Development of Micro-channel Arrays for Peripheral Nerve Recording

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Abstract: MicroTube Array (MTA) technology was developed to create an axon regeneration interface for exchanging motor and sensory data with residual nerves. Future clinical application will include sensory-motor transducers for individuals with limb amputation. In this pilot study, a small matrix (9) of MTAs 1, 3 and 5mm long with either 99um, 200um or 287um diameter MicroTubes (MTs) filling nerve cuffs of 3mm in diameter were implanted in tibial nerve of NZW rabbits and histologically evaluated after more than 6 months post-op. Full regeneration was observed in all 3 lengths for 287um MTAs, and for all three diameters of MTs with 1mm length. The remaining implants were mechanically dislodged during the healing phase. A second implant set was designed to include 12 platinum-iridium wire electrodes direct wired to a percutaneous connector. Successful recordings of useful amplitudes were observed during reflex righting behaviour for over 2 years before the anticipated wire breakage ended the experiments.

1 INTRODUCTION

Recent advances in the design of upper and lower extremity prosthetic limbs has underscored the need to provide more effective means for amputees to control these prostheses as well as to provide sensation from these devices. It is widely appreciated that these goals could best be met by establishing a permanent electrical interface with the trunk nerves in an amputee's residual limb (Mannard et al. 1974; Edell 1986; Riso 1999), however the development of the technology to achieve this has been elusive. Among the approaches that have been advocated and continue to be researched are: diverse designs of nerve cuffs (Loeb and Peck 1996; Naples et al. 1988; Walter et al. 1997; Grill and Mortimer 1998; Hoffer and Kallesoe 2000; Schuettler and Stieglitz 2000), intrafasicularly placed fine wire (Lefurge et al. 1991) and conductive polymer filaments (McNaughton and Horch 1996; Lawrence et al. 2002; Lawrence et al. 2004; Boretius et al. 2010), transverse penetrating arrays of microfabricated needle electrodes [e.g. Utah "Slant Array" (Clark et al. 2011; Wark et al. 2013)] and "sieve" (Kovacs et al. 1992; Bradly et al. 1997; Wallman et al. 2001; Lago et al. 2005) styled devices. While progress continues to be made (see for example Tan et al. 2014), none of these device designs has been shown to completely meet the needs of the prosthesis application in terms of numbers of independent recording channels for obtaining motor commands, provision for activating discrete sensory afferents for feedback of tactile and proprioceptive events, or device longevity (although cuff designs have been successfully deployed for other neuroprosthesis applications such as bowel and bladder control (Creasey et al. 2001) or FES based standing and walking for paraplegia (Schiefer et al. 2013).

The most useful device for an individual with amputation would provide sufficient information exchange between the nerve and prosthesis to enable return of complete and natural sensorimotor function. One approach is to embed the distal end of the residual nerve within a nerve cuff that contains properly designed and constructed MicroTube Arrays (MTAs). MTAs are small diameter Micro Tubes (MTs) that each contain neural recording and activation functionality for use in motor control and sensation. Under appropriate conditions, axons in all severed nerves will regenerate into and through small openings in MT devices.

In 1974, it was known that amphibian peripheral

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nerves would regenerate through small holes in implant structures from the work of Mannard and Stein (1974; Stein et al. 1975), but that approach was not successful when applied to mammals. In 1980 Edell showed that with biocompatible materials and design, the regeneration approach would work in rabbit peripheral nerves (Edell 1980; Edell et al. 1982). However, the selectivity of the recordings was limited by the open environment about the electrodes. In 1977, Loeb et al. (1977) published a method for isolating small groups of axons and improving signals in peripheral nerves by having them regenerate through MicroTubes, but was unsuccessful in making it work. The theory was sound - small diameter tubes increase signal amplitudes by increasing the effective extracellular resistance (Fitzgerald et al. 2008). However, the choice of dimensions (~10x15um) for the tubes was perhaps smaller than needed for the required support cells, collagen and capillaries, and the materials' surfaces may have triggered tissue micro-incompatibility.

