Simultaneous SPR and electrochemical sensing of an alkane thiol Self-Assembled Monolayer (SAM) - towards an optical biosensor.

A.K. Sheridan* 28, P. Ngamukot b, P.N. Bartlett c, J.S. Wilkinson a,
 a Optoelectronics Research Centre, University of Southampton, Southampton, SO17 1BJ UK,
 b Department of Chemistry, Faculty of Science, Chulalongkorn University, Phyathai Road, Patumwan, Bangkok 10330, Thailand;
 c School of Chemistry, University of Southampton, Southampton, SO17 1BJ UK

ABSTRACT

We present simultaneous electrochemical and waveguide surface plasmon resonance measurements demonstrating the electrochemically controlled desorption of a thiol monolayer from a gold electrode. Self-assembled thiol layers are used in biosensors to enable the attachment of lipids to the sensor. This work demonstrates the potential for development of the device for biological sensing applications. Simultaneous SPR electrochemical measurements are made during the thiol desorption and these results are discussed and compared to the results of a numerical simulation.

Keywords: Surface Plasmon Resonance, Waveguide, Electrochemistry, Biosensor, Alkane Thiol

1. INTRODUCTION

Surface plasmon resonance (SPR) is a sensitive technique for monitoring changes in refractive index at the surface of a sensor based on the formation of surface plasmons at the interface between a dielectric and a metal. Incorporation of electrochemical control, by using the gold area as both the SPR sensing area as well as an electrode for electrochemical reactions, allows electrochemical control, reversal of sensing reactions, as well as complementary information about electrochemical processes at the sensor surface [1]. This has predominantly been achieved using the prism or Kretschmann configuration. However, this system is bulky and not well suited to multisensor operation. These problems can be overcome by waveguide SPR sensors, where standard photolithography and ion-exchange are used to define the waveguides [2]. The use of integrated optics allows more complicated designs containing, for instance, reference channels, as well as allowing many devices on a single chip. An additional advantage is the reduced size of the sensor. We have recently demonstrated a combined waveguide SPR electrochemical sensor to study the electrochemical oxidation of a gold electrode surface [3]. In this paper we extend this work to investigate a self-assembled alkane thiol monolayer. Thiols are commonly used in biosensors to enable the attachment of lipids to the sensor surface. This enables them to be directly 'wired' to the electrode enabling electrochemical reactions to be carried out. In this paper we show that cyclic voltammetry in sulphuric acid results in thiol desorption from the gold surface. We use combined SPR and electrochemistry to investigate this reaction and compare the SPR and electrochemical data to the results of a numerical simulation.

2. FABRICATION

The planar waveguide structure shown in figure 1 was fabricated using conventional photolithographic techniques. Waveguides 3 \mu m wide were formed in 60 mm square Pyrex wafers by potassium ion exchange at 380 °C for 7 1/2 hours. The edges of the ion-exchanged substrate were chemo-mechanically polished, using Syton (Logitech) to allow efficient end-fire coupling of the incident light into and out of the waveguide. Prior to the deposition of the gold film, the sample were treated using (3-mercaptopropyl) trimethoxysilane [4] to enhance the adhesion of gold to the glass. The sample was refluxed in water, IPA and MPS in the ratio 48:1:1 for 15 minutes, then rinsed thoroughly with IPA, dried with nitrogen gas and baked at 110 °C for 8 minutes. This procedure was repeated, and then a 55 nm gold layer was deposited using thermal evaporation and patterned to form gold sensing areas 1 mm by 20 \mu m. The sample was then coated with a 1\mu m thick Teflon AF 2400 (n =1.31) isolation layer, which was patterned using lift-off. The fabrication procedure is described
in more detail in [5]. The gold areas were then cleaned using cyclic voltammetry in sulphuric acid as described below. The decanethiol monolayer was formed by self assembly from a 1 mM solution of decanethiol in ethanol over 12 hours using high grade ethanol. The sample was then washed in ethanol and dried.

3. EXPERIMENTAL

For the SPR measurements, TM light from a HeNe laser at 633 nm was first mechanically chopped at 100 Hz and then coupled into a polarization maintaining fiber for efficient coupling into the waveguides. The output from the waveguides was focused using a microscope objective lens and collected using silicon photodiodes. The signal (P_{sig}) and the reference (P_{ref}) powers giving the transmission (P_{sig}/P_{ref}) were recorded on a PC after noise reduction using lock-in amplifiers. The electrochemical measurements were carried out in 10 mM sulphuric acid using a Hg/HgSO_4 (mercury mercuro sulphate) reference electrode and gold wire counter electrode. The gold sensing area acts as the working electrode and is connected via photo-lithographically defined micro-wires and an edge connector to the potentiostat. A drop of sulphuric acid was placed onto the working electrode surface and the reference and counter electrode were held in the drop using clamps and connected to a computer-controlled potentiostat for data collection. This allows the current through the working electrode to be measured as the potential of the working electrode with respect to the reference electrode is controlled. The working electrode was ramped from 0 V up to 1.1 V and back down to 0 V repeatedly using a scan rate of 20 mV/s. To perform the theoretical simulations of the optical response of the device, a waveguide model was used, which is described in detail in an earlier publication [6]. The model approximates the structure as a planar slab waveguide. In practice waveguides are channels with graded index profiles, however this model provides useful insight into the behavior of the sensor. In the calculation values for the refractive index of Pyrex of 1.471 and ion-exchanged waveguides of 1.478 are used.

