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Optimization of silica surface with nanosize holes for immobilization of biomolecules and analysis of their interactions

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ABSTRACT

An evanescent-field-coupled waveguide-mode sensor of the Kretschmann configuration with a silica waveguide having nanoscale holes is an ideal tool for detection of bimolecular reactions. In the present research, an optimized surface of the sensor with cylindrical nanoscale holes was modified with sodium (1-{[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate) (Sulfo-EMCS) to facilitate the attachment of biomolecules; the resulting surface could be cleaned for reuse simply by changing the pH of the buffering solution. The modification is expected to be useful for wide range of molecular detection.

1. Introduction

The formation or immobilization of surface-assisted and self-assembled molecules to functionalize a sensor surface is a common technique in the development of sensors [1]. Attachment of biomolecules on the sensory surfaces is an important procedure in the development of sensor devices, and several strategies for surface immobilization biomolecules have been reported [2–6]. Although a number of sensors have been reported in which thiolated biomolecules are attached to gold surfaces [3,7–10], several other modifications are commonly used because of their lower cost [5,6,11–15]. Among several reported modifications, amorphous SiO_2 (silica) substrates are widely used as a platform for the detection of the interactions of labeled or nonlabeled biomolecules [5,6,14–16]. Silica surfaces have several advantages over metallic substrates, especially for studies on nucleic acid ligands [4]. The immobilization of nucleic acids on a silica substrate has numerous applications in the field of nanotechnology and for sensor developments [17].

There are two important parts in a biosensor. One is a biorecognition interface that can selectively detect an analyte, and the other is a transducer that converts the recognized message into an electronic signal that will appear on a screen or will be communicated to the user in some other way [3]. Bionanosensor systems based on various nanomaterials can be used effectively and efficiently in systems for detecting a range of bioactive substances and they allow the monitoring of interactions quantitatively and in real time. All biological events involve interactions of biomolecules such as DNA, RNA, or proteins with each other or with small molecules; such interactions are involved in important functions such as the storage, translation and transfer of genetic information, so it is vital to develop sensitive biosensors. Because of their large surface area and their ability to trap a wide range of molecules, silica-based nanocomposites have been successfully used as platforms for the development of chemical and biological sensors and similar devices, and such materials hold considerable promise for further developments [18]. Interfacing of biomolecules with silica is a prerequisite for the development of a biosensor. The most appealing route for the modification of a silica surface involves the covalent bonding with organic molecules, and this relies mainly on silanization [19]. In our previous study, we produced a silica waveguide having nanoholes and we examined it for use in an evanescent-field-coupled waveguide-

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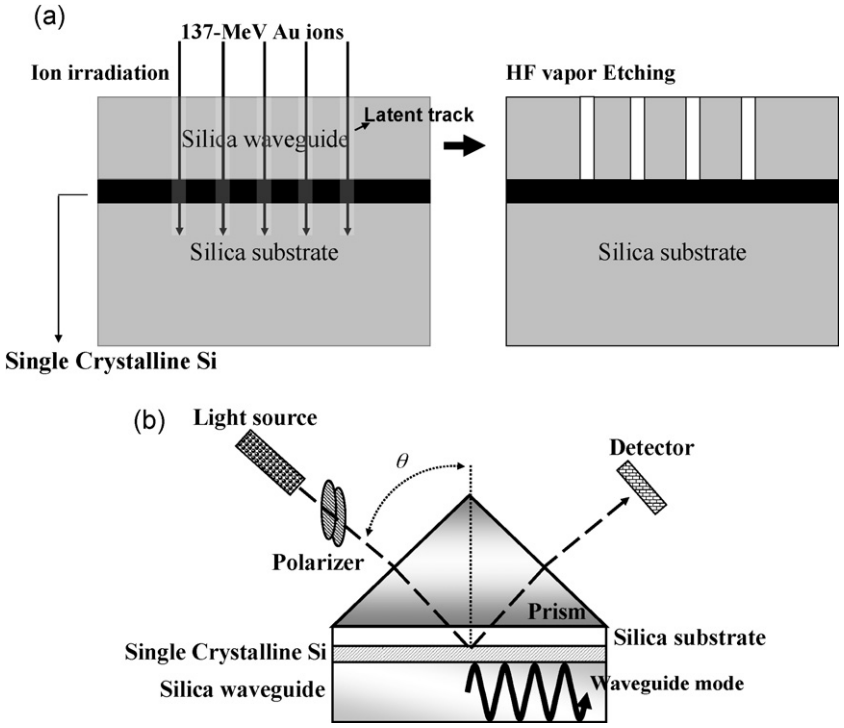

