A Microfluidic Device for Opto-Electrochemical Sensing

Y. Choi, U. Huh and T. J. M. Luo

Department of Materials Science and Engineering
North Carolina State University, Raleigh, NC, USA Mark_Luo@ncsu.edu

ABSTRACT

A microfluidic device that contains a silica film as optical transducer and electrochemical electrode was used to monitor how ferricyanide ion behaves within nanoporous matrix. This prototype device allows UV-VIS absorption spectroscopy and cyclic voltammetry to be performed at the same time. Significant reducing in leaching of redox molecules proved this device integrated with silica film can be utilized to explore biosensor and biofuel cell concepts.

Keywords: microfluidic, sol-gel, electrochemistry, light-guide, ferricyanide

1 INTRODUCTION

There has been a tremendous interest in combing biological systems, especially transmembrane proteins, for biofuel cells, biosensors, bioreactors, and biosolar cell applications. To integrate functionality of transmembrane proteins to devices, a platform compatible with proteins and phospholipids membrane is required. Recent success in soft materials has demonstrated the possibility of utilizing these complex biological systems (e.g. bacteriorhodopsin and ATPase)[1] for energy storage and conversion from a solid matrix. Further advances in this field requires a better control on bio-inorganic interfaces, and monitor the system through electrochemical and optical methods simultaneously.

In our effort to address the needs for current and future research, we report a microfluidic device that is integrated with an opto-electrochemical cell. This device is based on a zero alignment broadband sol-gel light guide.[2] It consists of an optical transducer, a microfluidic channel integrated with three electrochemical electrodes. This rapid prototyped device is easily constructed and compatible with most of commercial spectrophotometer and potentiostat. An organically modified silicate (ORMOSIL) film can be fabricated through sol-gel synthesis on a fluorine doped tin oxide (FTO) glass to interact with liquid (Figure 1). The advantages of such configuration are enhanced optical sensitivity and rapid fluidic diffusion for electrochemical sensing. Initial measurement was performed with a ferricyanide solution using cyclic voltammetry together with optical absorption spectroscopy to examine properties of sol-gel thin film. The results are comparable to a regular electrochemical cell and show rapid and sensitive response on absorption spectra.
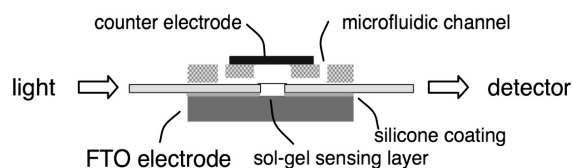

Figure 1: Side view of the electrochemical cell that consists of a silica optical transducer as the working electrode and a microfluidic flow cell. This microfluidic flow cell contains a platinum counter electrode and a silver reference electrode.

Sol-gel materials have been known for its ability to encapsulate biomolecules and transform to highly-selective biosensors.[3] The major advantage is the long term stability of proteins associated with the enhanced thermal properties of proteins in the sol-gel matrix.[4] One factor that affects the performance of sol-gel sensor is the response time required for analyte to reach equilibrium concentration inside the matrix,[5] therefore sol-gel as thin film at the range of sub-micron were constantly used to reduce response time. However, the short light path usually mean lower sensitivity. Planar thin film waveguide based sensors,[6] in the contrast, has the advantage of long light path and the short response time. Sol-gel in the form of thin film can act as waveguide to conduct light wave and serve as chemical sensors.[7] Thin film can be easily fabricated on cover glasses or silicon wafers by dip coating or spin coating.[8] Several groups also have reported using microscope slides or cover glasses as sensors[9] To easily integrate sol-gel thin film structures within a microfludic device that serves as electrochemical cell, thin cover glasses and conducting oxide coated glass were used here as substrate.

