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Tunable surface free energies of functionalized molecular layers on Si surfaces for microfluidic immunosensor applications

Ramchander Chepyala, Siddhartha Panda*

Department of Chemical Engineering, Indian Institute of Technology Kanpur, Kanpur, UP 208016, India

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ABSTRACT

Enhanced antigen–antibody interactions in microfluidic immunosensors can be effected by tailoring the surface free energies of the antibody immobilized surfaces to obtain the appropriate fluid–wall interactions. We report a systematic study to evaluate the surface free energies from contact angle measurements, using the LWAB method, of different antibody (anti-BSA, anti-PSA, and anti-CRP) surfaces, each immobilized separately on to non- and nanotextured Si surfaces via a stack of functionalized layers including aminosilanes of which three different types were used. The apolar surface free energy components were independent of the physical modification in the non-functionalized and the intermediate hydrolyzed surfaces where as they depended on the nature of the surface and the chemical modifications in the subsequent functionalized stages. Surface free energies of the different antibodies immobilized with the shorter chain length aminosilane (APTES) on non- and nanotextured surfaces were in the order of anti-BSA < anti-PSA < anti-CRP. A tunability of the surface free energy up to 9.6 mJ/m^2 was achieved which is reasonably significant when compared to the surface free energy window ($\Delta\gamma_s$ = 40 mJ/m^2) of biofunctionalized surfaces. This fundamental understanding of the surface energetics of the biofunctionalized surfaces can be utilized in modulating the surface properties to design efficient immunosensors.

1. Introduction

Studies on microfluidic immunosensors for point of care diagnostics have increased over the past several years. The miniaturized size, ability to precisely control and manipulate small quantities of reagents, lower analysis times, and efficient detection of analytes at low concentrations enable their use for early disease detection [1–3]. Several intermediate coupling molecules are used to immobilize the antibodies on to the substrate surfaces; and to achieve lower detection limits, high surface densities of properly oriented antibodies are desired. In this context, using a stack of functionalization stages (hydrolysis, silanization, cross-linking, and antibody immobilization) with and without site-directing intermediate protein molecules (protein-A and streptavidin), we studied the densities and orientations of antibodies immobilized on non-textured (i.e. smooth) and nanotextured Si surfaces and reported the role of surface nanotexturing in enhancing the orientation [4]. In addition to this we had earlier studied the effect of Si surface nanotexturing on the enhancement of antigen–antibody kinetics in mixed, non-flow systems [5] and the correlation of kinetics and conformations of immobilized enzymes [6]. In microfluidic immunosensors with surface immobilized antibodies, flow characteristics of the solution-containing antigens in the microchannels and thus the diffusive transport of antigens to these antibody-coated surfaces, depend on the interaction of the solvent fluid molecules with the surface immobilized molecules. Now, this interaction is governed by the surface free energies of the antibody layer, which in turn is governed by the surface free energies of the underlying layers. The surface free energies depend among other factors [7–14] on the physical topography [7] and the chemical nature [8–10], and many of the physicochemical processes at the solid–liquid interface can be well understood in terms of surface free energies [7,11–15]. Thus, a fundamental understanding of the surface energetics of the biofunctionalized surfaces is important in tailoring the surface properties to design efficient immunosensors.

There are many reported studies on the surface energetics of solids functionalized with different polymers [12,16], bacterial coated polymers [17,18], bacterial coated metal oxides [19], microbial cells and yeast cells on mineral surfaces [20], fabrics [21], lipid layers on mica [22] and glass [13,23], enzymatically modified cellulose surfaces [24] and of different alkylsilanes on Si [25–27]. And the focus of these works have been on understanding the effects of the underlying layers [12], film thickness [16], film structure and topography [23], silane chain lengths [25–27] on the solid surface free energies. However, there is very limited work on the surface energetics of antibody coated surfaces [28]. In this work,

* Corresponding author. Tel.: +91 512 259 6146; fax: +91 512 259 0104.
E-mail addresses: spanda@iitk.ac.in, siddpanda@gmail.com (S. Panda).

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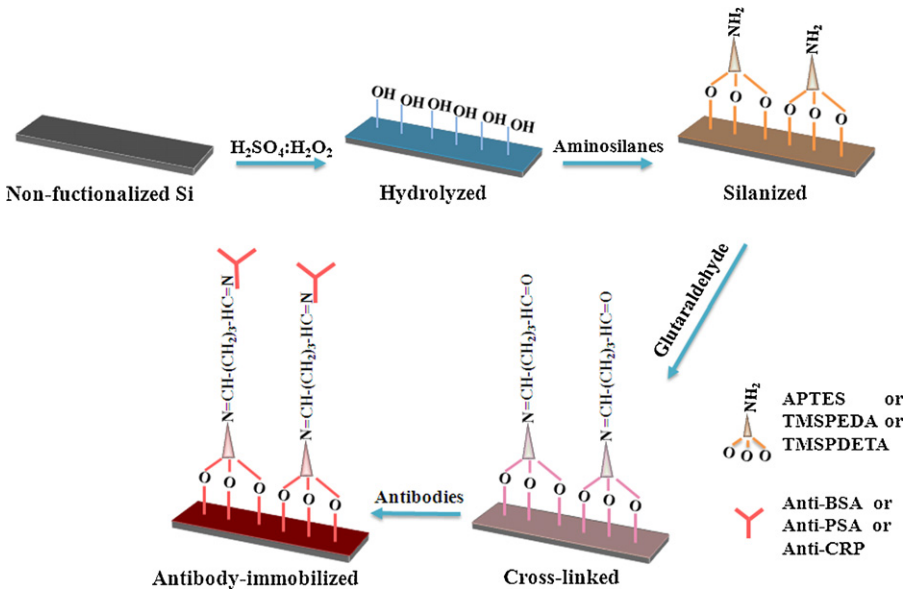

