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Regenerative Microchannel Electrode Array for Peripheral Nerve Interfacing

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***Abstract*—A regenerative microchannel scaffold capable of housing microelectrodes to form a high-throughput peripheral nerve interface is described. The regenerative microchannel scaffold was fabricated using polydimethylsiloxane (PDMS) and SU-8 as substrate and structural materials, respectively. This microchannel scaffold is capable of being integrated with a PDMS-based thin-film microelectrode array (MEA) to form a peripheral nerve interface. Preliminary *in vitro* characterization was performed using dorsal root ganglia cultures to evaluate biocompatibility and the ability of the microchannel design to direct and orient neurite outgrowth. Our findings provided evidence that the microchannel design will be effective in directing axon regeneration to the benefit of a neural interfacing device.**

I. Introduction

Nerve injuries and amputation can result in severe loss of function, pain, and a reduction in quality of life of an individual. Peripheral nerve interfacing has the potential to remedy this by helping modulate damaged nerve function to achieve functional outcomes like neural prosthetic control through stimulation, recording, or conduction blocking. However, current technologies fall short in terms of the number of functional electrodes and the recording reliability.

Many of the current peripheral interface designs for neural prosthetics often record electromyograms. As a result, this and other current designs allow limited control and are largely 'uni-directional' interfaces that do not permit information transfer to the nervous system to enable proprioception, tactile feedback, and other sensations. Next generation peripheral nerve prosthetics aim to interface with the limb's remaining nervous system, which remains viable and functional for years after injury [1]. However, the widely used interfacing cuff electrodes have limited capability because they are not in direct contact with individual axons, while other penetrating electrodes are too invasive and often damage nerves [2]. In most cases, there exists a tradeoff between the ability to selectively interface with single or few axons and the degree of injury to the nerve caused by interfacing [2].

A regenerative approach to peripheral nerve interfacing has the potential to overcome some of these challenges. In this approach, nerves are re-shaped by being encouraged to regenerate into specific electrode geometries. While theoretically, the regenerative approach is promising, current designs to attain stable regenerative interfaces have not been successful. The Sieve Electrode, the classic regenerative interface enables only a few viable electrodes because the measurable extracellular potential of the action potential (AP) is small [3, 4]. This low signal is caused by the low impedance extracellular volume surrounding axons and the resulting high ionic diffusion/dispersion [3]. In fact *all* peripheral nerve interfaces are forced to endure this challenge. It is possible to circumvent this by recording at the Nodes of Ranvier, which occur at least every 2mm [5] and is where the extraceullular potential of the AP is largest. However, this imposes a technically challenging spatial dependence on the nodes [2, 6] that is difficult to solve. All penetrating electrode designs as well as the Sieve electrode designs face these difficulties leading to compromised SNR situations, limiting peripheral nerve interfacing.

With these limitations in mind, our ***long-term goal*** is to create a novel regenerative neural interface to establish high-channel, bi-directional communication between an amputated nerve and a prosthetic limb. This interface would allow for intimate contact between electrodes and small groups of axons while enhancing regeneration. The ***objective*** of this project is to engineer a thin-film PDMS/SU-8 based regenerative microchannel scaffold with microelectrodes incorporated in the microchannels, the Georgia Tech Regenerative Electrode (GT-RE). Confining regenerated axons in microchannels will increase the extracellular potential resulting from an action potential (AP) [3, 4], thus, enhancing signal strength and recording capabilities.

In the present work, we have fabricated prototype PDMS/SU-8 based regenerative microchannel scaffolds as the housing basis for microelectrodes forming the GT-RE. Preliminary *in vitro* characterization was performed using dorsal root ganglia cultures to evaluate biocompatibility and the ability of the microchannel design to direct and orient neurite outgrowth.