This paper describes previously unpublished studies (DARPA project, 2006) demonstrating that MTAs can be made to work in rabbit peripheral nerves. Results are reported concerning an initial study that surveyed the effects of MT diameter and MT length on the ability of nerve fibers to grow into such devices and provide stabile recordable, multi-channel neural activity for use in prosthesis control. Robust regeneration into 100um diameter MTs was documented, the smallest diameter tested. Histological evidence suggests that MTs as small as 25um may be possible if properly designed. Within each MT there must be room for collagen, Schwann cells, and capillaries for mechanical, axonal, and metabolic support. These early findings have been corroborated in recent studies in other laboratories where successful neural regeneration has been reported (in rodents) into devices having channel sizes with cross sections of 100um x100um (Lacour et al. 2009) or as small as 70um x 20um rectangles (Stoyanov et al. 2013) and this results in the subdivision of a composite nerve into mini-fascicles which may enhance the separability of targeted nerve fibers by functional type.

2 METHODS

2.1 MicroTube Array Device Fabrication

MTAs were constructed within a Class 100 cleanroom from micro-polyimide tubing (Micro-Lumen) aggregated together within 2.5mm ID polyimide tubing. The interior diameters of the MTs were either 287, 203 or 99um (Fig 1). The bundled tubing was sliced transversely to produce micro-channel arrays having defined lengths of 1, 2 and 3mm.

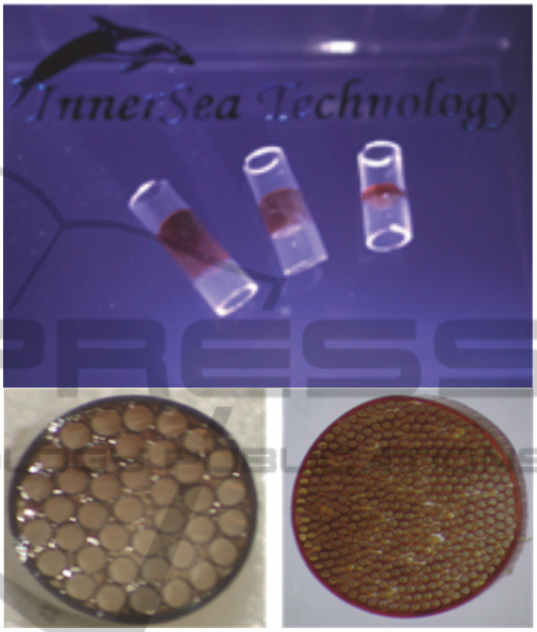

Figure 1: Top – photograph showing the microchannel arrays that were constructed using three different lengths of MTs. MTAs can be seen as the darken areas within the nerve cuffs); Lower - Photographs showing the cross sections of arrays that were comprized of the largest diameter tubes (287um dia.) and the smallest diameter tubes (99um dia.) used in this study. These arrays contained approximately 40 and 400 MTs, respectively.

2.2 Incorporation of Recording Electrodes into MicroTube Arrays

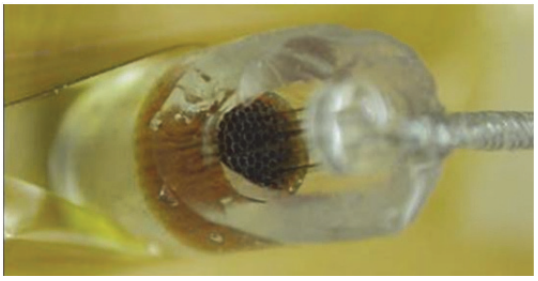

Figure 2: Magnified view of 3mm long, 203um diameter MicroTubes (brown) with 50um diameter insulated iridium microelectrode shafts placed halfway into 12 of the MicroTubes. The portal visible at the center of the picture is the exit port required for axon growth. Reference electrode coils and nerve cuffs were added to both the entry and exit chambers after this photo was obtained).

Fig. 2 shows a photograph of an assembled MTA nerve interface device in which 12 of the MTs are instrumented for neural recording and/or stimulation.

2.3 Experimental Design for in-Vivo Studies

The first part of this pilot study was aimed at determining the extent to which channel size (i.e. cross sectional area) would influence the ability of nerve fibers from a newly transected nerve to enter and grow through the channels. A second objective was to determine any effects of the channel length on neural ingrowth. To address these issues, nine New Zealand White rabbits (2.4-3kg) were implanted with a nerve interface device from a matrix of 9 devices representing combinations of the three MT sizes (287, 203 and 99um dia.) and three channel lengths (1, 3 and 5mm).