The silica film was synthesized using silica precursor that contains high density of amine functional groups, which enhance the electrochemical properties of optical transducer. For rapid prototyping purpose, this film was produced with a thickness equal to that of cover slides. Our studies have shown this silica film, with thickness < 1 μm, exhibited improved oxidation and reduction responses of ferricyanide, $Fe(CN)_6^{3-}$, compared to a blank FTO electrode. After treated with ferricyanide, the silica film remained electrochemically active even after 48 hours in phosphate buffer solutions. We observed significant

reducing in leaching of redox molecules and therefore allows us to explore this properties for other applications.

2 EXPERIMENTAL

2.1 Device configuration

The electrochemical device consists of microfluidic component and sol-gel optical electrode (light guide). The light guide electrode was prepared by attaching two 9 x 18 mm cover glasses (150 μm thick, n = 1.47) on a fluorine doped tin oxide glass (FTO electrode) using polydimethylsiloxane (PDMS, n = 1.4) as adhesive. Two cover glasses were separated by 3 mm spacing where ORMSIL light guide was formed. This electrode was mounted on a PDMS fluidic cell that was built with platinum and silver counter electrodes. The immersing buffer solution exhibits lower refractive index (n = 1.33), therefore, a total internal reflection at the gel-liquid interface was achieved. Wet gel was maintained at all time to preserve the integrity of silica gel. Since two cover glasses were used to couple light into the sol-gel layer, the sol-gel and glass interface should be smooth to reduce light scattering. The interface was examined under inspection microscope and no degradation was observed indicating good bonding between silica and cover glasses. Figure 2 illustrates the setup for this device when it was placed inside a UV-VIS spectrophotometer. Since the refractive index of PDMS adhesion layer and channel solution was lower than that of glasses and sol-gel, only two pieces of black polystyrene foam (2 x 2 cm) with 150 μm slit width were mounted at the both end of the device. This was to cut off stray light and to pass straight light through sol-gel electrode to the detector side under the condition that the incident beam and the light-guide was parallel.

2.2 Sol-gel synthesis

Silica precursor was prepared by mixing a methanol solutions of 0.1 M bis[3-(trimethoxysilyl)propyl]ethylene-diamine (enTMOS) with deionized water at a volume ratio of 1 :3. Each time fresh solution was prepared and used immediately. The mixture was injected into the space between two cover glasses on the surface of the FTO electrode with the help of a flat PDMS template. Surfaces of FTO and cover glasses were oxygen plasma treated before injecting the solution. Oxygen plasma treatment was found to improve the bonding between glass substrate and silica matrix, and improve wetability of surface for easy loading. In general, gelation occurred within 5 minutes of injection, followed by removal of the template resulted in integrated silica-light guide electrode. This procedure produced a sol-gel layer that has same thickness as cover slip. Then the light guide was kept moisture in a phosphate buffer and stored for future use.

2.3 Device operation

The fabricated device was mounted inside a Varian Cary 100 spectrophotometer with its three electrodes connected to an Obbligato potentiostat. During the experiment, a 1x phosphate solution containing 1 mM of ferricyanide was injected immediately followed by absorption spectroscopy and cyclic voltammoetry measurements. The voltages of cyclic voltammograms were measured based on silver pseudo electrode as reference. For kinetics study both measurements were performed every 30 minutes. For leaching experiment, flow cell was washed with a solution of 1x phosphate buffer several times and allowed ferricyanide to diffuse into the solution.
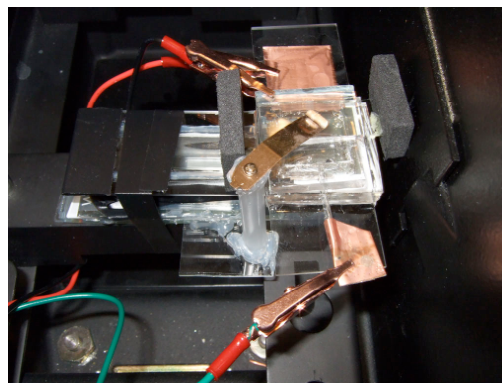

Figure 2: Photo of microfluidic device installed in the chamber of a UV-VIS spectrophotometer.

