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Microchannel-based regenerative scaffold for chronic peripheral nerve interfacing in amputees

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ABSTRACT

Neurally controlled prosthetics that cosmetically and functionally mimic amputated limbs remain a clinical need because state of the art neural prosthetics only provide a fraction of a natural limb's functionality. Here, we report on the fabrication and capability of polydimethylsiloxane (PDMS) and epoxy-based SU-8 photoresist microchannel scaffolds to serve as viable constructs for peripheral nerve interfacing through *in vitro* and *in vivo* studies in a sciatic nerve amputee model where the nerve lacks distal reinnervation targets. These studies showed microchannels with 100 μm × 100 μm cross-sectional areas support and direct the regeneration/migration of axons, Schwann cells, and fibroblasts through the microchannels with space available for future maturation of the axons. Investigation of the nerve in the distal segment, past the scaffold, showed a high degree of organization, adoption of the microchannel architecture forming 'microchannel fascicles', reformation of endoneurial tubes and axon myelination, and a lack of aberrant and unorganized growth that might be characteristic of neuroma formation. Separate chronic terminal *in vivo* electrophysiology studies utilizing the microchannel scaffolds with permanently integrated microwire electrodes were conducted to evaluate interfacing capabilities. In all devices a variety of spontaneous, sensory evoked and electrically evoked single and multi-unit action potentials were recorded after five months of implantation. Together, these findings suggest that microchannel scaffolds are well suited for chronic implantation and peripheral nerve interfacing to promote organized nerve regeneration that lends itself well to stable interfaces. Thus this study establishes the basis for the advanced fabrication of large-electrode count, wireless microchannel devices that are an important step towards highly functional, bi-directional peripheral nerve interfaces.

1. Introduction

Prosthetics aim to restore the functional capacity once held by an amputee. A number of neural prostheses have been developed with the goal to artificially substitute or mimic sensorimotor functions in patients. For instance, this goal can be accomplished via an interface with the peripheral nervous system by means of implanted electrodes capable of neuromuscular stimulation and neural signal recording. Stable bi-directional communication could allow for a natural control over advanced prosthetic limbs and other similar assistive devices for amputees.

State of the art peripheral nerve interfacing has centered around three main types of device designs: cuff, penetrating, and regenerative sieve electrodes. Cuff and penetrating interfaces face a large tradeoff between the ability to selectively interface with individual axons versus the amount of disruption to the nerve and long-term stability as a result of trying to get in close proximity to axons [1]. Cuff electrodes, which wrap around the nerve, cause minimal damage and provide long-term stability at the expense of stimulation/recording specificity due to a lack of direct contact with

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individual axons [1–4]. In contrast the Utah Slanted Electrode Array (USEA) penetrates the nerve and provides enhanced specificity through direct contact between the electrodes and axons. However, the insertion causes injury and the electrodes elicit chronic inflammation and scar tissue [5]. An alternative to combat these negative outcomes is a regenerative approach, where transected axons grow into specific geometries within close proximity of electrodes [6]. The hallmark Sieve Electrode is implanted so axons are forced to regenerate through its many perforations/holes making contact with ring electrodes [7]. However, this design has few viable electrodes because the extracellular potential of an axon's action potential (AP) is small. Furthermore, there is a spatial dependence of the electrodes to the nodes of Ranvier, which occur at least every 2 mm and are where the extracellular potential is largest [1,8–10].

Microchannels offer a unique opportunity to take advantage of the regenerative approach while circumventing issues with small APs and the node of Ranvier dependence, as detailed elsewhere [2,9,11–13]. Briefly, if a nerve regenerates through electrically insulating microchannels, small groups of axons can be isolated from each other and the surrounding low impedance extracellular fluid. Limiting the extracellular volume increases the extracellular resistance and from Ohm's law, increases the extracellular potential of an axon's AP [2]. Using microchannels with a length of 3 mm ensures a node of Ranvier will always be contained within the microchannels making them spatially independent of the nodes. Such conditions have been validated *in vitro*. APs from axons were recorded from inside a microchannel, the spatial position of the axons changed, and recordings taken again without any significant difference in AP signal amplitude [9].

However, the underlying success of a regenerative microchannel interface as a dependable solution for neural interfacing is not only its ability to detect high quality APs, but also a function of long term tolerance by the peripheral nervous system. Thus, the neural implant is inherently dependent on the chronic condition of the matured nerve post-regeneration. To prevent neuropathic complications post-implantation, the device must facilitate sufficient room for nerve maturation over time while in close contact with as many axons as possible to obtain clear neural signals. The device must also facilitate healthy nerve characteristics such as a maintained tissue architecture, established myelinated fibers, and prominent axon profiles through the regeneration and maturation phases [7,14,15]. Lacking such nerve characteristics has proven to yield poor nerve functionality, compressive axonopathy, and neuromas which result in poor translation of neural signal and loss of nerve utility [7,15–19]. The tendency of regenerating injured nerves to form neuromas is also a major clinical concern for amputees due to associated chronic pain as well as a major concern for specificity during neural interfacing due to characteristic random AP firing [20–24]. Neuromas not only negatively impact performance but also the tolerance of an implant by the host's PNS by creating a hostile environment not conducive to typical axon regeneration and maturation. They are abnormal in their physiological characteristics as manifested by a marked degeneration and absence of myelination and axons, myelin debris, a decrease in Schwann cell population, loss of nerve organization/structure and oriented axon growth, loose basal lamina structure surrounding axons, edema and swelling, and the overall decline of axonal health in a regenerating nerve [17,18,21–23,25]. If a regenerative microchannel interface is unable to support the maturation and chronic retention of healthy axons, then even if it can record action potentials, it still fails from a biological perspective due to these neuropathic complications.