Fig. 1. Schematic of surface functionalization of nontextured Si surfaces. A similar procedure is followed for functionalization of nanotextured surfaces (image not shown here).

we report a systematic study to evaluate the surface energetics utilizing three different antibodies immobilized on to non- and nanotextured Si substrates via intermediate functionalized molecular layers (hydroxyl, aminosilanes (where three types of different chain lengths have been used), bifunctional aldehyde). Thus, as the energetics of the terminal antibody layers are governed by energetics of the underlying layers, the effect of the underlying layers have been studied by using different topographies of the Si surfaces (i.e. physical modification) and different types of silanes (i.e. chemical modification).

Different methods to determine the surface free energies [8,29,30] broadly fall into three categories; macroscopic approaches based on surface tension components, macroscopic approaches based on the equation of state, and calculation of interfacial tensions directly from molecular properties [31]. After Zisman's [32] interpretation of contact angle data in terms of surface energies, Fowkes [33] proposed a surface tension components (STCs) approach for the dispersive solids where the total surface tension was expressed as the sum of the polar (i.e. dipole–dipole, hydrogen boding, etc.) and apolar interactions; and then van Oss et al. [34] expressed the total surface tension in terms of the Lifshitz–van der Waals (LW) and Lewis acid–base (AB) components.

In this work, we used the van Oss or Lifshitz–van der Waals Acid–Base (LWAB) method [28,34] where the total solid surface free energy is evaluated in terms of the Lifshitz–van der Waals (γ_s^{LW}) and the Lewis acid–base (i.e. the electron-acceptor γ_s^+ (acid) and the electron-donor γ_s^- (base)) components. We used two polar (water, glycerol) and one apolar (diiodomethane) probe liquids [35] to measure the contact angles on the different layers of functionalized non- and nanotextured Si surfaces [4] in a controlled environment and calculated the values of γ_s^{LW}, γ_s^+, γ_s^-, and the total solid surface free energy, γ_s^{Tot}. These probe liquids were chosen as they have sufficiently high surface tension components ($\gamma_l > 44\,mJ/m^2$) [20], and as the water–glycerol pair has a reasonably high value of ΔQ_r (i.e. $\Delta Q_r = \gamma^-/\gamma^+ = 14.6$) as per Holländer's criteria [36]. Evaluation of the surface free energies in terms of their components help in understanding the energetics of the subsequent layers from the bare Si surface to the antibody immobilized layer. Then we attempted to correlate the effect of energetics of the underlying layers on the total surface free energies. This knowledge of the surface free energies of the functionalized layers will be useful in deciding the appropriate choice of the surfaces and the molecules to be used in the different layers on the immunosensor surfaces so as to obtain optimum surface energetics to enable optimum interaction between the flowing fluid molecules and the surface bound antibody molecules.

2. Experimental

2.1. Materials

P-type Si wafers of ⟨1 0 0⟩ orientation with resistivity of 1–20 Ω cm were obtained from Wafer World Inc., USA. Ammonia solution 25%, hydrochloric acid 37%, hydrogen peroxide 30%, toluene 99%, sulphuric acid 98% were obtained from Qualigens Fine Chemicals, India; absolute ethanol 99.9% from S.D. Fine-Chem Ltd., India; aminopropyltriethoxysilane (APTES) 99%, N-[3-(trimethoxysilyl)propyl] ethylenediamine (97%) (TMSPEDA), N^1-(3-trimethoxysilylpropyl) diethylenetriamine (97%) (TMSPDETA), PBS buffer (pH 7.4) from Sigma–Aldrich Inc., Germany; glutaraldehyde 25%, glycerol 99% and diiodomethane 99% from Loba Chemie, India; anti-BSA IgG from Chromous Biotech, Bangalore, India; anti-PSA IgG, anti-CRP IgG from GeneTech Laboratories, Lucknow, India; de-ionized (DI) water (0–0.5 S/m) from Glen RO$^+$ Systems and zero-grade nitrogen from Sigma Gases & Services, India.

3. Methods

3.1. Stack preparation

The stack, a schematic of which is shown in Fig. 1, comprised a Si substrate which was hydrolyzed, silanized, and cross-linked by glutaraldehyde to the terminal antibodies. The Si surfaces used were either nontextured (i.e. without any physical modification) or nanotextured (i.e. with physical modification); three types of aminosilanes of different chain lengths (Fig. 2) were used, and three types of antibodies were used, thus resulting in various combinations of the layers in the stack. Information on the Si surface preparation (physical modification) and the surface functionalization with the different layers (chemical modification) are provided below.