Multiplexed optically thin impedance biosensors for multicellular constructs

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Abstract—**A series of optically thin electrically conducting indium-tin-oxide (ITO) bio-electrodes have been micro-fabricated using sputter deposition and incorporated into single and multi-cellular constructs. The surface nano-topography and optical absorption of the ITO bio-electrodes were examined as a function of sputter plasma oxygen feed concentration. The temperature was fixed at 400°C and the oxygen feed concentrations ranged from 0 to 10% during sputter deposition. Atomic force microscopy characterized the surface properties. The optical absorption was measured between 250 nm and 1200 nm. The layout and application of these electrodes for cardiomyocyte and a multilayer tissue a blood brain barrier construct are demonstrated using photolithography and rapid prototyping. Oxygen feed concentrations near 5% produced high quality stable electrodes. The optical properties were compatible with phase contrast, differential interference contrast, fluorescence, and near infrared microscopy.**

Keywords-Atomic Force Microscopy; Blood-Brain Barrier Model; Cardiomycoyte Biosensor; Cancer cell; Scanning Electron Microscopy; Impedance Spectroscopy; Optical Microscopy

I. INTRODUCTION

Under the right sputter deposition conditions, indium-tin-oxide (ITO) layers can conduct electric currents and pass optical photons [1]. As a result, ITO has many potential applications in biomedical devices where optical transparency and electrical conduction are required. The particular application of ITO to providing multiplexed cellular electrical impedance and optical microscopy measurements has many applications in high throughput microfluidic devices. The electrical and optical properties of indium-tin-oxide, however, are a complicated function of the microfabrication parameters. Despite this, few if any studies, have examined the role deposition parameters play on ITO as a multiplexed electrical and optical sensor in microfluidic devices.

The optical and electrical properties of indium-tin-oxide films vary with the oxygen feed concentration. Low oxygen feed concentrations produce good conducting layers and have relatively good optical absorption. Increasing the oxygen feed concentration produces ITO layers with better optical properties but at the expense of increasing resistivity. How the oxygen feed concentration influences the properties of the bio-electrode in a two or four electrode configuration in contact with an electrolyte solution, however, has not been considered to the best of our knowledge.

This study examined role that sputter deposition oxygen feed concentration plays on the optical absorption and surface properties of indium-tin-oxide biosensors. A series of bio-electrodes were fabricated from fused silica wafers sputter coated with indium-tin-oxide with oxygen feed concentrations ranging from 0 to 10%. Atomic force microscopy (AFM) measured the surface nano-topography and ITO film thickness. The incorporation of the electrode arrays into constructs for cardiomyocyte and a blood-brain barrier model are demonstrated.

II. METHODS AND MATERIALS

A. *Microfabrication*

Two inch, 100 µm thick, fused silica wafers (University Wafer, 1951) were cleaned with acetone and isopropanol and then dried at 120 °C. Indium-tin-oxide was deposited using a sputter coater (AJA International, Orion 8) with oxygen concentrations of 0.0, 2.5, 5.0, 7.5, and 10.0% at 400°C. After 300 seconds of sputter deposition, the ITO thickness was 100 nm. The ITO layer thickness was measured using a profilometer (Veeco, Dektak3). The surface topography was also measured using an atomic force microscope (Veeco, Nanoscope V). Multiple optical absorption measurements were acquired over several regions of the fused silica wafer with ITO by attaching it to a bottomless 96 well plate fitted with a silicon gasket (Grace Bio-Labs ProPlate MP) and measuring it with a plate reader (Molecular Devices, SpectroMax I3).

Electrode patterns were defined using positive photoresist (Shipley, 1813) spin coated (CEE, CB 100) at 3,000 rpm with a ramp speed of 1,000 rpm/sec for 60 seconds. Softbaking for 60 s at 110 °C was followed by exposure to 245 mJ/cm^2 using a mask aligner (Karl Suss, MA/BA 6). The exposed wafer was post exposure baked at 110 °C for 10 minutes. The exposed wafer was developed in a 4:1 deionized water MF 351 mixture for 70 seconds. Chemical wet etching was carried out (Transene, TE-100 ITO etchant) for 70 s at 40 °C. Stripper solution (Microchem 1165) at 70 °C and 70 s removed the photoresist. The wafer was dried on a hot plate at 120 °C for 240 s. The dielectric layer was formed by spin coating polyimide (HD MicroSystems, HD-8820) on the glass-ITO wafer using a ramp speed of 1,000 rpm/s at 3,000 rpm for 30 s. The polyimide was soft baked at 120 °C for 180 s exposed to 360 mJ/cm^2. After developing with TMAH (Shipley, RD6) for 70 s the wafer was rinsed with deionized water and dried. The polyimide was cured at 300 °C under a nitrogen atmosphere for 3 hours.