Our long term goal is to establish a large-electrode count microchannel interface for bi-directional communication within an amputated nerve. The basic design of the microchannel architecture is inspired by work from Dr. Fawcett and Dr. Lacour while at the University of Cambridge, and is shown in Fig. 1. The interface utilizes top and bottom polydimethylsiloxane (PDMS) layers with SU-8 walls forming series of adjacent microchannels. Prior to implantation, the array of microchannels is rolled onto itself forming the device filled with microchannel conduits, mimicking the general cross-sectional architecture of a nerve. The device is implanted so that the transected axons can regenerate through the microchannels and over the electrodes housed in the bottom PDMS layer for interfacing.

Here, we focus on the response of the regenerating nerve to an artificially imposed microchannel obstacle and separately the ability to obtain neural signals from these microchannels that are meaningful for the eventual goal of neural interfacing. We first validate cytocompatibility of the scaffolding materials through *in vitro* experimentation. We then explore the capability of axons in an amputated nerve that lacks distal reinnervation targets to regenerate through the device and mature in a PDMS and SU-8 based microchannel scaffolding. Furthermore, we characterize the regenerated nerve from a morphological perspective once the axons have grown out of the microchannel scaffolding and attempt to assess whether neuroma formation is a major concern by evaluating the characteristics described previously common to neuromas. Specifically we consider the presence or lack of organization within the nerve and oriented axon regeneration, Schwann cells and myelin deposition on axons, tight basal lamina structures around axons/Schwann cell units, and edema/swelling. Finally, we evaluate the ability to record single and multi-unit action potentials through microchannels permanently integrated with microwire electrodes in a terminal study after chronic implantation in the rat sciatic nerve. The chronic terminal electrophysiology experiment allows the testing of interfacing capabilities without needing to invest in the development of advanced wiring and packaging technologies for chronic continuous behavioral studies.

2. Materials and methods

2.1. Regenerative microchannel scaffold fabrication

To fabricate the regenerative microchannel scaffolds, a 40 μm PDMS (1:10 weight ratio Sylgard 184, Dow Corning) base layer was first spun on glass coated in titanium (10 Å) and gold (50 Å) for anti-adhesion. The PDMS base layer was then briefly treated with oxygen plasma to increase the adhesion between PDMS and SU-8. A 100 μm layer of SU-8 (SU-8 2100, MicroChem Corp) was then spun on top of the PDMS. The SU-8 was cured, exposed using an experimentally determined exposure dose of 520 mJ/cm^2, and developed forming the patterned microchannel walls on the PDMS base layer. The width and length of the microchannel walls were 20 μm and 10 mm, respectively. The width of the microchannels ranged from 50, 100, or 150 μm. The dimensions and spacing between the microchannel walls were controlled by the photolithography mask used for each microchannel type. The basic fabrication process of these steps is depicted in Fig. 2. Detailed methods for the fabrication of these steps can be found in literature [26].

Adding the top PDMS layer involved first spinning polyacrylic acid, a water resorbable layer, on another glass slide and drying it on a hotplate at 60 °C for 5 min. This was done twice. A 10 μm layer of PDMS was immediately spun on the polyacrylic acid (PAA) layers and partially cured on a hotplate at 65 °C for 4 min. During this time, the bottom PDMS layer with the SU-8 microchannel walls was treated with oxygen plasma to increase the adhesion between the two layers. The two glass slides were placed together with weight on top and baked on a hotplate at 60 °C for 30 min and then 90 °C for 1 h to ensure that the top PDMS layer was fully cured. Finally, the glass slide sandwich was soaked in water until the PAA dissolved and allowed for easy removal of the top glass slide. The basic process is depicted in Fig. 2. Once the layer of microchannels was removed from the glass slide, they were rolled perpendicularly to the direction of the microchannels and cut to 3 mm forming microchannel scaffolds with a cylindrical shape resembling that of the rat sciatic nerve. Each microchannel scaffold type was designed to contain a different number of microchannels based on the microchannel cross-sectional area. This was necessary in order to maintain a uniform rolled scaffold cross-section of approximately 1.5 mm across all three types of microchannel scaffolds. The 150 μm × 100 μm microchannel scaffolds contained 59 microchannels, the 100 μm × 100 μm microchannel scaffolds contained 84 microchannels, and the 50 μm × 100 μm microchannel scaffolds contained 143 microchannels.