A Microchannel-Scaffold Electrode Array for Peripheral Nerve Interfacing

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Introduction:

Injuries to the nerve can severally impair individuals, but peripheral nerves have limited regeneration ability. This means that there is potential for functional recovery. Implementing technologies for nerve interfacing can be used to guide nerve growth and introduce user control to advanced prosthetics. To this effect, studies have demonstrated that scaffolds, with appropriate topographical cues such as microchannels, enhance nerve regeneration through extended gaps [1, 2]. Current interfacing technologies fall far short of ideal. For example, cuff electrodes cannot directly interact with axons for directional control whereas other penetrating electrodes are too invasive [3].

Implementing this technology allows for directed guidance of axonal growth such that it passes over the electrodes to facilitate electrical recording and stimulation. Despite great interest in pursuing this technology, much more quantitative and qualitative information is needed to understand how nerves grow and respond to electric signals in microchannels.

Here, we present a novel device to further study the above-described phenomena. Additionally, we present work being done in developing the functional scaffold. This work will be crucial in attaining direct control of advanced prosthetic limbs through closed-loop mechanisms.

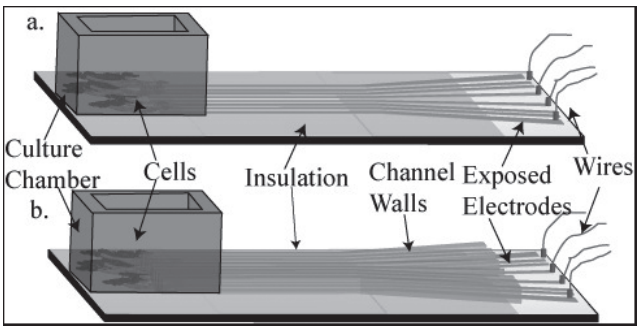

Figure 1: a) Schematic cartoon of MEA device. b) Schematic cartoon of mMEA array device.

Experimental Procedure:

Multielectrode Array (MEA) Fabrication. We fabricated a MEA device for comparison purposes prior to starting on the microchannel MEA (mMEA). Briefly, we patterned NR4-8000P photoresist for liftoff. Gold was deposited by an e-beam evaporator to a height 2000Å with titanium adhesion of 500Å. Following liftoff, the gold electrodes were insulated using SU-8 2007 leaving exposed areas for cell culture and wiring. Polydimethylsiloxane (PDMS) was mixed in a 10:1 base to curing agent ratio to make a cell culture chamber. Conductive epoxy was mixed in a 1:1 ratio of Part A to Part B to glue on microwires (Figure 1).

Microchannel MEA (mMEA) Fabrication. All steps for the standard MEA device were followed with the exception of patterning for microchannel walls. SU-8 2035 was used to pattern the walls before adding the PDMS chamber (Figure 1). Wall alignment was such that there is a gold electrode in each microchannel.

Electrical Evaluation of Devices. Impedance measurements at f = 1000 Hz were conducted using a Neurocraft ICM. Connectors were soldered onto the microwires to allow setup with a stimulation and recording system. One microwire was connected to a function generator and the other to a signal recorder. These were connected to MC Rack software, which reported the input signal and the recorded signal.

Cell Culture on Devices. After sterilization with 70% ethanol and UV exposure, the cell plating area was coated with poly-L-lysine and placed in an incubator overnight. Following this, cortical neuronal cells were seeded on the surface. Images of cell culture were taken three days after seeding.

Microchannel Scaffold. Fabrication of the microchannel scaffold was accomplished. Additionally, preliminary patterning of electrodes on PDMS was accomplished using methods similar to those described previously.

Results and Discussion:

***In vitro* Device Fabrication.** Successful fabrication of devices was accomplished for both the MEA and mMEA devices. Important fabrication concerns included sufficient undercut for electrode patterning, strong gold adhesion, and a smooth photoresist spin for microchannels. Additionally, microchannel walls were not extremely stable causing some to bend and fall over. This posed problems in preparation for cell culture as this caused stress to the walls.

