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HIGH ASPECT RATIO SU-8 STRUCTURES FOR 3-D CULTURING OF NEURONS

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ABSTRACT

This paper presents a simplified fabrication method for high aspect ratio SU-8 towers for culturing three-dimensional networks of neurons. The photosensitive epoxy SU-8 is an attractive candidate for these 3-D tower structures and substrates because of its mechanical stability, biocompatibility, and potential for high aspect ratio fabrication. Fabrication is effectively simplified by an unusual double exposure technique, allowing tower structures and an integral SU-8 substrate to be formed from the same epoxy deposition, thereby addressing column-to-substrate adhesion issues that have traditionally limited the aspect ratios achieved with conventional fabrication techniques. Aspect ratios up to 35:1 have been achieved, and successful cell culturing has been demonstrated.

INTRODUCTION

Two-dimensional neuron culturing has become routine for basic science studies ranging from toxicity to cell attachment and growth. However, it is critical to develop cellular systems for *in vitro* measurement that mimic the three-dimensional *in vivo* environment as closely as possible, for enhanced cellular survival as well as functionality in networks more representative of tissue-engineered structures and systems[1][2][3].

As a simple example of a scaffold for growth of three-dimensional cellular networks, consider an array of high aspect ratio polymeric towers extending normally from an integral polymeric substrate. This configuration facilitates neuronal cell growth on and between the towers in addition to enabling future perfusion, stimulation and monitoring capabilities via pores and electrodes. The high aspect ratio structures allow study of thicker brain slices, thereby more closely resembling the *in vivo* environment.

A straight forward approach to the three-dimensional structures would be to form polymeric towers adhered to a glass, polymeric or otherwise suitable substrate. A number of techniques exist to fabricate these structures. For example, a thick photoresist could be cast on a substrate, the towers photolithographically defined, and the photoresist developed to leave polymeric towers adhered to the substrate. The problem with this approach is that the adhesion durability between tower and substrate must remain high, even in aggressive (e.g. aqueous and saline) environments. In this work, we demonstrate a fabrication method in which towers and substrate are formed from a single, *unitary* polymeric piece, thus eliminating the issue and providing a three-dimensional micromachined scaffold particularly appropriate for cell culturing.

FABRICATION

The fabrication sequence is summarized in Fig. 1 and begins with patterned chromium on a glass mask as a temporary substrate. A one-millimeter-thick layer of SU-8 is spun on the patterned chromium side of the mask enabling good contact between the SU-8 and the pattern. The pattern used is a checkerboard later utilized in dividing the completed devices into individual arrays. After soft baking, the sample is then exposed to a relatively (5000mJ/cm^2 for a 700μm thick film) low exposure dose (200mJ/cm^2) from the bottom through the glass plate to cross-link a 300μm thick SU-8 plateau (still embedded in unexposed SU-8 film) immediately adjacent to the transparent portions of the underlying chromium mask. Only a 300 μm depth of SU-8 is crosslinked due to the low exposure dose utilized. Prior to developing, the sample is exposed from the top using a large exposure dose (5000mJ/cm^2) through a tower mask to crosslink 700μm tall SU-8 towers. The sample is then developed, removing the

uncrosslinked regions and resulting in tower arrays on polymeric substrates separated by the checkerboard pattern but still attached to the chromium glass mask. After development, a topside flood exposure is performed to further solidify the structure. Individual arrays suitable for culturing are then formed by separation of the individual tower-bearing polymeric substrates from the glass mask via an additional bake at 100°C for 40 minutes.

Structures over 700 μm tall with aspect ratios as high as 35:1 have been fabricated with good repeatability. Arrays of 8x8 and 16x16 towers with tower diameters ranging from 20 μm to 200 μm and edge-to-edge tower spacing ranging from 100 μm to 225 μm were fabricated using the technique outlined above. Fig.2 shows SEM views of a variety of fabricated geometires, while Fig. 3 shows a top view of the completed SU-8 towers.

