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Investigation of thin polymer layers for biosensor applications

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ABSTRACT

Novel biosensors made of polymers may offer advantages over conventional technology such as possibility of mass production and tunability of the material properties. With the ongoing work on the polymer photonic chip fabrication in our project, simple model samples were tested parallel for future immobilization and accessing conditions for applications in typical aqueous buffers. The model samples consist of a thin, high refractive index polyimide film on top of TEOS on Si wafer. These model samples were measured by in situ spectroscopic ellipsometry using different aqueous buffers. The experiments revealed a high drift in aqueous solutions; the drift in the ellipsometric parameters (delta, psi) can be evaluated and presented as changes in thickness and refractive index of the polyimide layer. The first molecular layer of immobilization is based on polyethyleneimine (PEI). The signal for the PEI adsorption was detected on a stable baseline, only after a long conditioning. The stability of polyimide films in aqueous buffer solutions should be improved toward the real biosensor application. Preliminary results are shown on the possibilities to protect the polyimide. Optical Waveguide Lightmode Spectroscopy (OWLS) has been used to demonstrate the shielding effect of the thin TiO_2 adlayer in biosensor applications.

1. Introduction

Development of low-cost biosensors requires low-cost materials (e.g. polymers) and mass production. The P3SENS project (Polymer photonics multiparametric biochemical sensor for point of care diagnostics, [1]) was aimed to design and produce polymer based biosensor for the point of care diagnostic. The main targets of this novel multi-analyte biochip are protein markers of the stroke that need to be detected in blood. The specificity of this biosensor is given by antibodies as receptor molecules, and its label-free detection technique is provided by the photonic waveguide layer (evanescent wave sensing).

Due to the waveguide requirement, a high refractive index difference should be achieved between the waveguide and the substrate layer. High refractive index glasses (dielectrics) are prevalent in waveguide layers in classical integrated optical devices, unlike the polymers. Polyimides (PIs) as shielding/protective and insulator layer are well known polymers in microelectronics [2], and even this function was used in sensor devices [3]; however as a waveguide layer do not play a significant role in sensor applications. The relatively high refractive index of polyimides (n = 1.6–1.7), good preparative characteristics (the layer thickness can be controlled in a wide range by spin coating technique) and low preparative and material costs were promising properties for waveguide application in the P3SENS biosensor project [4]. To reach a sufficient refractive index difference, the PI layer was combined with TEOS (tetraethyl orthosilicate) making a hybrid material system [5,6].

To bind the antibodies to the PI waveguide layer for biosensor applications, we used polyethyleneimine (PEI) as crosslinking molecule. This polymer can be used for crosslinking of biomolecules by means of the large number of free amino-groups, though mainly the polycationic property has been used in previous PEI applications in physical- and colloid-chemistry [7,8]. The PEI-based immobilization method also has many other advantages in binding receptor molecules: it is a simple method and it does not require expensive reagents and heat or plasma treatment (unlike the silanization). PEI has high adhesion affinity to organic and inorganic surfaces; it can be deposited easily on various surfaces from aqueous solutions. The details of our PEI based immobilization method will be published elsewhere.

Biosensors for point of care diagnostics need at least medium instrumental sensitivity, but high reliability measurements, which requires solid sensor structure. However, the biosensor will use aqueous solutions in the real application, therefore it is a very

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important point that the chip consists of stable materials in these environments. Consequently, the sensor requires appropriate stability of the waveguide in aqueous solutions and the deposition technique of the PEI should be optimized in these solutions as well.

In this work, we discuss the behavior of the PI in aqueous solutions, and we present our investigations of the PEI deposition on PI surface, and its stability. Our main measurement technique was in situ spectroscopic ellipsometry, which ensures many possibilities to study thin layers [9,10]. As the measurements immediately revealed a large drift of the measured signal for PI in aqueous solutions, the protection of the PI layer was also aimed. Thin layers of TiO_2 prepared by atomic layer deposition (ALD) was tested and found to be advantageous.

2. Materials and methods

2.1. Materials

The composition of the three solutions used was the following.

2.1.1. Borate buffer

The borate buffer was made from sodium tetraborate decahydrate purchased from Sigma–Aldrich, sodium hydroxide purchased from Renal, and Milli-Q ultrapure water produced by our Millipore Direct Q device (18.2 MΩ cm of resistance). Ultrapure water was used for all the preparations.

2.1.2. PBS

Phosphate buffered saline (PBS) tablets have been obtained from Sigma–Aldrich which yields a 10 mM phosphate buffer containing 27 mM potassium chloride and 137 mM sodium chloride, pH 7.4, at 25 °C.

