Sensing Nitric Oxide Neuronal Messengers Using Screen-Printed Carbon Micro-Electrode Arrays

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SUMMARY

This paper presents the development of a microfabricated electrochemical sensor for measuring neurotransmitter activity in brain tissue. Electrochemical sensing is used to detect neuronal messengers such as nitric oxide (NO). Sensing is accomplished using the principle of an oxidation-reduction (redox) reaction at the carbon electrode surface that produces a current signal proportional to the NO concentration. The carbon microsensors are patterned onto an array using a carbon polymer ink that is screen-printed onto electrically conductive traces. Thick SU-8 photosist serves a dual-role, acting both as an insulating layer and also as a mold for the carbon ink. Surface profiling showed that our process produces mesa-shaped electrode structures. The electrochemical activity of the sensors is tested potentiostatically and NO sensitivity is demonstrated. Sensitivity to various interferents is also tested to determine electrode selectivity. Microfabricated sensor arrays would provide a novel experimental means to study the spatial and temporal neurochemical activity across brain tissue.

Keywords: nitric oxide, dopamine, screen printing, carbon micro-electrode array.

INTRODUCTION

Neuronal communication occurs through a combination of electrical and chemical signals. A major area of interest in the field of neuroscience involves determining and interpreting the ways by which neurons and ensembles of neurons communicate with each other in the brain. Conventional approaches rely on recording electrical signals from neurons, however, the chemical activity of neurons is also important in understanding physiological and pathophysiological mechanisms.

Nitric oxide (NO) is a membrane permeable gas involved in neuronal modulation as well as pathophysiological processes [1]. Although it was first identified as an endothelium-derived relaxing factor, NO plays an important role in the peripheral and central nervous system. NO acts as a neuronal messenger as well by a product of brain injury [1-4]. Carbon fiber sensors for in vivo and in vitro recordings have elucidated the role of NO during brain injury [5]. Other sensors monitor current on glassy carbon electrodes, or measure light produced from NO interactions [6,7]. Because of the gaseous nature of NO, an array of sensors should provide a more complete picture of its impact across an entire tissue. Multiple sensor sites can also provide a better method for studying NO diffusion and its effect on various regions of brain tissue.

Application of microfabricated devices to study electrical signaling in neural tissue has been a growing field over the past decade [8,9]. However, the technology for neurochemical sensing is not as developed. We use conventional microfabrication technologies along with screen printing techniques to develop a novel chemical sensor for measurement of neurotransmitters. The screen printing technology provides a rapid, inexpensive, and reproducible method for depositing thick, stable films. One common thick film used in sensor development is carbon ink. Carbon inks are used to create a variety of sensors ranging from lead sensors to biosensors [10-12]. Carbon ink generally contains graphite particles, organic polymers acting as a binding agent, and other components for adhesion and dispersion [13]. When a potential is applied between the carbon site and the surrounding solution, a redox reaction between NO and carbon is known to occur. The resulting current is proportional to the NO concentration, which allows for nitric oxide measurement.

FABRICATION

Standard microfabrication processes are used to create the sensor substrate and the fabrication steps are illustrated in Figure 1.

Aluminum Traces

A double polished, 4-inch fused quartz wafer (Valley Design Corp., Westford, MA) having a nominal thickness of 500 µm is used as a substrate to provide good ink adherence. More importantly, the transparent properties of quartz should allow for combined optical and neurochemical sensing. Electron beam evaporation is used to deposit 0.5 µm of aluminum directly on the quartz substrates. Photolithography is next utilized to pattern the aluminum layer, and an acid etch removes the unwanted, exposed metal from the wafer (Figure 1a(i)). The aluminum traces serve as connectors between the carbon sites and external bonding pad connections.
Figure 1. Schematic of fabrication steps. (a) Cross-sectional diagrams of fabrication. (i) Aluminum is evaporated on fused quartz wafer and patterned. (ii) SU-8 is spin coated and patterned to open contacts to aluminum. (iii) Screen printing is used to apply carbon ink. (iv) Completed carbon sensor. (b) Layers used to create carbon sensors with a zoom of finished die. The square array is approximately 4mm wide.

SU-8 Photoresist

Subsequently, the thick SU-8 photoresist is spin-coated onto the wafer to provide insulation between the aluminum and the carbon sites (Figure 1a(ii)). The SU-8 (MicroChem Corporation) also serves as a mold for the deposited carbon ink. The quartz wafers are dehydrated, and then a primer layer of hexamethyldisilazane (HDMS) is spun on to promote photoresist adhesion. The SU-8 is spin coated using a two-step process. A slow spin at 500 rpm for 15 seconds spreads the SU-8 across the wafer, and the second spin at 4000 rpm for 30 seconds results in a 25 μm thick dielectric layer of SU-8. The wafer is then pre-baked on a hot plate at 100°C for 1 hour before it is placed in an oven with a temperature of 90°C for an additional 3 hours. After the pre-bake is completed, the SU-8 is photolithographically exposed to open contacts to the aluminum traces. The wafers are exposed for 3 minutes and 45 seconds using a Karl Suss exposure/aligner. Next, the SU-8 is baked for an additional 30 minutes on a hot plate at 100°C and then placed in a developer for 30 minutes. Finally, the wafer is removed from the SU-8 developer, and a post-bake of 5 minutes is performed in an oven at 120°C.

Carbon Deposition

Next, the carbon ink (7102 Dupont Electronic Materials) is deposited on the exposed aluminum contacts. Individual carbon sensing sites are formed through a screen printing technique (Figure 1a(iii)). A 25 μm brass shim (Precision Brand) forms the screen for the carbon ink. To construct the screen, photoresist is applied to both sides of the brass shim for etch protection, and photolithography is performed to reveal the brass regions where holes are to be etched. A nitric acid solution (50% nitric acid, 50% water) is used to completely etch through the exposed brass. The screen is mounted on a screen printer and aligned with the fused quartz substrate. Next, a squeegee is used to apply the carbon ink across the brass screen. Carbon ink is forced through the holes in the screen onto the aluminum contacts. The ink is then cured at 120°C for 5 minutes.

**CARBON MICRO-ELECTRODE ARRAY**

Several patterns of carbon arrays were designed to determine which device placed the most electrodes in the regions of interest in the tissue. Each carbon site is slightly less than a millimeter in diameter and the sensors are separated roughly by 0.5 millimeters (Figures 1b and 2b). As seen from the profilometer scans (Figures 2a,c), the ink dries giving a rough