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Microfabrication procedure of PDMS microbeam array using photolithography for laminin printing and piconewton force transduction on axons

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Abstract— **The purpose of this paper is to introduce our design for transducing forces on the order of tens of piconewtons by optically measuring deflection of a microfabricated beam tip as it pulls on an array of flexible structures such as axons in an array of laminin-printed neurons. To achieve this we have designed polymeric beams with spring constants on the order of 10pN/μm. We have fabricated circular microbeams with Sylgard® polydimethylsiloxane (PDMS). The elastic modulus of PDMS was determined experimentally using a microscale and a micrometer at different concentrations of curing agent and base agent and found to be on the order of 100kPa. The designed geometry is a 100x100 tapered microcone array with each beam having a length of 100µm, and a base diameter of 10 µm. A SU-8 negative photoresist is etched using photolithography and used as a mold for PDMS soft lithography. PDMS was injected into the mold and the array peeled from the mold.**

Keywords **– Neural array, microbeam, force transduction**

I. INTRODUCTION

The primary objective of this paper is to demonstrate a method for design and fabrication of a highly compliant microbeam array for measuring cell stiffnesses. The highly compliant beams may also be used to print proteins such as laminin as an array on a cover slide for cell printing.

Living cells change shape in response to applied mechanical forces e.g. [1]. The extended processes of axons known as neurites have been shown to be initiated *in vitro* by localized application of tensile forces [2]. While this has been achieved previously with glass [3, 4], we have chosen the soft material polydimethylsilixone (PDMS) for our microbeams for its mechanical compliance, optical transparency, manufacturability and biocompatibility [5]. Some other attributes of PDMS compared to the other polymers are their usability over a high temperature range (-100°C to 100°C), low chemical reactivity, and non-toxicity [6]. PDMS has a relatively small modulus of elasticity, 300kPa, making it favorable because the stiffness value of axons ~10pN/µm [7], since the stiffness value of the microbeams should be on the same order to be able to measure the deflection optically, where the resolution is limited to ~0.5μm. By measuring the deflection and knowing the stiffness values of the beams both analytically and experimentally, the mechanical properties of the axons may be determined. A variety of reasons exist for the utility of such force-deflection measurements. These include understanding the effects that various cytoskeletal proteins have in determining the mechanical properties of axons [8], to understanding the role that various pharmaceuticals play in affecting neural growth characteristics [9] to understanding the effects that various neurotoxins have on neural growth characteristics [10].

Laminin is a large, basement membrane glycoprotein with diverse biological functions including differentiation, migration, and adhesion of cells. Its cell binding ability (via membrane-bound integrin receptors) makes laminin an effective substrate coating for stimulating and enhancing cell migration and neurite outgrowth. Laminin-5 is a basement membrane extracellular matrix macromolecule that provides an attachment substrate for both adhesion and migration in a wide variety of cell types, including epithelial cells, fibroblasts, neurons and leukocytes [11].

II. DESIGN STRATEGY AND MICROFABRICATION

A. Strategy

A mechanical PDMS 100x100 microbeam array for optically transducing forces in parallel in a neuron array will be aligned above the cell array. By lowering the microcone array at a certain rate with the help of the cones at the corners microcone array will adhere to the cell array. After the adhesion occurs; the cells will be stretched unidirectionally (Figure 1). We propose to determine the stiffness of cells such as axons by optically measuring the deflection of an array of beams pulling on the cells according to

$$F_{\text{cell}} = F_{\text{beam}}, \tag{1}$$

where F_{beam} is the force on the microbeam and F_{cell} is the force on the axon. The forces may be written in terms of deflections and the stiffness

$$\int_0^{x-x_{\text{beam}}} k_{\text{cell}}(x)dx = \int_0^{x_{\text{beam}}} k_{\text{beam}}(x)dx, \tag{2}$$

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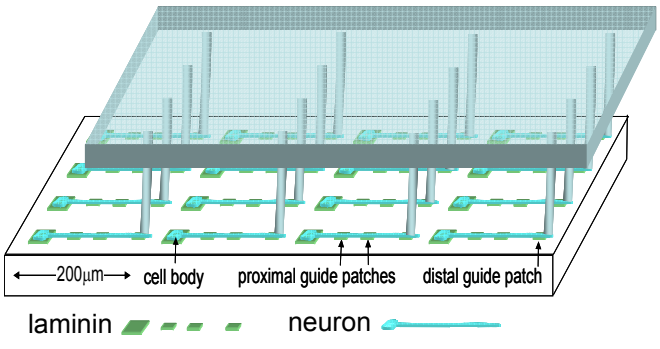

Figure 1 A mechanical micro beam array depicting PDMS cones in a 4x4 array for optically transducing forces in parallel in a neuron array.

