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Covalent Attachment of Multilayers on Poly(tetrafluoroethylene) Surfaces

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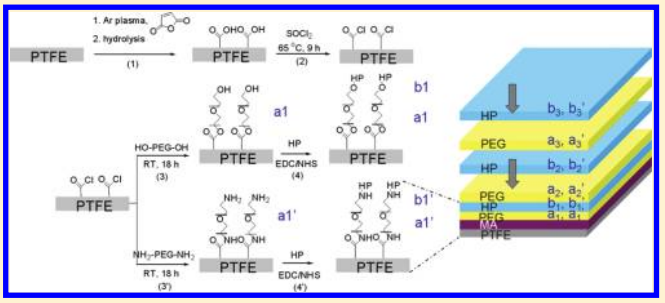
Supporting Information

ABSTRACT: These studies demonstrate a new approach of producing multifunctionalized coatings on poly(tetrafluoroethylene) (PTFE) surfaces by covalent attachments of multilayers (CAM) of heparin (HP) and poly(ethylene glycol) (PEG). This process can be universally applied to other covalently bonded species and was facilitated by microwave plasma reactions in the presence of maleic anhydride which, upon ring-opening and hydrolysis, provided covalent attachment of COOH groups to PTFE. These studies showed that alternating layers of PEG and HP can be covalently attached to COOH-PTFE surfaces, and the volume concentration and surface density of PEG and HP on the PTFE surface achieved by the CAM were 7.02−6.04 × 10^{-3} g/cm^3 (2.1−1.8 × 10^{-7} g/cm^2) and 9.3−8.7 × 10^{-3} g/cm^3 (2.8−2.6 × 10^{-7} g/cm^2), respectively. The CAM process may serve numerous applications when the covalent modification of inert polymeric substrates is required and particularly where the presence of bioactive species for biocompatibility enhancement is desirable.



INTRODUCTION

Although many polymeric materials serve in biomedical applications ranging from implants[1−3] to artificial organs[4,5] or tissue regeneration[2,6,7] to drug delivery systems,[7,8] their biocompatibility continues to be an ongoing challenge.[9] One of the common approaches is to modify polymer surfaces in contact with biological agents. For example, layer-by-layer (lbl) deposition processes[10−14] have been employed, but the instability and consequently effectiveness may be limited due to noncovalent interlayer bonding. Other approaches, including UV surface grafting[2] or end-capping of anticoagulants molecules to polymers[6] were also employed, but achieving controllable biochemical and morphological features is not obvious. While covalent attachments[2,5,15−19] of molecules or macromolecules to polymers that exhibit anticoagulant characteristics offer significant advantages, inertness of polymeric substrates and the low surface energies impose other limitations. Ideally, one would like to covalently attach multilayered structures that are stable, exhibit suitable effectiveness against formation of blood clots inside blood vessels obstructing the flow (thrombosis), and are antifouling, thus facilitating dual and longer protection.

The approach developed in this study deviates from the previous attempts and utilizes a covalent attachment of multilayers (CAM) by reacting alternating layers of heparin (HP) and poly(ethylene glycol) (PEG) species while maintaining useful polymer attributes. These reactions will be sequentially conducted by covalent bonding of functionalized PEG and HP to COOH-modified PTFE. The latter will be accomplished by previously developed microwave plasma reactions in the presence of maleic anhydride (MA) capable of generating COOH groups.[12,20−23] An ultimate goal is to engineer robust multilayered covalently bonded coatings on PTFE surfaces which will potentially exhibit useful antifouling and antithrombotic properties for extended periods of time, especially for in vivo and in vitro blood platelet deposition and blood compatibility.

EXPERIMENTAL SECTION

Covalent Attachment of Multilayers (CAM). PTFE specimens were purchased from McMaster-Carr (Atlanta, GA), cut to 7 × 7 mm squares, washed with a 1:1 mixture of acetone and isopropanol, and dried at room temperature. Plasma reactions were conducted using open reactor conditions, as described elsewhere.[20−22] The PTFE substrate and 1.5 g of solid maleic anhydride (MA; Sigma Aldrich) 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 19.99 Pa (150 mtorr), followed by purging it with Ar gas at a flow rate of 3.0 mL/min to reach a steady state pressure of 33.30 Pa (250 mtorr). 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 7 s. Under these conditions, the reaction chamber pressure increases continuously

