Polymeric Surfaces with Anticoagulant, Antifouling, and Antimicrobial Attributes

*Min Yu, Marek W. Urban**

Summary: Recent advances in developments of anticoagulant, antifouling, and antimicrobial polymeric surfaces with the focus on surface modifications of polymeric materials as well as the influence of nanosize materials on interactions with biological systems are discussed. Controllable surface modifications using microwave plasma reactions in the presence of maleic anhydride provides a platform for attaching acid groups to inert polymeric surfaces which serves as a reactive anchor for further reactions. To effectively attach bioactive species inhibiting interactions with biological systems it is often necessary to attach a molecular spacer that facilitates mobility to terminal bioactive species.

Keywords: biomaterials; microwave plasma reactions; nanotechnology; surface modifications

Introduction

Continuous demands for materials with anticoagulant, antifouling, or antimicrobial properties have stimulated new research avenues leading to the development of surface modifications of polymers utilized in various bio-medical areas.[1–4] Depending upon applications, poly(tetrafluoroethylene) (PTFE), expanded PTFE (ePTFE), poly(ethylene terephthalate) (PET), poly(dimethylsiloxane) (PDMS), poly(urethane) (PU), poly(propylene) (PP), poly(vinyl chloride) (PVC), poly(methyl methacrylate) (PMMA), high and low density poly(ethylene) (HDPE), (LDPE)[1–3] are of particular importance. Unfortunately, the majority of these materials in contact with bio-systems become problems for microbial growth and/or adsorption of bioorganisms. One approach to alleviate and control interactions of bioorganisms with polymeric surfaces while maintaining useful bulk characteristics is to modify their surfaces. Among various polymer modifications, a simple addition of bioactive molecules to a polymer matrix during processing[2,3] has been utilized, but the effectiveness of these approaches often relies on the rates of diffusion of bioactive species to the surface. Other approaches involved surface physisorption using layer-by-layer deposition,[5–7] dipping,[2,8] and self-assembled monolayers (SAMs),[4,9] which appear to be useful, but offer less stability and uniformity compared to covalently bonded species. Since covalent bonding to surfaces using grafting-to[2,10] and grafting-from[2,3,11,12] reactions offer significantly greater control over the surface chemistry as well as surface morphologies, various synthetic paths were utilized. However, key components for successful covalent attachments of other species are to create a reactive surface group as well as a molecular spacer. While a surface reactive group facilitates an anchor for further reactions, the presence of a spacer provides mobility to bioactive species attached to the end of the spacer. This paper discusses recent advances in surface modifications of selected polymers that exhibit anticoagulant, antifouling or antimicrobial properties as well as new developments in

School of Polymers and High Performance Materials, Shelby F. Thames Polymer Science Research Center, The University of Southern Mississippi, Hattiesburg, MS 39406
E-mail: marek.urban@usm.edu

nanomaterials and their interactions with biological systems.

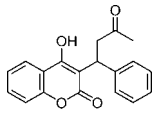
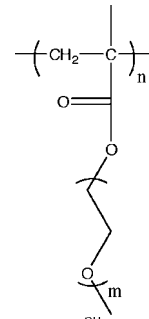
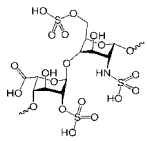
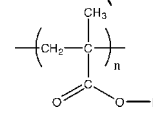
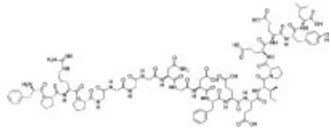
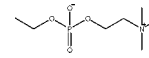
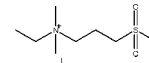
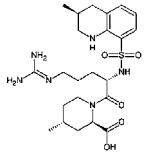
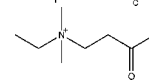
Modifications of Polymer and Nanomaterials Surfaces