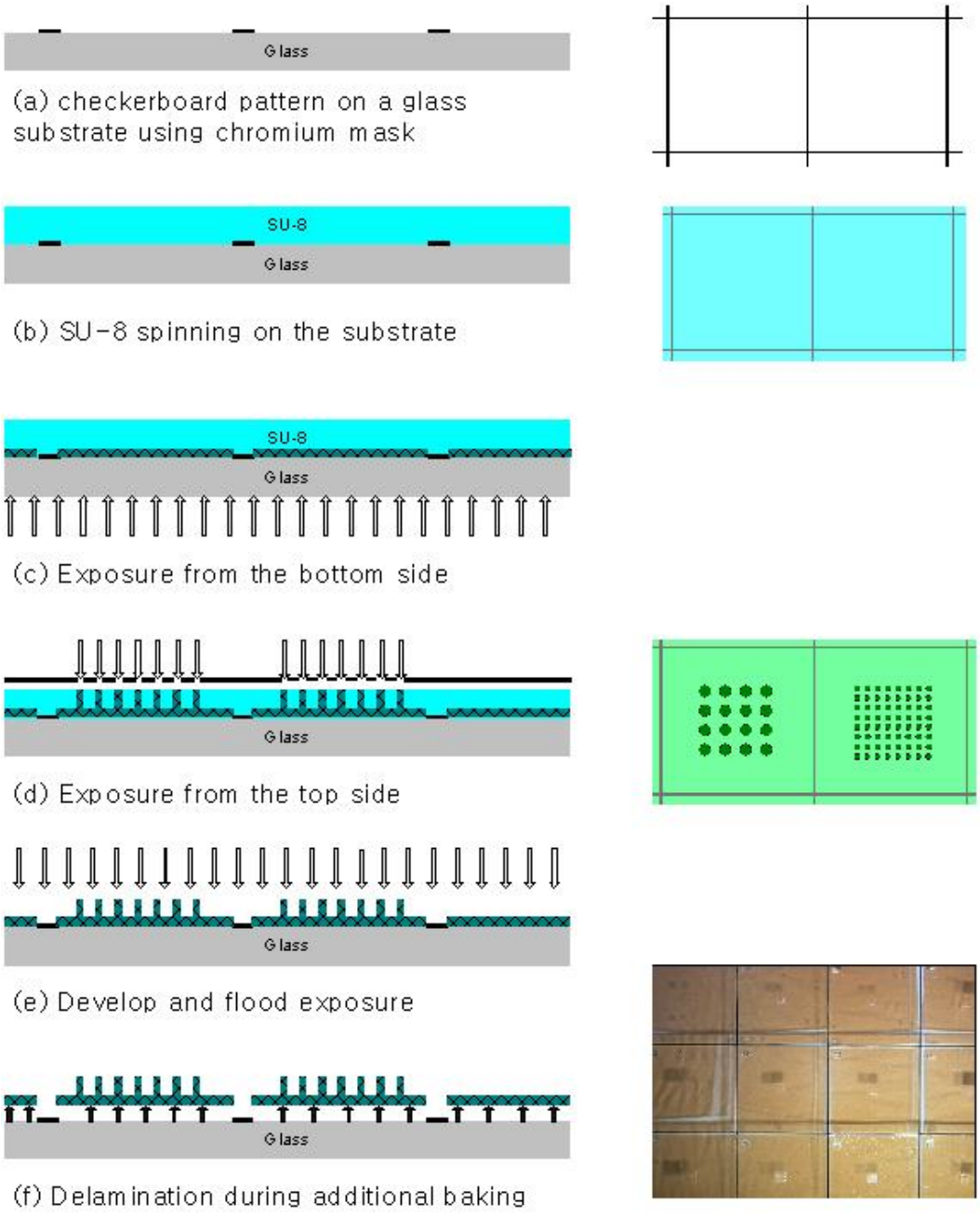

Fig. 1: Fabrication Scheme

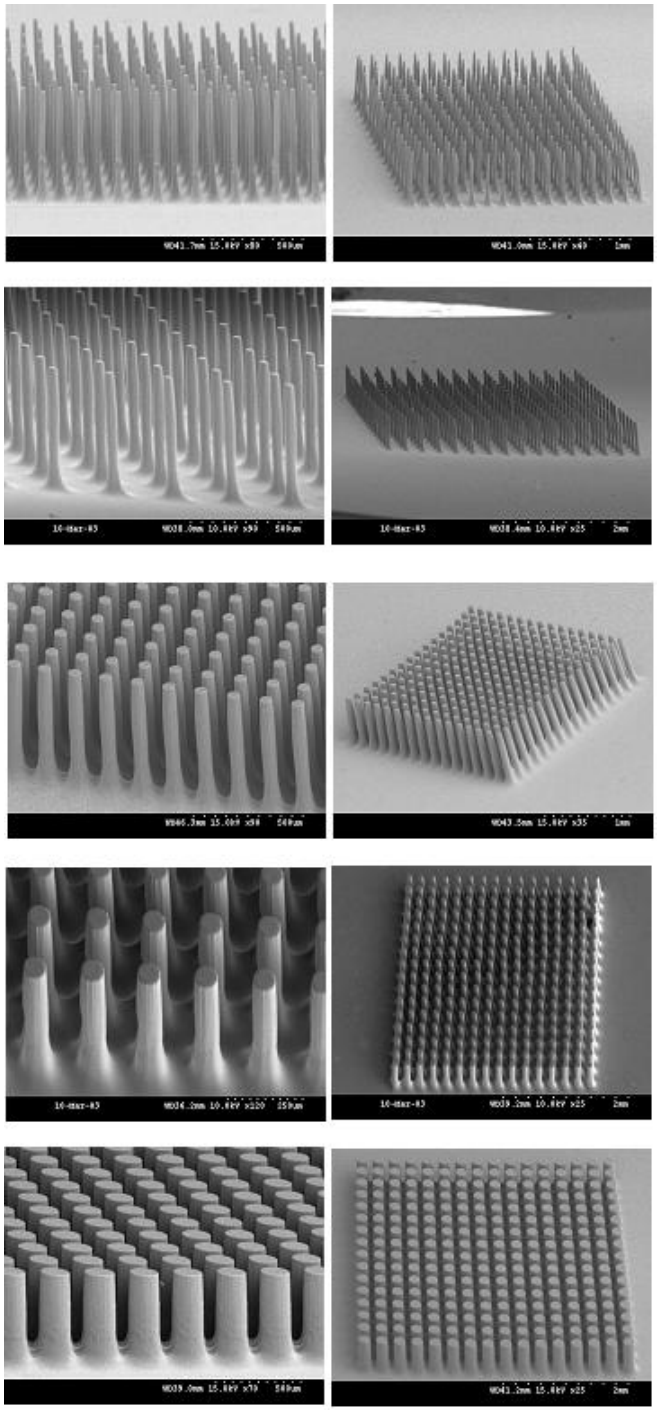

Fig. 2: SEM micrograph of fabricated 3-D scaffolds. The towers are 700 in height and range from 20~200 diameter. Both 8x8 and 16x16(shown) arrays have been fabricated

