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17

Development of a Neural Interface for PNS Motor Control

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1. Introduction

1.1 The design process

Engineering disciplines offer powerful analytical techniques which are applicable in a wide range of problem solving applications. As an emerging field, biomedical engineering draws on the tools of the classical engineering fields, such as mechanical, electrical, and chemical engineering, to address problems originating in complex biological or clinical systems. The real art of biomedical engineering comes in the creativity it takes to reframe these ill-defined biological or clinical problems into ones which can be addressed using the tools offered by classical engineering disciplines. Because the range of clinical and biological problems is so broad, it is impossible to give examples of every possible problem and their appropriate solution sets. However, developing a general framework in which complex, multilevel problems can be addressed can help formalize the process by which these problems are conceptualized and prevent them from becoming overwhelming in their complexity. This framework consists of the 5 following steps:

1. Identify a precise, well-defined problem (may be a sub-problem of a much larger problem)
2. Identify desired endpoint/solution to problem
3. Identify a set of reasonable assumptions based on current knowledge of the field
4. Based on the problem statement, desired endpoint, and assumptions, use engineering tools to design potential solutions
5. Identify optimal solution based on desired endpoint and the pros/cons associated with each solution
6. Repeat as necessary

By way of example, we will walk through these steps, demonstrating how it can be applied to neural prosthetics to see how it generates a new and promising approach to solving problems currently preventing progress in this field. The use of neural prosthetics is complex, involving many different therapeutic goals and technical approaches to achieving them. The above process will be repeated several times as we re-frame our biological problem and think through possible solutions, but it will differ slightly each time as it is used to address new problems arising at each decision point in the design process.

1.2 Defining the problem: Appropriately selecting a patient population and defining their clinical need

There are an estimated 1.7 million Americans living with limb loss [1] and many more suffering from peripheral nervous system (PNS) injury without expected motor recovery [2, 3]. Although these conditions result in significant loss of function, many patients do not take advantage of the powered prosthetics available to them, due mostly to the fact that the pros (restoration of partial function) do not outweigh the cons (cost and discomfort) [4-6]. The mechanical capabilities of prosthetic devices have become sophisticated, but motor tasks are generally driven by gross anatomic movements or low bandwidth myoelectric couplings, making them cumbersome [10-12]. For example, NASA has developed a robotic hand that approximates human dexterity, but commercially available prosthetic technology can currently control an extremely limited number of joints at a time. Amputees, therefore, are unable to benefit from the advanced hand [10]. For such prostheses, communication with the user is the weakest link in the chain of components that includes electronics, computing, and actuators, all of which are adequate for the application [10-12].

Neuroprosthetic devices aim to correct this deficiency by placing a prosthetic directly under neural control. Achieving this goal would give the user control over multiple joints simultaneously, restoring intuitive control over the prosthetic appendage. Restoration of real-time, dexterous control, however, requires high bandwidth communication between user and device. The neural interface is the point at which this communication occurs (reviewed recently in the popular press [13]). In the case of sensory prostheses, such as a cochlear implant [14, 15], the neural interface is designed to insert signals into the nervous system by stimulating the nervous tissue, while in the case of motor prosthetics [10, 16, 17], the purpose is to extract signals from the nervous system by recording the activity of the nervous tissue. Long-term efforts are aimed at creating hybrid systems capable of two-way communication with the nervous system for restoring full function to amputees as well as to other patient groups [18, 19].

While this technology has advanced rapidly, these devices have yet to perform at the level necessary to justify their use in large-scale clinical trials [8, 9]. The major hurdle to progress in the clinical advancement of neuroprosthetic devices is the development of neural interfaces capable of efficient communication with the nervous system [8, 11, 20]. In part, this is because neural interfaces are developed using performance objectives which do not have direct clinical corollaries. This rift between technological advancement and clinical advancement is exemplified by the examples of cochlear and cortical implants. Cochlear implants, using a neural interface with electrodes designed specifically for stimulating the cochlear nucleus, are widespread because they restore function and are relatively non-invasive. The size, positioning, and composition of the electrode contacts, however, make them unusable in recording applications. In comparison, other neural interface designs are used primarily in basic science research because they can both stimulate and record and can be used with multiple different tissue types from both the central nervous system (CNS) and PNS. However, they are not employed on a large scale clinically because it is difficult to justify their invasiveness relative to their clinical benefits.

Technologies exist that are capable of recording neural activity from both the PNS and CNS [21, 22], but they face problems acquiring large enough numbers of independent and appropriately tuned neural signals to provide reliable dexterous control. Current reviews of

neural interface designs highlight the following functional criteria as primary bottlenecks [8, 20, 23, 24]:

- Obtaining stable, long-term recordings of large populations of neurons,
- Developing computationally efficient algorithms for translating neuronal activity into command signals capable of controlling a complex artificial actuator, and
- Determining how to use brain plasticity to incorporate prosthetics.

While it is nearly impossible to address each of these points optimally at the same time, it may be possible to address them within a single, clinical context by focusing design considerations on an appropriately selected patient base and a precisely identified clinical goal. For example, for the purpose of this case study, we will focus on amputees and others with severe PNS injury as our patient population and on restoring motor control through the use of neuroprosthetics as our clinical goal. Our subsequent design decisions can therefore be guided by the need to acquire neural information specifically about motor intention rather than simply acquiring neural signals generally.

1.3 Microelectrode arrays basics

The neurons of the PNS and CNS communicate using bioelectric events called action potentials (APs). To connect to the nervous system in a biologically relevant manner, we must integrate electronics and nervous tissue to transform these bioelectric signals into electronic signals which can be interpreted using computers. The gold-standard method to measure the activity of electrogenic cells uses electrodes pulled from metal wires and glass tubing and is not logistically adaptable to long-term or *in vivo* use. Although alternative methods, such as voltage-sensitive dyes, have been used, these methods are unsuitable for *in vivo* applications because of the infrastructure involved and the toxic side effects.

Microelectrode arrays (MEAs) were developed to overcome many of these problems by recording from multiple sites in a non-invasive way. Neuronal activity is recorded by placing a conductor, which is connected to a recording device, in the vicinity of a neuron's soma where neuronal APs create their largest transmembrane currents [25, 26]. This rapidly changing current creates voltage transients that can be transmitted along the conductor and subsequently recorded. A conductor will transmit all such activity along its length so that regional specificity is achieved by insulating the entire conductor except the points from which you want to record activity (Fig. 1 A).

MEAs are not, however, without limitations. Extracellular MEAs have been used with some success in recording from large populations of neurons, but they record only a fraction of the transmembrane voltage and record from the space surrounding the electrode rather than from the space inside of an individual neuron. Therefore, the extracellular voltage, referred to as the extracellular voltage trace (EVT), at any point is the sum of the activity of any neurons close enough that their APs can be detected (Fig. 1 B-C) [27] as well as background noise [28]. As a result, they suffer from poor signal fidelity, and single electrodes can wind up unintentionally recording from multiple neurons (Fig. 1 D). The interpretation of data from multiple neurons recorded on a single channel is a complex problem that has been likened to trying to understand the function of an orchestra without any knowledge that the final sound is generated by different instruments playing simultaneously [25]. Similarly, the most meaningful interpretations of neuronal activity depend on knowing the activity of single cells, due to the nature of information exchange through APs.

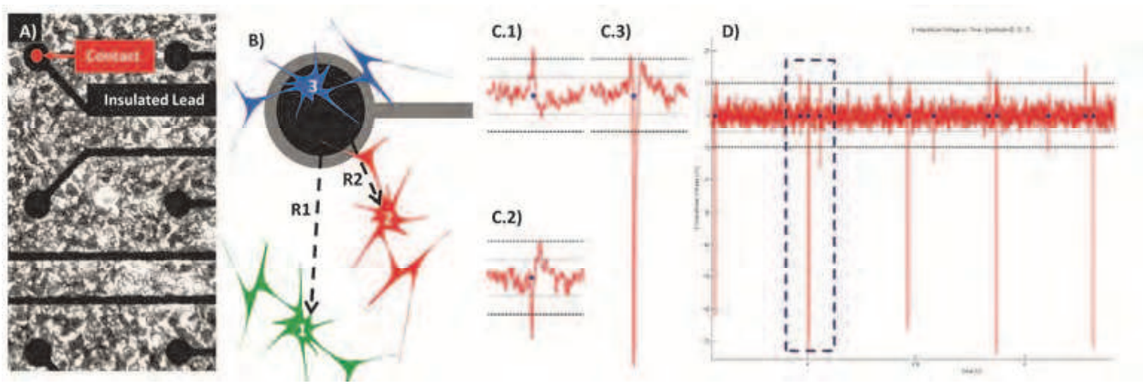

Fig. 1. Recording and interpretation of EVTs using an MEA. A) Phase microscopy of cortical neuron cell culture on commercially available MEA. B) Schematic representation of potential neuron locations relative to contact pad on MEA. C) Example segments of EVT recorded from a single electrode, showing three distinct spike morphologies (small-positive, small-negative, and large-negative), indicating the likely presence of three nearby neurons. Threshold levels used for spike detection are indicated by black dashes. D) Example EVT data recorded over 2 seconds, demonstrating temporal relationship of spike events.

