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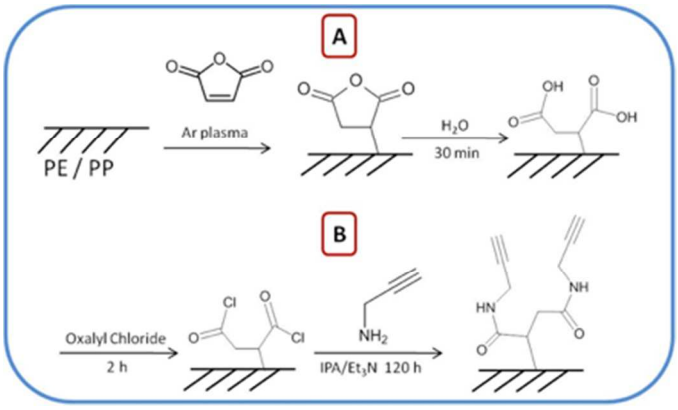
TOC

MICROWAVE PLASMA (A) AND CLICK CHEMISTRY (B)
ON POLYMERIC SUBSTRATES



Simple Click Reactions on Polymer Surfaces Leading to Antimicrobial Behavior[+]

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Abstract

In these studies we developed a simple reactions that combined microwave plasma reactions in the presence of maleic anhydride with alkyne click chemistries to achieve a platform for unlimited possibilities for further surface modifications of aliphatic polymer surfaces. Using this approach, we covalently attached ampicillin (AMP) to polyethylene (PE) and polypropylene (PP) substrates. As a result, high efficacy against microbial film formation, manifested by efficient antimicrobial activity against *S. aureus* with a 97-99.8% decrease of bacterial growth, was achieved. This simple and clean process allows functionalization of any polymeric substrate without adverse affects on bulk polymer properties.

Polymer surfaces, particularly aliphatic polyethylene (PE) and polypropylene (PP) in contact with biological systems, are susceptible to adverse macroscopic processes among which antimicrobial film formation represents a serious problem. Simple devices, such as catheters, tubing, or other instruments in contact with biological systems may lead to infections, which accounts for over one hundred thusand deaths annually in the U.S. alone. One approach to alleviate this situation is to chemically modify surfaces of commodity polymers in such a way that their use is not only safe in biological environments, but also prevent deadly infections. Although non-covalent and covalent surface modifications have been utilized to attain desirable surface properties while maintaining bulk attributes, the majority of physico-chemical approaches ranging from layer-by-layer deposition,[1] chemical etching,[2] or radiation grafting,[3] appear to offer relatively limited antimicrobial effectiveness. In the layer-by-layer approach, multiple layers are deposited onto surfaces by dipping, and multi-layers are held together by opposite electrostatic charges. However, the resulting films may suffer from mechanical and chemical instability.[4] Chemical etching alters hydrophobicity of aliphatic polymer surfaces resulting in surface oxidation and morphological changes, whereas radiation grafting sources in the presence of monomer uses infrared, visible light, ultraviolet, and γ radiation as well as high energy electron to graft species to/from the surface.[3] In contrast, microwave plasma surface reactions offer a fast, solventless, and sterile method for covalently attaching carboxylic acid groups to almost any polymer substrate. In this simple process, excited ionized gas is utilized within 0.5–10 sec. to create reactive groups in the presence of a desirable monomer, thus providing controllable conditions for covalently attaching desirable chemical entities.[5] When maleic anhydride is used as a monomer, resulting surface modifications is the formation of –COOH groups that are covalently attached to a polymer backbone. Their use was recently demonstrated by a covalent attachment of bacteriophages.[6]

If aliphatic polymer substrates, such as PE and PP, exhibit –COOH surface groups, their presence facilitates an opportunity for further reactions. Since click chemistry[7, 8] represents one of the highly efficient (>95%) synthetic routes of achieving high reaction yields in relatively short time under mild conditions, we combined highly efficient microwave plasma reactions and click chemistry to create antimicrobial PE and

PP surfaces. While the first step generates acid groups, the second step is not limited to "click" chemistry. It may also include Huisgen 1,3-dipolar cycloaddition,[9] Diels-Alder reactions, [10] nucleophilic substitution with epoxy and aziridine compounds, [11] dihydroxylation,[7] and thiol-yne reactions.[9] Notably, surface reactions utilizing cycloaddition click reactions yielded high efficiencies with Cu,[8] Au,[12] Si,[13] and carbon nanotube[14] surfaces have shown promising results, and so did biofunctionalization of Si surfaces with "clicked" biotin and glucose.[15] Thus, the attachment of bioactive molecules can be particularly important in creating synthetic-biological interfaces. Other unique attributes of click chemistry also broke new grounds for selective reactions with complex dendrimers,[16,17] hydrogels,[18] vesicles,[19] and nanoparticles.[20]