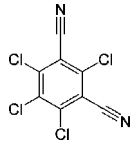
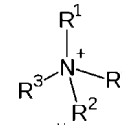
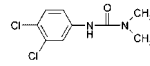
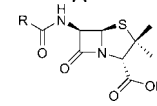
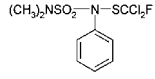
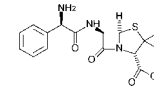
Due to surface energies, the majority of polymeric materials exhibit highly thrombogenic surfaces, and consequently may adsorb fibrin, thrombus, or other proteins which result in clot formations.[3] To minimize this process anticoagulant agents that inhibit adhesion of platelets and coagulation of the blood have been utilized.[3,13–16] Heparin (HEP) surface-functionalized poly(carbonate-urea) urethane cardiovascular grafts is an example of a aqueous-based process resulting in bioactive surfaces at a high HEP surface density.[13] Recombinant-hirudin was covalently immobilized on PET surfaces via the carboxylic acid groups which showed binding and inhibition against thrombin adsorption.[14] Other antithrombotic agents include warfarin,[3,17] argatroban,[3,18] and bivalirudin,[3,19] but their anticoagulant functions are only effective when these species are released to the blood stream. For example, warfarin inhibits the synthesis of vitamin K thus minimizing blood coagulation, whereas hirudin, argatroban, and bivalirudin are direct thrombin inhibitors that bind to thrombin active sites. Table 1 provides a comprehensive list of chemicals utilized in preventing coagulant, fouling, and microbial processes.

Biofouling resulting from protein adsorption and cell adhesion to materials is also another major area of interest. To minimize surface attachments of biological organisms, additive of biocides such as chlorothalonil, diuron and dichlofluanid to a polymer matrix is utilized. However, due to their toxicity, a number of studies focused on modifications of polymer architectures as well as surface modifications of poly(ethylene glycol) (PEG),[20–24] PDMS,[25–27] fluoro-containing,[28–32] zwitterionic phosphorylcholine (PC),[33–35] sulfobetaine (SB),[36,37] and carboxybetaine (CB)[38,39] polymers. The adhesion strength (A) of biomolecules to polymer surfaces is related to the surface energy (γ) and a polymer matrix modulus (E), which are related via $A = (\gamma E)^{1/2}$.[40] For example, hydrophilic PEG with a low surface energy exhibits good biocompatibility and resistance to biofouling and provides a high activation barrier for protein adsorption as well as steric repulsions which are also important for protein resistance.[30] PEG-functionalized polymer brushes synthesized by surface-initiated atom transfer radical polymerization (ATRP) have been intensively examined which resulted in surfaces with a significant resistance to protein adsorption and cell adhesion.[20–24] In contrast, elastomeric polymers such as PDMS exhibit favorable low modulus, but its high surface energy results in increasing protein adhesion strength. In response to this drawback novel polymer architectures and surface modifications of PDMS[25–27] have been investigated and one example is zwitterionic phosphorylcholine polymer which resembles the structure of natural membrane lipids. These materials, upon grafting onto PDMS using photo-induced polymerization, exhibit an excellent surface hydrophilicity and antifouling properties.[27] Fluoro-containing polymers also exhibit low surface energy, but their high modulus limits their applications. Consequently, novel polymer architectures[28–32] were synthesized such as hyperbranched fluoropolymer-PEG network,[28] and a PTFE membrane grafted by poly(ethylene glycol) methacrylate (PEGMA) via surface-activated plasma treatments were developed, thus providing good biofouling resistance.[32]

The development of antimicrobial surfaces is also of great significance. Various polymers[2,11,41,42] such as PDMS, PP, ePTFE, PVC, PMMA, and PE utilized in biomedical implants and medical devices serve as a playground for bacterial and other microorganisms growth. Thus, modifications of polymer surfaces with antimicrobial species to inhibit this growth are essential. Although antimicrobial agents such as metals (silver and silver-based

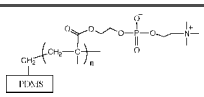
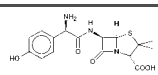
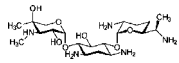
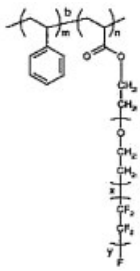
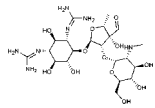
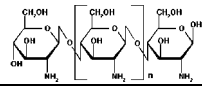
Table 1.
Chemical species utilized in preventing coagulant, fouling, and microbial processes.

Anticoagulant		Antifouling (Continued)	
Name	Chemical Structure	Name	Chemical Structure
Warfarin		Poly(oligo (ethylene glycol) methyl ether methacrylate)[21]	
Heparin		Zwitterionic polymer	
Hirudin		• Phosphoryl -choline (PC)	
		• Sulfobetaine (SB)	
Argatroban		• Carboxybetaine (CB)	

Antifouling		Antimicrobial	
Name	Chemical Structure	Name	Chemical Structure
Chlorothalonil		Metals: Silver ion, Tin, Mercury	Ag^{+}, Sn, Hg
		Quaternary ammonium	
Diuron		Beta-lactams • Penicillin	
Dichlofluanid		• Ampicillin	

(Continued)

Table 1.
Continues.