MEAs are fabricated using standard integrated circuit (IC) and micro-electromechanical systems (MEMS) manufacturing techniques, which build MEAs on rigid substrates, such as glass or silicon [29]. In standard designs, a conducting layer of metal is deposited on top of the substrate and formed into the appropriate electrode contact and lead pattern using microlithography. Several layers of thin-film insulating material, usually silicone dioxide (SiO_2) or silicone nitride (Si_xN_y), are then deposited to insulate the conducting layer except at the sites of intended cell contact [30]. These electrodes can detect changes in voltage secondary to cellular depolarization at the conducting contact site, transmitting it along the conducting wires underneath the insulating layers to an amplifier and recording device [29]. As microfabrication technology has evolved, so have the means of generating features of varying size and shape. Standard lithographic techniques have reached new lower limits on the feature size they are able to create, and soft lithography has allowed the use of new materials and techniques to reduce cost. Such design decisions are eventually made based on the end use of the electrode, such as desired performance characteristics and the cell type or tissue type it is meant to interact with.

1.4 Cell-surface interactions

The first important interface is the cell-electrode interaction in which an electrogenic cell communicates with a substrate electrode. Fig. 2 A-B is a schematic of a neuron resting on top of an electrode in a planar electrode array [31]. Current can either be forced into the cell by the electrode, or cellular depolarizations can be detected by the electrode, depending on whether the electrode is stimulating or recording from the associated tissue. In Figure 2 the current originates in the cell and disperses into the culture environment and the electrode contact pad.

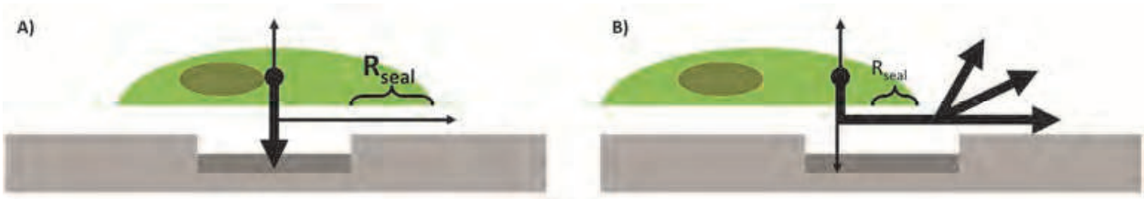

Fig. 2. Geometry of cell-electrode interactions. A) Good electrode coverage results in a high R_{seal} and more voltage observed at the electrode contact. B) Poor electrode coverage results in a low R_{seal}, greater dissipation of current into the culture environment and less voltage observed at the electrode contact.

The current in the sealing gap between cell and electrode arises from the lower cell membrane, and the resulting potential field is measured by the electrode. This current is divided into a portion that flows from the cell to the underlying electrode and a portion that leaks out around its boundaries (through the sealing gap) and into the medium. The relative amount of current traveling via each of these two paths is a function of the membrane characteristics of the cell, the material characteristics of the electrode, and the resistance of the sealing gap (R_{seal}) [31]. A high R_{seal} means that more of the current associated with the cellular depolarization is transmitted directly to the electrode (Fig. 2 A), while a low R_{seal} allows more of the current to leak from the sealing gap into the culture medium, escaping detection by the electrode (Fig. 2 B). R_{seal} is related to cell-electrode geometry (size, shape, and relative positions of the cell and electrode). Finite element solutions, based on a lumped RC-circuit model of the cell-electrode interface, identify two regions of R_{seal} based on this geometry: one in which the electrode is not entirely covered and R_{seal} is low, and one in which the electrode is completely covered and R_{seal} is high and dependent on the position of the electrode relative to the rest of the cell body [32]. The range of resistances is larger in the latter case, indicating that the relative positions of the electrode and cell body play an important role in determining R_{seal} regardless of whether the electrode is completely covered. High quality cell-electrode contacts, where quality is determined by electrode coverage and sealing, will permit selective and reliable recording [33]. Experiments have similarly demonstrated that the sealing resistance increases when the electrode is completely covered by the cell and drops off sharply when any portion is exposed directly to the medium [31].
Of the parameters in this model, R_{seal} is the only one related to mechanical design of the electrode. The practical implications of these findings are that maximizing recording fidelity means maximizing electrode area in such a way that electrode coverage, and therefore R_{seal}, is preserved. This is a goal easily accomplished using larger cells, as they can effectively cover larger electrodes, but which is very difficult for smaller structures such as axons.

1.5 Performance objectives

A complete neural interface should provide a control signal that restores natural movement to paralyzed body parts or prostheses without extensive learning. Most upper limb amputees want a prosthesis that requires less visual attention to operate, has sensory feedback, and can execute multiple movements simultaneously [34]. In other words, control should emerge from the voluntary intent to carry out an action, rather than the retraining of other actions. Researchers believe that with an efficient neural interface, efferent motor nerve signals carrying command information outward from the CNS could be used to control actuators in an artificial limb and to selectively stimulate afferent sensory neurons that carry sensory information towards the CNS, thus providing sensory feedback [34].

Because of the massive amounts of information exchanged between end organs and the brain during seemingly common tasks, future rehabilitative applications, like graded neuromuscular control or for prosthetic vision, will depend on the availability of large-scale, selective neural interfaces [33]. This requires that the neural interface record efferent nervous signaling with enough specificity to reproduce fine motor control [12]. Ideally, the device would acquire and distribute these signals in the least invasive way possible. At present, these are all major challenges. The design of the neural interface component of a neuroprosthetic device should be based on achieving the above by manipulating electrode design, material properties, surface chemistry, and implantation site.

Extracting motor control signals from the firing patterns of populations of neurons and using them to reproduce motor behaviors in artificial actuators are the two key operations that a clinically viable neuroprosthesis needs to perform [20]. It is easy to define success as simply improving the adhesion of nerve cells to an electrode array in order to improve signal acquisition *in vitro*; however, it is much more useful to set the endpoint for determining success as improved functionality by the end-users. Eventually, the goals of neural interfaces are for patients to use the system for restoring mobility and sensory functions associated with the activities of daily living and enhancing their quality of life and independence [21]. This means usability of the signal acquired by a neural interface, as well as signal fidelity, should be included in the definition of success.

Existing neural interface designs are as diverse as the nervous system itself, including multiple cortical, deep brain, spinal, non-invasive, and PNS-based approaches to stimulation and recording. Additional complexity is added by the exploding number of algorithms designed to analyze and decode the information recorded from neural interfaces. Consequently, there are essentially limitless pairings of technologies, nervous system targets, and disease states, which may potentially be addressed with technologies labeled as neural interfaces. The ultimate adoption of neuroprosthetic devices into mainstream clinical practice will depend on whether or not they improve significantly on current prosthetic designs' functionality. In this case, successful device function requires the integration of the nervous system, implantable device, and computational algorithm. In other words, the interface is one link in a chain of components that must work together to form a functional end product. As such, it should be designed with the links on either side in mind in order to facilitate these components integration into a whole.

Despite the speed with which computers execute complex algorithms, the human nervous system is far superior at tasks such as complex motor control [24]. The main processing task for algorithms associated with neural interfaces is decoding: deriving motor commands from neural impulses which can be used to drive prostheses. There are several approaches to decoding, including neural networks, pattern-recognition algorithms, and hybrid filters. None of these methods is clearly superior, as each carries an individual set of pros and cons, involving the amount of information that can be simultaneously processed, the amount of time it takes to do so, and the accuracy with which they can predict motor intention [10]. The types of algorithms employed to decode neural motor volition are an important constraint when designing a neural interface. Questions pertaining to the rate at which usable data can be acquired and the speed at which it can be translated into motor intention will drive decisions regarding implantation site and the target number of neural recordings, especially as more effort is put into including signal processing components on the implants themselves as a way of reducing the bandwidth required to transmit the acquired signal. As a means of illustrating the importance of having the appropriate amount of relevant data, imagine trying to read a computer screen with only a small number of pixels (Fig. 3). The

more complex the message, the larger the number of pixels required to read it. Sadly, there is no exact answer to the question "how many independent neural signals are required to restore dexterous control?" because the answer is dependent on the nature of the signals and what type of device they are used to control. While small populations of highly tuned neurons can accurately predict movement parameters, highly tuned neurons are rare in a random sample of cortical cells. Because motor information is represented in this highly distributed way, large samples of recorded cortical neurons are preferred [20, 34, 35]. It has been estimated that recordings from 500 to 700 cortical neurons would be needed to achieve 95% accuracy in predicting one-dimensional hand movements [36]. The minimum number of recordings required to transform thoughts into a reasonable range of motions most likely exceeds 1000 [10], a number presently exceeding the capabilities of cortical probes.