4. RESULTS AND DISCUSSION

Figure 2a shows the oxidation and reduction of an untreated gold electrode by cyclic voltammetry in acid (dark line) together with the waveguide SPR measurements (dotted line). The current peaks associated with the oxidation (1 V) and reduction (0.4 V) of the gold can be seen clearly. The optical measurements show a drop in the transmission at potentials above 0.9 V, corresponding to the oxidation of gold and a sharp increase in the transmission as the reduction of the gold takes place. The shallow decrease of the transmission between 0 V and 0.9 V is thought to be due to changes in the anion concentration at the surface of the gold associated with the double layer charging process. This is discussed in more detail in [5]. The cyclic voltammetry was repeated for 10 cycles in order to clean the gold of any surface impurities before deposition of the decanethiol.

Figure 2b shows both the electrochemical (solid dark line) and the SPR (dotted line) response to cyclic voltammetry in acid for a gold electrode coated with a decanethiol monolayer over ten cycles. The first electrochemical cycle shows a small oxidation current and correspondingly a very small reduction current with a peak of ~17 nA at 0.4 V. For subsequent cycles, larger oxidation and reduction currents are observed, until reproducible cyclic voltamograms are obtained with peak currents of 85 nA, similar to those expected for an untreated gold film. The first optical cycle also shows only a small drop in transmission compared to the data for an untreated gold surface. The optical data also shows that the transmission at the end of each cycle (0V) increases for each subsequent cycle. The increase is largest for the first run, where a change of 2% is observed. After ten cycles the shape and magnitude of both the optical and electrochemical data are similar to that measured for the untreated film (figure 2a).

These results show that cycling in acid over 10 cycles causes the thiol to be desorbed from the gold surface. It has been shown that sufficiently large negative or positive potentials applied to the working electrode can result in the oxidative or reductive desorption of an alkane thiol layer from the gold surface [7]. The results shown in figure 2b demonstrate that
the decanethiol film is removed from the gold surface by oxidative desorption during cycling in acid. The processes involved in the reaction will be discussed in more detail elsewhere [8]. Porter et al. have shown using a different thiol (mercaptoethanol) that electrochemical oxidation shows three oxidative processes which occur sequentially resulting in the cleavage of the C-S bond [9]. Figure 2b shows that initially a small oxidation and reduction current are measured. This is because the thiol layer blocks the flow of charge to the gold working electrode, however as coverage of the thiol is not perfect, some gold is available to take part in the oxidation/reduction reaction. This results in a small oxidation and reduction current and correspondingly, a small change in the optical signal. At around 1.0V some desorption of the thiol film takes place. It is likely that electrochemical oxidation of the thiol takes place initially at defects in the thiol coverage. This results in a larger area of gold available to be oxidised and therefore larger oxidation and reduction currents. After ten cycles the thiol is completely stripped off and the CV is almost identical to that of the untreated gold film. The increase in the transmission at 0V in the optical data is due to the change in effective index close to the surface of the gold which determines the amount of light coupled to the surface plasmon. A decrease in the effective refractive index results in a decrease in the light coupled to the lossy surface plasmon and an increase in the power of light in the waveguide.

In order to understand the reaction dynamics in more detail we have calculated the area of gold exposed by the removal of the thiol during the experiment. The surface area of the electrode can be calculated by assuming that the charge under the reduction peak corresponds to the desorption of one oxygen atom from one gold substrate atom. The integral of the area of the reduction peak over time gives the charge passed during the reaction and by using the result that 400 μC corresponds to 1 cm² [10], the area of the gold electrode can be calculated. This is a useful measurement for examining the desorption process in more detail. The value of the area of gold calculated for the untreated gold electrode is 9.4x10⁻⁴ cm². This is larger than the actual value of the electrode area, 6x10⁻⁴ cm² due to surface roughness of the film introduced by the evaporation process.

Figure 2 shows Cyclic Voltammetry (solid line) and SPR (dashed line) measurements on a) gold electrode b) decanethiol coated gold electrode.

The figure shows a linear relationship demonstrating that both the SPR and electrochemical measurements are able to detect the removal of the thiol layer in real time, and that the magnitude of the changes for each measurement are in good agreement. This is important for the development of this device into an enzyme based biosensor, where the attachment and removal of lipids, which have a similar size and refractive index as the thiol layer, will be monitored.

Figure 4 shows theoretical results for the transmission versus analyte refractive index for a device 1 mm long and 55 nm thick. The solid line shows the response of the SPR sensor for a bare gold film and the dotted line shows the response for a gold film with a self-assembled thiol layer. The layer was assumed to be 10 nm thick with a refractive index of 1.46 [11]. Both graphs show the typical surface plasmon resonance with a minimum for the plain gold film at a refractive index of 1.36. The additional thiol layer shifts the resonance to lower refractive indices, resulting in an increase in the
transmission (at an analyte index of 1.3) when the thiol is removed from the gold film. This is in agreement with the optical change measured. The magnitude of the change can not be compared as the model is for a planar waveguide structure whereas in practice we have graded index channel waveguides.

**CONCLUSIONS**

In this paper we have shown that a self-assembled thiol layer can be removed from a gold electrode by oxidative desorption by cyclic voltammetry in acid. We show that the removal of the thiol layer can be monitored in real-time using both SPR and electrochemical techniques and we examine the desorption process by comparing the fractional coverage of gold calculated from the area of the reduction peak with the change in SPR transmission. Both the SPR and electrochemical measurements give similar results for the magnitude of the change seen on removal of the thiol. This demonstrates that electrochemical control coupled with sensitive optical measurements. Work is underway to develop this device into a biosensor for detection of the enzyme phospholipase \( \mathrm{A}_2 \), which is implicated in inflammatory diseases such as asthma and cystic fibrosis.

**ACKNOWLEDGMENTS**

The Optoelectronics Research Centre is an Interdisciplinary Research Centre partially supported by the UK Engineering and Physical Sciences Research Council.

**REFERENCES**

10. R.K. Burshtein, Electrochimica 3 349 (1967)