Developing Nanoscale Electrodes for Sensitive Detection of Brain Cell Activities

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Abstract:

Vertical nanopillar electrode arrays have garnered much attention due to their applications in the study of the electrical behavior of neurons. Previous methods for measuring neuronal activity include the use of planar multielectrode arrays (MEAs); however, neuronal mobility and inefficient neuron-electrode interactions on flat substrate surfaces make it difficult to monitor the activity of specific neurons over extended periods of time. Vertical nanopillar arrays serve as neuron traps by effectively pinning the neurons and also offer a non-invasive measurement technique, thus allowing for long-term study. The aim of this work is to develop a novel electrode device composed of a patterned MEA fabricated on quartz and glass substrates using photolithography and subsequently electrodepositing vertically aligned Au nanopillars of varying diameters (200-600 nm) on the MEA. Using optical microscopy and scanning electron microscopy, it was noted that the specified quality and morphology of the devices were maintained throughout processing.

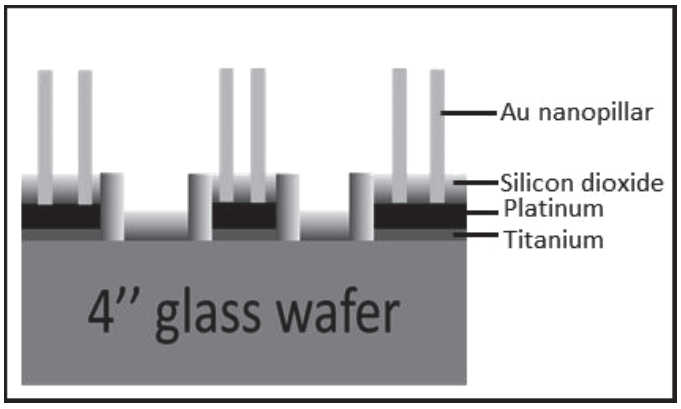

Figure 1: Representation of the vertical nanopillar electrode device.

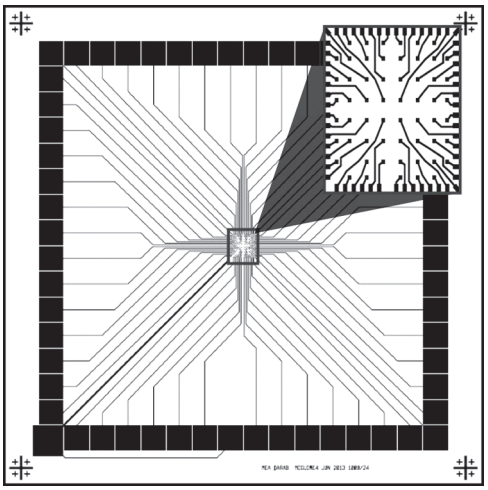

Figure 2: Electrode design pattern.

Introduction:

One of the main problems in measuring action potentials (AP) of neurons is neuronal mobility. Syntheses of many current nanostructures used to address this problem involve complex procedures and loading the neurons can be difficult and time consuming [1]. Other methods to control migration include the use of chemical modification or patterned deposition of proteins to promote neuron-electrode adhesion; however, it is difficult to control how many neurons are attached to each electrode [1].

Accurate AP measurements require efficient coupling of the measuring electrode and cell membrane. Intracellular methods such as patch clamping provide accurate measurements but are invasive and therefore reduce cell life, limiting recording times to only a few hours [2]. Extracellular techniques such as multielectrode arrays (MEAs) have lower signal quality due to the infrequent one-to-one neuron-electrode interactions [2].

Combining the extracellular and intracellular measurement technologies of multielectrode arrays and vertically aligned nanopillars, respectively, provides a non-invasive technique for accurate AP measurements. Vertical nanopillar arrays serve as low throughput intracellular recording electrodes, effectively pinning the neurons for long-term studies [2]. Previous work by Xie et al. has shown that neuritic protrusions wrap the nanopillars with a thin layer of membrane to improve interactions [1]. It is also proposed that vertical nanopillars serve as focal adhesion substrates, allowing for stronger anchorage of the cell matrix than on flat surfaces leading to preferential adhesion of neurons to the nanopillars [1].

