacer is essential. For that reason terminal COCl surface groups were reacted with 200 and 600 equiv wt PEG to produce a hydroxyl terminated spacer tethered to the PP surface. This is shown in Figure 3, step 3-4. The next step involved PEN attachement to the end of the OH-terminated PEG via esterification reactions with the hydroxyl groups acting as the nucleophile (step 3-5). In an effort to attach GEN, starting with step 3-3′, epoxy terminated PEG was attached directly to COOH groups, followed by GEN reactions to epoxy entities on the PEG-MA-PP surface. This is illustrated in Figure 3, steps 3-4′ and 3-4″.

Spectroscopic evidence for the occurrence of these surface reactions is shown in Figure 4a and b, where traces A, B, and C illustrate ATR-FTIR spectra recorded from the surfaces of PP, MA-PP, and PEG-MA-PP, respectively. Upon completion of microwave plasma MA reactions, two bands at 1775 and 1710 cm^{-1} are detected on the surface of MA-PP (trace B) which are attributed to the formation of anhydride and acid carbonyl groups on the PP surface. Upon converting COOH to COCl, and reacting with PEG (trace C), the bands at 1725 and 1100 cm^{-1} due to ester carbonyl and ether linkages (C−O−C), respectively, are detected, thus signifying that PEG is covalently attached to the PP surface.

Step 3-5, shown in Figure 3, illustrates reactions leading to the attachment of PEN, whereas Figure 5, traces A, B, and C displays ATR-FTIR spectra recorded from the PP surfaces (trace A), PEN (trace B), and PEN-PEG-MA-PP surfaces (trace C),

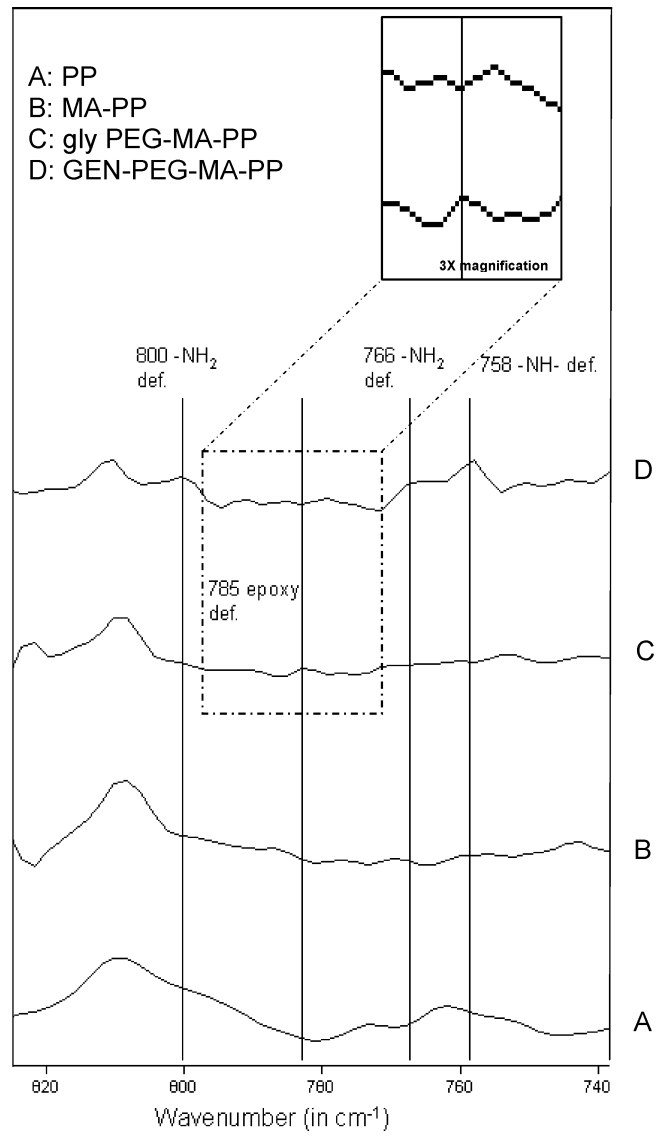

Figure 6. ATR-FTIR spectra of (A) PP, (B) MA-PP, (C) glycidyl PEG-MA-PP, and (D) GEN-PEG-MA-PP in the 880−720 cm^{-1} region.