III. Results And Discussion

Figure 1 shows the AFM images of 10µm×10µm regions of ITO sputtered fused silica with plasma oxygen concentrations ranging from 0-10%. Increasing the plasma oxygen concentration from 0 to 10% tends to produce smoother surfaces. Figure 2 shows the corresponding optical absorption spectra of ITO coated fused silica wafers with varying sputter plasma oxygen concentrations. Increasing the oxygen feed concentration decreased the optical absorption.

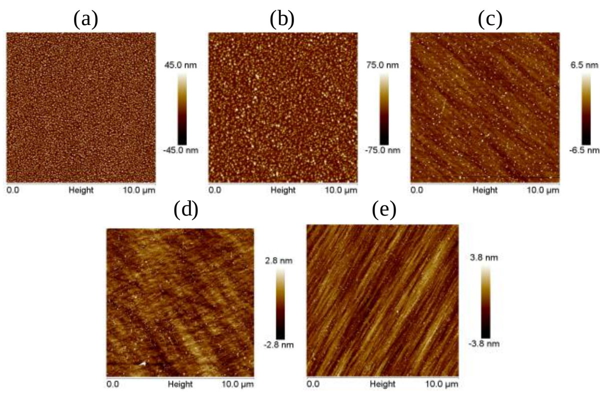

Fig. 1. Representative AFM images of ITO sputtered fused silica surfaces with plasma oxygen concentrations at (a) 0%, (b) 2.5%, (c) 5.0%, (d) 7.5%, and (e) 10%. Increasing the plasma O_2 concentration from 0 to 10% tends to produce smoother surfaces.

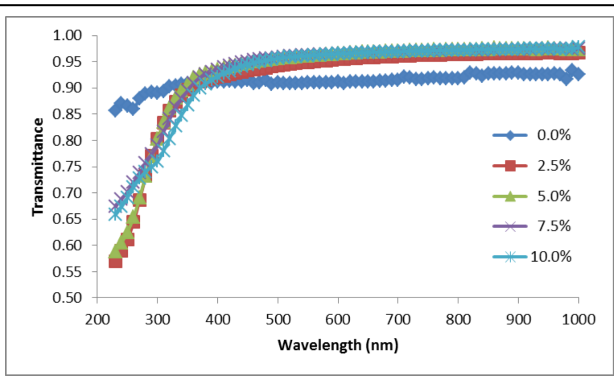

Fig. 2. Optical absorption spectra of ITO coated fused silica wafers with varying sputter oxygen feed concentrations. Increasing the oxygen plasma concentration increases the transmittance above 400nm. Below 300 nm increasing oxygen concentrations decrease the transmittance.

The incorporation of the bioelectrodes into arrays for cellular measurements are shown in Fig. 3. The incorporation of electrode microelectrodes in conjunction with the optically thin ITO electrodes permits the ITO bioelectrodes to be used to quantify cardiotoxicity. Interacting multicellular constructs are currently being examined using rapid prototyping. The construct shown in Fig. 3b permits the electrode to be incorporated into a blood-brain barrier model. The bio-electrodes are incorporated into the bottom layer to quantify neuro toxicity both optically and electrically.

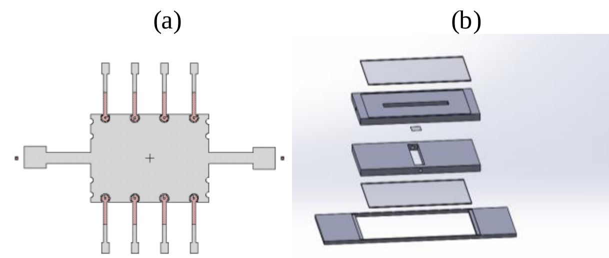

Fig. 3. Integration of bio-electrode into constructs for multicellular measurement systems. (a) Including gold micro-electrodes allows the arrays to be used as a cardiomyocyte drug toxicity system. (b) An assembly of channels separated by a micro-porous membrane allows the electrode arrays to be used in a blood-brain barrier model with separate compartments for endothelial and neural cells.

The surface topography plays in important role in cellular attachment and adhesion. The nanoscale surface properties are a critical determinant of cellular attachment. Understanding how the sputter deposition parameters influence cellular attachment is, therefore, important to their biomedical application. The nanoscale topography influences cellular attachment directly or indirectly by influencing protein absorption. The results of these measurements show that on the scale of a biological cell, the surface roughness of ITO is highly dependent on the oxygen plasma concentration.

IV. Conclusion

Increasing the sputter plasma oxygen concentration from 0 to 10% produced an overall decrease in surface roughness. Above 400 nm, increasing the oxygen concentration also increased the overall transmissivity. Bellow 500 nm, a complex transmissivity pattern was observed. The feasibility of incorporating these bio-electrode arrays into single and multi-cellular constructs has also been demonstrated.

Acknowledgment

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References

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