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Immobilization of polysaccharides on a fluorinated silicon surface

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Abstract

A self-assembled monolayer (SAM) of fluoroalkyl silane (FAS) was deposited on a silicon surface by chemical vapor deposition (CVD) at room temperature under 1.01×10^5 Pa nitrogen. Using this new approach, the quality and reproducibility of the SAM are better than those prepared either in solution or by vapor phase deposition, and the deposition process is simpler. In this modified CVD process, the silane monomers, instead of the oligomeric species, are the primary reactants. Full coverage of the silicon surface by FAS molecules was achieved within 5 min. Heparin and hyaluronan, two naturally occurring biocompatible polysaccharides, were successfully covalently attached on the FAS SAM/Si surface by photo-immobilization. Atomic force microscopy (AFM) revealed the morphologic changes after the immobilization of heparin and hyaluronan, and X-ray photoelectron spectroscopy (XPS) confirmed the change in chemical compositions. Such combination of coatings is expected to enhance the stability and biocompatibility of the base material.

Keywords: Heparin; Hyaluronan; Silicon; Fluoroalkyl silane; Chemical vapor deposition; Photo-immobilization

1. Introduction

Fluoroalkyl silane (FAS) coated surfaces are both highly hydrophobic and oleophobic [1], and they have demonstrated superb wear resistance and friction reduction. Thus modified surfaces have proven to be super-hydrophobic and super-stable on the surfaces of polymer and silica fillers (in denture composites) [2,3]. FAS coatings have found applications in adhesion control, low-soil coatings, lubricating treatment, textile treatments, and protective coatings [4–6]. They have also been found to be capable of improving the particle trapping efficiency of glass air filters [7], and to reduce bacterial infection [8] on biomaterials. FAS coatings have shown good biocompatibility, which has been attributed to their extreme low surface energies (~8 mJ/m^2) [9–13].

The surfaces of metals, semiconductive materials and polymers have often been modified by the deposition of a self-assembled monolayer (SAM) of FAS. Traditionally, such self-assembly on solid surfaces was performed in solution [14–18]. However, there are several shortcomings to this approach: (1) the quality of the SAM is strongly affected by the quality of the solvents, especially the water content, even at very low concentration; (2) most of the organic solvents are toxic to a certain extent; (3) some surfaces (e.g., polymers) are not compatible with organic solvents due to possible swelling, molecular rearrangements and even dissolution; (4) depositing SAMs on tiny delicate devices (e.g., AFM tips, silicon electrode arrays) is extremely challenging, since the coating solution tends to be trapped inside holes, gaps or edges, and many areas could not be coated at all [4]. Furthermore, some alternate structures, including inverse micelles and lamellar phases, can form in the deposition solutions, which are detrimental to the performance of the final SAMs [19]. Adsorption of silane oligomers on the solid surfaces, formed by hydrolysis and condensation reactions in solution, has often been found to occur, slower than but competitive with SAM formation at the liquid–solid interface [16].

These problems can be avoided by using a chemical vapor deposition (CVD) technique. In the literature, CVD of FAS SAMs has been performed under vacuum and/or at elevated temperature [2,7,20–26]. Sometimes the temperature can be

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as high as 190 °C [27]. However, high temperature (≥90 °C) often resulted in SAMs with poor quality and reproducibility [4]. Organic solvents, which formed vapor together with FAS molecules, were even sometimes used as the dilution media during the CVD deposition process [2,11,28]. Excess amounts of FAS molecules on the surface after CVD were typically removed by subjecting the surfaces to a vacuum at elevated temperature for a few hours. Aggregates of FAS molecules were often observed on the FAS SAM modified surface when formed by the CVD process. We found that a very smooth, uniform and reproducible FAS SAM can be formed on silicon surfaces at room temperature without using vacuum and organic dilution solvents, as evidenced by the AFM and XPS results. This CVD process is simpler than those adopted previously as well [2,7,20–26].

In this paper, FAS SAM on a silicon surface prepared by this simplified CVD process is reported. The deposition process was performed under an ultra-dry nitrogen atmosphere to minimize water interference. The vapor pressure of FAS13_Cl ($CF_3(CF_2)_5(CH_2)_2SiCl_3$) is approximately 40 Pa at room temperature [4], which is high enough to facilitate the SAM formation, and full surface coverage was achieved within only a few minutes. In the literature, plasma was often combined with CVD techniques to produce coatings on solid surfaces. Such plasma-enhanced coatings typically demonstrate less pure structures and much lower stability, although they are much thicker (hundreds of nanometers to tens of microns) than the SAMs (1–3 nm), furthermore the plasma sources often pose a high risk of damaging the samples to be coated [29].

