A novel neuroprosthetic interface with the peripheral nervous system using artificially engineered axonal tracts

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Objective: *We have previously described a technique developed in our laboratory to create transplantable living axon tracts of several centimeters in length. In this paper, we describe how these engineered neural tissue constructs can be used to create a novel neuroelectrical interface with the regenerating peripheral nervous system, to potentially enable afferent and efferent communications with prosthetic devices.*
Methods: *Using continuous mechanical tension, we have generated axon tracts of up to 10 cm in length, spanning two populations of neurons* in vitro. *We have now adapted this stretch-growth paradigm to include a mechanically compliant multi-electrode array that is attached to one of the neuron populations. Once the desired axon length has been reached, the neuroelectrode construct is completely embedded in a supportive hydrogel matrix and affixed to the transected sciatic nerve.*
Results: *Building upon our previous work with peripheral nerve repair, we have designed our neural interface to ensure transplant stability and firm attachment to the electrode array substrate.*
Discussion: *Our preliminary findings indicate that the interface not only maintains its orientation, but also is conducive to host nerve ingrowth. Our ongoing analysis seeks to characterize transplanted neuronal survival, synaptic integration, and functional connectivity. This research provides an opportunity to evaluate an entirely new approach in restoring motor and sensory functions of patients with peripheral nerve damage.* [Neurol Res 2008; **30**: 1063–1067]

Keywords: Neural interface; neurally controlled prosthesis; tissue engineering; peripheral nerve regeneration

INTRODUCTION

The coupling of electrical devices to the human nervous system has long been the realm of science fiction. However, technological advancements of the past several decades have now made this phenomenon a reality. The cochlear implant, the first device to truly interface the nervous system with the external environment, was developed in the 1960s. However, the direct neural control of prosthetic limbs is a far more nascent field. In 1999, a seminal study described the control of a robotic arm using signals directly derived from the rat motor cortex[1]. Since then, there have been a number of exciting advances reported in the literature, in both central nervous system (CNS)- and peripheral nervous system (PNS)-based approaches.

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In this review, we describe a 'hybrid' neuroprosthetic interface architecture consisting of a biological tissue coupled to electrical substrates, which is currently being developed in our laboratory. Our group has developed a method of creating transplantable axonal tracts of unparalleled length *in vitro*. The initial goal of this 'nerve in a dish' was to bridge large deficits in the spinal cord or PNS. In this paper, we describe how it may also be used to establish a direct, biological connection between severed peripheral nerves and electrode arrays, thus re-enabling afferent and efferent signalings between the PNS and prosthetic devices.

OVERVIEW OF EXISTING TECHNOLOGIES

The ideal neuroprosthesis should be a functional facsimile of the amputated limb, i.e. it must facilitate continuous bidirectional communication between the CNS and the external environment. Currently, the vast majority of efforts in this area focus exclusively on the re-establishment of motor control, relying on visual

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feedback to guide the movement of the prosthesis. However, to achieve a truly 'normal' interaction with the surroundings, tactile feedback is vital. Additionally, from a clinical and rehabilitation standpoint, it is important to have an architecture that minimizes surgical complexity and recovery time, provides a hospitable environment for nerve survival and lends itself to rapid learning.

Over the past several decades, a variety of architectures that target both the CNS and PNS have been developed. CNS-based approaches attempt to restore motor function by directly deriving commands from the patient's motor cortex. Two major strategies have emerged to accomplish this. The first is a non-invasive technique that obtains a movement intent via surface (scalp) electrodes over the motor cortex[2,3]. Using this approach, which entirely avoids the risks associated with surgery, patients have demonstrated the ability to perform such tasks as cursor manipulation and even basic word processing. However, the poor information transfer rates associated with this technique makes its translation to the control of more sophisticated systems immensely challenging in the near future[4,5]. To produce complex signal integration, more invasive methods have been developed, such as chronically implanted microelectrodes into the motor cortex or spinal cord to locally record activity from a select population of neurons[6–10]. Neuronal population decoding algorithms are then used to decipher the recorded signals in real time. This approach has yielded considerable success in re-enabling motor control; indeed, a version of this system is currently the subject of a pilot clinical trial. Nonetheless, this approach has a number of drawbacks, including substantial computational complexity, significant clinical risk arising from the chronic implantation of electrodes in healthy neural tissue, and signal attenuation and/or remapping due to scar formation. Moreover, findings from functional magnetic resonance imaging studies indicate that there is extensive and dynamic overlap of the cortical representations of different limb regions, adding additional difficulty to implant positioning and signal decoding[11]. Additionally, there is still no clear approach to relay sensory signals/feedback.