respectively. As shown in trace C, the presence of the β-lactam carbonyl and amide carbonyl due to PEN is manifested by the bands at 1750 and 1650 cm^{-1}. The band at 1730 due to carbonyl of ester linkages between the PEN and PEG indicated that PEN was covalently attached to the PP surface.

As depicted in Figure 3, steps 3-3′ and 3-4′ involve reactions of epoxy terminated PEG (step 3-3′), followed by GEN reactions (step 3-4′). Figure 6, traces A, B, C, and D, illustrate ATR-FTIR spectra recorded from PP, MA-PP, PEG-MA-PP, and GEN-PEG-MA-PP surfaces, respectively. The weak band at 785 cm^{-1} due to ring-breathing deformation of monosubstituted epoxides shown in trace C indicates the presence of the epoxy groups, which is not detected on the surface of GEN-PEG-MA-PP (trace D) due to ring opening and further reactions with amine groups of GEN. The primary and secondary amine deformation bands at 800, 766, and 758 cm^{-1} of the attached GEN are also detected in trace D, thus confirming reaction steps illustrated in Figure 3.

To determine the effectiveness of the above surface reactions against *S. aureus* and *P. putida* bacteria, controls of PP, MA-PP, PEG-MA-PP, and (glycidyl)PEG-MA-PP along with GEN-PEG-MA-PP and PEN-PEG-MA-PP specimens were incubated in a *S. aureus* and *P. putida* liquid media. The results of these