II. Methodology

A. Future System Overview

The overall design being pursued, illustrated in Fig. 1, aims at engineering a GT-RE that will be functionally evaluated *in vivo* through the stimulation of and single unit recordings from regenerated axons in a rat sciatic nerve amputee model. The GT-RE will consist of microchannels composed of top and bottom PDMS layers and SU-8

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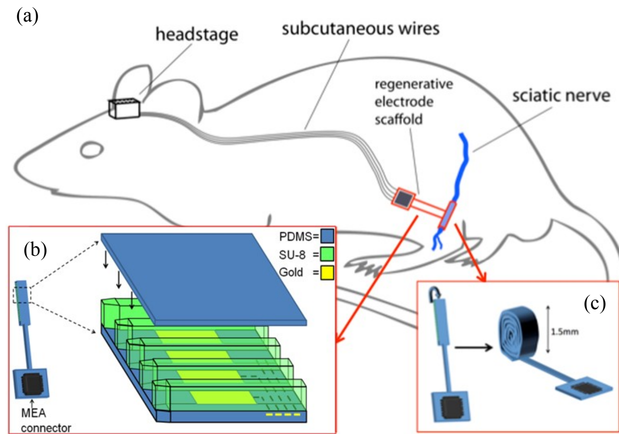

Fig. 1. System overview of future concept. (a) Implantation schematic. (b) Depiction of microchannels. (c) Depiction of final scaffold after rolling.

polymer walls with incorporated gold electrodes (Fig. 1b). The PDMS top and bottom layer thicknesses will be 10μm and 40μm, respectively. Microchannels walls will have a width and height of 20μm and 100μm respectively; the widths of the microchannels will range from 50 to 150μm and will be optimized in a future *in vivo* study. The microchannel lengths will be 3mm. Finally, the layer of microchannels will be rolled to form the overall scaffold with a total radius of 1.5mm (Fig. 1c). The implantation setup involves mounting the proximal stump of a transected sciatic nerve to one end of the scaffold and allowing the nerve to disassemble and regenerate through the microchannels as shown in Fig. 1a. The regenerating axons in the nerve are expected to integrate with the microelectrodes inside the microchannels. The microelectrodes will be wired to the integrated 'on-board' electronics by a short PDMS cable. The 'on-board' electronics will help to reduce recording noise and power for stimulation, as well as allow for multiplexing which will reduce the number of wires needed. The I/Os of the electronics will be wired to a percutaneous head stage through subcutaneous wires.

B. Regenerative Microchannel Scaffold Fabrication

In the ultimate design, the microchannels will be fabricated on top of a PDMS-based thin-film MEA, whereas in the current study, for characterization purposes, we fabricated the microchannels with thicker top and bottom PDMS layers on a thin PDMS film as an analogy to the PDMS-based MEA.

To fabricate the regenerative microchannel scaffold, a 70μm PDMS (Sylgard 184, Dow Corning) base layer was first spun on glass, in which an MEA would be fabricated [7] if electrodes were to be incorporated into the scaffold. The PDMS base layer was treated with oxygen plasma to increase the adhesion between PDMS and SU-8. A 100μm layer of SU-8 (SU-8 2100, MicroChem Corp) was then spun on top of the PDMS. The SU-8 was cured, exposed, and developed forming the patterned microchannel walls on the PDMS base layer. The width and length of the microchannel walls were 20μm and 10mm respectively. The width of the microchannels ranged from 50-150μm. Finally a 70μm PDMS cover layer was spun on glass and then bonded onto top of the SU-8 walls to form closed microchannels. The basic fabrication process is depicted in Fig. 2.