Although click chemistry has been utilized in a variety of model synthetic studies, the major limiting roadblock is the ability to react to inert polymeric surfaces. In view of the previous microwave plasma reactions leading to –COOH functionalization of aliphatic polymer surfaces, this study examines simple two-step surface reactions that formulate a new platform for almost any polymer surface reactions using click chemistry without adverse affects on bulk polymer properties. Figure 1 schematically depicts this simple two-step process: 1) microwave plasma reactions that lead to COOH formation and 2) covalent attachment of alkyne moieties. The ultimate outcomes are azide surface groups to which many entities may be "clicked." Selected examples of 'desired click molecules' include peptides utilized in stem cell adhesion,[21] fluorophores for labeling hydrogels,[22, 23] biotin,[24] bacteriophages,[25] or DNA,[26] to name just a few. These studies focus on the attachment of antibiotics, such as ampicillin (AMP) with Gram (+) and (-) attributes to offer antimicrobial functions on PE and PP surfaces. It should be noted that the efficiency of these surface reactions is primarily determined by the very first microwave plasma reaction step and is substrate dependent. For example, previous studies have shown that for PP,[27] concentration levels of COOH groups is in the range of ~7.0 mg/m^3 at ~0.2 μm from the surface, whereas for polytetrafluoroethylene (PTFE) surfaces these values are diminished to ~ 2 mg/m^3.[28]

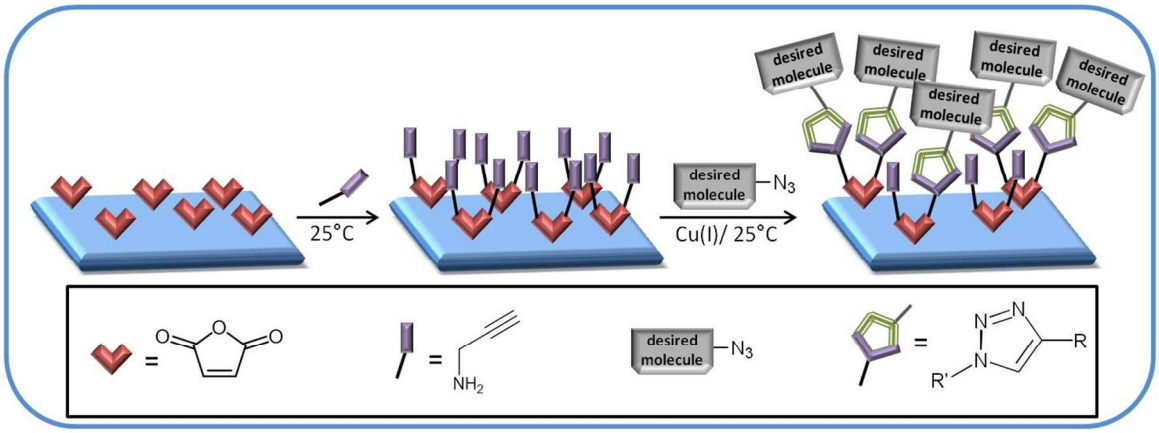

Figure 1: Schematic diagram of 'clickable' polymeric surfaces exhibiting alkyne functionalities for 'clicking' any azide-containing molecule.

Figure 2 illustrates the two-step process in which MA was reacted to PE and PP (A) to obtain surface –COOH groups, followed by their conversion to acid chloride. The second step relies on reactions of propargylamine (B) to obtain alkyne functionalized polymeric surfaces to which any azide containing molecule may be reacted.

