

# PARYLENE TECHNOLOGY FOR MECHANICALLY ROBUST NEURO-CAGES

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We present a novel process to produce parylene cages for the *in vitro* study of cultured neural networks. For the first time, a neuro-cage fabrication technology is demonstrated that is scalable to high density cage arrays and able to withstand the chemical and mechanical rigors of supporting cellular cultures for long-term study.

Hippocampal neurons are known to play a role in learning and memory, thus efforts have been taken to study the development and plasticity of hippocampal neuron networks. Previously, it has been difficult to study these populations in detail. *In vivo* measurements are ideal for observing neurons in their natural environment; however, it is beyond our ability to understand the complex recordings obtained from tissue and additionally, observations of individual neuron behavior is difficult. To greatly improve the ability to research neurons, many have developed *in vitro* techniques involving patterned extracellular electrode arrays [1, 2]. Even so, neuron mobility and the lack of specific connections between neurons and electrodes severely limit our interpretation and understanding of these systems. To perform long term studies of complex neuronal interactions, neuro-wells were designed [3, 4]. Dissociated neurons are placed in each well and held in close proximity to a specific extracellular electrode. While this device drastically improved our ability to perform neural research, the complexity of fabrication and difficulty in scaling the design have inhibited further device development. Parylene technology is uniquely suited to solving these problems and also allows for the integration of microfluidics.

4×4 arrays of cages arranged on 100 μm centers have been fabricated (Figs. 1-6). This spacing is selected to match the range of axonal growth in cultures. Each cage consists of a loading hole and 8 tunnels radiating from the cage base. These tunnels allow for the outgrowth of axons and dendrites.

Several technological innovations were required for fabricating neuro-cages. First, in order to provide robust mechanical anchoring of the cages to the substrate, XeF<sub>2</sub> gas phase etching was used to create undercut anchoring structures with large exposed surface area. Thus, neuro-cages are able to root themselves solidly into the silicon substrate and survive sterilization and cell culture treatments (Figs. 7-11). In addition, a high-aspect ratio etching technique using a modified Bosch-like process was adapted to patterning the parylene structural material. Oxygen plasma etching alone is only able to produce structures with a 1:1 aspect ratio. Aspect ratios of 2:1 or greater are possible using ICP (inductively coupled plasma) -assisted etching techniques.

The successful growth and development of rat hippocampal neurons on parylene surfaces has been demonstrated in [5]. Mechanical robustness in terms of culture support and survivability in chemical treatments have been verified. Currently, neuro-cages are being further optimized for neural cultures. The next step is to integrate platinized gold electrodes for non-destructively recording and stimulating trapped neurons. In this manner, specific cells can be targeted for study without interfering with cell growth. It is hoped that the device can aid in the design of future *in vivo* studies.

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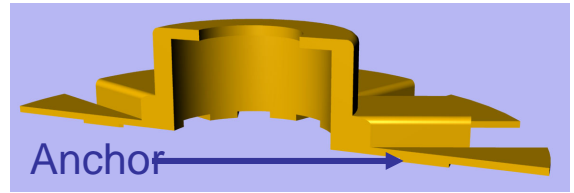