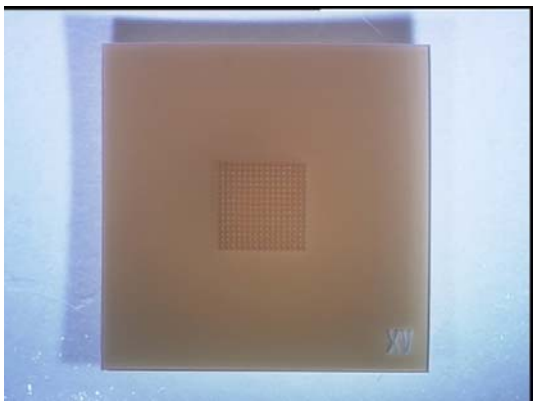

Fig. 3: Top view of the completed towers

CELL CULTURING

This section describes how the SU-8 tower based substrates were tested for cell culture. First, the substrates were detoxified by dry heating at 110°C for three days, then by wet heating in DI water (PURELAB Plus, U.S. Filter, Lowell, MA) at 45 °C for one day, followed by a one hour drying at 45°C in an oven (Economy Oven, Model 1320, VWR Scientific, West Chester, PA). Once dry the surfaces were sterilized under UV light for 2 hours (Class II A/B3 Biological safety Cabinet, Forma Scientific, Inc., Marietta, OH). Upon sterilization, the surfaces were incubated in a 0.005% poly-L-lysine, 70,000 – 150,000 Daltons molecular weight, solution (Sigma Chemical Co., St. Louis, MO), at 37°C in an incubator (CO2 water jacketed incubator, Forma Scientific, Inc., Marietta, OH) for 1 day, at the end of which the poly-L-lysine coating solutions were aspirated away using a Pasteur pipette inside the bio-safety hood.

Surfaces prepared in the above manner were seeded with embryonic day seventeen primary cortical neurons at a seeding density of 900 cells/mm2. (The cells were obtained from rat embryos following an animal usage protocol (99-04) which has been approved by the Institute Animal Care and Usage Committee (IACUC) of the Georgia Institute of Technology.) The cells were plated in Neurobasal Medium supplemented with 2% B-27 (both from Invitrogen Corporation, Grand Islant, NY) and 500 μM L-Glutamine (Cellgro, VA). The first media change was done one day post plating by removing half the media and replacing it with 1.25 times fresh media. A similar media change was done approximately every 5 days until day 20 when a terminal live dead assay (Live/Dead Viability/Cytotoxicity Kit, Molecular Probes, Eugene, OR) was performed.

Fig. 3, A and B show phase contrast microscopy pictures (Nikon Eclipse TE 300, Marietta, GA) after one day of neural culture on the polystyrene control (6 well cell culture plate,

Corning Incorporated, Corning, NY) and SU-8 substrates respectively.

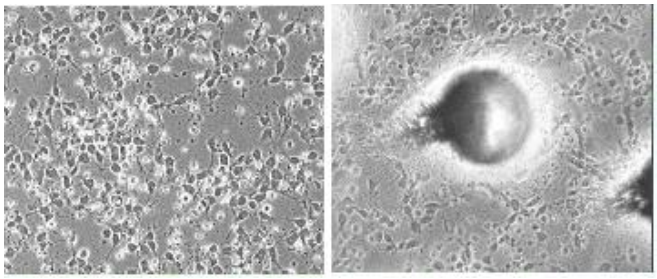

Fig. 3: Phase contrast microscopy (200X magnification) of neural culture on a polystyrene control (A) and around a tower on a SU-8 substrate (B). Both figures A and B show neurons having formed cell processes

Fig. 4, A and B show fluorescent microscopy pictures after twenty days of neural culture on the same two surfaces. As can be seen, the neurons were viable in a network around the towers even after 20 days in culture on the SU-8 surfaces, indicating the potential of these structures for use in MEMS based 3-D neural interfaces. Several sterilization, detoxification, and surface treatment schemes were tried (data not presented) before the ones used in this cell culture study were selected for cell culture.

Thus we have developed a specialized process for fabricating micro-scaled SU-8 structures, and detoxifying, surface coating and sterilizing them for use in neural cell culture applications.

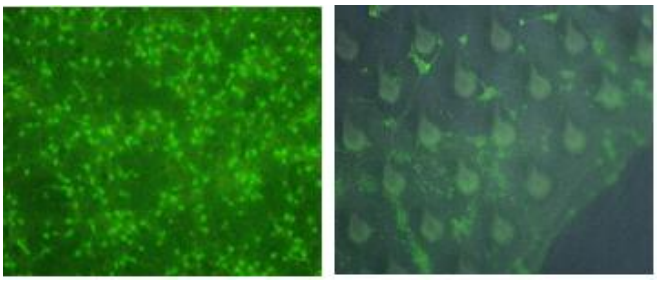

Fig. 4: Fluorescent microscopy of neural culture on a polystyrene control (A) at 200X magnification and around a set of tower on a SU-8 substrate (B) at 100X magnification. In both A and B, the bright green colored spots are the neuronal cell bodies and the green mesh-work are the neural processes. Microscopic imaging on SU-8 substrates is challenging because of an apparent auto-fluorescent property of SU-8

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