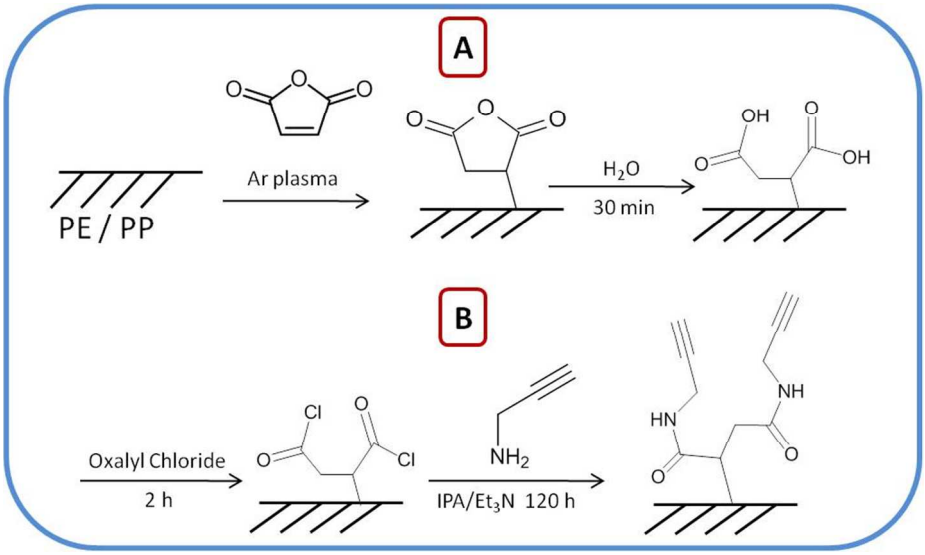

Figure 2: Surface reactions leading to the formation of alkyne surface groups; Microwave plasma reactions in the presence of MA (A) and alkyne functionalization using propargylamine (B).

Figure 3-(a)-A, B, C, and D illustrates chemical structures that develop as a results of reactions depicted in steps A and B of Figure 2. These features labeled as A,

B, and D, were determined using FT-IR spectroscopy. While Traces A in Figure 3-b and 3-c represent PE and PP substrate spectra, respectively, the 1712 cm^{-1} band in Traces B in these Figures corresponds to the C=O vibrations of –COOH groups due to maleic anhydride reactions and its subsequent hydrolysis. Traces C in Figure 3-b and 3-c represent the spectra recorded after amidation reactions with PPA on PE and PP, where the C=O bands at 1660 and 1530 cm^{-1} manifest amide I and II formations due to C=O and N-H functionalities, respectively. Click reactions with azide functionalized AMP are illustrated in Figure 3-a, and the spectra of those surfaces are shown in Traces D of Figure 3-b and 3-c in which the β-lactam moiety of ampicillin is manifested by the band at 1770 cm^{-1} due to C=O stretching vibrations.