As this study was intended only as an initial survey, only one animal could be assigned to each of the nine permutations of channel size and channel length. All of the animal studies were reviewed and approved by the MIT- IACUC. With the animal fully anesthetized and using sterile technique, the sciatic nerve on one leg was exposed just proximal to the knee, and the tibial nerve component was isolated and transected taking care not to injure the peroneal and sural nerve components. The proximal end of the isolated tibial nerve was inserted into the MTA device and secured using microsutures applied through the epineurium. The distal segment of the transected nerve was then inserted into the distal opening of the nerve interface.

While this animal model is not a full amputation model, it was selected because it allows for monitoring of the progress of the nerve regeneration. The experimenter can readily observe when the regenerating nerve has traversed the nerve interface device and reconnected to the distal nerve target tissues. Thus, any return of ankle extensor function, either volitional or reflexive, denotes successful motor nerve regeneration through the device. Sensory nerve regeneration can be accessed by applying toe pinch and noting a return of flexor withdrawal of the limb.

Activity can be conveniently elicited by placing the animal prone on a table and gently rolling the head and shoulders from side to side. Leg extension will ensue as the animal acts to maintain balance. A second technique that was used to evoke activity in the ankle extensor muscle (and thus tibial nerve activation) involves the “drop reflex” whereby the animal is held in the air and then lowered towards the table so that the foot extends in anticipation of falling.

3 RESULTS

3.1 Assessment of Nerve Regeneration

After the tibial nerve regeneration was relatively stable as judged by the return of voluntary and reflex control of ankle extension function, each animal was euthanized and the devices retrieved for histological examination. Figure 3 shows the results seen with one of the implants (99um channels and 1mm length). The tissue was fixed in formalin and then the attached nerve was pulled out of the interface device, embedded in paraffin and stained. Not all of the fine micro-fascicles would slide out of the MicroTubes. However, for the most part, the micro-channel array pattern is evident.

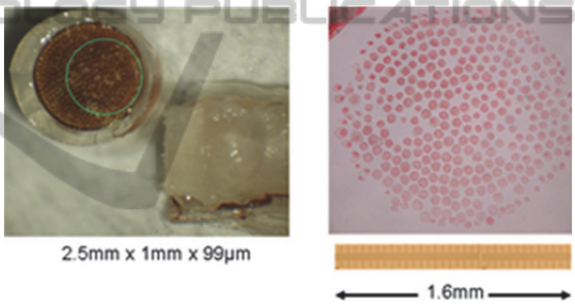

Figure 3: Left – Photograph of explanted 1mm long and 99um diameter MicroTube array assembly after regenerated peripheral nerve was removed. Green circle depicts the region from which the nerve mini-fascicles shown at the right were withdrawn. Right – Micrograph depicting the core of neural tissue that had grown through the micro-channel nerve interface.

Among the 9 implanted animals there were three mechanical failures most likely caused by fixation of the implants by connective tissue (which occurs rapidly) in a location that was not ideal mechanically. Straightening of the leg, perhaps when ear scratching, or during cage changes during the early healing phases could have generated sufficient stress on the interface to result in the nerve pulling out. In one instance (5mm long, 203um diameter array), partial regeneration occurred through a collapsed cuff where the distal nerve had been pulled out but the sutures held. The sutures collapsed the cuff so only a small opening was available for nerve regeneration to traverse on the distal side. There was good histology obtained from

the small subset of MTs that could support regeneration in the limited space.

Most of the MTs were filled with myelinated and (probably) non-myelinated axons, collagen and fibroblasts, and most importantly, capillaries (see summary in Table 1).

Table 1: Matrix of MT diameters and lengths studied.

Length\Dia	287 µm	203 µm	99 µm
1 mm	Regeneration	Regeneration	Regeneration
3 mm	Regeneration	Pulled Out?	Pulled Out?
5 mm	Regeneration	Partial*	Pulled Out?