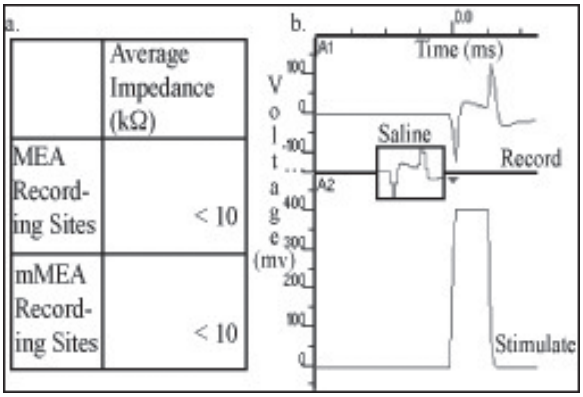

Figure 2: a) Impedance testing. b) Feasibility testing of stimulation and recording.

Impedance Testing. Impedance measurements showed that all recording sites had impedance to the cell culture chamber of less than 10 kΩ, with most under 2 kΩ (Figure 2). This illustrates that action potentials given off by nerve cells can be recorded. Additionally, impedance measurements on the device with microchannels showed no difference compared to the device without microchannels, illustrating that addition of walls did not damage electrode viability.

Signal Processing. We were able to illustrate the feasibility in simultaneously stimulating and recording of the device. Nerve cells need a stimulus to fire an action potential. Previously, in action potential studies, external stimuli have been used. Here we can stimulate and record simultaneously. As a control, we tested the system in saline and saw that there was good signal transduction over the devices (Fig.2).

Cell Culture on Devices. Cortical neuronal cells take approximately 20 days to form a neural network. Due to this time limitation, cell stimulation and recording was not achieved. Despite this, cell culture was established. Interestingly, the cells preferentially adhered to SU-8 substrate (Figure 3). Utilizing this, we can choose materials for better directional growth.

Microchannel Scaffold. Preliminary gold electrodes were successfully patterned on PDMS substrate (Figure 4). After gold patterning, following microchannel wall fabrication, the final scaffold can be achieved. The final scaffold will be similar to the scaffold presented in Figure 4 with the addition of electrodes.

Conclusions:

Here we have presented significant progress towards developing a system for peripheral nerve interfacing. We have developed a novel platform for better understanding nerve growth and interfacing in microchannels, as well as a system to serve as a control. We further demonstrated electrode viability and functionality in stimulation and recording. We further demonstrated proof of concept by seeding cortical neuronal cells. We hope that this device will serve as a platform for amassing quantitative information necessary to develop a system for use *in vivo*.

Additionally, work towards attaining a fully operational microchannel-scaffold electrode array shows promising results. This scaffold will need to be integrated with the necessary wiring and implantable chips for future use.

Acknowledgements:

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References:

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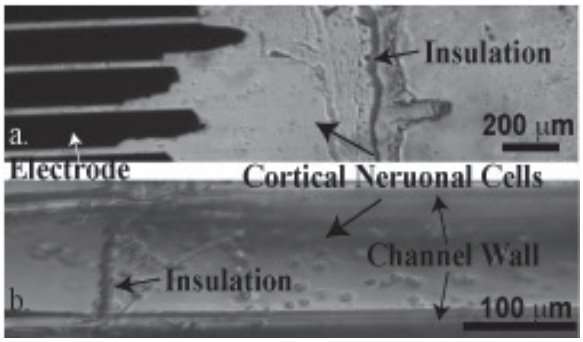

Figure 3: a) Nerve cells on MEA device. b) Nerve cells on mMEA device.

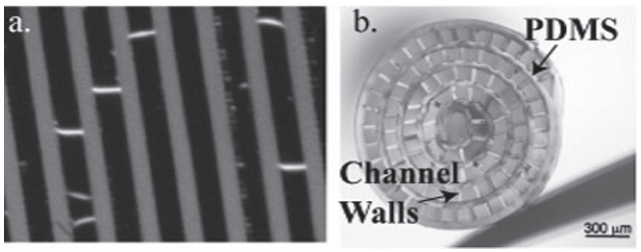

Figure 4: a) Preliminary patterning of electrodes (darker lines) on PDMS. b) Rolled scaffold without electrodes.