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A new simple tubular flow cell for use with variable angle spectroscopic ellipsometry: A high throughput in situ protein adsorption study

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ABSTRACT

A simple and novel flow cell design is presented here for use with variable angle spectroscopic ellipsometry (VASE) to study the adsorption of liquid-borne species on reflective surfaces. The flow cell allows a sample as large as 6 mm × 75 mm to be probed point by point and at any common ellipsometric angle of incidence, unlike other designs. Using our flow cell system with VASE, combinatorial films of $Al_{1-x}Nb_x$, $Al_{1-x}Ta_x$, and $Al_{1-x}Ti_x$ ($0 \leqslant x \leqslant 1$) were tested in situ for fibrinogen affinity along their 75 mm long compositional gradients. Fibrinogen adsorption on the films was found to be closely correlated to the various surface oxide fractions, with high alumina content at the surface leading to low amounts of adsorbed fibrinogen for each binary library. Adsorbed amounts measured in situ were in agreement with previously obtained values found using ex situ techniques.

1. Introduction

It is generally accepted that surface interactions of biomaterials begin with the adsorption of proteins and that by understanding this initial event, the cascade of host responses can be better controlled. Indeed, the types, orientations and conformations of proteins adsorbed to a surface are extremely important [1]. Of course, greater insight into the protein–surface interaction is the key to improving products for many industrial applications, such as biomedical implant devices, additives to reduce surface fouling in the food industry, biosensors and drug delivery systems.

An ellipsometer measures the relative change in the state of polarization between polarized light parallel and perpendicular to the plane of incidence upon reflection from a sample [2]. This change in polarization state is expressed in terms of the ellipsometric angles Psi (Ψ) and Delta (Δ). These angles are sensitive to the measurement arrangement and the optical properties of the sample; in particular, the wavelength of light, the incidence angle, film thickness(es), and the optical constants (refractive index n and extinction coefficient k) of the ambient, film(s) and substrate. Analysis becomes increasingly more complex for systems that contain more layers and generally, more knowledge of the system is required for reliable analysis. State-of-the-art ellipsometers are variable angle, spectroscopic instruments which are fully automated and include sample motion stages, allowing for data acquisition in a short period of time across an entire sample.

Ellipsometry has a thickness resolution on the order of Angstroms, is noninvasive, requires no chemical treatment with labels for protein studies, and is capable of measuring film growth in situ (in a liquid environment) on a time scale (seconds) relevant to the kinetics of biological processes. Due to these reasons, it has been well-established as an effective tool for determining protein adsorption amounts for both ex situ [3–5] and in situ [6–8] studies. Ex situ measurements may be useful for judging how protein adsorption amounts depend on protein concentration or solution pH, for example, but the results obtained may be skewed by the rinsing and drying procedure. Of course, in situ measurements are more technically involved since a flow cell system is required, but the true nature of the protein–surface interaction is better determined since there is no intervention between the adsorption and measuring steps. For this reason, in situ experiments provide more valid results, since they involve measuring in place without disruption or transfer to another medium. Furthermore, ex situ studies force one to measure protein adsorption at one particular moment of an experiment. On the other hand, in situ ellipsometry experiments allow one to measure protein adsorption in real-time

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at any point on a sample, changing conditions when desired, and hence, allows the user much more experimental freedom and capability without intrusion. In situ testing can be especially beneficial when using costly proteins and antibodies since many one-step ex situ experiments can be condensed into one continuous in situ study.

Besides ellipsometry, other in situ analytical techniques have been reported to be successful in determining protein adsorption amounts on various substrates. Among the most widely used are: quartz crystal microbalance (QCM) [8–10], optical waveguide light spectroscopy (OWLS) [8,11–13] surface plasmon resonance (SPR) [10,14,15] and scanning probe microscopy (SPM) [16] techniques such as atomic force microscopy (AFM) [17] and scanning tunneling microscopy (STM) [18]. Each method has its advantages and disadvantages. For instance, AFM and STM are very straightforward imaging techniques but they are both affected by surface roughness effects. OWLS and SPR are akin to ellipsometry in that they are sensitive optical techniques but OWLS requires highly transparent surfaces while strictly noble metal substrates are required for SPR. On the other hand, the substrates for QCM studies can be somewhat freely chosen since they need not have reflective or transparent optical properties. However, the measurement is inherently sensitive to water–protein coupling. Furthermore, AFM, STM and QCM techniques are not suited to high throughput studies.

Quite often an in situ technique is combined with another to complement its deficiencies. Hook et al. [8] adsorbed albumin, fibrinogen, and hemoglobin, as well as antibodies, onto TiO_2-coated substrates. They used water-insensitive optical techniques (OWLS and ellipsometry) to observe the 'dry' adsorption amounts and QCM to observe the amounts which included bound water in order to provide insight into the hydrodynamic state of the adsorbed adlayers. In addition, SPR and AFM are highly complementary in situ techniques since AFM can directly visualize surface coverage from the top while SPR can optically monitor protein adsorption kinetics from the bottom through an underlying noble metal substrate. Chen et al. [19,20] interfaced the two techniques into one instrument for simultaneous measurement and Green et al. [21,22] and others [23,24] have since used the combined system for detailed studies.