Fig. 1. (a) Schematic showing the formation of latent tracks in the waveguide by swift-heavy-ion irradiation and etching of the latent tracks. (b) A waveguide-mode sensor. The sensor uses sensing plates consisting of a silica substrate, a single crystalline Si layer, and a SiO_2 waveguide layer.

mode sensor, in which thermally oxidized silicon layer formed the silica waveguide [20]. We have shown that the sensor had a high sensitivity with good resonance. The production of uniform cylindrical nanoholes improves the sensitivity of detection as a result of an increase in the internal surface area available for molecular adsorption. Furthermore, the nanoporous nature of the surface, in addition to increasing the sensitivity, is also capable of inducing selectivity on the basis of molecular size [5]. In the present study, we demonstrated the modification of a silica surface containing nanoscale pores by silanization and subsequent treatment with Sulfo-EMCS (sodium 1-{[6-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)hexanoyl]oxy}-2,5-dioxopyrrolidine-3-sulfonate) as a crosslinking agent. This modification has advantages over our previously modified surfaces, as we are able to reuse the chip in monitoring a concentration-dependent signal simply by changing the buffering conditions.

In the present research, to permit comparative analyses with our previous modifications, we used the RNA-aptamer generated against human coagulation factor IX, that we had used in our previous studies [14,15,21]. Factor IX has ability to interact with factors XIa, VIIIa, Va and factor VIIa/tissue factor complex in the coagulation cascade, and therefore, they are vital in the overall coagulation process. Anti factor IX agents are shown to have reduced bleeding risks in animal models, as the better reagent than heparin, and thus factor IX is an important target in the blood coagulation cascade.

2. Experimental

2.1. Reagents and biomolecules

We purchased 3-(triethoxysilyl)propan-1-amine (3APTS) from Sigma–Aldrich (Tokyo, Japan). Sulfo-EMCS crosslinker was purchased from Pierce Biotechnology (Rockford, IL). Factor IX was purchased from American Diagnostica (Stamford, CT).

A 33-mer stable RNA-aptamer, previously selected against factor IXa [21], was enzymatically synthesized by in vitro transcription using T7 RNA polymerase on a synthetic DNA template. A template with the T7 promoter region (in italics) 5′-*AGTAATACGACTCACTATAGG*GATGGGGACTATACCGCGTAATGCTG-3′ was synthesized to generate the double-stranded DNA. By using this template DNA and the primers 5′-AGTAATACGACTCACTATAGG-3′ (forward) and 5′-$(T)_{24}$ATGGGGAGGCAGCATTACGCGGTATA-3′) (reverse), a PCR reaction was performed with a commercial PCR kit (Ex *Taq* kit, Takara Bio, Shiga, Japan). RNAs were prepared from the PCR products as reported previously [14]. The resulting transcribed RNA (57-mer) had a 33-nucleotide moiety that specifically recognizes human factor IXa at its 5′-end, and 24 nucleotides of A residues at its 3′-end. For the negative reaction, a single-stranded DNA molecule with 20 bases (dT_{20}) was synthesized chemically.

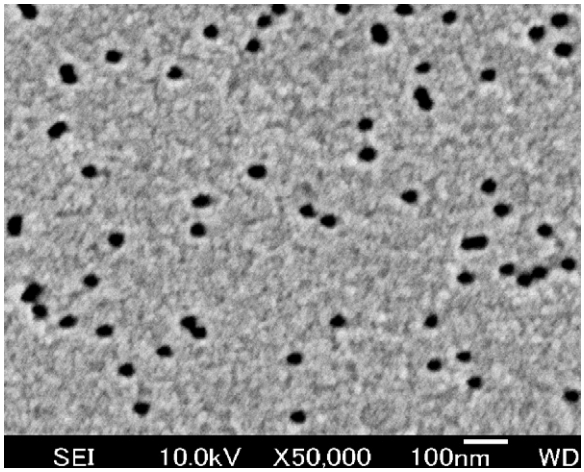

Fig. 2. SEM image of the sensor chip with cylindrical holes used in the present experiment. Scale is shown by the horizontal bar.