3.2 Speculation regarding Minimal Micro-Channel Sizes

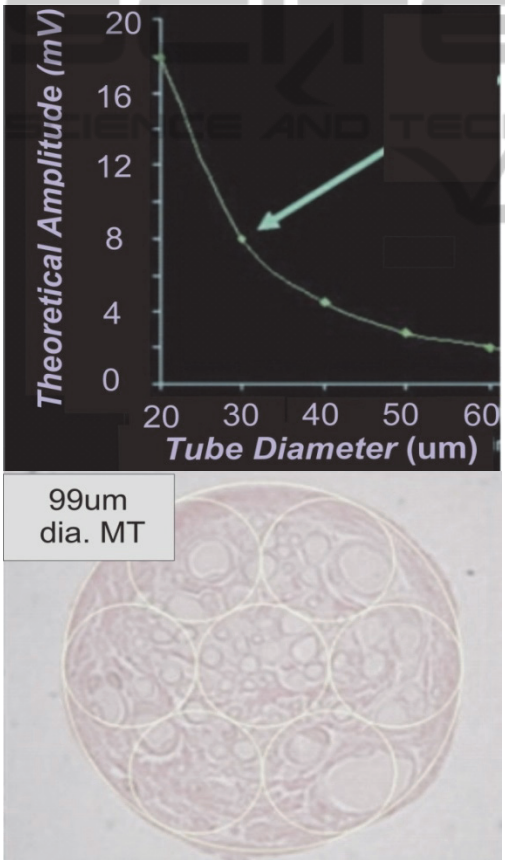

Figure 4: Upper – Theoretical graph showing the effect of micro-channel diameter for a 3mm long MT vs. the expected signal amplitude recordable from a single node of Ranvier positioned at the center of a MT with either a larger or smaller diameter. Lower – micrograph showing regenerated neural tissue that was removed from one MT of an explanted 3mm long and 99um dia. MTA assembly. Capillaries can be seen in all "sampled" regions delineated by the 33um dia. superimposed circles.

Figure 4 (*upper*) shows a graph of the theoretical signal amplitude that could be recorded from one node of Ranvier that is centered in 3mm long MTs of different diameters. The micrograph (*Fig. 4 lower*) showing the neural tissue that regenerated into a 99um dia. tube, was overlayed with small (yellow) circles having 33um dia., and it can be seen that there is a sufficient density of axons, capillaries and support cells within each drawn circle to suggest that regeneration would be successful for MT diameters of this small size (33um). Notably, if this regeneration were successful, then the use of small MTs on the order of the 33um dia. could be expected to afford axon signals in the range of 8mV as indicated by the green arrow in the figure.

3.3 Recordings of Neural Activity

Based on results from the regeneration studies with the 203um diameter x 3mm long MTA, and the fact that the effects of the presence of 50um wires within the tubes was unknown, we decided to use these intermediate sized tubes as the basis for developing the neural interface array that incorporated integral electrodes.

Three devices were constructed and implanted. Each of the implants was successful. The first two animals showed signs of regaining muscle function, and yielded good recordings after 6 weeks. The third animal showed promising recordings after 4 weeks, but shortly thereafter the percutaneous connector that was used to access the electrodes failed. Useful recordings were able to be obtained throughout the 7 months post implantation that the animals were studied. Examples of recorded neural activity from the first animal are shown in Figs. 5, 6 and 7.

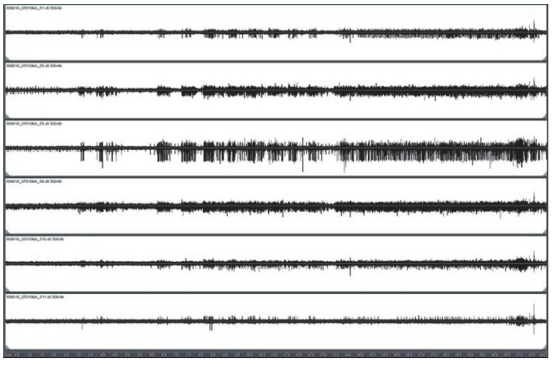

Figure 5: Example waveforms from rabbit R06015 approx. 3 months post-implantation. While immature, axons were beginning to exhibit robust responses to the righting reflex (~6-13s. middle time section of graph) and steady resistance to manipulation (~13.5s-end). (Max ampl. ~ 180uV).

For this animal, six of the eleven recording channels yielded high quality signals (Fig. 5) while four channels produced lower amplitude signals and one channel had a failed lead at the time of implantation.

The capacity for selective channel recording was clearly evident as there are frequent occurrences of activity present on one channel that does not occur on neighboring channels. In addition to demonstrating freedom from channel crosstalk, this shows that the recorded activity originates with axons contained within each specific tube and is not due to contaminating artifacts such as EMG from muscle tissue that's surrounds the implant (since such activity would be expected to contaminate all of the channels simultaneously if not excluded by the differential recording instrumentation). There is also a considerable amount of nerve activity that occurs approximately at the same time among different channels (Fig. 5). This is due to recruitment of motor units or afferent feedback that is synergistic since the neural waveforms are not coincident as would be expected if they were from the same neurons.