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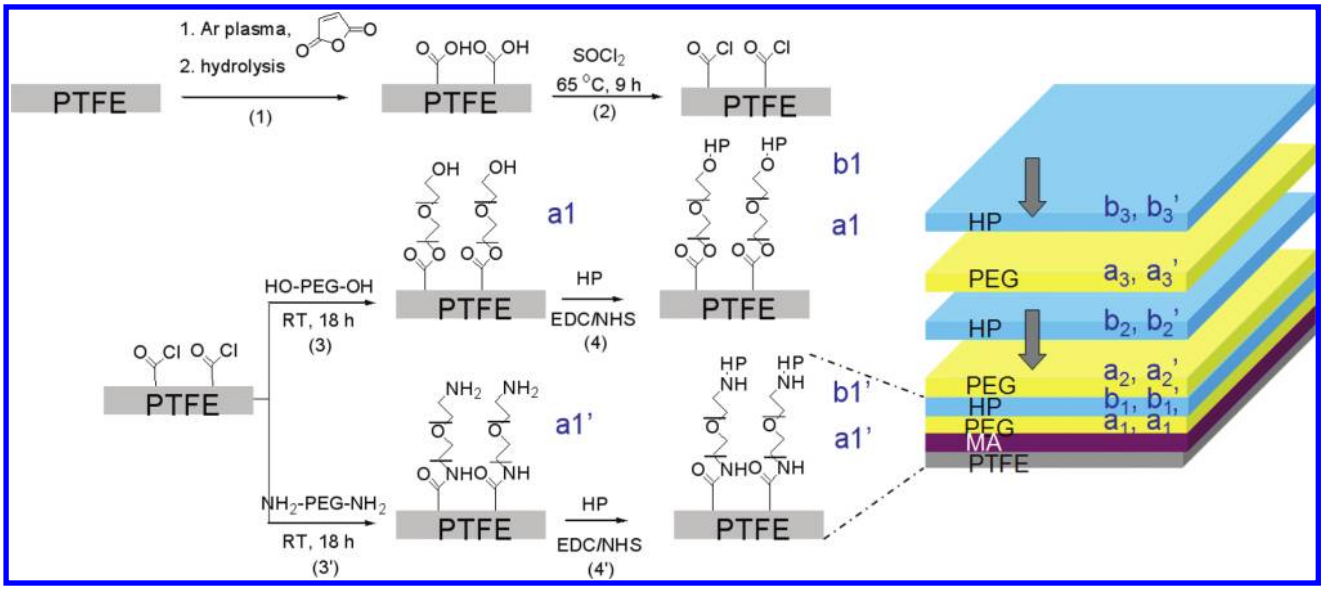

Figure 1. Schematic diagram of CAM process; covalent attachment of alternating OH- PEG (a1-a3), OH-HP (b1-b3), NH_2−PEG (a1′-a3′), and NH_2−HP (b1′-b3′) layers.

during the microwave plasma discharge. In an effort to maintain the plasma environment during longer exposure times, a vacuum was applied continuously to maintain pressure conditions during the experiment. To ensure the covalent attachment of MA to the PTFE surface and that all of the MA were converted to COOH groups, the specimens were washed in DI water for 30 min, followed by boiling in water for 20 min. After drying, the specimens were kept in the desiccator under ambient conditions.

The CAM process involves covalent attachment of alternating HP and PEG layers. To attach the first PEG layer (PEG-MA-PTFE), the COOH surfaces were converted to acid chloride (COCl) using thionyl chloride ($SOCl_2$; Sigma Aldrich) under reflux conditions at 65 °C for 12 h. The sample was removed from the flask and washed with chloroform in order to eliminate excess thionyl chloride. The acid chloride PTFE surfaces were then placed in a chloroform solution of either hydroxyl (M_w = 600 g/mol) or amine terminated PEG (M_w = 2000 g/mol). To determine the possibility of reactions of both PEG ends to PTFE surface, OH stretching (OH-terminated) and NH bending (NH_2-terminated) normalized vibrations of unreacted and surface reacted PEG IR spectra were compared. When one end PEG is reacted, in an ideal scenario the band intensity diminishes by half. Spectroscopic analysis has shown that ∼20−35 mol % of PEG exhibit dual end reactions. If all COOH surface groups from MA were consumed by reactions with PEG, molar volume concentrations of PEG should be equal to MA (Figure 3, panels A and B). As shown, this is not the case, and the efficiency of PEG reactions is 65−80%. The reactions were carried out in a sealed flask at room temperature for 24 h. A small amount (1−2 drops) of triethylamine was added to the reaction flask in order to neutralize hydrochloric acid generated during the reaction. The samples were washed with chloroform several times to remove unreacted PEG, dried, and kept in the desiccator. The attachment of HP onto the PEG-MA-PTFE surface was carried out by activating the COOH groups of the 1% w/v HP (HP diaminated Na^+ salt; Sigma Aldrich) with 0.05 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; Sigma Aldrich) in a pH 4.6 buffer solution for 3 h, and then the PEG-MA-PTFE specimen was reacted with the activated HP for 24 h. The specimen was then removed from the reaction, washed with the buffer solution for 20 min, rinsed with DI water, dried, and kept in a desiccator. The next PEG and HP layers were attached to the HP-PEG-MA-PTFE surface by repeating the reactions described above.