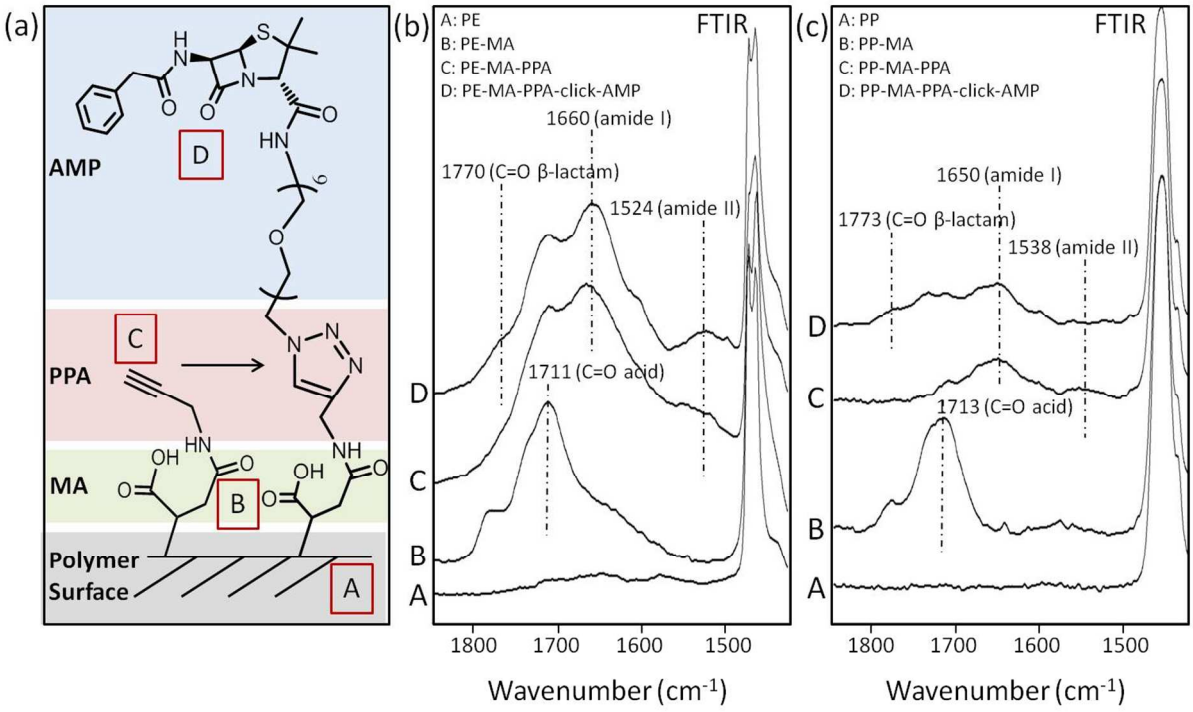

Figure 3. (a) Reaction sequences on PE and PP substrates leading to AMP clicked surfaces; (b) ATR-FTIR spectra of A: PE, B: PE-MA, C: PE-MA-PPA, D: PE-MA-PPA-AMP; (c) ATR-FTIR spectra of A: PP, B: PP-MA, C: PP-MA-PPA, D: PE-MA-PPA-AMP.

Figure 4 illustrates a sequence of complementary Raman spectra recorded after each step leading to the formation of alkyne moieties. While Trace A of Figure 4 represents the reference spectrum of PP, Trace B shows the Raman spectrum of PP after MA attachment. Trace C of PP-MA surfaces after reaction with digylcidyl ether PEG having terminal epoxide groups to which PPA was reacted. However, no alkyne bands are observed. Trace D shows the spectrum of PP-MA in which PPA was reacted via carbodiimide coupling chemistries using DCC. Again, no alkyne bands are observed. Trace E illustrates the spectrum of PP-MA to which PPA was attached by converting the surface –COOH groups to –COCl using oxalyl chloride (OC) prior to addition of PPA. The band at 2127 cm^{-1} in Figure 4, Trace E corresponds to C≡C groups present on the polymer surface.

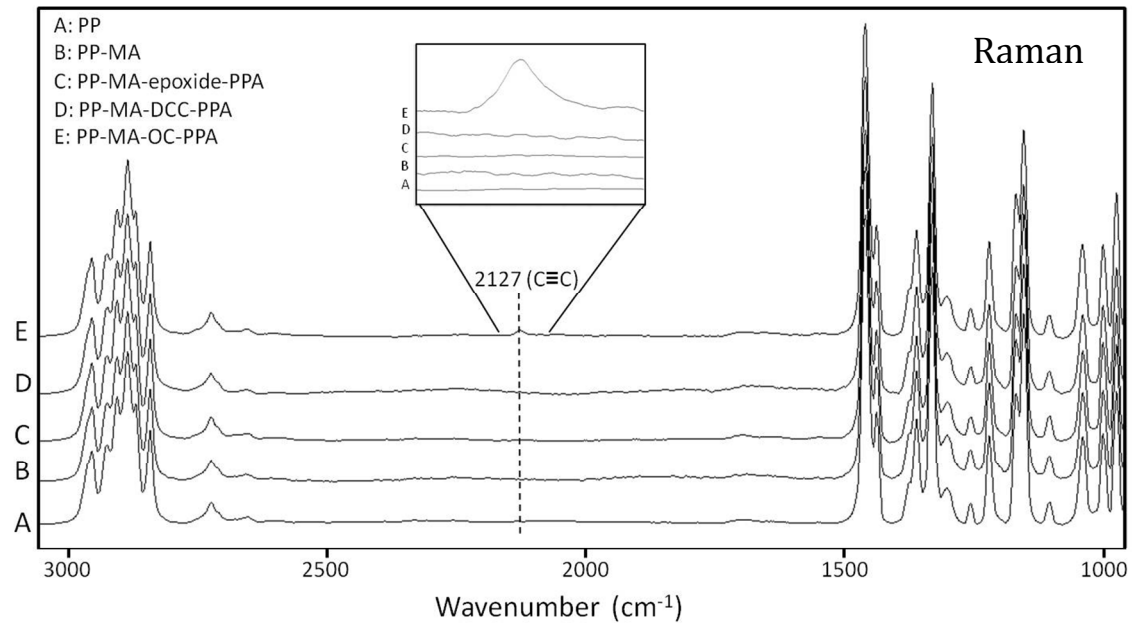

Figure 4. Raman spectra of A: PP; B: PP-MA; C: PP-MA-epoxide-PPA; D: PP-MA-DCC-PPA; and E: PP-MA-OC-PPA.