Alternatively, interface approaches outside the CNS typically take one of two forms: (1) electrodes that are implanted in or around the damaged peripheral nerve or (2) electrodes that are implanted within, or on the surface of, skeletal muscle[12]. Nerve electrodes, the form of which can vary from encircling nerve cuffs[13] to intrafascicular penetrating electrode arrays[14,15], exhibit both enhanced signal selectivity and high signal-to-noise ratio. However, this approach still has its drawbacks: the materials used are often of poor biocompatibility, the electrodes themselves can be extremely damaging to the already traumatized nerves and there is a reduced likelihood of chronic interface due to nerve degeneration.

Electromyogram-based myoelectric prosthesis systems have met with remarkable success in recent years. In particular, the targeted innervation of the brachial plexus nerves into the pectoral muscles has allowed for the real-time control of multi-jointed prosthetic limbs[16,17] and the transmission of sensory modalities including touch and pain[18] to the CNS. Although with extremely beneficial practical applications for patients in the near future, this approach has clear limits; not only must healthy muscle tissue be compromised to provide a target for regeneration, but also recovery time and first indications of reinnervation can be in the order of several months.

PROPOSED APPROACH

Although tremendous advancements have been made, there is no approach to date that directly integrates with the nervous system while leveraging the processing abilities of the brain and spinal cord. In contrast to all other strategies for neural interface development, we propose to exploit a novel method of engineering nervous tissue constructs as a means of interfacing a multi-electrode array (MEA) with regenerating peripheral nerves. The use of living neural tissue, which may be coupled to the MEA to form a stable interface before transplantation, provides an enticing target for host axon ingrowth and synaptic integration. By directly accessing the transected nerve, we eliminate the need for interpreting computationally complex neural signals in the CNS. Instead, upon integration, simple operant conditioning should allow the implantee to control the prosthesis with ease[19]. Thus, our approach builds upon current interface architecture capabilities and holds enormous promise to provide both motor control and sensory feedback for normal function.

Stretch-induced axon growth

The central feature of the proposed neural interface is an engineered axonal tract created *in vitro* by the controlled separation of two integrated populations of neurons[20–22]. This newfound method of axon growth was discovered in our laboratory while studying the biomechanics of axonal trauma. Using this technique, we have grown CNS and PNS axon tracts of up to 10 cm in length and containing over 106 axons. Immunocytological analysis of these axons reveals a normal complement of neuronal cytoskeletal proteins. Findings from a scanning electron microscope revealed the tendency of the fibers to coalesce into aligned axon bundles. Finally, electrophysiological studies have verified the ability of these elongated axons to conduct normal action potentials[23].

The PNS axon tracts have also been transplanted into a rat sciatic nerve lesion, and robust host regeneration into the graft was observed[24]. Compound action potentials could be conducted across this transplanted nervous tissue bridge and the treated animals demonstrated some restoration of limb function.

Neural interface

On the basis of these findings, we propose to couple this novel tension-grown tissue engineered nerve

construct to an electronic interface at one end and allow it to integrate with the proximal nerve stump at the other, thus providing a natural, bidirectional pathway of communication with the CNS. We hypothesize that the regenerating nerve will either integrate with the ''free'' end of the interface or grow along the axon tract towards the MEA and the attached cell population. The use of elongated axons accords us freedom in the placement of the interface in the limb. Upon integration, the natural, willful stimulation of the interfaced nerve will enable us to map its motor representation on the MEA. Similarly, stimulation of the electrode array will allow us to determine the representation of sensory fibers, as well as their corresponding modalities.