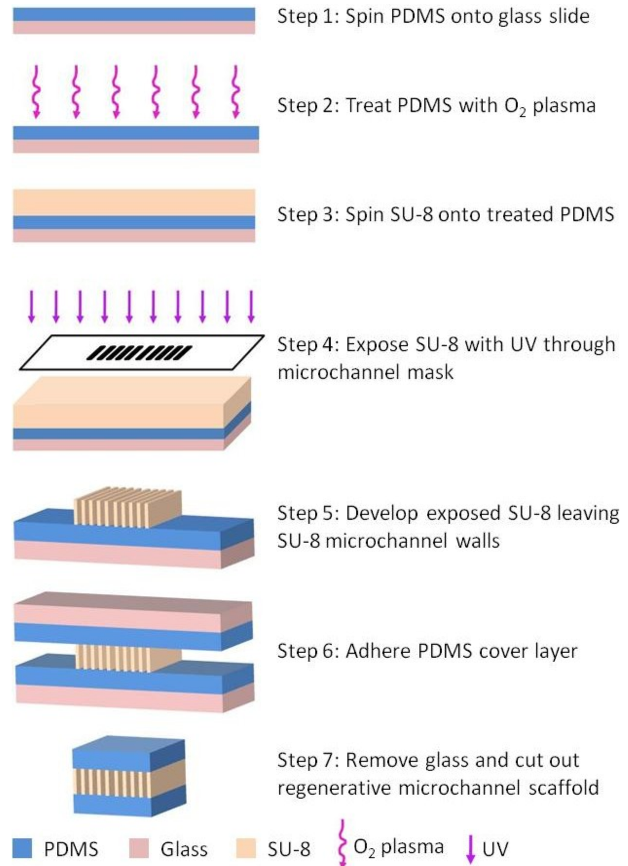

Fig. 2. Regenerative microchannel scaffold fabrication process (not to scale).

C. Dorsal Root Ganglia Culturing In Vitro Experiment

Regenerative microchannel scaffolds lacking the PDMS cover layer were used for the *in vitro* experiments. Once fabricated, the open regenerative microchannel scaffolds were placed at the bottom of tissue culture wells and coated with Poly-D-Lysine and then Laminin to facilitate DRG adhesion onto the scaffolds for the culturing experiment. DRG's were explanted from the spinal cords of P1 rat pups. The nerve roots were removed and the DRG's were placed on the scaffolds at the entrance to the microchannels. For the first several hours, the DRG's were incubated with only a thin layer of DMEM/F12 media with 10% FBS and 50 ng/mL nerve growth factor (NGF) (Roche). Afterwards, the wells were fully covered with the same media. The media, including NGF, were replaced every two days for a total of seven days. After seven days the DRG's were fixed with 4% paraformaldehyde in PBS for 20 min and washed three times with 1X PBS. To visualize neurite outgrowth, cells were tagged overnight at 4°C with the primary neurofilament 160 kDa (NF160, 1:500, mouse IgG1, Sigma) to stain for the

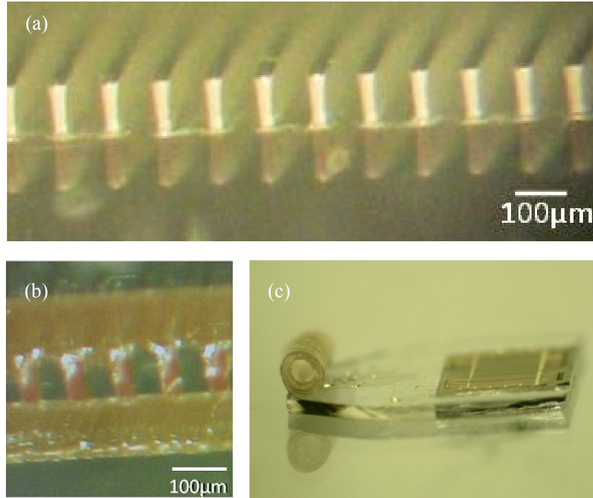

Fig. 3 (a) Regenerative microchannel scaffold prior to PDMS cover layer bonding (open version). (b) Regenerative microchannel scaffold with PDMS cover layer. (c) Prototype IC-integrated PDMS-based MEA (nonfunctional) with the electrode shaft rolled into a scroll proving the concept in Fig. 1c. Microchannels were not incorporated in this device.

neurites. The secondary antibody goat anti-mouse IgG1 Alexa 488/594 was used. Cell nuclei were labeled with DAPI (10µM, Invitrogen). The fluorescently labeled cells and nuclei were visualized using a Zeiss upright microscope and the images were captures with an Olympus digital camera.

III. Results

A. *Regenerative Microchannel Scaffold Fabrication*

Fig. 3a shows the fabricated prototype regenerative microchannel scaffold prior to the bonding of the PDMS cover layer (open version). In this sample the microchannel widths were 75µm. Note, in this image the channel walls are reflecting on the PDMS base layer. It can be seen that the patterning of SU-8 channel walls on the PDMS base layer is successful and the SU-8 is capable of forming 20µm by 100µm channel walls.

Fig. 3b shows the fully fabricated prototype regenerative microchannel scaffold before being rolled as it would be when implanted. The microchannels are clearly visible and in this sample have a width of 50µm. Bonding between the PDMS cover layer and the SU-8 channel walls can be seen by the lack of a visible division between the SU-8 and PDMS. This validates the fabrication process design and its ability to produce microchannels in PDMS using SU-8 walls.