In this work, MEA devices are designed and fabricated on glass and quartz substrates. These substrates allow for observation of the nanopillars and neurons on top of the device using optical microscopy. Vertically aligned gold nanopillars are electrodeposited on the surface of the MEA (Figure 1). Gold

is chosen because it is a good conductor, unreactive in most aqueous reagents and has good self-assembly chemistry for surface functionalization. This device will allow for both long-term and multiplexed measurements of the AP of individual neurons at the synapse.

Experimental Procedure:

Two masks with multiple square die, each of edge length 20 mm were designed (Figure 2) and used to pattern four-inch quartz and glass wafers with electrode (10 nm Ti/100 nm Pt) leads and pads using standard photolithography methods. The sizes of the inner contact pads were varied to facilitate variation in nanopillar diameter. The substrate's surface was passivated with a 1.8 μm SiO_2 layer deposited by low pressure chemical vapor deposition. A reactive ion etch was used to expose the outer contact pads of the device for wiring bonding during packaging. The wafer was diced into pieces. A simple electrical test was performed for each piece to confirm electrical connection using a digital multimeter. Electron-beam lithography was used to make holes of diameter 200-600 nm to facilitate pillar growth. A 24K pure gold solution (Gold Plating Services) maintained at 65ºC was used for electroplating.

Results and Discussion:

Optical microscopy and SEM images show that the MEAs maintained the features of the original design (Figure 3 and 4) with clear lines and only small changes (1-2 μm) in feature size. These changes were expected since the device fabrication involved several processing steps. The electrical test confirmed all MEA devices were functioning as expected, indicating that the passivation layer had been successfully etched away from the surface of the outer contact pads.

Attempts to etch through the oxide following e-beam lithography were unsuccessful because the layer of e-beam resist used was too thin. The high etch rate of the resist led to significant reduction in oxide thickness, creating a surface that was not conducive to nanopillar growth. Nevertheless, dummy devices were used to confirm the gold plating conditions.

Conclusions and Future Work:

Patterned MEAs were successfully designed and fabricated using standard photolithography techniques. However, additional work needs to be done in optimizing the technique used in preparing the MEA surface for nanopillar growth. Once these challenges are overcome, nanopillar arrays of varying diameters (200-600 μm) will be electrodeposited on the MEA. In the future, we also hope to vary nanopillar shape and composition to determine how these properties affect action potential measurements in neurons. These nanopillar electrode devices have the potential to serve as high-sensitivity probes for detecting membrane potentials at the synapse level which will help in understanding the long term behavior of neuronal circuits and thus provide insight into the mechanics of the brain.

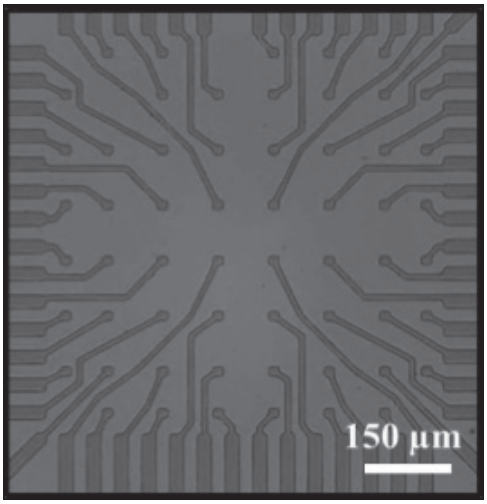

Figure 3: Optical microscopy image showing the center of the MEA, where nanopillar arrays will be grown.

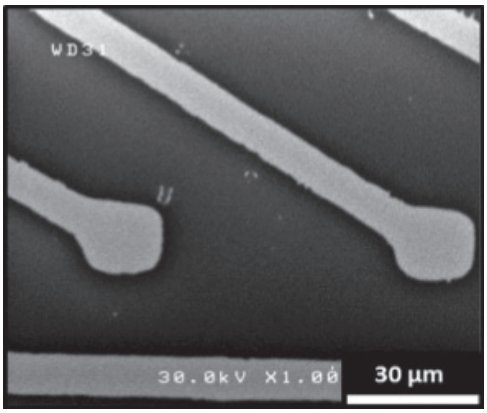

Figure 4: SEM of inner contact pad of the MEA device.

Acknowledgements

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References:

[1] Xie, C., et al. Nano Lett. Noninvasive neuron pinning with nanopillar arrays 10, 4020-4024 (2010).

[2] Xie, C., et al. Nature Nanotech. Intracellular recording of action potentials by nanopillar electroporation 7, 185-190 (2012).