Surface PEGylation *via* Native Chemical Ligation

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Statement of Purpose: Recently, surface modification using poly(ethylene glycol) (surface PEGylation) is of interest, because it prevents the non-specific adsorption of biomolecules which can interfere with desired functions of biomedical devices like implant. The surface PEGylation have been investigated by various approaches, such as grfting of functional polymers, adsorption of polyelectrolyte. For example, langer et al. showed the surface PEGylation onto the silicon substrate using functional copolymers containing PEG and silane groups.[1]

On the other hand, the introduction of native chemical ligation techniques to join unprotected peptides next to a cysteine residue has greatly facilitated the synthesis of proteins of moderate size.[2,3] Moreover, native chemical ligation chemistry have successfully been applied to various organic and bio-organic reactions. However, there are no reports for applying native chemical ligation to surface chemistry. Herein, we introduce a method for surface PEGylation of titanium substrate by phosphonic acid SAM formation on the substrate, followed by native chemical ligation of PEG.

Methods: *Materials* $NaPO_3H$-$(CH_2)_{11}$-$(OCH_2CH_3)_3$-NH_3 (PA-C_{11}-EG_3-NH_3, COS Biotech, Inc., Korea), ethyl 3-mercaptopropionate (TCI), succinic anhydride (99%, aldrich), 4-dimethylaminipyridine (TCI), Fmoc-Cys(Trt)-OH (novabiochem), mPEG-amine (sunbio) were used as received.

Phosphonic acid SAM on titanium was prepared by dipping the titanium substrate in PANa-C_{11}-EG_3-NH_3 solution (1 mM) at room temperature. After 1-day immersion, the sample was taken out and washed by deionized water for several times.

Ethyl 3-mercaptopropionate-succinic acid (EMPSA) was synthesized by previous report.[4] EMPSA coating on phosphonic acid SAM was performed by dipping the sample into the EMPSA solution (0.2 mmol/mL) at room temperature for 24 hrs. The cys-PEG was synthesized by reacting the Fmoc-Cys(Trt)-OH (100 mg/mL) with mPEG-amine (640 mg/mL) at room temperature for 24 hrs. For surface PEGylation, the EMPSA-functionalized sample was dipped in alkaline cys-PEG solution (1 mg/mL, pH 8.0). After 1-day reaction, the PEGylated substrate was taken out and rinsed with deionized water.

Results:

The reactions on the surface were characterized by ellipsometry, contact angle goniometry, and X-ray photoelectron spectroscopy (XPS). The thickness of the phosphonic acid SAMs was measured to be 15 Å by ellipsometry, and the static water contact angle was 41° (Fig 1a). After the reaction of amine groups with EMPSA, the thickness of the SAMs was increased to 20 Å, and the water contact angle of the sample was also increased to 80° (Fig 1b). Changes of thickness of the layer and the water contact angle were supportive to the reaction of EMPSA with amine-terminated SAMs. After the EMPSA coating on the SAMs, the surface of the titanium was PEGylated by cys-PEG. The water contact angle changed to 64° after the PEGylation, indicative of relatively hydrophilic nature of the PEGylated surface (Fig 1c).

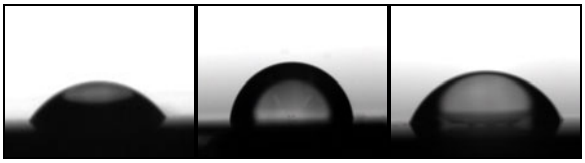

Figure 1. Water contact angle images of (a) phosphonic acid SAM-coated, (b) EMPSA-coated, and (c) PEGylated Ti substrates.

The C(1s) region of the XPS spectra (Fig 2) further confirmed the chemical transformation from thioester to PEG by native chemical ligation: in addition to peaks at binding energies of 284.4 eV (C-C) and 288.3 eV (C=O), we observed an additional peak from C-O at 286.0 eV.

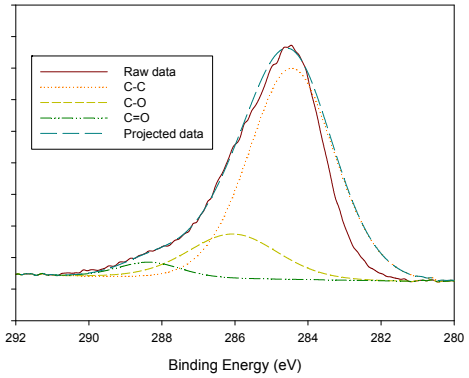

Figure 2. High resolution XPS spectra (C(1s) region) of PEGylated Ti substrate.

Conclusions: In summary, we fabricated a PEGylated Ti substrate by phosphonic acid SAM formation and subsequent PEGylation through native chemical ligation. We clearly showed that our approach was suitable for the generation of PEGylated Ti substrate by contact angle measurement and XPS spectroscopy. We believe that the method described here is widely applicable to biomedical devices.

References:

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