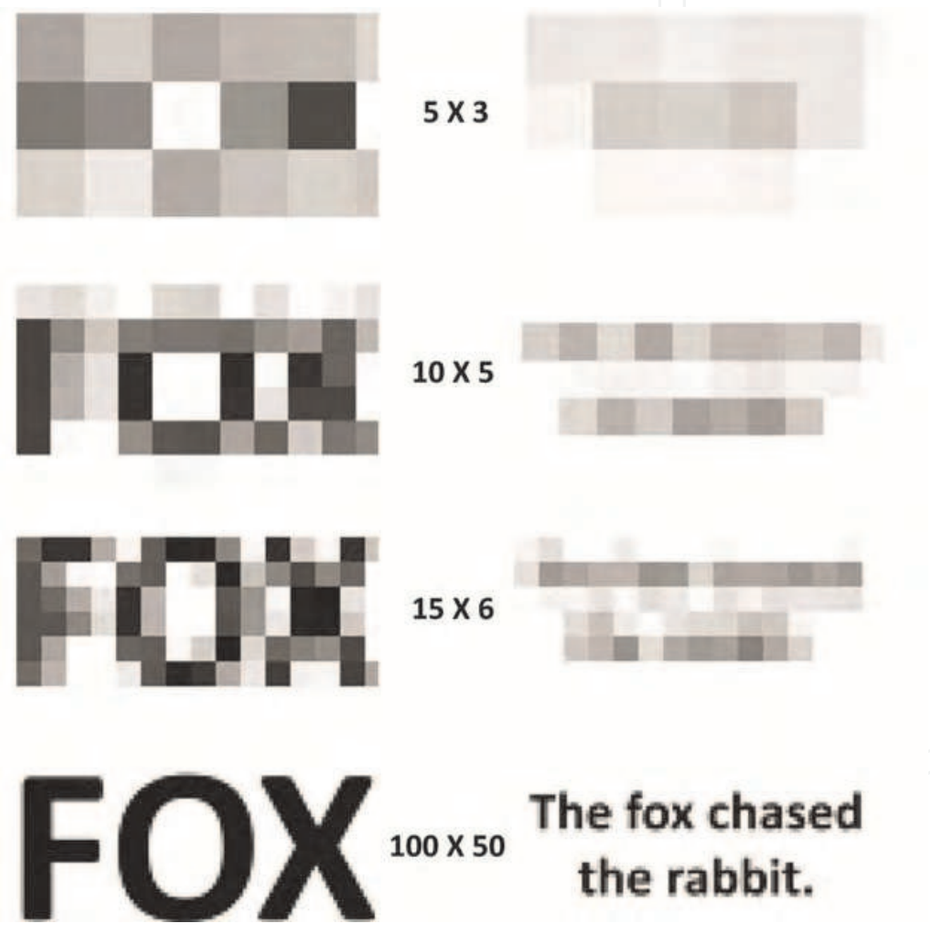

Fig. 3. More complex messages require a larger number of independent signal sources (in this case pixels) for proper decoding

The nervous system that handles this information consists of peripheral nervous structures and central nervous structures. Each region encodes, processes, and transmits information in a unique form, and therefore, has unique anatomic and physiologic characteristics which employ not only a highly dynamic and adaptive information structure, but also a highly dynamic physical environment. Recent findings suggest that the task of designing a device that acts in the same way and feels the same as the subjects' own limbs might be

accomplished by designing devices which capitalize on the brain's ability to undergo experience-dependent plasticity and assimilates the prosthetic limb as if it were part of the subject's own body [20]. Electrode design and placement should aim to benefit from neural plasticity rather than combat it. Electrodes intended for use in a neural interface should be designed appropriately for their intended site of implantation, based on the characteristics of the target contact neural cell types and the coding structure of the neural information.

Due to the need for highly specialized algorithms to interpret the recorded neural signals, the design, size, material choice, and interface surface have to ensure temporally stable transducer properties of the neuron-electrode interface throughout the lifetime of the implant. Electrodes need to not only be non-destructive to the surrounding tissue and record from the same or similar set of neurons, but need to avoid encapsulation, inflammation, and other "foreign-object responses" which would alter its ability to record neural signals. Neural interfaces therefore have to fulfill high demands with respect to biostability and biofunctionality [21].

Finally, it is important to consider the attitudes of the end-users of neural interface technology. Fully paralyzed patients anxious to gain enhanced physical abilities may be willing to accept the risks of brain surgery and to live with hardware implanted in their brains, but most healthy people would probably not. Amputees and spinal cord injury patients are likely to fall along a range of attitudes regarding the concept of implanting devices in their brains. The key to success in restoring human capabilities will be gaining access to motor and sensory signals in an unobtrusive way [36].

1.6 Design decisions

As an exercise in engineering design, we are going to walk through a small amount of biological background and then discuss how this plays into designing a new interface. Because neuroprosthetic devices, and even neural interfaces specifically, involve multiple components, there is a hierarchical sequence to how design decisions need to be made. Every design decision made in the process comes with a set of benefits and drawbacks. While some aspects of our design are flexible, and the drawbacks they introduce can be minimized in the actual decision, others are more rigid and the drawbacks they introduce need to be mitigated in subsequent design decisions. Over the course of this chapter, we will see how each decision draws from those before it and affects those that follow.

Based on our goal of restoring dexterous control of powered prosthetic devices to our patient population, we need to answer the following questions: What type of information do we want from the nervous system? Where are we going to get that information from? How are we going to get it? To help us answer these questions, it helps to establish a conceptual framework by which to organize current approaches to interfacing with the nervous system. Based on the evolution of the field of neural interfaces so far, it is helpful to consider several different aspects of neural interface design:

- The intended function of the neural interface (signal recording or signal insertion)
- The site of implantation (CNS or PNS)
- Target electrogenic cell type (myotubes or neurons)
- The invasiveness of the implant (the degree to which it is destructive to the native tissue)

2. Design decision #1: Signal recording vs. signal insertion

The nervous system is a bidirectional communication system, with efferent signals carrying command information outward from the CNS and afferent signals carrying sensory information toward the CNS. Neural interfaces can be designed to restore function in

instances where one or both of these transmission pathways have failed. Generally, "input prostheses" are designed to insert information into the nervous system (supplementing the afferent pathway), while "output prostheses" are designed to record information from the nervous system (supplementing the efferent pathway on the nervous side).

Input prostheses are designed to restore function in individuals with sensory disabilities by selectively stimulating nervous tissues to which natural nervous stimulation or response to environmental cues has been lost. The most advanced of these has brought the perception of sound to ~40,000 deaf individuals by means of electrodes implanted in the cochlea. Similar attempts are underway to provide images to the visual cortex [24], and the direct electrical stimulation of both cortical sensory areas and peripheral nerves has shown promising results in restoring the sensation of touch.

The major goal of output prostheses is recording neural signals with the intention of later translating them into meaningful command functions. These commands can then serve as a means to control disabled body parts or devices, such as computers or robotic limbs [37]. Current efforts to achieve this have been slowed by the need to record from large numbers of neurons (arguably thousands depending on the site of recording), which is a challenging biological problem, compounded by the need to analyze and interpret these neural signals in real time.

2.1 Decision summary

While simultaneous restoration of sensory and motor function in prosthetics is the ultimate goal, it is unclear that this is achievable using a single interface design. It is far more likely that this will ultimately be achieved using a combination of techniques (consider the earlier discussion of cochlear vs. cortical implants), due to the fact that electrode modifications made to optimize the recording or stimulating modality frequently come at the expense of the other modality. As such, we choose to limit our future design considerations according to our clinical goal of restoring motor control (Fig. 4).