The independence of the recording channels in this same animal is more readily apparent in Fig. 6 which shows two different epochs of nerve activity (left and right columns) that were recorded simultaneously using electrodes #3 and #8 (compare upper vs. lower panels).

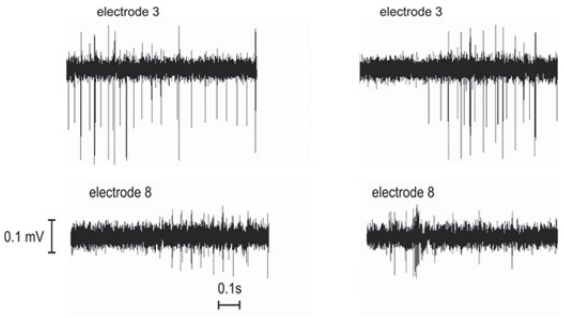

Figure 6: Example of recorded neural activity from animal R06029 electrodes 3 and 8. LEFT - simultaneous recordings showing distinct differences in activity levels and type. RIGHT - recordings from another episode of activity again showing little correlation other than similar timing of general heightened activity. Data acquired 6 weeks post-implant.

3.4 Qualities of the Recorded Axons from Regenerated, Maturing Axons

The recorded nerve activity showed a wide range of action potential amplitudes as well as spike durations. It isn't known at this point why some channels yielded higher amplitude recordings than others. We can offer several speculations to explain these effects: 1) Possibly the amplitude disparity is due to the nerve fibers not being fully regenerated in one channel verses another, as newly regenerated fibers are known to have smaller diameters, and thinner myelination than more mature fibers and thus have lower amplitude action potentials. 2) With regard to the range of action potential durations - The design of the microtube constricts the small axonal currents so that large signal amplitudes are recorded from non-myelinated fibers as well as myelinated fibers though the time course of the waveforms are markedly different. It is worth noting that the ability to record from non-myelinated fibers may be desirable for neurophysiology studies as small caliber autonomic fibers are normally difficult to record. The small diameters, thin myelin, and closely spaced nodes of the recently regenerated axons are associated with slow nerve conduction velocities and the action potentials from small fibers (and particularly from unmyelinated immature fibers or autonomic nerve fibers) tend to be of long duration. 3) Some of the largest recorded activity may represent compound action potentials formed from the superposition of quasi-simultaneous nerve discharges within the tube structures. 4) There is a wide distribution of fiber diameters in any peripheral nerve so that even a normal nerve has a wide distribution of amplitudes and action potential duration due to the fiber diameter distributions.

3.5 Utility of Combined Activity from Different Channels

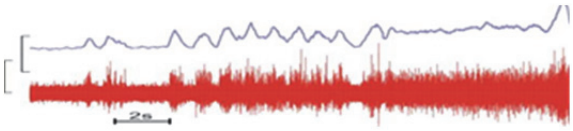

Figure 7: Composite signal from combining channels 1,2,3,9,10,11 yields a more predicable response than single channels due to the effective increase in motor unit pool being recorded. Note pulsed responses due to rhythmic righting reflex and relatively steady amplitude due to continuous force on leg, followed by relatively vigorous response at end. Amplitude bar: lower trace approximately 85uV; upper trace was a smoothed/rectified image of lower trace where the amplitude bar is about 8.5uV.

There have been several instances where recordings of 'whole nerve' have been useful for neuro-prosthetic devices (Haugland and Sinkjaer 1999; Sinkjaer et al. 1999; Riso and Slot 1996). Using the microtube array it is possible to select various combinations of channels to create the

highest quality composite signals. Fig. 7 shows an example of combining channels 1,2,3,9,10, and 11 from one experiment to achieve such a composite signal to
better represent the intended contraction of a particular tibial n. innervated muscle or group of muscles than each waveform alone.

An estimate of the composite signal intensity (solid line) is shown above the raw neural waveforms. With smaller tubes, more 'muscle specific' reconstruction of the efferent activity could be achieved. In addition, tubes that contained afferent nerve fibers could be designated for stimulation for cutaneous or proprioceptive sensation. Since axons tend to aggregate by function in peripheral nerves, this approach should be robust.