Surface Characterization. Spectroscopic analysis of HP immobilized PTFE surfaces was conducted using attenuated total reflectance Fourier transform infrared (ATR FT-IR) spectroscopy. 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 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 for spectral distortions using Q-ATR software.[24] 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. Internal reflection IR imaging (IRIRI) experiments were conducted on a Varian Stingray system with a Ge internal reflection element allowing spatial resolution of about 1 μm or better. This system consists of a Varian FTS 7000 spectrometer, an UMA 600 FT-IR microscope with a focal plane array (FPA) image detector, and a semispherical Ge crystal. IRIR images were collected using the following spectral acquisition parameters: under sampling ratio of 2, rapid scan speed of 5 kHz, and 8 cm^{-1} spectral resolution. Image processing was performed using the Environment for Visualizing Images (ENVI) software (Research Systems, Inc., version 3.5). When appropriate, baseline correction algorithms were applied to compensate for baseline deviations which were accomplished by a built-in application software supplied by GRAMS/AI v7.02 (Galactic Ind.).[25] Variable angle ATR was employed to determine the volume concentration of newly formed species after each step of the reaction as a function of depth by using Ge (50 × 20 × 3 mm) crystals and angles varying from 35° to 60°. Since quantitative ATR-FTIR depth profiling requires knowledge of the extinction coefficient for each of these bands,[26] various concentration standards of heparin were prepared, and plots of the absorbance of the 1620 cm^{-1} OH bending bands as a function of concentration were generated. The extinction coefficients of the bands due to HP is 6778.6 L/mol-cm, whereas the 1710 cm^{-1} extinction coefficient of C═O vibrations of COOH groups and 1080 cm^{-1} of C−O−C of PEG were previously determined to be 544.32, and 778.58 L/(mol cm),[27] respectively. Using a double Kramers−Kronig transformation (KKT) and previously developed algorithm for quantitative analysis using ATR-FTIR spectroscopy,[26] concentration levels of COOH groups resulting from the microwave