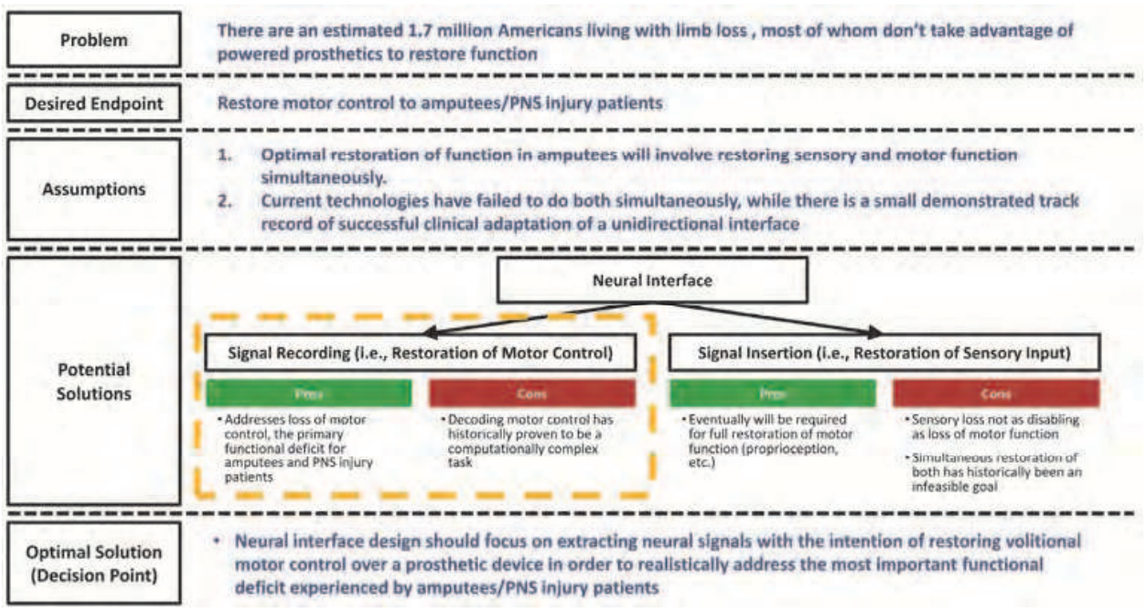

Fig. 4. Design process summary diagram after Decision #1

3. Design decision #2: Neural target (CNS vs. PNS)

The most important design constraint is the one over which we have the least control: the nature of the neural target our interface is designed to interact with. We can manipulate the rest of the components of the neuroprosthetic system, but the biology of the brain and nervous system is largely unalterable. The CNS typically refers to the nervous tissue contained within the skull and spinal column. The PNS refers to nervous tissue found outside of these locations which carry motor and sensory information to and from the CNS, such as the brachial and lumbar nerve plexuses or the radial and sciatic nerve trunks. The intention to perform an action is born in the cortex of the brain, is processed through multiple regions of the brain and spinal cord, and is transmitted along the axons of the PNS, finally arriving at the neuromuscular junction (NMJ) where it triggers the depolarization and contraction of the specific muscle cells required to perform the desired action. There is continual debate on where in this chain of transmission is the best location from which to derive a useful motor signal, and therefore, to target with a neural interface [8, 20, 22, 38-40].

3.1 Accessibility

One of the primary differences between the CNS and PNS has to do with accessibility of neural signals. With regards to the motor system, for example, the motor cortex of the CNS contains large cell bodies (upper motor neurons) while the portions of the PNS that are relevant to motor control consist mostly of axons from alpha motoneurons (lower motor neurons). The cell bodies in the motor cortex are large enough so that their depolarizations can be sensed using microelectrodes inserted into their vicinity, while the depolarizations occurring in lower motor axons are small enough so that they can only be sensed in aggregate (remember our earlier point about electrode sealing and cell size).

Historically, neural interfaces have been designed with the intention of communicating directly with cortical tissue [41]. Most of these efforts use penetrating MEAs to record the depolarization of cell bodies [11, 20-22]. With these designs, electrodes located at the end of micron scale spikes are inserted directly into the CNS, such as in the primary motor cortex or spinal cord [12]. While there are a number of benefits to this approach (most notably that it is technically simple to record a neural signal from a region where the large neuronal cell bodies may be accessed), progress is confounded by the complicated encoding of information in cortical brain regions [37] and by the highly invasive nature of implanting foreign devices in the CNS [41-46]. In the cases where these neural interfaces have been used *in vivo*, the surgical technique used for implantation strongly influences the long-term results. Most implantable microelectrode designs, which make direct contact with cortical tissue, enable good quality recordings to be made for several months, but recording quality deteriorates over time, due most likely to electrode encapsulation by fibrous tissue and cell death in the vicinity of the electrode [20]. Additionally, the size of electronic devices meant to be implanted inside of a living animal's skull is another design constraint that limits the functionality of cortical implants [10]. Thus, complicating issues for these electrodes include poor long-term recording due to fibrous encapsulation, inflammation, death of surrounding neurons, and insufficient data transfer and decoding ability to interpret signals recorded at the cortical implantation site [41, 45, 46].

The PNS may provide an especially useful point of access for neural interfaces in the case of restoring motor function to amputees and spinal cord injury patients. Amputation removes the structural elements of the limb, but the ends of lesioned nerves remain and continue to carry motor and sensory information from the removed limb. Even lesioned peripheral nerves that are not permitted to regenerate continue to demonstrate a normal pattern of discharge for the muscles that they originally innervated [47]. Both central and peripheral motor and somatosensory pathways retain significant residual connectivity long after limb amputation.

3.2 Encoding

Recent work has shown that motor information is represented in the cortex in a highly distributed way [20, 35]. Discoveries using fMRI indicate that cortical representations of different upper-limb regions are not demarcated into the discrete topographic areas of the classic homunculus. There are multiple foci representing a given limb movement and overlapping representations of disparate limb regions [34, 35]. What was thought at one time to be a simple topographical map superimposed on the cortex is far more complex. Each action originates with a few neurons in the motor cortex that trigger a large neural network that, in turn, coordinates the activities of several effector muscles. This neural network synthesizes input from thousands of tactile, positional, and visual sources with motor intention from the primary motor cortex to derive controlled motor output. Transforming this tangled mesh of millions of bioelectric signals into graceful movements is a routine accomplishment of our sensorimotor system but is not currently reproducible using computers [10]. As discussed previously, neurons which are highly tuned to motor intention are rare in a random sample of cortical cells. The farther synaptically removed from the periphery a neuron is, the more its activity is determined by its peers rather than by the environment, and the more it encodes cognitive abstractions rather than direct representations [25]. Because cortical motor information is represented in this highly distributed way, it may take recordings from hundreds to thousands of cortical cells to restore a reasonable range of motion [10, 20, 34-36].

In comparison to these complex CNS representations, PNS motor encoding is relatively simple. Peripheral nerves are organized somatotopically at both fascicular and subfascicular levels [34]. Each motoneuron synapses with many muscle fibers, constituting a motor unit. The neuromuscular junction is a one-to-one synapse, meaning that the excitation of a motoneuron produces the same frequency of action potentials in all muscle fibers in the motor unit. Graded contraction is produced by increasing the number of motor units activated and by increasing the frequency of action potentials [21]. Each muscle is controlled by up to a few hundred motor neurons [22], implying that each limb is supplied by several thousand. This high degree of organization in the neuromuscular system is achieved through the bidirectional interactions of myotubes and motoneurons. The final system architecture is the result of a highly interactive process between motoneurons and myotubes, involving the exchange of several known, and potentially more unknown, growth factors and cell adhesion molecules.

In the case of walking and other complex motor programs, complex signal processing occurs between the cortex and actuator muscles, and there is integration of sensory input from the visual and vestibular portions of the brain, which facilitate coordinated movement.

Recording from cortical sites may record movement intention but would not contain any information regarding the massive integration of environmental cues with motor intention that go into developing a useful motor signal. Recording or inputting signals in the PNS and allowing them to proceed through the many processing steps occurring naturally may result in improved communication. For example, cochlear implants are one of the most successful neural interfaces currently in clinical use. They restore hearing to the deaf by directly stimulating the nerve cells in the cochlea which fail to respond to normal sound stimuli. Attempts to stimulate more central areas of the cortex associated with hearing have been less successful, however, probably because of the loss of important signal processing in the periphery [24].