B. *Microfabrication*

Photolithography will be used to fabricate the mold for soft lithography. 1/8" thick borosilicate glass is to be spin-coated at 500rpm 100rpm/s for 10 seconds and 750rpm 300rpm/s for 30 seconds with a negative photoresist SU-8. It will be soft baked at 5 min at 65°C and then ramped to 95°C for 20 min and slowly cooled. It will then exposed to UV light using the mask. Post baking was performed to selectively cross-link the portions exposed to UV-light. The SU-8 is developed with PGMEA solution.

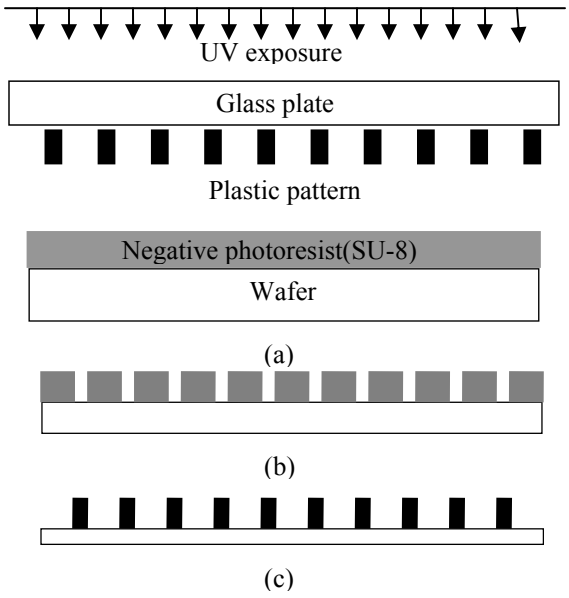

Figure 2. Photolithography and soft lithography processes. (a) shows the UV exposition of the spin coated SU-8 on a glass substrate (b)is the SU-8 mold,(c) shows the obtained PDMS microbeam array from SU-8 mold lithography

PDMS prepolymer (Sylgard 182, Dow Corning) was prepared by mixing the resin with curing agent at 1:10 ratio in a mixer attached to a hydraulic dispenser (Mixpac System 200). It was then kept ready in a petri-dish at room temperature to pour onto the SU-8 mold. After pouring it onto the mold, it is degassed for about 60 minutes to eliminate bubbles. They are then put it into the oven to be cured at 80°C.It is then simply peeled off the SU-8 negative photoresist.

To improve and compare the surface quality of the PDMS using another material, silicon will be used as an alternative mold. Silicon is etched using deep reactive ion etching process (DRIE). SU-8 negative photoresist is used as a mask for DRIE process. Same thickness is obtained by adjusting the exposure time of the gas.

III. Results

A. *Mechanical Properties of PDMS*

Elastic modulus of the PDMS is dependent on the ratio of curing agent to base agent. The effect of curing agent was determined calculating the elastic modulus at 5%, 10%, 15% and 20% curing-agent to base-agent ratio. Four different 3.75mm long cantilever macro scale PDMS beams with 0.5mm diameter were fabricated by using curing-agent at four different ratios (5%, 10%, 15% and 20%). The beam was placed horizontally and lowered on a microscale with a resolution of 1μN so that only the tips of the beams touched to the microscale. Tip forces and tip deflections were observed. The elastic modulus was determined by using analytically derived deflection formula for circular cross-sectional beams and applying a certain known tip force and tip deflection using

$$k = \frac{3E\pi r_A^4}{4L^3}. \qquad (3)$$

The elastic modulus was then determined for each beam. Experiments were repeated three times in order to decouple the manufacturing errors from measurement errors.