2.1.3. PEI

The 5% PEI solution was made from 50 (m/V)% aqueous stock solution of 750 g/mol average molecular mass hyperbranched PEI, purchased from Sigma–Aldrich. The pH of the solution was set by dropwise addition of cc. HCl. Working solutions were prepared by serial dilution.

2.2. Model samples

The model samples were fabricated at the VTT laboratories in a form identical to the layer thicknesses and layer structure of the proposed sensor. At the first step, TEOS was deposited in 2100 nm thickness on the Si substrate. The PI brand was Evonik P84® (from the HP Polymer GmbH). The PI layer was prepared on the Si/SiO_2/TEOS sample using spin coating technique from 6% N-ethyl-2-pyrrolidone solution. The process was performed to obtain the PI layer in 800 nm thickness, and finally post bake (250 °C, 30 min) in order to fully eliminate the remaining NEP. In some of the samples the PI was subsequently coated with TiO_2 in 10, 50 and 100 nm using atomic layer deposition (ALD) technique. See also Fig. 1. The model samples were prepared in 15 mm × 30 mm size, fitting into our flow-cell for the in situ ellipsometric measurements.

2.3. Spectroscopic ellipsometry

Spectroscopic ellipsometry (SE) is a mature, high sensitivity and depth resolution optical measurement method for investigation of surfaces and thin layers [11]. It can be used on solid/liquid interfaces with an appropriate liquid cell, where surface changes, surface layer growth, macromolecular deposition might take place. In situ SE using flow-cell provides possibilities to study these phenomena in different environments in high time resolution and in long measurements (days) [12]. In this study the PI stability in aqueous solutions and the deposition of the PEI on the PI were investigated by this technique.

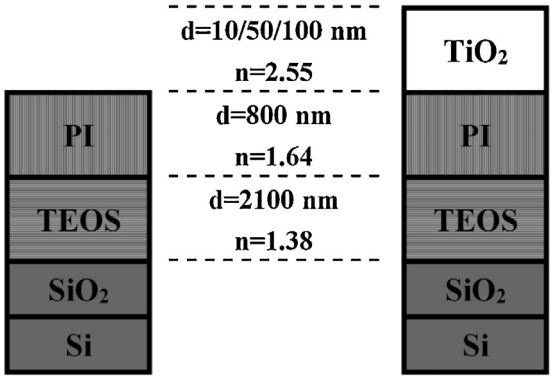

Fig. 1. Structure of the model samples with their nominal parameters; *d*: thickness of the layer, *n*: refractive index of the layer.

SE is able to investigate thin layers determining their dielectric properties. It can provide such layer parameters like thickness and refractive index. The acquired raw ellipsometric measurement parameters Δ and Ψ (change of phase and amplitude of polarized light) might give a first sight information about changes in the sample (Δ being usually more sensitive); for the quantitative result the ellipsometric spectra should be evaluated by an appropriate optical model. Our evaluation procedure was carried out as it follows. First, the optical model of the sample was constructed, considering our prior knowledge about its layer structure (Fig. 1.). Then initial values were assigned to each layer parameter (layer thicknesses, refractive indices), which were iterated in order to minimize the value of MSE (Mean square error). The calculations resulted values for the unknown layer parameters, which provided the best fit (the lowest MSE). We used CompleateEASE, version 4.64 software to control the measurements and to carry out the evaluations.

2.4. In situ ellipsometric measurement method

The measurements were performed using a Woollam M-2000DI rotating compensator spectroscopic ellipsometer in the wavelength range of 193–1690 nm, produced by J.A. Woollam Co., Inc. A home-made flow-cell (0.2 ml) was used for the in situ experiments [13]. The measurements were performed at the incidence angle of 75°, close to the Brewster-angle of the silicon substrate. The applied solutions were driven through the cell by a peristaltic pump (Reglo Digital from Ismatec, 0.201 ml/min) using a Tygon® tube.

The stability investigation of the PI and the PEI deposition process were performed in aqueous buffer solutions. Borate buffer provides proper pH for the "mushroom" conformation of the PEI which will be important in the aspect of the further antibody immobilization, while the buffer also provides the aqueous environment for the stability examinations. We also investigated the PI stability in pH 7.4 PBS aqueous solution to examine its function at neutral pH. During the in situ measurements Δ and Ψ spectra were recorded as a function of time with 80 s time resolution. For the PEI deposition measurements we applied 20 s time resolution.

2.5. AFM

After the in situ ellipsometric experiments the model samples were dismounted from the cell and dried using high purity nitrogen blow. Smaller pieces were cut and fixed onto the AFM specimen holders. AFM images were collected using a SmartSPM 1000 system (AIST-NT). Silicon cantilevers with a nominal tip curvature radius of 10–25 nm were used to acquire topographic images in tapping