NEURAL INTERFACE DESIGN

Our recent efforts have been focused on optimizing the neural interface design, with the goal of maximizing biocompatibility, construct stability, and host ingrowth. With this in mind, we have decided to adopt a flexible MEA as our electrode substrate to give us mechanical compliance and a plethora of input/output channels. This MEA, which serves as the movable (or towing) surface during stretch-growth, is treated with a bioadhesive substrate upon which the embryonic dorsal root ganglion (DRG) neurons are plated. The cells are then allowed to adhere and integrate before being subjected to stretch-growth. Once grown to the desired length, the entire neuroelectrode construct is completely embedded in a hydrogel. We have designed these three-dimensional constructs considering neural tissue survival and mass transport requirements[25]. The construct is then placed in an ensheathing tube to provide structural support. The transected nerve stump can then be inserted into the proximal (open end) of the tube and affixed by suturing the epineurium to its walls (*Figure 1*).

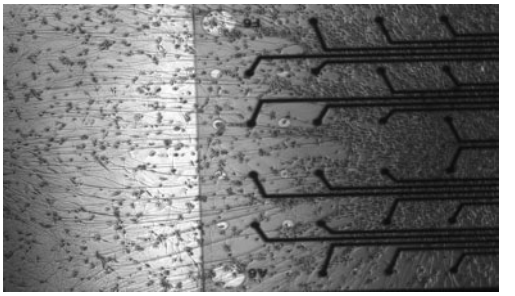

Figure 2: Dissociated embryonic DRG neurons on a FlexMEA being subjected to stretch-growth at 1 mm/day for 2 days *in vitro*

In undertaking this endeavor, we have divided the project into a series of discrete and complementary stages. In the first stage, our aim was to confirm if a flexible electrode array system could be incorporated into our existing stretch-growth mechanism. Embryonic DRG explants were plated on a commercially available 32-channel FlexMEA array (Multi Channel Systems GmbH) that was pretreated with poly-L-lysine and collagen (*Figure 2*). After 5 days *in vitro*, the axons were stretch-grown at the rate of 1 mm/day using the FlexMEA as the towing membrane. After 7 days, robust axon bundles aligned in the direction of elongation were observed, proving that the FlexMEA could be adapted into our stretch-growth paradigm.

In the second stage, static cultures of E16 DRG explants were plated on FlexMEA electrodes. After 5 days *in vitro*, these cultures were completely embedded in an agarose matrix and inserted into NeuraGen tubes of 4 mm inner diameter (Integra Lifesciences Corp., Plainsboro, NJ, USA). The neural constructs were then sutured to a transected sciatic nerve (*Figure 3*) with the nerve carefully positioned