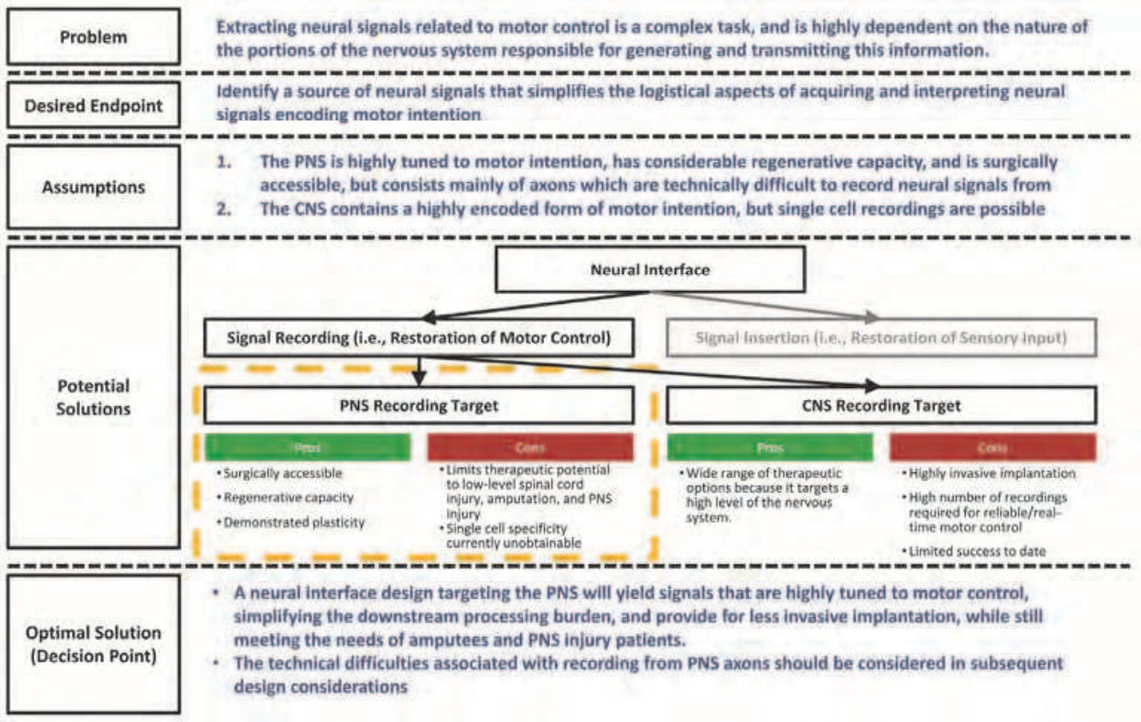

Fig. 5. Design process summary diagram after Decision #2

3.3 Decision summary

Many studies have centered around interfaces with the cerebral cortex because it was believed that motor intent and sensory percepts were more readily accessed there than elsewhere in the brain [37]. The benefits of a CNS interface include a more diverse set of applications, as it targets a higher region of the nervous system. However, recording from the CNS yields a highly encoded neural signal, as at that point the nervous impulses have not undergone the extensive processing performed in the cerebellum and spinal cord. The complicated nature of this encoding exacerbates the problems associated with decoding it and increases the number of signals required to establish reliable motor control. Due to its comparative physical accessibility, the discrete encoding of motor and sensory signals, and

the regenerative capacity of peripheral axons [34, 47, 48], the PNS may represent a more convenient location for accessing neural signals.

Neural interfaces that target the PNS pose a good compromise between the benefits and drawbacks of many types of neural interfaces [38, 39]. Rather than placing microelectrodes into delicate nervous tissue, one could consider interfacing through the PNS and exploiting its capacity to "remap" motor and sensory functions, effectively outsourcing some of the requirements for efficient communication to the native neural structures. An MEA-based neural interface that targets the PNS improves on current technology by taking advantage of the specific nature of the PNS in managing motor control. Regenerating peripheral axons naturally re-innervate tissue different from their original locations. With training, patients are then able to assimilate the new axon distribution and regain limb function. Through rehabilitation, the patient may be able to learn to control particular motor functions directly by using the portions of the nervous system specifically designed for such a task. Taking advantage of naturally occurring neural plasticity in the PNS would reduce the need to interpret complex neural processing using computational tools from a small region of the brain or spinal cord, a process better handled by the CNS [12]. Essentially, based on what is known about motor coding in the periphery, by tapping into the PNS, there may be less need to decipher the "neural code." However, subsequent design decisions will have to be made which mitigate the technical difficulties of recording from axons due to their small size.

4. Design decision #3: Myotubes vs. neurons

4.1 Myotube signals as a proxy for neural signals

Attempts to use lesioned nerves directly as a source of information for prosthetic devices in human amputees have met with limited success, due in part to the low signal amplitude. In one set of experiments, the signals obtained from normal nerves via cuff electrodes during functional activity are in the range of 10-50 μV [47, 49]. However, the group also observed that larger motor signals can be obtained by allowing the lesioned nerves to innervate isolated slips of host muscle from which electromyographic (EMG) signals can be recorded by wire electrodes and that these larger EMG signals correlated with the smaller EMG signals in the nerves. This indicates that recording from a regenerated neuromuscular interface may allow the acquisition of stable, naturally amplified signals from lesioned nerves [47]. Furthermore, this is an excellent example of how motor neurons and myotubes communicate bidirectionally to promote each other's survival [50] and axonal regeneration. For example, denervated muscle strips attract the ingrowth of lesioned nerve fascicles. It has been shown that in the absence of neuromuscular synaptic transmission, nerve sprouts are generated as a form of self-repair and that both denervated and inactive muscle fibers release at least one sprouting factor. Three of the different proposed sprouting molecules, neuroleukin, insulin-like growth factor, and neural cell adhesion molecules, can be viewed as muscle-derived retrograde signaling molecules. These muscle-derived sprouting factors may be capable of diffusion for considerable distances [51]. Sprouts form in response to several stimuli but most notably in response to the sprouting factor secreted by partially denervated or paralyzed muscle [51].

Historically, the most clinically successful means of establishing a control signal for powered prosthetic devices has been recording the EMG activity of residual muscles [52,

53]. This is accomplished using residual muscles that were related to the activity of the prosthesis prior to amputation or by using EMG activity recorded from other unrelated muscles that have been retrained for prosthetic control [54]. More recently, a technique titled "targeted muscle reinnervation" (TMR) has been developed, in which the residual peripheral nerves left after an amputation are rerouted to muscles left useless by the loss of the limb [55]. These nerves regenerate onto the new musculature allowing the amputee to contract them by trying to perform actions with the missing limb, and providing a new EMG source from which more intuitive control over a powered prosthetic may be derived [56]. All currently available technologies depend on EMG recordings made at the skin's surface, and while muscle-implantable electrodes have been shown to be stable for long periods of time, such devices are almost exclusively used for functional electrical stimulation (FES) rather than EMG recording (with the notable exception of devices intended for diagnostic purposes) [22].

By using myotubes as the electrogenic cell type in a cultured probe approach, we may address several of the shortcomings of current neural interface designs. The increased size and depolarization amplitude of myotubes relative to neurons may make it easier to record depolarization events (Fig. 6 A-C). The favorable geometry and improved electrode sealing will relax some of the constraints on surface characteristics of the electrode itself, making it easier to design cell-favorable biointerfaces (Fig. 6).

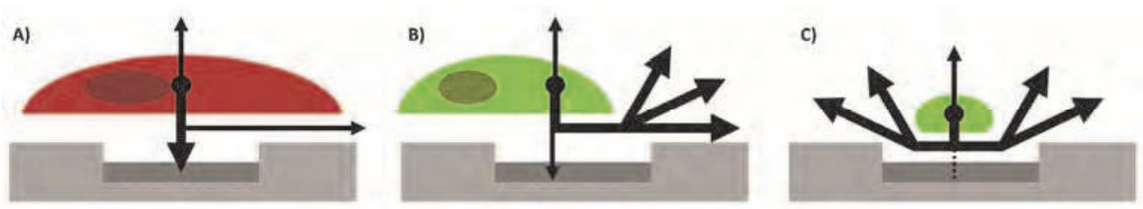

Fig. 6. Cell-electrode contacts shown in cross-section for A) myotube with good electrode coverage, B) neuronal cell body with reduced electrode coverage, and C) a neuronal axon, with minimal electrode coverage. The poor electrode coverage exhibited by the neuron and axon (B, C) allows current to leak into the surrounding environment. Complete sealing is more easily achieved using myotubes, due to their larger size.

