

PARYLENE TECHNOLOGY FOR MECHANICALLY ROBUST NEURO-CAGES

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ABSTRACT

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.

Keywords: parylene, mechanical anchoring, neuro-cage

1. INTRODUCTION

Hippocampal neurons are known to play a role in learning and memory, thus it is interesting 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 extremely difficult to obtain and understand the complex recordings from tissue. Additionally, observations of individual neuron behavior are difficult. One strategy to investigate neurons is to use *in vitro* techniques such as patterned extracellular electrode arrays [1, 2]. Even so, neuron mobility and the lack of specific connections between neurons and electrodes severely limit our ability to study these systems. To enable long term investigation of complex neuronal interactions, neuro-wells were designed [3, 4]. Each well contains a single dissociated neuron that is held in close proximity to an extracellular electrode. While this device drastically improved neural research technology, difficulties encountered in fabrication and scaling the design have inhibited further device development. Parylene devices have been fabricated that are uniquely suited to solving these problems (Figure 1). Here, improvements to mechanical anchoring, first explored in [5], over cages developed in [6] are described in detail.

2. EXPERIMENTAL

4×4 arrays of cages arranged on 100 μm centers have been fabricated as a test bed for hippocampal neuron cultures (Figure 2a). This spacing is selected to match the range of axonal growth in cultures and neural networks of this size are known to produce connections of sufficient complexity to warrant detailed study. Each parylene cage consists of a loading hole (15 μm in diameter) and 8 tunnels (15 μm long, 5 μm wide, and 0.5 or 1 μm high) radiating from the cage base (30 μm in diameter and 15 μm high) (Figure 2b & c). These tunnels allow for the outgrowth of axons and dendrites. Parylene cages are anchored to the substrate and sit on a SiO₂ surface.

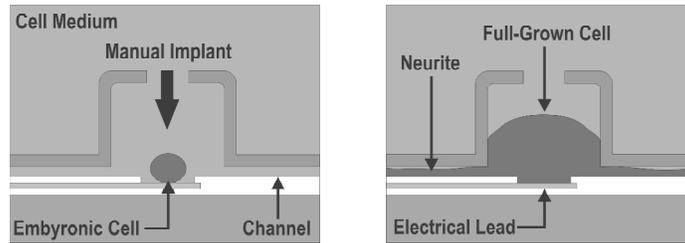


Figure 1. Neuro-cage concept showing (a) a loaded embryonic neuron and (b) a mature, trapped neuron

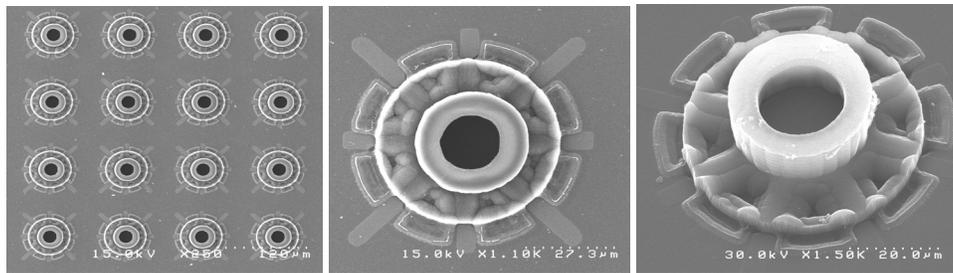


Figure 2. SEM images of (a) a neuro-cage array, (b) top view of a cage, (c) tilted view of a cage

Several technological innovations were required for fabricating neuro-cages. The overall cage structure is depicted in Figure 3. First, in order to provide robust mechanical anchoring of the cages to the substrate, XeF_2 gas phase etching was used to create anchoring structures with large exposed surface area. Shallow pits measuring $0.5 \mu\text{m}$ deep are created by etching and undercutting the silicon substrate through a SiO_2 mask. Then, these structures are filled with parylene. The resulting mushroom-shape of structures is seen in Figure 4 and the etched silicon surface in Figure 5. Thus, the parylene neuro-cages are able to root themselves solidly into the silicon substrate through mechanical interlocking. This enables cage survival through sterilization and cell culture treatments.