Antifouling		Antimicrobial	
Name	Chemical Structure	Name	Chemical Structure
Phosphoryl-choline-PDMS[27]		• Amoxicillin	
		Aminoglycosides • Gentamicin	
PEG-fluoropolymer[30]		• Streptomycin	
		Chitosan	

compounds, tin, mercury),[2,43–47] phenols,[48] and chitosan,[2,49] have been known for many years, more recently quaternary ammonium compounds[2,12,50,51] and antibiotics were utilized on polymer surfaces.[11,41] While metals cause the release of K^+ ions and cytoplasmic membrane from bacteria, quaternary ammonium and chitosan binding to negatively charged bacterial surfaces cause the leakage of intracellular constituents. Attachments of poly(quaternary ammonium) (PQA) to PP surfaces using photochemical synthesis and ATRP, were successful and molecular weight and the density of the QA can be controlled.[51] Antibiotics, on the other hand, provide different mechanisms of prevention and the common classes are aminoglycoside and beta-lactam-based antibiotics. Aminoglycosides, such as gentamicin and streptomycin, prevent bacteria protein synthesis, whereas beta-lactams, such as penicillin (PEN), amoxicillin, and ampicillin (AM), inhibit bacterial cell wall formation. Penicillin[11,52] and ampicillin[41] were also successfully attached to the ePTFE surfaces and these modifications involved plasma reactions and grafting through carboxylic acid groups. These studies showed high effectiveness against both gram positive and negative bacteria and microwave plasma reactions and the attachment of bioactive molecules are illustrated in Figure 1. As indicated earlier, the first step is the attachment of an anchor molecule through the reaction of maleic anhydride to form acid groups, followed by reactions of a spacer to provide mobility to the active antibiotic molecule attached to the other end of the spacer.

Understanding of interactions of nanomaterials with biological systems is also very timely, and particularly the influence of nano-size and shapes on cell cytotoxicity are of significance. Recent studies that utilized carbon nanotubes and fullerenes indicated that interactions of these species with living cells or other biosystems are complex and not well understood. Although cytotoxicity of carbon nanotubes and fullerenes have been attributed to size,[54,55] shape,[56,57] surface functionalizations,[58,59] and even the choice of a test method,[60,61] further detailed studies are needed to resolve these issues. There is a common agreement that these materials,

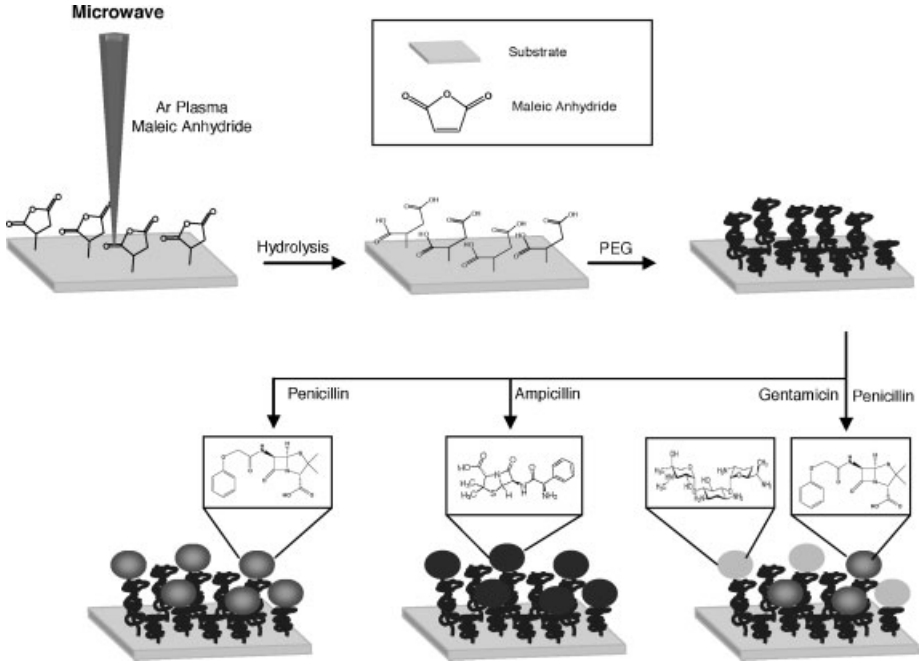


Figure 1.
Schematic diagram of Ar microwave plasma reactions in the presence of maleic anhydride leading to the formation of −COOH groups, followed by PEG attachment and bioactive molecules to functionalized surfaces.[11,41,42,53].