4.2 Decision summary

There are three primary ways in which using myotubes rather than neurons and targeting the PNS rather than the CNS will improve on current neural interface designs: 1) the current understanding of cell-electrode contact suggests that the increased physical size and transmembrane current of myotubes will improve electrode sealing [31, 32, 57], 2) the bi-directional communication between myotubes and motoneurons may promote growth of axon collaterals from the native PNS into the cultured probe [34, 47], and 3) current knowledge about neural signaling suggests that targeting the PNS for neural interface implantation will simplify the algorithms involved in decoding motor intention by specifically targeting neural signals that are highly tuned to motor intention and targets a portion of the nervous system where motor intention has already undergone cerebellar processing [11, 21]. We will use cultured myotubes as a biological signal amplifier to record neural signals carried in α-motoneuron axons. Recording selectively from α-motoneuron axons is not feasible with traditional approaches since penetrating electrodes

depend on proximity to the relatively large neuron cell bodies [30, 41] (Fig. 6 A-C). Neurites are much smaller and are correspondingly more difficult to record from [30] (Fig. 6 C). Using our approach, the myotube amplifies the signal traveling down the α-motoneuron axon by virtue of coupling through the neuromuscular junction (NMJ) in much the same way a loud speaker amplifies the voice of someone speaking into a microphone.

As discussed above, while the idea of using a myotube adaptor to amplify neural signals does use the regeneration of motoneuron axons onto denervated myotubes as a way of amplifying neural signals, the currently used techniques rely on recordings made on the surface of the skin. These recordings are therefore of large populations of myotubes in their native environment of functional muscle tissue. Recording from myotube populations in this manner may improve the signal acquisition by increasing the amplitude of neural signals, but it likely reduces the specificity of recording (i.e., it is no longer possible to precisely identify individual nerve fibers when they fire). Subsequent design decisions will need to mitigate this loss of specificity, and aim to increase the number of independent signals our device is likely to be able to record.

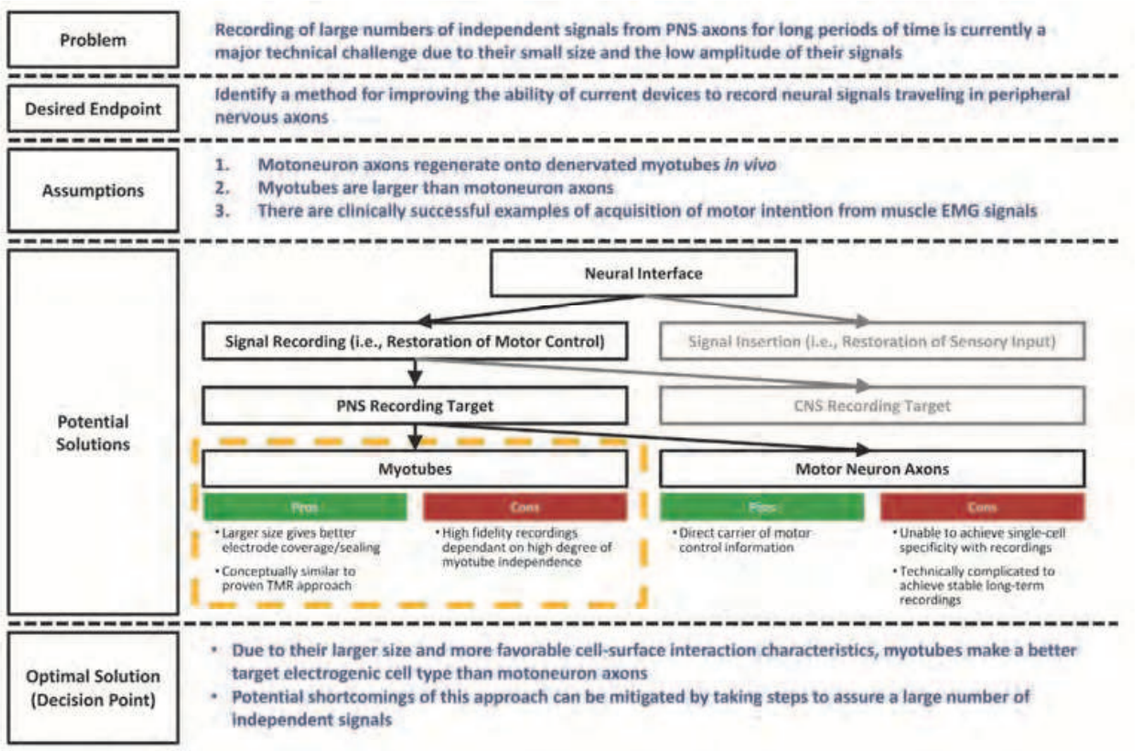

Fig. 7. Design process summary diagram after Decision #3

5. Design decision #4: Invasive vs. noninvasive

Some neural interfaces are designed to record from large populations of neurons in a completely non-invasive way, as in electroencephalography (EEG)-based and myoelectric neural interfaces. At the other end of the scale are neural interfaces designed to record from axons with single cell specificity which have regenerated through the

electrode, requiring that the axons be transected prior to electrode implantation. The trade-off between the two is the degree of specificity of the neural recordings. Generally speaking, recording specificity increases with increasing invasiveness, where specificity refers to the ability of an electrode to differentiate between closely related signal sources and invasiveness refers to the extent of tissue damage the electrode is likely to cause. The benefit of specificity is that it increases the number of independent neural signals available from a given neural substrate. In general, a low number of signals and low specificity is acceptable for robust use and limited functionality, while high numbers and selectivity are required for improved spatial resolution and high functionality [21]. While highly specific recordings are believed to be required as a prerequisite for decoding fine motor control from neural signals, the risks associated with an invasive implantation procedure and potential tissue damage and loss of functionality in the long-term need to be considered in the design of any interface. There is some debate regarding the numbers of neuronal recordings necessary to resolve fine motor control, and the relative number will differ depending on the site of recording.

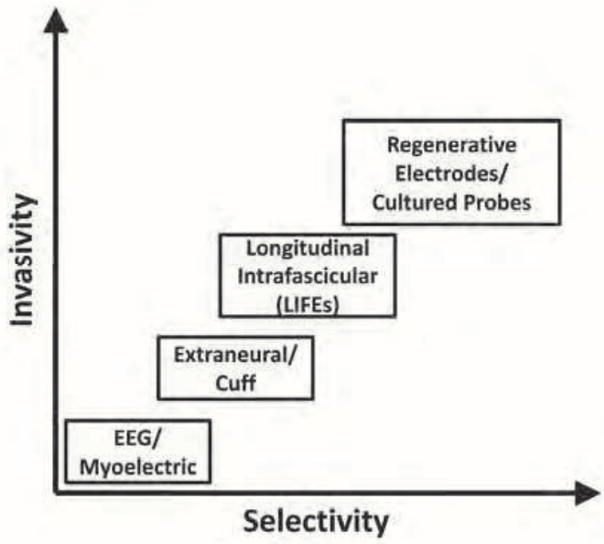

Fig. 8. Invasive PNS interfaces schematized by selectivity and invasiveness (Modified from [58])

Extraneural electrodes, which are relatively noninvasive and nonspecific, attach to the outside of peripheral nerves. The most popular current examples are cuff electrodes [21, 22, 49, 59], which attach to the outside of nerve bundles and record the electroneurgraphic signals of the impulses traveling down the enclosed fascicles (large, related axon clusters). Flat-interface nerve electrodes [21, 22] are a modified cuff designed to separate fascicles for improved recording from individual fascicles within the larger nerve bundle but are still only capable of recording a relatively low number of independent signals because the distance between the recording electrode surface and the axon precludes the identification of single spikes [21, 60]. Other notable problems with this technology are that the electroneurographic (ENG) activity recorded from a nerve with electrodes placed around its periphery is dominated by the excitation of large myelinated fibers and those located at superficial locations.

Intraneural electrodes, which are more invasive but more specific [21, 22], are inserted directly inside of the peripheral nerve where the recording sites can make nearly direct contact with the axons transmitting information. Notable examples include longitudinal intrafascicular electrodes [39], but also a number of other penetrating electrode designs have also been deployed in the PNS [61].

Regenerative electrodes, which are highly invasive and highly specific, are placed in the gap of peripheral nerves that have been fully transected and record from axons which regenerate through the electrode [62]. Problems with this type of interface are currently being addressed by redesigning the recording sites to be tubular rather than planar [63-66]. These tubular recording sites are frequently fabricated by rolling arrays of parallel microchannels, or microgrooves, with incorporated substrate-embedded MEAs into cylindrical constructs for implantation [67, 68]. Generally, axonal regrowth occurs from the proximal end of the transected nerve to the distal end, spanning the natural course of axonal regeneration. However, one group has recently speculated on the idea of a blind "endcap" design, in which regenerating axons would enter and not exit [66]. Furthermore, at least two groups are currently working to address the problem of enhancing such blind-ended axonal regeneration and stabilizing its long-term survival and viability by incorporating biological elements into the endcap, such as growth factors, as are frequently used within the context of artificial nerve conduits [69] [68], pools of living exogenous neurons [66], and even muscle cells [70-72].