Fig. 3c shows a prototype IC-integrated PDMS-based MEA. The MEA has a shaft approximately 30µm in thickness lacking microchannels. This shaft has been mechanically rolled into a scroll using forceps proving the basic concept of the design in Fig. 1c. Additionally, the silicon die of a 16-channel amplifier (3.1mm x 4.3mm x 380µm, RHA1016, Intan Technologies, UT) has been integrated in the thick PDMS layer, and the MEA was fabricated overlay with via-bondings on contact pads of the die [8].

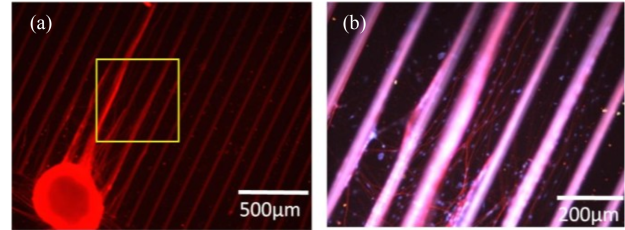

Fig. 4. DRG tissue culture on 100µm open microchannels. (a) Image of DRG, non-neuronal cells, and neurites stained for cell bodies. (b) Magnified view of boxed area in (a) showing cell bodies (red) and cell nuclei (blue).

B. *Dorsal Root Ganglia Culturing In Vitro Experiment*

One example of the scaffolds lacking the PDMS cover layer fabricated for *in vitro* DRG culturing is shown in Fig. 3a. This open scaffold has microchannel widths of 75µm. The cultured DRGs adhered well to these open scaffolds. Fig. 4a and 4b show a DRG cultured on a scaffold with 100µm microchannel widths. Neurite extension (shown in red) and migration of non-neuronal cells (nuclei shown in blue) on the regenerative microchannel scaffold was observed corroborating the scaffolds ability to support growth of multiple cell types. Furthermore, the neurites extended and non-neuronal cells migrated in an oriented fashion along the direction of the microchannels.

IV. Discussion

A prototype regenerative microchannel scaffold using PDMS as the base and cover layers and SU-8 as the microchannel walls has been successfully fabricated. Additionally, the first step in developing an integration method for on-board electronics to aid in signal extraction has been made and further validated the device's capability of being rolled to form a three-dimensional scaffold.

The substrate and structural materials of the scaffold have been shown to be non-toxic by supporting the growth of multiple cells types, DRG neurites and non-neuronal cells. Furthermore, the capability of the microchannel design to guide and direct DRG neurite outgrowth and non-neuronal cell migration along and through the microchannels has been verified. These results show that the microchannel design provides a method to guide regenerating axons and can be used as a novel platform to incorporate electronics for chronic recording and stimulation from small specific groups of axons.

We are currently working towards optimizing microchannel dimensions through a series of *in vivo* nerve regeneration studies. Concurrently, we are engineering the next generation neural interface by integrating a microelectrode in each microchannel to form a high-throughput electrode array. Such a microchannel-scaffold electrode array, the GT-RE, has potential to significantly enhance the efficacy and reliability of peripheral nerve

interfacing. This is based upon the rational that confining an axon to a microchannel limits the volume of the low impedance extracellular fluid and matrix surrounding the axon. Limiting the extracellular volume effectively increases the extracellular resistance and following from Ohm's Law, increases the extracellular potential [3]. Using a 3mm microchannel will also ensure that a node of Ranvier is somewhere in the channel allowing the incorporated electrodes to be spatially independent of the nodes, which is a major advantage. Furthermore, encouraging the axons to regenerate in small numbers through numerous microchannels allows for highly selective recording and stimulation based upon electrical and spatial isolation.

Finally, this microchannel design can lend itself to a number of other applications. These include using the incorporated electrodes to induce nerve conduction blocking for pain modulation, stimulation of specific nerve fiber types using high frequency wave forms, [9, 10] and monitoring the extent of nerve regeneration through the scaffold using impedance testing for non-amputee cases. Our hope is to use this novel design to influence the field of neural interfacing as a whole, regardless of the application.

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