Fig. 2 3D Cross Section Through Anchor

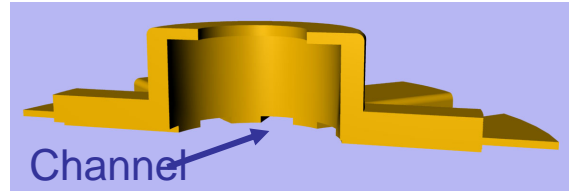


Fig. 3 3D Cross Section Through Channel

Fig. 1 Process Flow

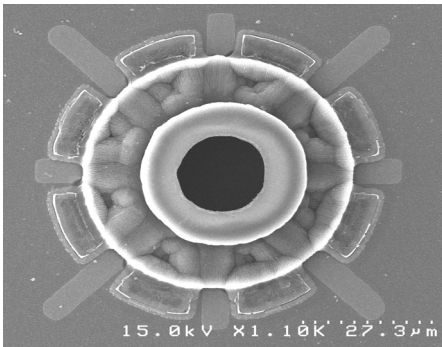


Fig. 4 Top View of Cage

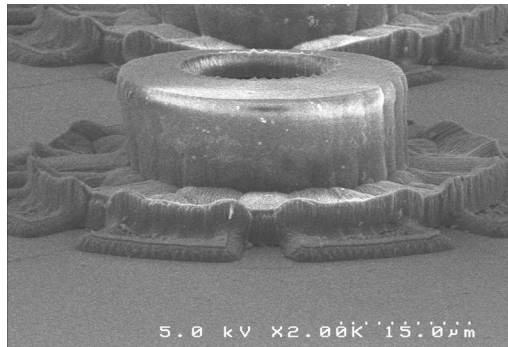


Fig. 5 Tilted View of Cage

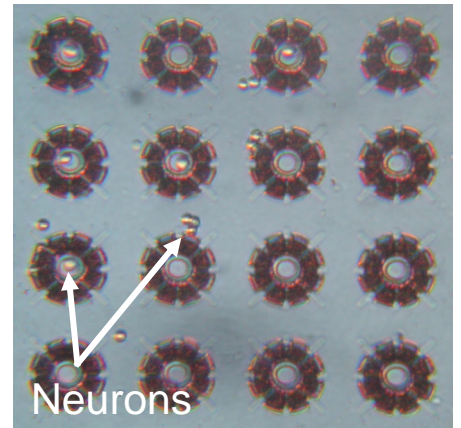


Fig. 6 Partially Loaded Array

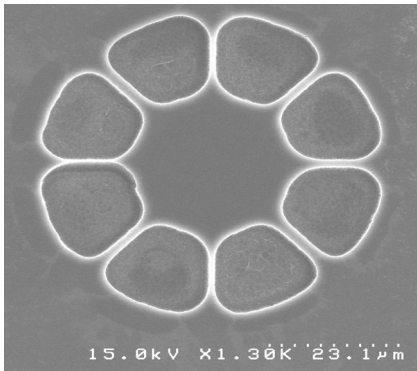


Fig. 7 Anchors Etched in Substrate

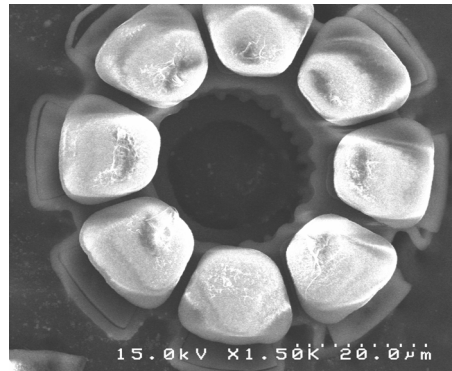


Fig. 8 Upside-down Cage

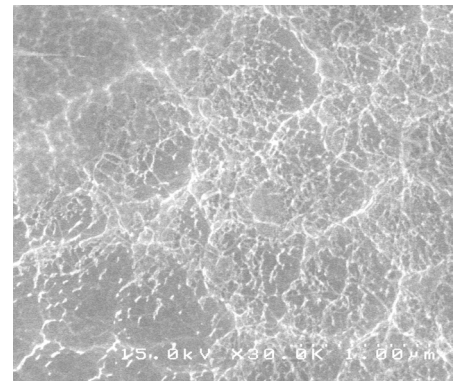


Fig. 9 Roughness in Anchor Area

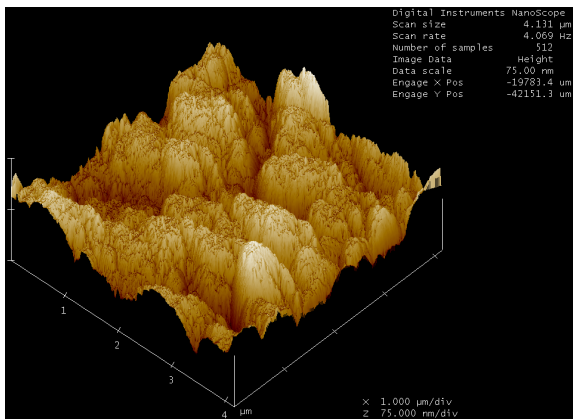


Fig. 10 AFM of Anchor Area

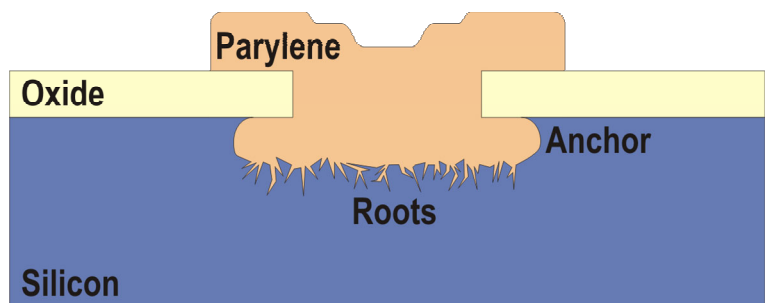


Fig. 11 Anchoring Method