4 DISCUSSION AND CONCLUSIONS

4.1 Choice of Animal Model

Rabbits have long been accepted as adequate models for peripheral nerve repair work in humans. Rats and lower animals, particularly amphibians, regenerate more robustly than rabbits and higher animals, and thus, the results obtained with those preparations are not as readily extrapolated to the human condition. Because the studies presented herein were performed using an animal model in which the nerve interface served as a bridge between the transected ends of the tibial nerve, for experimental expediency, it might be argued that the successful regeneration of the nerve into the device may depend strongly on the ability of the proximal nerve to reconnect to the distal portion of the transected nerve and thus may not be applicable for interfacing to the truncated nerves in the amputee's residual limb.

At issue here is not whether the transected nerve will regenerate into a MTA. So long as the distal end of the MTA remains sufficiently open, the nerve fibers will exit the device and form into a disorganize mass as a neuroma. It is generally best, however, to avoid the occurrence of a neuroma because neuroma tissue frequently is overly sensitive to mechanical stimulation. This can result in painful sensations unless specific tactics are used to shield the neuroma from such stimulation.

One solution to prevent the formation of a neuroma in the absence of the distal nerve segment for attachment, is to provide small pieces of target tissue (e.g. muscle or skin) at the distal exit of the nerve interface. This target tissue allows the regenerated nerve fibers to terminate onto appropriate end organs and has a stabilizing effect on the nerve. A possible contra-indication with applying this strategy, however, is that the receptors present in the target tissue may generate activity in the connected nerve that could conflict with the intended nerve recording or stimulation paradigm. Further research in this area is warranted.

4.2 Importance of Channel Size

Smaller (more narrow) channels favor improved separability of axons so that efferent fibers to muscles that normally would control different functions don't co-mingle within the same channel. Additionally, segregation of the motor units associated with any given muscle into smaller groups allows for finer resolution for the derived control signal. Where desired, perhaps to achieve a more robust control signal, the activity of those channels could always be recombined. Furthermore as previously stated, the recorded signal amplitude is larger for smaller diameter channels.

With regard to electrical stimulation to provide sensory feedback, it would be best to be able to selectively activate afferent fibers that shared a common modality and region of referred sensation. While there is some evidence from microneurography studies in man (Stoyanov et al. 2013; Ochoa and Torebjörk 1983), that suggests grouping of sensory axons in peripheral nerves by modality, and end receptor location, presently too little is known about the extent of such grouping as they course, for example, from the brachial plexus, down the arm, and to the wrist and fingers to be able to suggest optimal channel sizes.

Ultimately, selection of an "optimal" channel size might depend on the intended application for the nerve interface and the particular morphology of the targeted nerve. The best strategy currently would seem to be to develop a modular system that would be scalable and adaptable to accommodate different size nerves and different locations within the amputee's residual limb. Large trunk nerves are intrinsically divided into small fascicles by perineurium, and within the small fascicles, axons aggregate mostly by function. As the axons near their targets, they further segregate into individual fascicles before exiting the nerve trunk. With a scalable system, the level of amputation would not present significant difficulty as it is simple to deploy individual interface devices to each of the divided

nerve fascicles. Most fascicles are 0.5-2mm diameter. Some fascicles are larger, but can easily be divided as needed to accommodate a standard MTA interface sub-unit of perhaps 2.5mm diameter.

An additional benefit of employing the micro-channel approach to nerve interfacing is that the same micro-channel could be used for both motor and sensory functions as desired. This feature may be particularly important for groups of axons destined for a muscle, as motor nerves carry a great deal of sensory information about the muscle target. Once the physiological function of each micro-fascicle is determined, information can be combined as appropriate.

4.3 Design Considerations for MTA Devices

It should be noted that in spite of its use in this pilot work, polyimide is NOT a suitable long term implant material – it undergoes slow hydrolytic degradation and will over time fail as an insulator. Further, the wall thickness is far too great for use in a good clinical device. The open area occluded by wall thickness can completely prevent regeneration. While in these simple single fascicle experiments the open area of the devices can be kept to at least the open area of the fascicle, in a practical device for human shoulder, arm or leg amputation applications might require many of these devices and result in unnecessary bulk. A better solution would be to use silicon dioxide or titanium dioxide MTs fabricated on low-power integrated circuit substrates. Such substrates would assist to mechanically stabilize the nerve interface, and the integrated electronics could be insulated reliably (Edell 2004) to survive implantation for decades with minimal bulk.

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