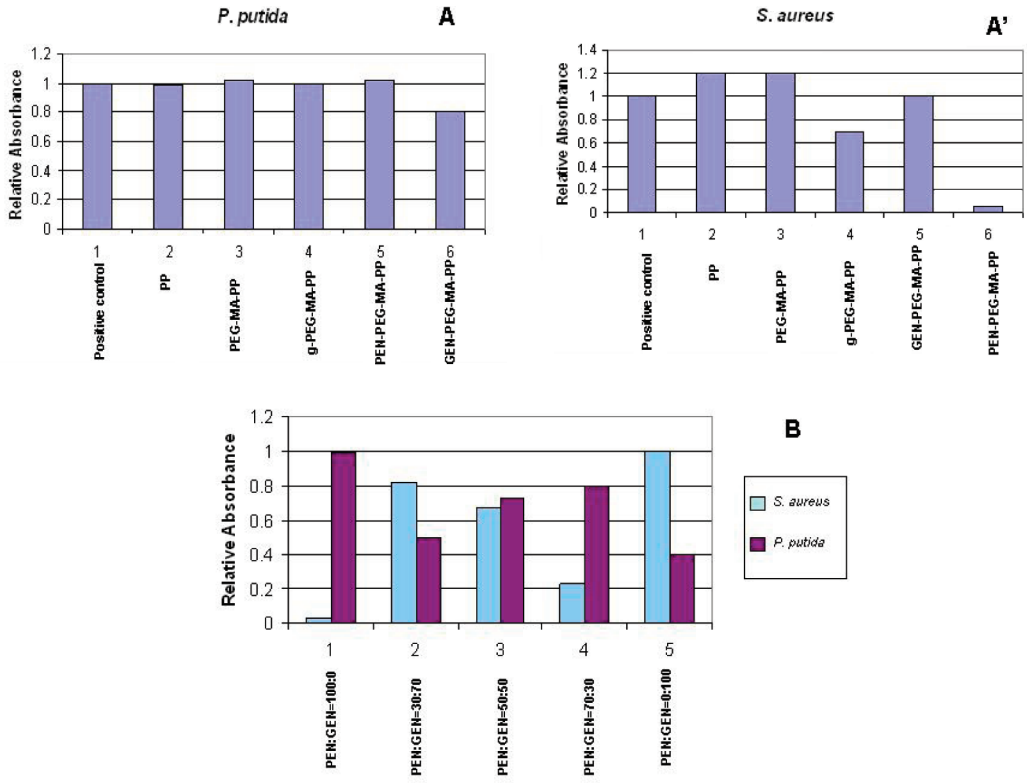

Figure 7. Optical density (absorbance) recorded at 600 nm for *P. putida* (A): positive control (1), PP (2), PEG-MA-PP (3), glycidal-PEG-MA-PP (4), PEN-PEG-MA-PP (5), and GEN-PEG-MA-PP (6); and *S. aureus* (A′): positive control (1), PP (2), PEG-MA-PP (3), glycidal-PEG-MA-PP (4), GEN-PEG-MA-PP (5), and PEN-PEG-MA-PP (6); (B) Optical density (absorbance) recorded at 600 nm for *S. aureus* and *P. putida* cultures: PEN/GEN = 100:0 (1), PEN/GEN = 30:70 (2), PEN/GEN = 50:50 (3), PEN/GEN = 70:30 (4), PEN/GEN = 0:100 (5).

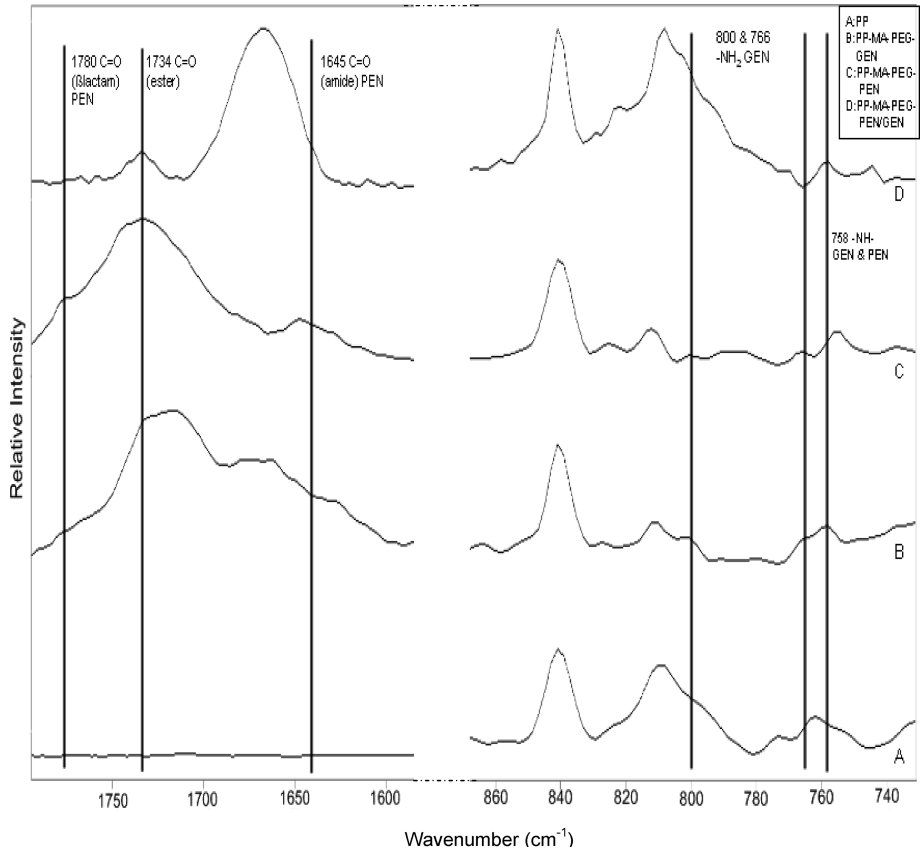

Figure 8. ATR-FTIR spectra of (A) PP, (B) GEN-PEG-MA-PP, (C) PEN-PEG-MA-PP, and (D) GEN/PEN-PEG-MA-PP.

experiments are shown in Figure 7A and A′, respectively, and illustrate that the lowest absorbances are detected for these cultures after exposure to PEN-PEG-MA-PP and GEN-PEG-MA-PP, thus manifesting their effectiveness against the proliferation of *S. aureus* and *P. putida*, respectively.