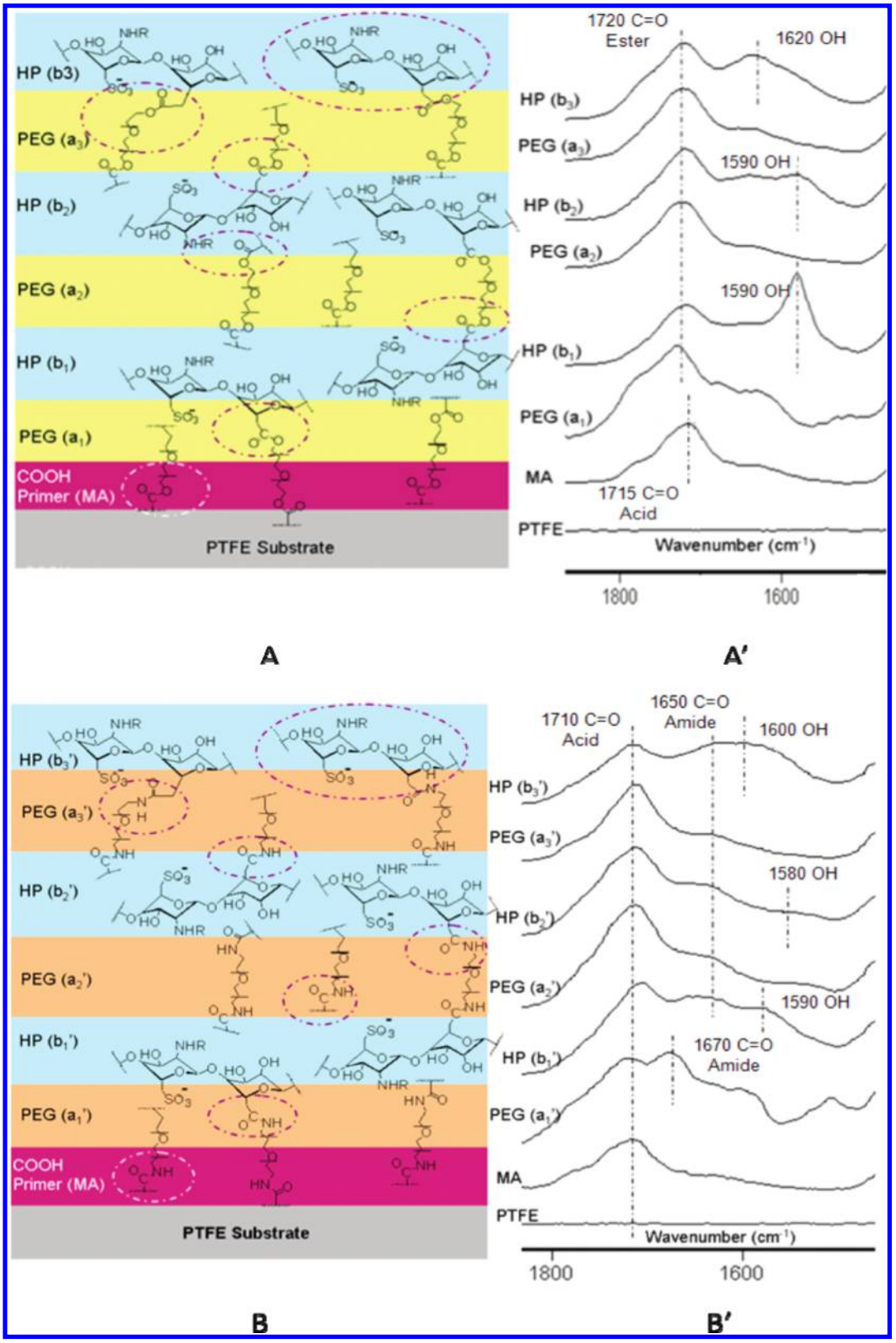

Figure 2. A-CAM process of modifying COOH-PTFE surfaces using OH-PEG: a_1-PEG; b_1-HP; a_2-PEG; b_2-HP; a_3-PEG; b_3-HP, (A′)-ATR FT-IR spectra of PTFE, MA, PEG (a_1 layer), HP (b_1 layer), PEG (a_2 layer), HP (b_2 layer), PEG (a_3 layer), and HP (b_3 layer). Circles indicate covalent linkages, B-CAM process of modifying COOH-PTFE surfaces using NH_2-PEG: a_1'-PEG; b_1'-HP; a_2'-PEG; b_2'-HP; a_3'-PEG; b_3'-HP, (A′)-ATR FT-IR spectra of PTFE, MA, PEG (a_1' layer), HP (b_1' layer), PEG (a_2' layer), HP (b_2' layer), PEG (a_3' layer), and HP (b_3' layer). Circles indicate covalent linkages.

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plasma modifications, PEG reactions, and HP attachment were determined.

RESULTS AND DISCUSSION

The primary objective of these studies is to create robust PTFE surfaces coatings containing HP and PEG. Figure 1 depicts a schematic diagram of the process which involves the covalent attachment of multilayers (CAM) of HP and PEG. To ensure covalent bonding of the first PEG layer, MA was reacted to the PTFE surface using microwave plasma conditions[23,29] (step 1), followed by its hydrolysis producing COOH-PTFE surfaces. The resulting groups were converted to acid chloride by reactions with thionyl chloride ($SOCl_2$) (step 2), followed by grafting either α, ω-OH or NH_2−PEG (step 3 and 3′), generating first a_1 and a_1' PEG layers. The remaining distal ends of the PEG bearing OH or NH_2 groups were then reacted with heparin in the presence of EDC and NHS (step 4 and 4′) resulting in b_1 and b_1' HP layers. Using this approach, two layers composed of PEG (a and a′) and HP (b and b′) were attached to the PTFE surface. This process was repeated three times to create six alternating PEG and HP layers that are chemically anchored to the PTFE surface and/or to each other. Although Figure 1 depicts uniform CAMs, at molecular scales the layers are likely non homogeneous, which is actually desirable since HP and PEG do not mask each other action.