Although the analysis of ellipsometry data can be relatively complex and reflecting surfaces are required, it is more versatile than OWLS or SPR since there is not a strict requirement on the type of adsorbing substrate. If the adsorbing substrate is optically transparent, a reflecting layer can be placed below it, and non-noble metals can be used just as easily as any other metal.

For in situ ellipsometric studies, a cell is required to hold the sample stationary in a liquid environment. When designing a cell, consideration must be given to a variety of issues. In particular, the volume of the cell should be as small as possible in order for the absorption kinetics to be responsive to liquid exchange and to minimize the costs of the proteins used. For larger open cells [25,26], stirring of the liquid is necessary to speed up mixing and migration of proteins to the surface. For this reason, flow cells are desirable since a small cell volume with rapid liquid exchange is achieved. Using a flow cell, Ortega-Vinuesa et al. [27] found that adsorption amounts of a number of serum proteins onto silicon was always larger under flow than in stagnant conditions, and that a range of shear rates did not noticeably affect these amounts. In addition, Jakobsen et al. [28] and Karlsson et al. [26] each found that increased flow rates led to a decrease in the amount of adsorbed proteins and that different flow rates used in the rinsing and cleaning steps did not affect residual protein amounts. However, one has to be aware of the shear forces to which the proteins will be exposed under laminar or turbulent flow within a flow cell since denaturizing effects can occur.

Another important design consideration is that high quality stress-free windows oriented perpendicularly to the incoming and outgoing light beam are required in order to prevent ellipsometric measurement errors. Of the few ellipsometric flow cell studies that are reported in the literature involving protein adsorption, all make use of flow cells with flat window panes such that one [8,12,25,26,29,30] or a few angles (multiple plane windows) [31] are possible. The problem with this type of design is that the angular placement of the windows limits the number of possible angles to one or a select few. In the present study, clear fused quartz tubing was used for the flow cell body so that the full range of possible angles is retained. To our knowledge such a flow cell design has yet to be reported in the literature. This is surprising considering its simplicity and relevance to variable angle ellipsometry. Alternatively, Benjamins et al. [7] successfully used a set of light guides in order to channel incident and reflected light beams through the air/solution interface, allowing for variable angle ellipsometry. However, a single wavelength ellipsometer was used with a non-flow measuring cell and so it is unclear how well this method would work with a spectroscopic instrument and a flow cell. Furthermore, the calibration of this technique is rather complicated.

The aim of this paper is to show that our simple tubular flow cell is suitable to monitor the kinetics of protein adsorption at surfaces with variable angle spectroscopic ellipsometry (VASE).

2. Materials and methods

2.1. Flow cell and pumping system

Fig. 1a shows pictures of the flow cell. A clear fused quartz pipe (Technical Glass Products Inc., OH, USA) of dimensions 8 mm inner diameter × 10 mm outer diameter × 146 mm in length, functions as the main body of the cell. An Al semi-rod, which rests inside of the quartz pipe, houses the sample in a recess such that the sample surface lies exactly along the centerline of the pipe. The effective liquid volume of the flow cell is about 3.7 ml. Fig. 1b shows a schematic cross-sectional view of the flow cell. The beam strikes the sample along the axis of the quartz pipe, allowing the incoming and outgoing beams to pass through the pipe at normal incidence for any angle. Of course, this is true only if the beam diameter is sufficiently small enough at the point of entry/exit of the flow cell – if it is too large then there will be unwanted refractive effects due to the curvature of the cell. In order to reduce the beam diameter our setup uses refractive optics to focus the beam down to about 150 μm, or about 400 μm at the point of entry/exit. Another added benefit of using the refractive optics is that it allows increased measurement resolution on our combinatorial sample.

From an ellipsometry standpoint, it is important that the beam not refract at any of the four quartz interfaces. Two stainless steel spring clamps fix the position of the semi-rod inside the pipe and keep the sample firmly in place. The quartz pipe is held in place on its base with two brackets, while the bottom portion of the base is received into a groove on the ellipsometer stage (picture not shown). The base is held tightly in place on the stage with clamps as well. It is very important that the sample does not move parallel to the scattering plane during testing – the clamping and bracketing ensures this. The flow cell is connected to the input and output pump lines via stainless steel Ultra-Torr® union vacuum fittings (Swagelok, OH, USA). One end of each union provides a tight o-ring seal around the quartz pipe while the other end seals around an Al insert that has a threaded bore hole. A VacuTight™ nut-and-ferrule (Upchurch Scientific Inc., WA, USA) screws into the bore hole to provide a tight seal for the tube connection against the back of the insert.