The elastic modulus of PDMS in a prototype design was determined at different four curing agent concentrations of 5%, 7.5% 10% and 20% resulting in moduli of 300 ± 20, 410 ± 30, 510 ± 20, 610 ± 25 kPa (Figure 3). It is highly dependent on the curing agent up to %10 of curing agent. However, adding more curing agent shows only slight changes on the elastic modulus. Adding curing agent less than 7% prevents PDMS to get fully solidified. Therefore, the optimum value to obtain the lowest modulus of elasticity and solid beam is between 7-10%

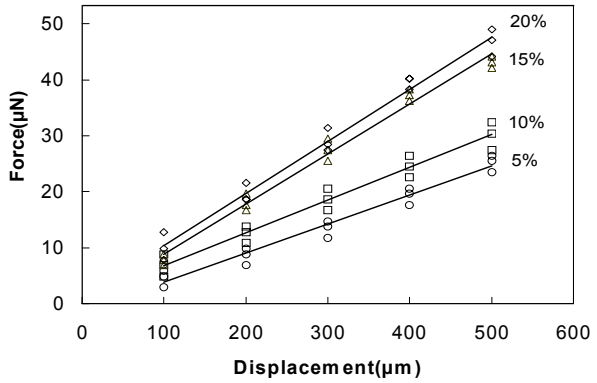

Figure 3 Single PDMS beam stiffness as a function of curing agent percentage

B. Microbeam array using soft lithography

The designed geometry is a 100x100 tapered microcone array with each beam having a length of 100μm, and an approximate base diameter of 10 μm.

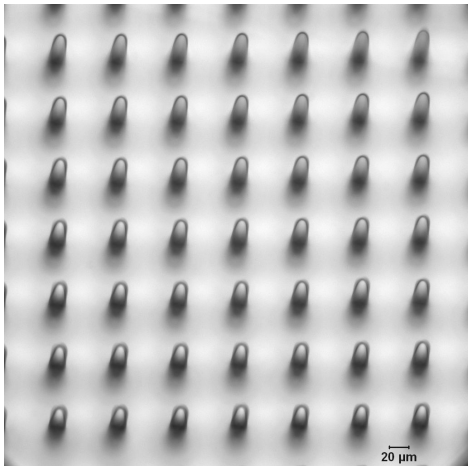

Figure 4 Microbeam array which will be used for growing axons and laminin printing for neuron attachment onto the glass

The same geometry is obtained using DRIE to obtain smoother microbeams.

C. Laminin Printing

Having obtained microbeam array, it will be used to print laminin on a cover slide. It will be used as a pattern for cell attachment onto the cover slide. Natural mouse laminin is the base substance. It is mixed with 0.05M TRIS and 0.15M NaCl in the Petri-dish such that the concentration of the laminin in the mixture is 20μg/ml. The mixture is deposited onto the patterned side of the microbeam array for 30 minutes and dried with a nitrogen stream [12]. A mass of 10g is put on the microbeam array to have full contact between the array and the cover slide.

IV. Future Work and Discussion

The primary objective of this project is to build neuronal-based bio-sensor device. Our design has the advantage in that it allows us to direct the growth direction and growth rate of the signal-carrying axons. Compared to previous neuron-based sensors, this has the advantage in that cell-cell interaction may be directly controlled mechanistically rather than relying on axons to follow printed guides.

The low modulus of elasticity, biocompatibility and transparency of PDMS makes it the best candidate to transduce forces on axons. Modulus of elasticity of PDMS increases with adding more curing agent. The optimum value for having a solid beam with the smallest modulus of elasticity is between 7% and 10%. To check the affect of miniaturization of PDMS beams, a mechanical testing will be performed to obtain the elastic modulus at smaller beams. The nerve cells will be printed such that each nerve cell body sticks to only one laminin spot. The microcone array will align above the cell array. By lowering the microcone array at a certain rate with the help of the cones at the corners microcone array will adhere to the cell array. After the adhesion occurs, the cells will be stretched which will accelerate the neurite growth and direct the growth to electrically active sites for sensing applications.

Acknowledgement

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