Figure 2, A and B, shows covalent linkages (circled) between each layer (left) and the resulting ATR FT-IR spectra (right) obtained after the covalent immobilization of each layer. The first MA layer is obtained by COOH-functionalization of PTFE manifested by the band at 1715 cm^{-1} due to C═O of COOH-PTFE entities. As shown in Figure 2A, the reactions of COOH functionalized PTFE with OH-PEG lead to ester formation (C═O), and the alternating layers of HP and OH-PEG also produce ester linkages. ATR FT-IR spectrum of the first PEG layer (a_1) shows the band at 1720 cm^{-1} due to ester linkages[30,31] resulting from reactions between COOH-PTFE and OH-PEG entities. The band at 1070 cm^{-1} due to C−O−C of PEG was also detected which is shown in Figure S-1 of the Supporting Information. Although before subsequent reactions each specimen was stored under dry conditions (desiccator at 50 °C), hydrophilic nature of PEG makes it impossible to eliminate all residual water molecule which is manifested by the weak broadening at 1650−1635 cm^{-1}. The first HP layer (b_1) was attached in the next step which is manifested by the presence of the band at 1720 cm^{-1} due to ester linkages that result from reactions between COOH groups of HP and OH of PEG as well as the band at 1590 cm^{-1} due to OH bending modes of HP. These results confirm the covalent attachment of OH-PEG and HP layers on the COOH-PTFE surface. Since the ester bands of the second (a_2 and b_2) and third layers (a_3 and b_3) are identical to those produced in the first layer (a_1 and b_1), enhanced intensities of the bands at 1720 cm^{-1} of ester linkages (PEG and HP reactions) and at 1590 and 1620 cm^{-1} due to OH bending vibrations of heparin are also detected, confirming covalent attachments of OH-PEG and HP layers to COOH-PTFE surface.

An alternative path of reactions to produce CAM is shown in Figure 2B, where reactions of COOH-PTFE and NH_2−PEG were employed to give amide formation (C═O—NH), also producing the alternating layers of HP and NH_2−PEG amide linkages. ATR FT-IR spectra shown in Figure 2B illustrate the

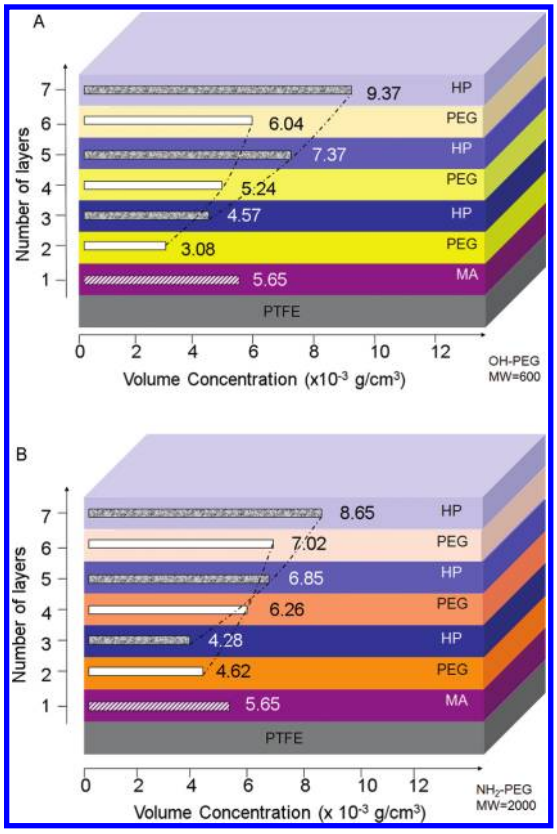

Figure 3. Quantitative representation of a number of alternating HP and PEG layers plotted as a function of their respective volume concentrations: A−OH-PEG process (Figure 2, A−A′); B-NH_2−PEG process (Figure 2, B−B′). To quantify surface volume concentrations intensities of the bands responsible for the above surface reactions were measured and used to quantify each layer. Since quantitative IR analysis requires knowledge of extinction coefficients of these bands, they were determined using transmission experiments of individual components and determined using the Beer−Lambert's law. In order to accomplish ATR quantitative analysis, the spectra were corrected for optical effects and interactively refined to minimize differences resulting from optical effects using Urban-Huang algorithm.[24] This interactive process is used in conjunction with numerical double Kramers−Kronig transformation (KKT) to obtain ATR spectra for reliable quantitative analysis. Using corrected ATR spectra, linear absorptivity is obtained to allow calculations of volume concentrations from Beer−Lambert law.