The simultaneous presence of PEN and GEN on the surface of PP provides an opportunity for creating surfaces with controllable levels of gram "+" and gram "−" antimicrobial activities. For that reason we pursued the simultaneous attachment of both PEN and GEN to glycidyl terminated PEG-MA-PP surfaces with the GEN/PEN ratios of 100:0, 70:30, 50:50, 30:70, and 0:100. Figure 3, step 3-4″ illustrates these reactions, while Figure 8 verifies the PEN and GEN attachment. As shown in Figure 8, traces A, B, C, and D, illustrate ATR-FTIR spectra recorded from PP, GEN-PEG-MA-PP, PEN-PEG-MA-PP, and PEN/GEN-PEG-MA-PP surfaces, respectively. As seen in trace D, the bands at 1780 and 1645 cm^{-1} due to PEN β-lactam carbonyl and amide carbonyl as well as the bands at 800 and 766 cm^{-1} due to the primary and secondary amine deformation bands of GEN are detected, thus confirming PEN and GEN attachment to PP surfaces.

In view of these data the main question is if indeed these surface reactions prevent from microbial gram (+) and (−) attacks on PP surfaces. The results of the antimicrobial analysis in the presence of gram "+" *S. aureus* and gram "−" *P. putida* bacteria are depicted in Figure 7B. As expected for *P. putida*, the lowest optical density (absorbance) is detected for PEN/GEN = 0:100 (specimen #5), but when the concentration ratio of the PEN/GEN decreases, the absorbance of the liquid cultures increases, thus indicating that a fewer bacteria are killed. The mirror image antimicrobial activity for *S. aureus* bacteria are also shown in Figure 7B. It should be noted that the same specimen GEN-PEG-MA-PP in Figures 7A (#6) and B (#5) was used, but the relative absorbance values vary because in each experiment the absorbance values were normalized to different controls. The same trends are detected for other PEN/GEN ratios, and the visual depiction of the antimicrobial activity are illustrated in the Supporting Information, Figures S-1, S-2, and S-3.

Conclusion

A new approach was developed to create antimicrobial coatings on the surface of PP that target *P. putida* and *S. aureus* proliferation through the attachment of both PEN (+) and GEN (−) antibiotics, respectively. While microwave plasma reactions in the presence of MA served to create carboxylic acid groups on the PP surface, two parallel synthetic paths were developed to accommodate reactions of PEN and GEN. While the attachment of PEN was proceeded by converting COOH to COCl, followed by the reactions with PEG, GEN was attached to diglycidyl PEG through the epoxy groups with proceeding reactions of diglycidyl PEG via the COOH of maleic acid on PP surface. The effectiveness of the PEN and GEN functionalized PP surfaces were tested against the proliferation of *S. aureus* and P. putida bacteria and showed that PEN-PEG-MA-PP effectively eliminates *S. aureus*, whereas GEN-PEG-MA-PP is effective against *P. putida*. Furthermore, by controlling concentration levels of each antibiotic on the PP surfaces facilitated a means for creating PP surfaces responsive to different strengths of antimicrobial activity.

Acknowledgment. These studies were partially supported by the National Science Foundation Materials Research Science and Engineering (MRSEC; DMR 0213883) and Major Research Instrumentation (DMR 0215873) Programs. The authors thank Dr. S. Heinhorst's Laboratory at USM for assistance in determining antimicrobial properties.

Supporting Information Available. Visual depiction of the antimicrobial activity. This material is available free of charge via the Internet at http://pubs.acs.org.

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BM8013473