however, exhibit antimicrobial properties.[55,62,63] For example, highly purified single wall carbon nanotubes showed antimicrobial activities against *Escherichia coli* (E. coli) and their cytotoxicity resulted from a direct contact with a bacterial cell membrane, causing severe cell membrane damage.[62] Ideally, one would like to control cytotoxicity while maintaining antimicrobial functions and thus surface modifications of multi-wall carbon nanotubes (MWNT), fullerenes (C_{60}), carbon nanofibers, and ferromagnetic nanotubes (FMNT)[64] are important. Controlling these properties of nano-materials will open up many potential applications in various fields of medicine. For example, Figure 2, A illustrates an example of introducing a flexible PEG variable length spacer between the MWNT surface and PEN which provides mobility to the terminal antibiotic PEN, thus enhancing PEN activity against the formation of microbes. As seen, each step of surfaces reactions was followed by IR analysis and revealed the presence of ester linkages due to reactions between PEN and PEG functionalities. This is illustrated in Figure 2, B.

Similar results were obtained for fullerenes which are illustrated in Figure 3, A and B. While Figure 3, A illustrates a sequence of events leading to the attachment of PEN, Figure 3, B shows ATR FT-IR spectra of each step. As shown, the presence of C=O groups due to amide, ester, and β-lactam entities are detected. The same observations were obtained for carbon nanofibers and FMNT (not shown).

The main question, however, is the effectiveness of these surface modifications. Microbial analysis of MWNT, C_{60}, carbon nanofibers, and FMNT containing surface-terminated PEN revealed highly effective anti-bacterial activity against gram + *Sta-Staphylococcus aureus* (*S. aureus*). For example, Figure 4, A-A' and B-B' illustrate antimicrobial activity results for MWNT,

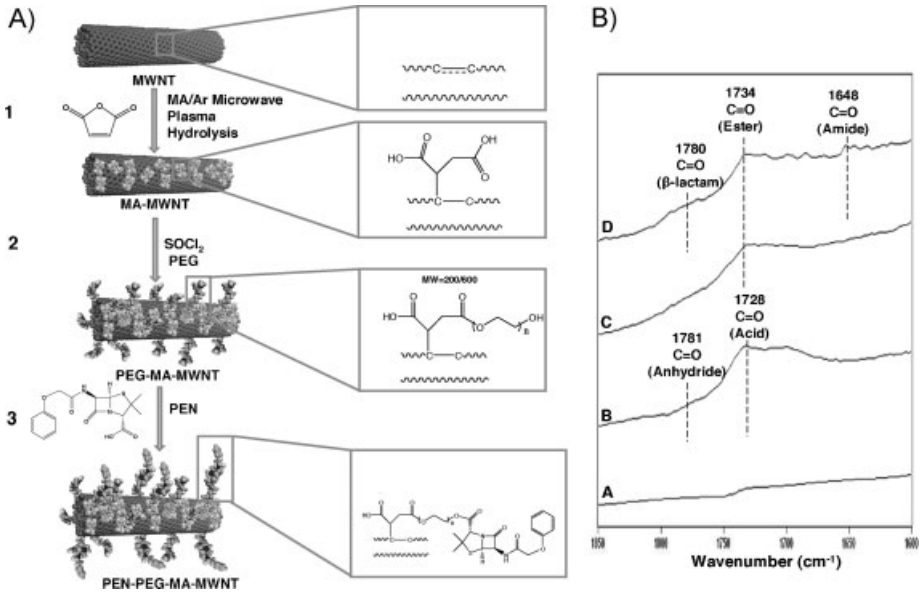


Figure 2.

A. Schematic diagram of surface reactions on MWNT: Step 1, Ar microwave plasma reaction and hydrolysis of maleic anhydride (MA)-modified MWNT; Step 2, conversion of acid group and PEG reaction; Step 3, PEN reaction. **B**. Attenuated total reflectance Fourier transform infrared **(**ATR FT-IR) spectra of (A) MWNT, (B) MA-MWNT, (C) PEG-MA-MWNT, and (D) PEN-PEG-MA-MWNT.