Fluorinated surfaces prevent the adhesion of most chemical and biological substances [30]. Although fluorinated surfaces showed good biocompatibility, their biocompatibility still needs to be improved for in vivo applications [31–36]. Inertness and low surface energy are characteristic advantages of fluorinated surfaces. However, these very characteristics make it difficult to functionalize them with chemical or biological species. While there are published clinical results on heparin modified fluorinated surfaces to enhance their biocompatibility, the heparin is either ionically bound or claimed covalently bound without defined, reproducible procedures [31–34,36].

In this paper, heparin and hyaluronan were successfully covalently immobilized on a FAS SAM modified silicon surface. Heparin and hyaluronan were first modified by attaching photosensitive groups on them, and then under UV illumination, they were covalently immobilized on a FAS SAM coated silicon surface. So far such UV-activation based photo-immobilization has only been applied to organic surfaces with an abundance of C–H, C–N groups [37–40]. We demonstrate here that this photo-immobilization technique can also be applied to fluorinated surfaces, which is regarded as the most inert among all the organic surfaces. The combination of heparin (or hyaluronan) and FAS SAM is expected to bring enhanced stability and biocompatibility to the substrates [41,42]. Our objective is to make long-term implantable neuroprostheses, and satisfactory biostability and biocompatibility are two extremely important criteria. The in vivo experiments to evaluate the biostability and biocompatibility on animals are ongoing.

2. Experimental

2.1. Materials

One-side polished N type silicon (1 1 1) wafers (test grade, with resistivity 1–2 Ω cm and thickness 475–575 μm) were purchased from Wafer World Inc. (West Palm Beach, FL). Deionized water (DI water) with a resistivity of ≥18 MΩ cm was obtained by a Barnstead Nanopure Systems (Dubuque, Iowa). Trichloro (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)silane (FAS13_Cl, $CF_3(CF_2)_5(CH_2)_2SiCl_3$, 97%), isooctane (anhydrous), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (water-soluble carbodiimide, WSC), 4-azidoaniline hydrochloride, heparin (sodium salt from porcine intestinal mucosa, ∼170 USP units/mg), and hyaluronic acid potassium salt (extracted from the human umbilical cord, named hyaluronan throughout this paper) were purchased from Sigma–Aldrich (St. Louis, MO). They were used as received.

2.2. FAS13_Cl SAM on silicon by CVD

The pre-cut silicon pieces (approx. 1.2 cm × 1.2 cm) were rinsed with ethanol, acetone and then oxidized by the RCA (named after the company Radio Corporation of America, where the procedure was first developed) method [43], resulting in hydroxyl groups on top of the silicon oxide layer [44,45]. (Such treated silicon pieces are referred to as RCA Si hereafter.) The silicon pieces were then blown dry with nitrogen before SAM deposition. The CVD process was performed in a glove box purged with nitrogen (extra dry). Two drops of FAS13_Cl (∼50 μL) was placed in an enclosed glass container (∼500 mL in volume) with RCA Si inside, and the silicon pieces were taken out after predetermined durations. To remove excess FAS13_Cl molecules physically adsorbed on the surface, the modified RCA Si was rinsed with isooctane twice, and blown dry with nitrogen.

2.3. Photo-immobilization of heparin and hyaluronan on FAS13_Cl modified silicon

Heparin, 4-azidoaniline hydrochloride and WSC at a weight ratio of 18.2:7.75:10 were dissolved in DI water to yield a 0.5% solution [38]. The pH of the solution was adjusted to 4.70–4.75 with 1N NaOH at room temperature. The solution was stirred at 4 °C for 24 h, resulting in the aryl azido-modified heparin solution. 0.2% aryl azido-modified hyaluronan solution was prepared by the same means except that the weight ratio of hyaluronan, 4-azidoaniline hydrochloride and WSC was 42:17:28 [37]. FAS13_Cl SAM/Si pieces were immersed in an aryl azido-modified heparin (or hyaluronan) solution in a Teflon container with 3 mm height of solution above them. They were then illuminated with a mercury vapor UV lamp (175 W, Regent Lighting, Burlington, NC) for 5 min at a distance of 10 cm. The heparin or hyaluronan immobilized FAS13_Cl SAM/silicon samples were immersed for 48 h in DI water, which was refreshed every 12 h, and the samples were rinsed by DI water every 12 h.