3 RESULTS AND DISCUSSION

Our first results show that the minimum response time of this device was 5 minutes for both UV-VIS spectroscopy and cyclic voltammetry. Both measurement shows increasing in intensity during each 30 minute interval. Compared with the device that contains a blank FTO electrode, oxidation peak and reduction peaks of 1 mM ferricyanide are 50 and 60 μA (Figure 3). It required 3 hours for this 150 μm thick silica layer to reach the concentration equivalent of 1 mM of ferricyanide solution. The silica film can continuously attract ferricyanide ion for another 3 hours (Figure 4b). Enhanced ferricyanide signals due to the presence of silica film has increased the currents to 70 and 90 μA. After flushed with phosphate buffer, only cyclic voltammograms showed decrease in signal with each scan. The ability of trapping ferricyanide was observed even after 48 hours continuous immersion in PBS buffer (Figure 4c). The equilibrium loading of ferricyanide was estimated to be 0.2 mM in the silica matrix. Because the electrochemical properties of silica light guide result from the trapping of $Fe(CN)_6^{3-}$, the enTMOS light guide was not electrochemically active if it was only treated with phosphate buffer (Figure 4c).

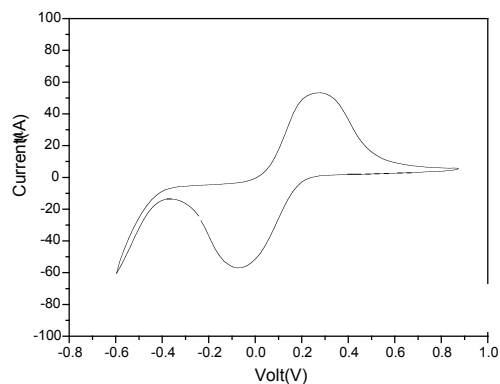Figure 3: Cyclic voltammograms of $Fe(CN)_6^{3-}$ measured in the liquid cell that contained a blank FTO electrode.

UV-VIS spectroscopy with background correction was utilized at the same time to monitor the reaction between ferricyanide and silica matrix. Ferricyanide exhibits characteristic absorption peak at 420 nm as indicated on Figure 5. Absorption peak was observed immediately after the injection indicating rapid diffusion nature of the film. This observation was also validated through electrochemical measurement (Fig 4b). After 30 minutes, peak at 420 nm became maximum indicating concentration of ferricanide increased due to concentration gradients. It is interesting to note that the absorption decreased to 50% of initial intensity during 3 hours of continual observation while absorption band (480 ~ 680 nm) steadily increased. The UV-VIS spectroscopy shows that the chemical shift from 420 nm to absorption band at 480 ~ 680 nm is caused by interactions between ferricyanide and silica matrix. The existing interactions are strong that silica optical film remained electrochemically active at least 48 hours after immersed in a phosphate buffer solution. The absorption band between 480 and 680 nm did not decrease when rinsing with phosphate buffer (not shown). Both measurements show the binding between ferricyanide and enTMOS silica matrix is strong, but it does not affect the electrochemical property of ferricyanide. In addition, we found no significant loss in optical sensitivity when silica layer was directly fabricated on top of FTO electrode. Since the refractive index of FTO is higher than that of enTMOS layer, the optical measurement was performed at zero order mode where internal reflection was minimized during the measurement.