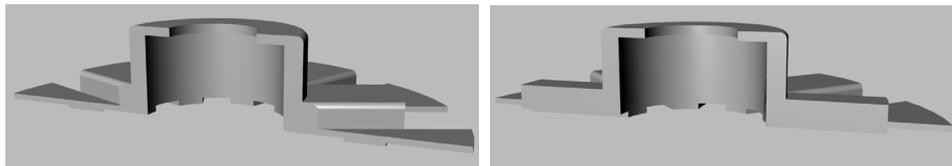


Figure 3. 3D cross section renderings of cages through (a) anchors and (b) tunnels

In addition, a high-aspect ratio etching technique using a modified Bosch-like process was adapted to pattern 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.

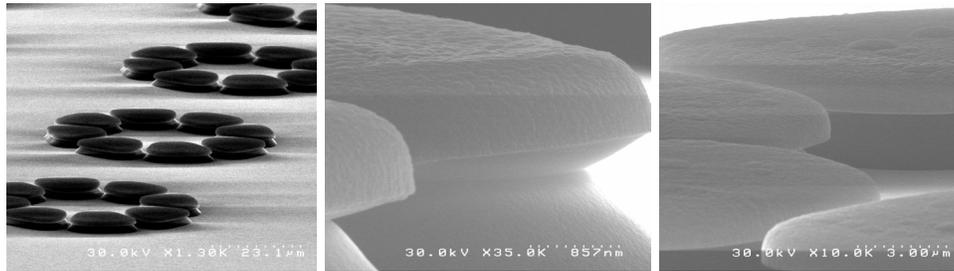


Figure 4. SEM images of (a)-(c) released parylene anchor structures

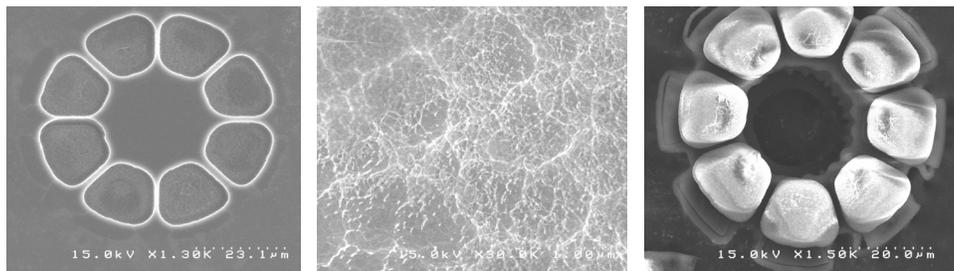


Figure 5. SEM images of (a) etched anchors in Si, (b) etched surface roughness, and (c) upside-down cage

3. RESULTS AND DISCUSSION

The surface roughness and undercut that result from XeF_2 etching are extremely important in keeping the neuro-cages firmly attached to the substrate during sterilization and culturing. Cages survive the Scotch tape test and removal of the cages by pressure sensitive adhesive film can only be accomplished when the SiO_2 overhang is etched away. A cage removed using this technique is shown in Figure 5. The quality of cage adhesion to the substrate by mechanical anchoring far surpasses that achieved by chemical adhesion promotion techniques.

The compatibility of neuronal cultures with parylene neuro-cage arrays has been demonstrated in [6] and is seen in Figure 6. Here, cultures have been sustained for up to 3 weeks. Cleaning techniques are now being developed to remove old neural cultures such that the arrays can be reused for repeated culture experiments.

4. CONCLUSIONS

The successful growth and development of rat hippocampal neurons on parylene surfaces has been demonstrated. Mechanical robustness in terms of culture support and survivability in chemical treatments has 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. In addition, similar structures can be used to trap and investigate the long term behavior of other types of individual cells or cellular networks.

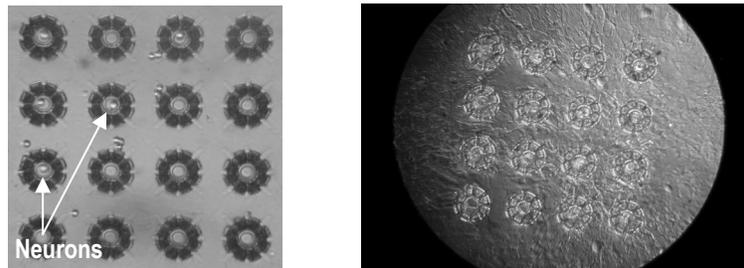


Figure 6. (a) Optical image of partially loaded cage array and (b) Nomarski image of 3 week old culture

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