Immobilization of Therapeutics on Metal Surfaces Using Self-Assembled Monolayers

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Introduction: Bio-organic molecules have been attached to self-assembled monolayers (SAMs) on metals for various biomedical applications [1]. Oligoethylene glycol is one of the molecules that is commonly attached to SAMs for resisting protein adsorption on metals [1]. Recently, RGD peptides and BMP growth factors were attached to SAMs on titanium to improve biocompatibility [2,3]. In this study, we have immobilized therapeutics on titanium (Ti) and gold (Au) through SAMs for drug delivery applications.

Methods: Hydroxyl (-OH) terminated SAMs were formed on Ti and Au surfaces by using 2mM solutions of (11-hydroxylundecyl) phosphonic acid in water and 11-mercapto-1-undecanol in ethanol, respectively. Flufenamic acid (FA), an anti-inflammatory, auto-fluorescent, fluorinated drug was used for immobilization. FA was refluxed in thionyl chloride for 1 hour. After rotary evaporating the excess thionyl chloride, 40 mM tetrahydrofuran (THF) was added. The -OH terminated SAMs coated Ti and Au substrates were then immersed in the solution followed by the addition of 120 mM of triethylamine. The substrates were incubated in the solution for 48 hours at room temperature. Upon withdrawal from the solution, the substrates were extensively rinsed in THF and double distilled-water to remove physically adsorbed drug molecules. The substrates were then characterized using X-ray photoelectron spectroscopy (XPS), fluorescent microscopy (FM), atomic force Microscopy (AFM), and contact angle measurements.

Results / Discussion: XPS spectra showed a large and symmetrical P 2p peak at 133.6 eV after the formation of phosphonic acid SAMs on Ti. A ratio of 2 was observed between the O 1s peaks at 531.4 eV and 533 eV and this confirms the covalent attachment of SAMs on Ti. On gold, XPS S 2p doublet was observed at 161.9 eV (S $2p_{3/2}$) and 163.1 eV (S $2p_{1/2}$). The contact angles of SAMs coated titanium and gold surfaces were 54.6° ± 2° and 27.6° ± 4.3°, respectively. After the immobilization of FA on SAMs (FA_{imm}SAMs) coated Au and Ti surfaces, the 1s peak for the unique element fluorine was observed at 688.5 eV on both metal substrates (Fig. 1).

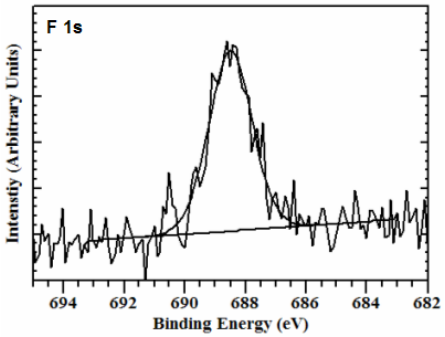

Figure1. F 1s XPS spectrum for the FA_{imm}SAMs on titanium

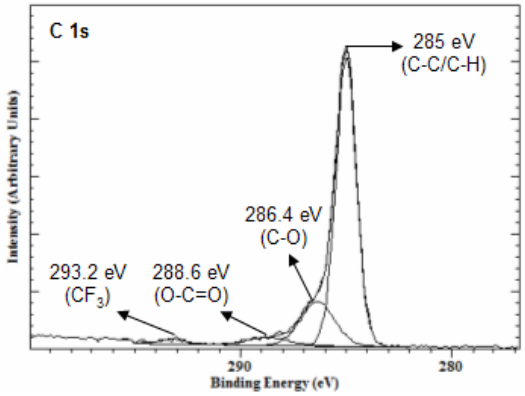

Figure 2. High resolution C1s XPS spectrum recorded for FA_{imm}SAMs on titanium

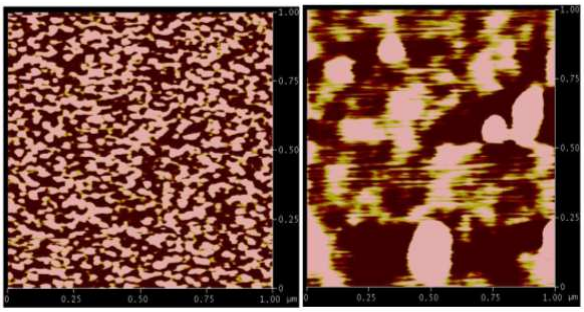

Figure 3. AFM contact mode height images (1µm x 1µm) of gold substrates before and after drug immobilization

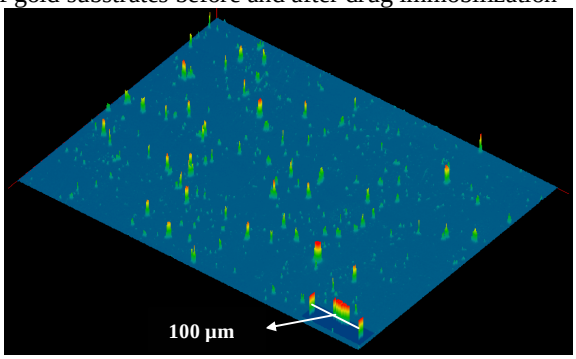

Figure 4. FM 3-dimensional plot for the FA_{imm}SAMs on gold (fluorescence intensity is plotted in Z-axis)

The formation of an ester bond between the SAMs and FA is confirmed by the appearance of the 288.6 eV binding energy C 1s peak. The peak at 293.2 eV is assigned to -CF_3 of the FA molecules (Fig. 2). The RMS roughness values of the metal substrates before and after drug immobilization were 0.66 nm and 0.74 nm, respectively (Fig 3). The auto fluorescence capability of FA was used to determine the distribution of FA on metal substrates (Fig. 4).

Conclusions: Therapeutics were successfully attached to SAMs coated Ti and Au substrates.

References: (1) Annu. Rev. Biophys. Biomol. Struct. 1996; 25:55-78 (2) Langmuir 2003; 19:200-204 (3) Langmuir 2006; 22:8197-8204