presence of the band at 1710 cm^{-1} due to C═O of COOH entities of the COOH-PTFE layer (MA) resulting from the microwave plasma reactions, and at 1670 cm^{-1} due to amide linkages,[30,31] produced from the reactions of COOH-PTFE and NH_2−PEG (a_1'). The band at 1080 cm^{-1} due to C−O−C of PEG (not shown) is also detected. The next reacted layer is HP (b_1'), which is also manifested by the bands at 1650 cm^{-1} due to NH_2 linkages resulting from the reactions of COOH of HP and NH_2 of PEG, and the 1590 cm^{-1} band attributed to OH bending vibrations of HP, confirming covalent attachments of NH_2−PEG and HP layers. Again, as subsequent layers are reacted, the band intensities increase, indicating that the multiple layers of NH_2−PEG and HP were covalently attached to the COOH-PTFE surfaces. Hydrolytic stability of the PTFE surfaces produced by the CAM process containing OH- and NH_2−PEG was determined by exposing each specimen to boiling water for 20 min while stirring. The IR analysis shown in Figure S-2 of the

Supporting Information show that the C═O bands due to ester and amide linkages before and after boiling are undistinguishable, thus indicating chemical stability of the CAM layers.

Since reactions leading to CAM formation were conducted in solutions, it is also of interest to determine concentration levels of each layer reacted to the PTFE surface. Figure 3, A and B, illustrates the summary of the quantitative analysis obtained by measuring the volume concentrations of each layer of OH- and NH_2−PEG and HP multilayers shown in Figure 2, A and B. We employed again ATR FT-IR analysis which allows us to quantify surface concentrations by measuring intensities of the bands of interest after covalent immobilization of each layer. While details regarding surface measurements and extinction coefficient were published elsewhere,[12,23,24] concentration levels of the species of interest are expressed by the mass of a given species per volume (g/cm^3) or per unit surface area (g/cm^2). In these studies, we utilized the bands at 1715, 1080, and 1620 cm^{-1} which are characteristic of COOH, C−O−C, and O−H bands of MA, PEG, and HP layers, respectively.

Figure 3A illustrates surface volume concentration changes of COOH-PTFE, OH-PEG, and HP after each step in the CAM process shown in Figure 1. The volume concentration of the COOH layer is 5.65×10^{-3} g/cm^3 (1.7×10^{-7} g/cm^2), whereas for the first OH-PEG and HP layers they are 3.08×10^{-3} g/cm^3 (9×10^{-8} g/cm^2) and 4.57×10^{-3} g/cm^3 (1.4×10^{-7} g/cm^2), respectively. As anticipated, ATR FT-IR spectra obtained after attachment of each layer show increased intensities of the bands at 1100 and 1620 cm^{-1}. Since in the ATR FT-IR depth profiling experiments a Ge crystal was employed, giving an approximate penetration depth of ∼280 nm, each subsequent volume represents the summation of the last and previously deposited layers. After attaching six alternating layers, the overall volume concentrations of HP are 9.37×10^{-3} g/cm^3 (2.8×10^{-7} g/cm^2). Similar trends are observed for NH_2−PEG, as illustrated in Figure 3B, where the volume concentrations of the initial NH_2−PEG and HP layers are 4.62×10^{-3} g/cm^3 (1.4×10^{-7} g/cm^2) and 4.28×10^{-3} g/cm^3 (1.3×10^{-7} g/cm^2), respectively. After attaching all subsequent layers, the volume concentrations of the HP are 8.65×10^{-3} g/cm^3 (2.6×10^{-7} g/cm^2). Comparison of these data with the recently reported[32] minimum surface concentrations levels of 1×10^{-7} g/cm^2 for HP to be effective anticoagulant agent indicates that the CAM approach not only exceeds these levels but also the presence of multilayers will extend the lifetime of anticoagulant and antifouling surfaces.

In summary, these studies showed for the first time that the CAM process conducted on inert PTFE surfaces will result in the formation of stable alternating PEG and HP layers, which was achieved by creating covalently attached COOH "primer" layers on the surface of PTFE using microwave plasma reactions in the presence of MA, followed by subsequent reactions of alternating layers of functionalized PEG and HP. While these CAM coatings may exhibit many useful biomedical properties, future studies will include in vivo and in vitro determination of blood platelet deposition and blood compatibility.

■ ASSOCIATED CONTENT

Supporting Information. Additional experimental information including additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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