Finally, *in vitro* designs have also been proposed, such as the "cultured probe" approach [21, 22, 33]. Cultured probes are a specific type of MEA in which electrogenic cells, typically neurons, are cultured on top of electrode arrays to promote the formation of an *in vitro* functional biointerface. These are electrodes on which nervous tissue has been cultured prior to implantation to develop a good seal with the electrode. The neurons are frequently grown in a way that one or a group of cells contacts only one electrode site. The hope is that upon implantation, the cultured neural tissue will functionally integrate with native neural tissue, establishing a link between electrode and the native nervous system. The theoretical advantage of this design is that the electrode-cell interface may be established and optimized in the laboratory, and because it is biologically active, it will actively promote the ingrowth of axon collaterals from the native nervous tissue. If both of these benefits prove possible, this technique may yield a very selective and efficient interface [22, 33].

5.1 Surface modifications and selectivity

The theoretical advantage of directly inserted probes is that they provide neural signals that are immediately available and do not depend on the variable ingrowth of axon collaterals from the native nervous system. By comparison, the benefit of a cultured probe is that it allows a custom environment to be made to achieve a wide range of desired cellular behavior. Deciding on an MEA design requires consideration of the intended function of the device, how this affects the manufacturing process, and in turn, the cost of the device. In our case, we are trying to create an interface that allows for acquisition of a large number of independent signals from a myotube culture.

One of the most unique aspects of skeletal muscle cells is the large morphological change that myoblast cultures undergo when fusing into contractile myotubes during development. As differentiation and maturation occurs, singly-nucleated myoblasts first

adhere to the substrate, then align and fuse into multinucleated myostraps, and finally mature into contractile myotubes, which can be several orders of magnitude larger than their precursor myoblasts [73]. Each step of this process is instructed by each cell's genetic program, communication with neighboring cells [74], and interactions with the chemical [75], physical [76], and electrical [77, 78] extracellular environment. In skeletal myotube cultures, two basic morphologies are usually present: 1) branching multipolar and 2) spindle-shaped bipolar. Because the multipolar myotube is a single, continuous cell, it contracts as a single unit. In the case that two separate myotubes are next to each other but are not fused, they retain the ability to contract independently of one another. Great progress has been made in the field of skeletal muscle tissue engineering, and the wide range of potential applications is reflected in the wide range of tools employed to control myotube growth [79, 80].

Researchers have begun using a number of techniques to induce the formation of patterned neural circuits in order to study neural network function in this simplified context [30, 81-84]. Generally speaking, these techniques can be divided into two groups based on mechanism: 1) mechanically-based techniques, in which the topography of the electrode itself is in some way modified to cause directed cell adhesion or neurite outgrowth [81, 84] and 2) chemically-based techniques, in which chemical guidance cues are patterned onto the electrode surface [30, 82, 83]. The tendency of many cell types to respond in a predictable fashion to topographical features is well known [85]. The scale nature of the topographical modification is important in determining how different cell types will react to the feature or even what portion of the cell will react to the feature. If a feature is too small, the cell will exhibit only a very small reaction to it, while if a feature is too large, a cell may not interact with it enough to show any significant change in behavior.

5.2 Decision summary

We have determined that in order to use a neural interface to acquire motor intention, it must be able to perform the following tasks: 1) acquire a large number of independent signals and 2) remain stable over a wide range of times and mechanical conditions. We have determined that interfacing with myotubes, rather than neuronal cell bodies or axons, is the best way of doing this. Based on our selected cell type and the desired goal for the interface, the optimal interface design will likely be achieved using an implantable, cultured probe technique to maximize the number of potential independent signals that can be acquired. We aim to use tissue engineering tools to direct myotube formation to specific sites and to preserve the independence of individually formed myotubes. These two goals will be accomplished by using topographical cues to both improve cell adhesion to individual electrode contacts and to facilitate the ordered fusion of myocytes into myotubes, thereby creating a predictable pattern of cell-electrode contacts over the entire culture. Using myotubes as the electrogenic cell type alters how we approach electrode design. Because myotubes fuse in a linear pattern, longitudinal trenches will be used to guide myoblast alignment and fusion to specific electrode sites. Because the myotubes are large and have increased depolarization amplitudes relative to neurons, these topographical features can be larger than those used in strictly neuronal applications. Because of the increased size of skeletal muscle cells, the electrodes meant to interface with this cell type can have a larger feature size. This frees us to use a photoresist as our mask and insulator, significantly simplifying the manufacturing process.

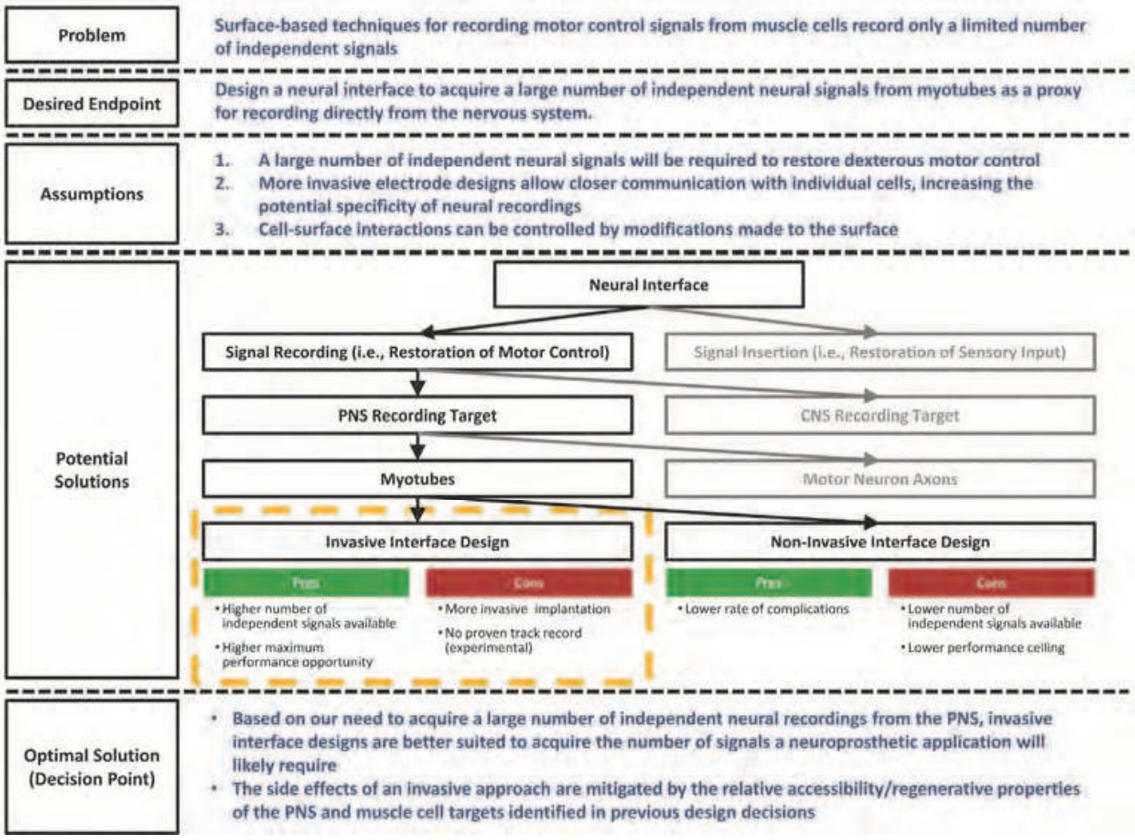

Fig. 9. Design process summary diagram after Decision #4

6. Experimental design

6.1 Proof of principal

In the preceding sections, we hypothesize that an MEA can be designed to guide myotube formation to specific electrode sites and can record myotube extracellular action potentials (EAPs) in a selective manner. It may, therefore, be possible to use this technology as a novel type of neural interface targeted to record from regenerating PNS axons, thereby recording motor intention along its final common pathway. Such an approach to neural interfaces would render the cultured probe effectively a cell-based biosensor [86]. Prior to being deployed in animal studies, these technologies are developed and tested *in vitro* in the form of modified planar MEAs [81, 87-92]. Much of the work done to date interfacing MEAs with cells *in vitro* has been performed with applications in two cell systems [86, 93]: 1) neurons, where the ability to identify the activity of single cells in spike trains through a process called "spike-sorting" is used to identify patterns of population activity and network dynamics and 2) cardiac myocytes, where spatial and temporal resolution allow the measurement of transduction velocity through sheets of linked cardiomyocytes. Although the design decisions we have made to this point have been based firmly on existing scientific knowledge, much of what we have proposed has never been demonstrated explicitly. Because restoring volitional motor control is such a complex clinical problem, the most appropriate next step is to use a highly reductionist system to provide *in vitro* proof of principal for the basic tenants underlying our proposal:

