Strong Resistance to Protein Adsorption by (Tridecafluoro -1, 1, 2, 2-tetrahydrooctyl)triethoxysilane Nanofilm

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Statement of Purpose: Implant devices that are in constant contact with blood would eventually develop a coating of coagulation proteins that can lead to the undesirable thrombus formation, which likely compromises the intended functions of the implant devices. The blood-contacting surfaces can be modified with specific molecules, such as fluorocarbons, that are regarded to be good at retarding non-specific protein adsorption. (Tridecafluoro -1, 1, 2, 2-tetrahydrooctyl) triethoxysilane (TTS) ($C_{14}H_{19}$ $F_{13}O_3Si$) is a fluorocarbon-containing silane that potentially can be used as an anti-fouling molecule due to the large number of terminal fluorine atoms (2 per methylene group for a total of 13) at the functional end. Previously, the potential of TTS for protein resistance has been indicated in a limited fashion [1]. The purpose of the current studies is to examine the effectiveness of TTS nanofilm coating on silicon surface, the properties of the TTS nanofilm coating including thickness and wettability, as well as the effectiveness of TTS nanofilm coating on resisting the adsorption of four model proteins, namely, bovine serum albumin, fibrinogen, immunoglobulin G and fibronectin.

Materials and Methods: Silicon wafer (University Wafer), after rinsing with HPLC grade toluene (Sigma), was placed in TTS solution (Gelest, 200 mM in HPLC grade toluene (Sigma-Aldrich)) for 4 hours, followed by rinsing with fresh HPLC grade toluene.

TTS-modified silicon wafer was placed in individual solutions of bovine serum albumin (BSA, Sigma), immunoglobulin G (IgG, Sigma), fibrinogen (Fg, Calbiochem) and fibronectin (Fn, Calbiochem) at a concentration of 0.1 mg/ml in phosphate-buffered saline (PBS) for 1 hour, followed by rinsing in deionized water to remove non-adsorbed protein molecules and a stream of nitrogen gas for drying. Bare, non-modified silicon surface was used as a control.

Silicon surface before and after modification with TTS, and TTS-modified silicon surface before and after the adsorption of test proteins were characterized by: (a) Water contact angle goniometry for surface hydrophilicity (OCA 15+; Data physics) using sessile drop method; (b) Ellipsometry for film thickness (SE 850; Sentech Instruments) using 1.46 as the refractive index of silicon oxide, TTS and protein layers; (c) Optical microscopy (Labophot-2; Nikon) operated in bright-field mode to provide visual evidence; (d) Fluorescence spectrophotometry (F-2500; Hitachi) to probe the adsorption of FITC-BSA, indicated by the emitted fluorescence intensity at 485 nm.

Results and Discussion: Bare, non-modified silicon was moderately hydrophilic (40.5 ± 1.5°) but became much hydrophobic after TTS modification (115.8 ± 1.6°). Non-modified silicon surface became more hydrophilic after exposure to solution of BSA, Fg and IgG (10°-25°), which is probably due to the presence of protein layer. TTS-modified silicon maintained the surface hydrophobicity before and after exposure to proteins as water contact angle changed very slightly (<5°), suggesting very few, if any, protein molecules are adsorbed to TTS-modified surface.

The thickness of TTS film was determined to be 14.8 ± 0.7 nm. The thickness of the protein layers on non-modified silicon ranged from 11.5 nm for both BSA and Fg, to 21 nm for Fn. In comparison, no substantial increase in the thickness of the protein layer on TTS-modified layer after exposure to BSA and Fg was observed. The thickness of the protein layer was reduced by ~67% and ~75% for Fn and IgG, respectively, on TTS-modified surface as compared to non-modified surface. The results suggest that TTS is quite effective in resisting the protein adsorption, particularly BSA and Fg.

From the digital images, protein particles (BSA, Fg, IgG and Fn) were observed on the surface of bare silicon wafer. In comparison, no or very negligible amount of protein particles was observed on the TTS modified surface (data not shown). The results suggest that most, if not all, of the protein molecules were rejected due to the presence of TTS nanofilm,

The fluorescent intensity was high on bare silicon wafer but was substantially reduced on TTS-modified surface. Comparing to TTS-modified surface, the ratio of fluorescence intensity was about sixteen times stronger on bare silicon than on TTS-modified surface, suggesting a high extent of FITC-BSA adsorption on the former surface.

Conclusions: A hydrophobic TTS nanofilm with a thickness of about 15 nm was obtained. The TTS nanofilm is highly effective in resisting protein adsorption, particularly for BSA and Fg. The presence of long, terminated fluorocarbon chain is the likely reason that leads to surface hydrophobicity and lower surface energy, which contribute to the property of resistance of protein adsorption. The use of fluorocarbon-containing silane molecules can be extended to instruments and devices that are based on silicon, quartz and glass.

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