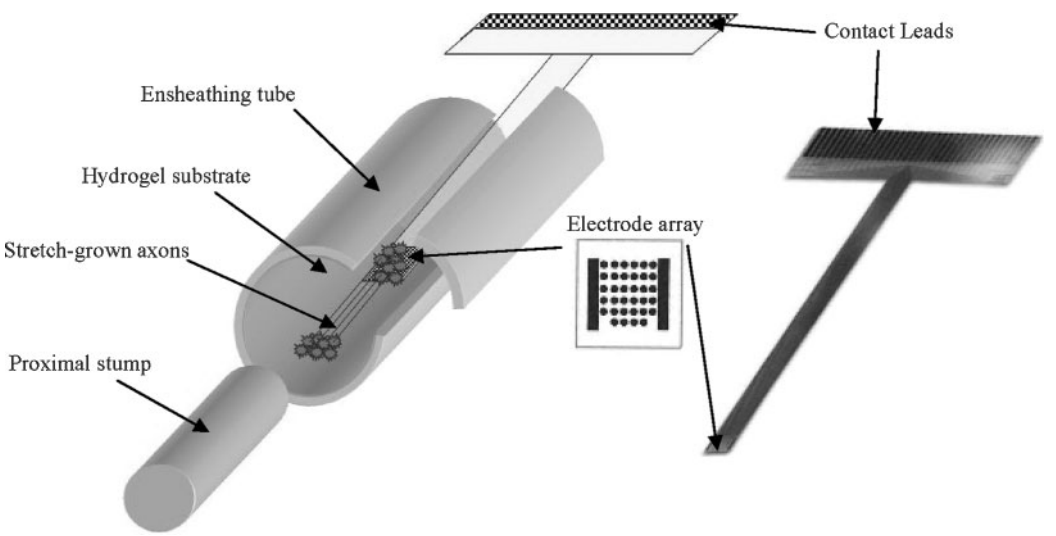

Figure 1: Schematic of the proposed neural interface. The electrode array serves as the movable substrate (left). Upon elongation, the construct is embedded in a hydrogel substrate, placed in a resorbable tube for structural support, and then sutured to the proximal nerve stump. Image of actual FlexMEA array, courtesy Multi-Channel Systems (right)

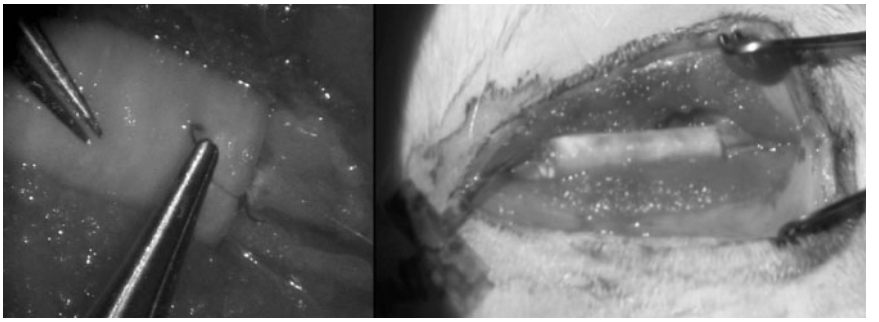

Figure 3: Neural interface at the time of implantation. Ensheathing NeuraGen tube being sutured to the proximal stump of the sciatic nerve (left). Macroscopic view of the interface (right). Contact leads of the electrode array are exteriorized for easy access by the recording equipment

immediately adjacent to the electrodes and left for 2 weeks *in vivo*. Upon removal, we observed that the nerves remarkably maintained their position within the neural constructs despite vigorous movement by the animals. We also found preliminary evidence of host axonal tract survival and vascularization within the constructs, a necessary condition for regeneration and restoration of function.

Encouraged by these results, we are currently pursuing the transplantation of neural interfaces, consisting of 1-cm elongated axons attached to FlexMEAs, to the transected rat median nerve. Our immediate goal is to quantify and maximize the host ingrowth and synaptic integration after 1 month *in vivo*. We will also test for functional integration with the interface by stimulating the proximal stump (in the anesthetized animal) and recording evoked signals using the FlexMEA.

FUTURE DIRECTIONS

In this paper, we describe a novel hybrid neuroprosthetic interface platform, consisting of biocompatible electrodes coupled to neural tissue constructs. For this to become clinically feasible, however, much work remains to be done. In addition to looking for markers of host axon ingrowth and synaptic integration, it is essential to determine whether meaningful motor commands and sensory stimuli can be communicated using this system. We are currently developing behavioral paradigms to test this.

With clinical applicability in mind, we are concurrently developing strategies to incorporate adult human DRG neurons into our design. These neurons were obtained from patients who underwent cervical and thoracic ganglionectomies. We have previously demonstrated our ability to adapt these ganglia to our stretch-growth paradigm and to keep them electrophysiologically active for several weeks *in vitro*[26]. We are thus confident that a clinically relevant version of our neural interface will soon be a reality.

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