In summary, a ORMOSIL light guide combined with three-electrode microfluidic flow cell was demonstrated and this device was successfully utilized to study the electrochemical and optical properties of the silica film, which will be used as a platform for testing new biosensor and biofuel cell concept. Current work includes reducing film thickness to allow rapid solvent exchange as well as incorporating functional enzyme for high selective biosensing.
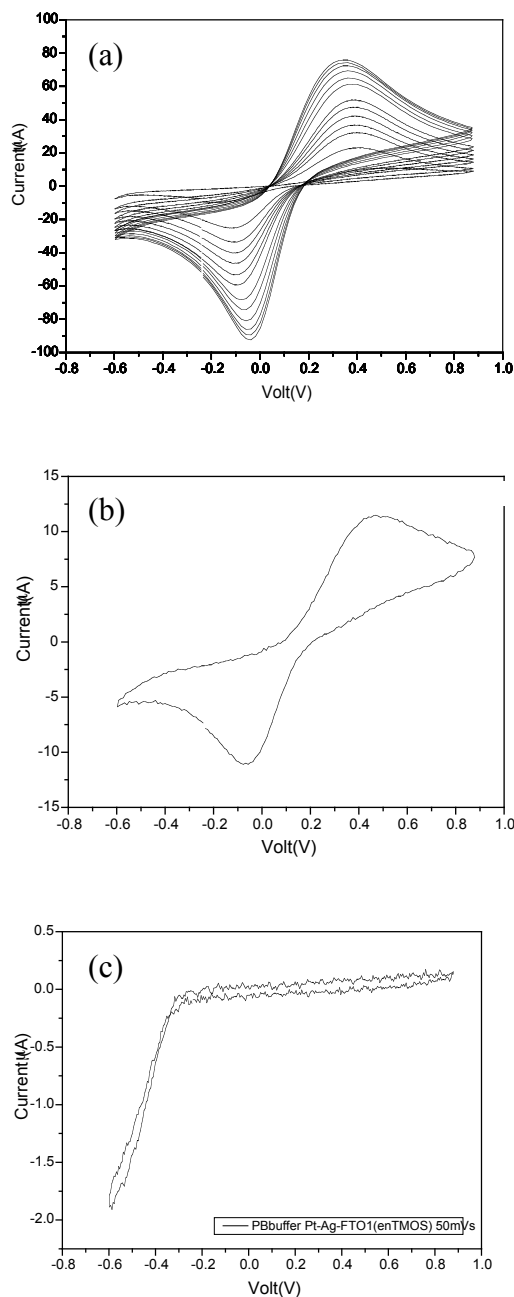

Figure 4: Cyclic voltammograms measured in the liquid cell with an enTMOS coated light guide electrode in (a) 1x phosphate buffer that contains 1 mM $Fe(CN)_6^{3-}$ for a period of 6 hours with each scan at 30 minutes interval (b) after immersed in a 1x phosphate buffer for 48 hours, and (c) 1x phosphate buffer without $Fe(CN)_6^{3-}$ ion.

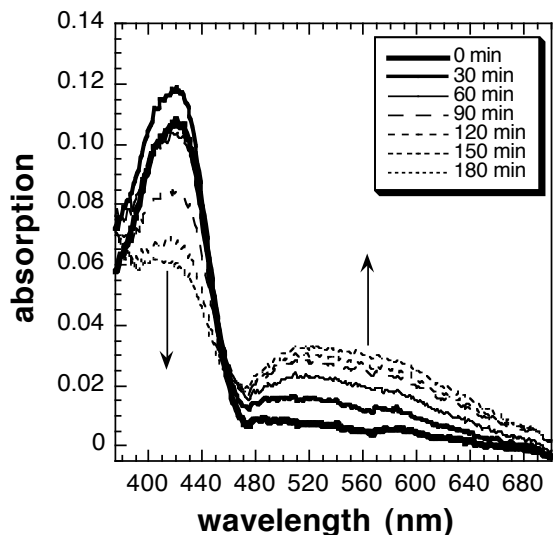Figure 5: Sequence of UV-VIS absorption spectra taken from the enTMOS light guide shows time dependent changes when solution of 1 mM $Fe(CN)_6^{3-}$ was injected in the liquid cell.

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ACKNOWLEDGEMENTS

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