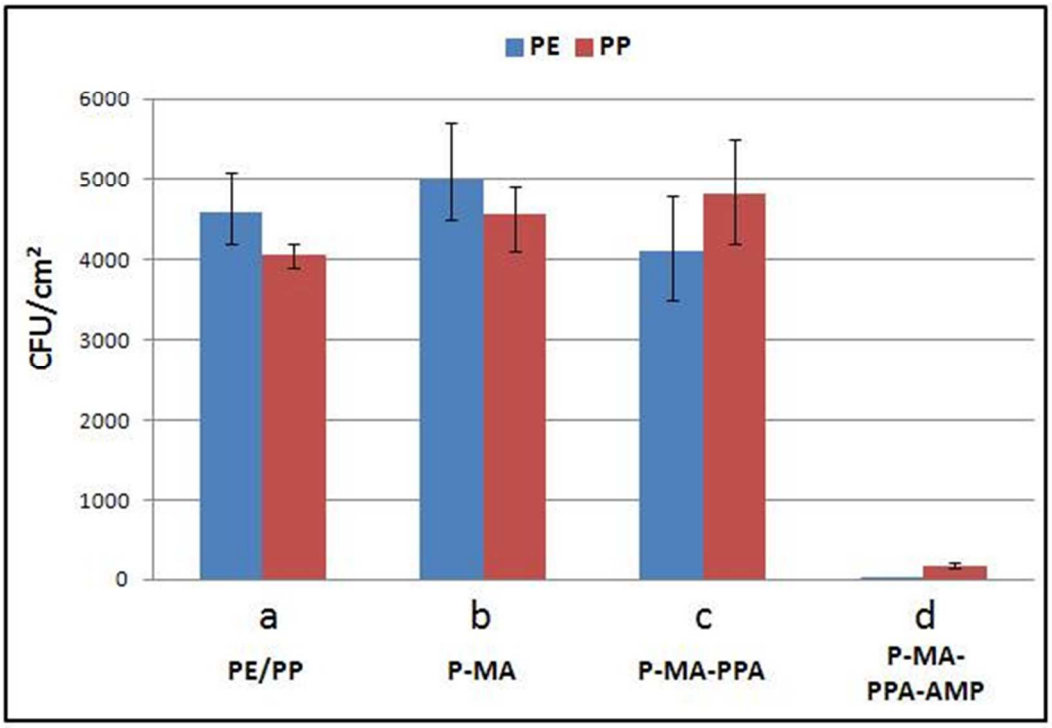

Figure 5: Antimicrobial activity against *S. aureus* of (a) PE (blue) and PP (red) surfaces; (b) PE-MA and PP-MA surfaces; (c) PE-MA-PPA and PP-MA-PPA surfaces; (d) PE-MA-PPA-AMP and PP-MA-PPA-AMP surfaces.

While spectroscopic analysis clearly identified molecular entities reacted after each step of this simple two-step sequence, an ultimate goal of these studies was to examine the effectiveness of covalently attached AMP to polymer surfaces against *S. aureus* bacteria. For that reason, PE-MA-PPA-AMP and PP-MA-PPA-AMP surfaces were tested for antimicrobial activity against *Staphylococcus aureus.* The results of the analysis are shown in Figure 8 in which the colony forming units (CFU) per cm^2 were enumerated using a drop plate method for PE/PP (a), PE-MA/PP-MA (b), PE-MA-PPA/PP-MA-PPA (c), and PE-MA-PPA-AMP/PP-MA-PPA-AMP (d) surfaces. Analysis of the data indicates that “clicked” AMP on both PE and PP facilitates a major enhancement of the antimicrobial activity manifested by a drop of CFUs from 4000-5000 for PE-MA-PPA and PP-MA-PPA specimens to 10-100 for PE-MA-PPA-AMP and PP-MA-PPA-AMP specimens.

In summary, these studies showed a simple sequence of “clicking” molecules onto aliphatic polymeric surfaces containing COOH groups. The latter was achieved by solventless microwave plasma surface reactions. This step was followed by functionalization with alkyne species to which any azide containing molecule can be easily reacted. To illustrate the efficacy of these chemistries against microbial film formation, a model system was examined in which azide functional AMP was synthesized and clicked onto PE and PP surfaces. These studies have shown that such modified surfaces exhibit highly efficient antimicrobial activity against *S. aureus* with a 97-99.8% decrease of bacterial growth.

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