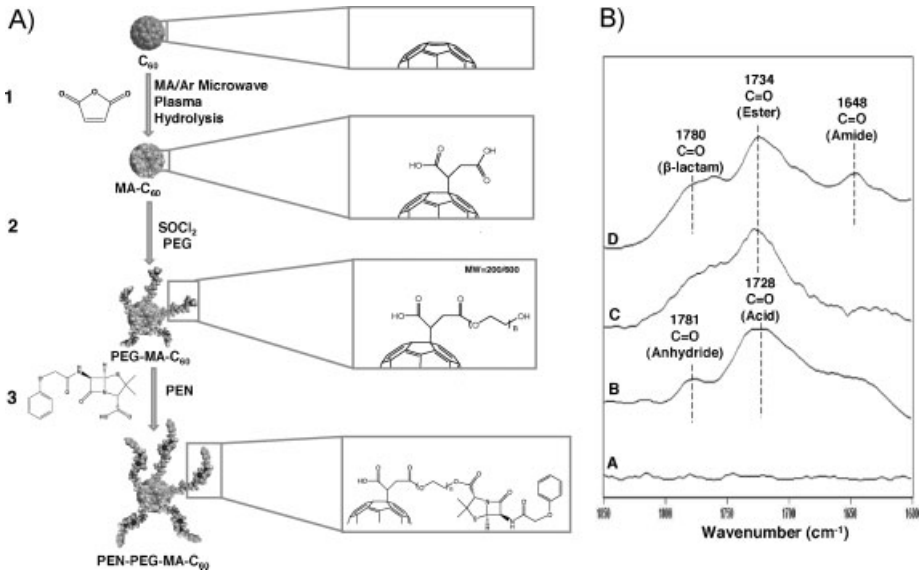


Figure 3.

A. Schematic diagram of surface reactions on C_{60}: Step 1, Ar microwave plasma reaction and hydrolysis of maleic anhydride (MA)-modified C_{60}; Step 2, conversion of acid group and PEG reaction; Step 3, PEN reaction. **B**. ATR FT-IR spectra of (A) C_{60}, (B) MA-C_{60}, (C) PEG-MA-C_{60}, and (D) PEN-PEG-MA-C_{60}.

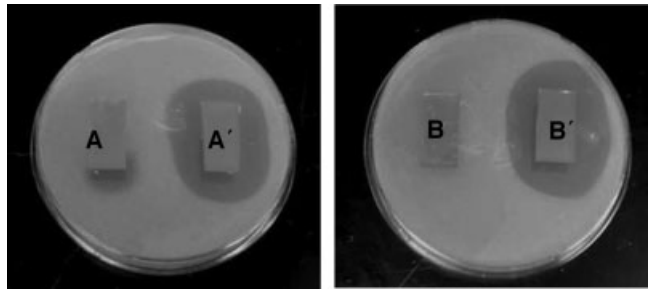


Figure 4.
Photographs of agar plates containing (A) MWNTs, (A′) PEN-PEG-MA-MWNTs, (B) C_{60}, (B′) PEN-PEG-MA-C_{60} after incubation with *S. aureus* for 16 h at 37 °C.

MWNT modified with PEN, C_{60}, and C_{60} modified with PEN, respectively. As seen in Figure 4, A, unmodified MWNTs only slightly inhibit the bacterial growth, which is consistent with the data reported earlier.[55,62,63] In contrast, upon covalent attachment of the PEN-terminated PEG spacer, antimicrobial tests demonstrate the inhibition of bacterial growth around the specimen and its surroundings. This is shown in Figure 4, A', where significant area surrounding the specimen becomes clear. The same results were obtained for the PEN-modified C_{60} specimen and are illustrated in Figure 4, B-B'. Again, the bacterial growth is only inhibited when PEN molecules are covalently attached to the surface. Our initial studies also indicated that these surface modifications may also alleviate potential cytotoxicity of nanomaterials.

In summary, functionalized with the flexible PEG spacer PEN-terminated nano-objects exhibit significantly enhanced antimicrobial activities. Although many nano-shapes, such as tubules, spheres, hollow particles, rods, and fibers exhibit many promising applications in biotechnology, particularly for drug delivery systems, biosensors, medical instrumental or devices, and early detection methods, the challenge is not only to make these materials resistant to microbial attacks, but also design their surfaces to be simultaneously non-toxic, anticoagulant and antifouling.

Conclusions

Although significant efforts have been made in polymer modifications leading to anticoagulant, antifouling, and antimicrobial properties, synchronous orchestrated efforts are required in all areas to achieve simultaneous anticoagulant, antifouling, and antimicrobial properties of materials used in contact with biosystems. The area that is of particular importance and great challenge is to understand the interplay between cytotoxicity, anticoagulant, antifouling, and antimicrobial properties of surfaces in nanomaterials.

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