- Topographical cues can direct myotube formation and activity to specific sites
- This affects their electrical independence

6.1.1 Structured myotube cultures

The goal of producing large numbers of independently identifiable myotubes requires that myotube activity be guided to specific locations. Myotube morphology *in vivo* suggests the choice of topographical modifications in the form of parallel grooves as a means of inducing myotube alignment and directing formation to specific locations. Using a video analysis algorithm which analyzes myotube contraction and coordination from captured video files [94], we can analyze myotube contractile activity on unstructured and topographically patterned surfaces (Fig. 10 A-B), allowing us to be among the first researchers to investigate

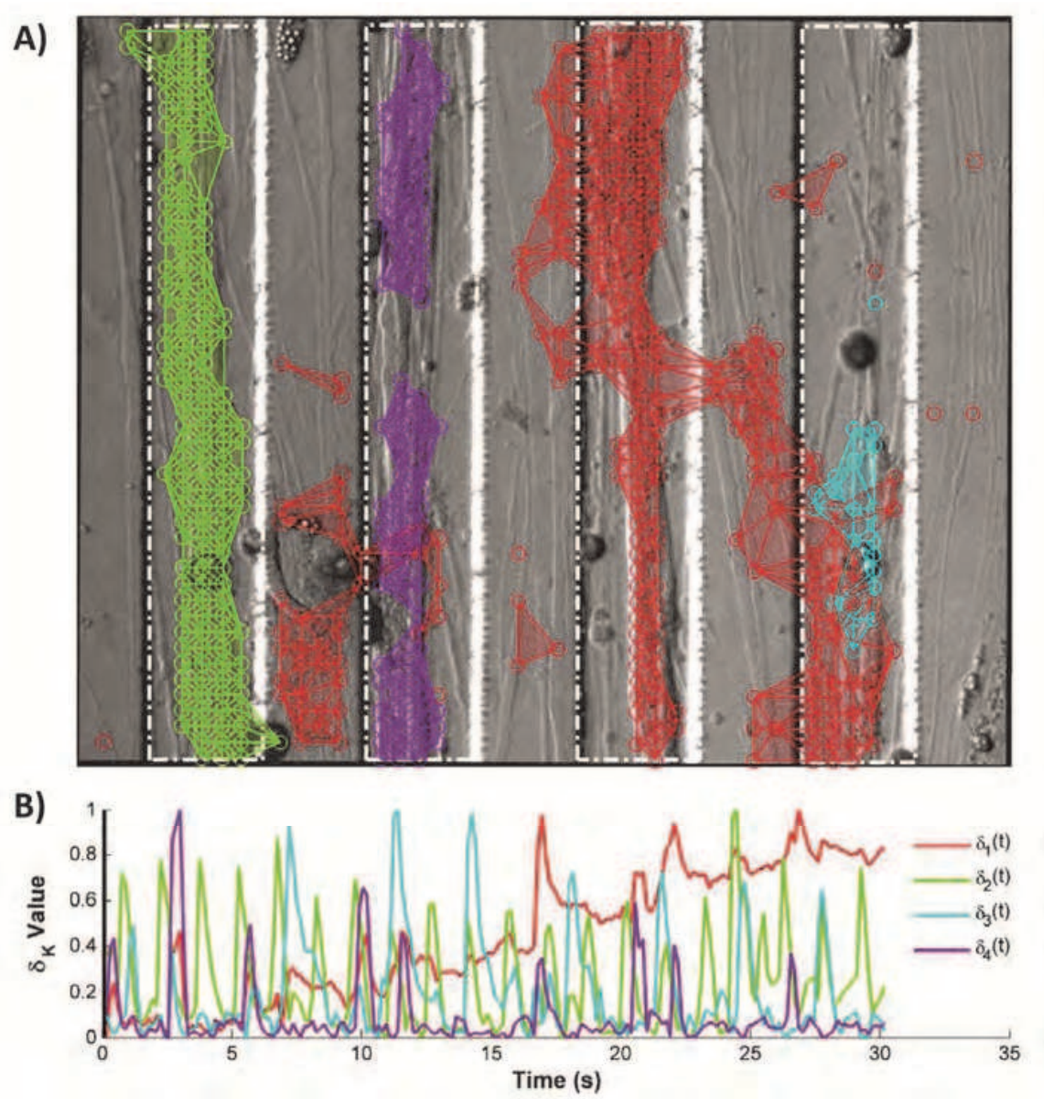

Fig. 10. The effects of microscale topographical trenches on myotube independence. (A) Example labeling of active myotubes in 100 μm trenches (each cell is color coded) (B) Cellular contraction activity tracked over time.

the effect of topography on myotube independence. We have found that rat myotube growth in microscale trenches causes a more rapid onset of spontaneous contractions and changes the spatial distribution of myotube activity compared to cells cultured on unstructured substrates. The percentage of total contractile activity increases within the trenches (Fig. 10 A), with few myotubes spanning multiple trenches. Overall, this indicates that the trenches successfully direct myotube formation to specific sites and facilitates the formation of 1:1 specificity between myotube contractile activity and trench location.

6.1.2 Detecting myotube and neuronal Extracellular Action Potentials (EAPs)

Work in this field to date has focused on neurons and cardiac myocytes [86, 93]. We have expanded on this foundation by successfully culturing primary rat skeletal myoblasts on MEA surfaces and differentiating them into spontaneously contractile myotubes (Fig. 11 A), which generate EAPs detectable with the underlying electrodes (Fig. 11 B). The shape of an EAP is a function of its spatial relationship with the electrode [26, 95], giving each bioelectrically active cell a unique EAP signature. Spike sorting is used to classify EAPs as coming from specific cells (or "units") and allows identification of the activity of several separate units recorded on the same electrode. Neurons have a stable interaction with surface electrodes (Fig. 11 C) and so have a stereotyped EAP morphology (Fig. 11 D). Myotubes are highly dynamic, and therefore, produce EAPs with arbitrary shapes, complicating detection (Fig. 11 B). Using commercially available MEAs and similar spike sorting algorithms, we find that the maximum and minimum μV deflection are on the same order of magnitude between cell types (Fig. 11 E) and that when this is normalized by electrode-specific recording characteristics, myotubes exhibit a slightly higher signal to noise ratio (SNR) (Fig. 11 F), indicating greater ease in identifying them over background. This may be due to their larger size facilitating electrode sealing [31, 96].

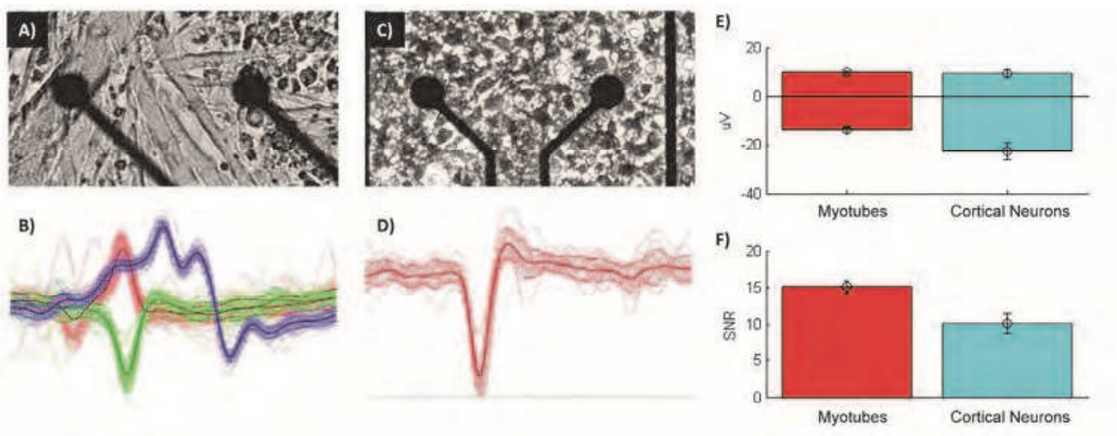

Fig. 11. Myotube vs. neuronal EAPs. Culture morphology and EAPs following spike sorting for (A-B) myotubes and (C-D) cortical neurons. (E) Quantification of EAP amplitude and (F) SNR for myotubes (red) and cortical neurons (blue). Myotube n=154, neuronal n=26, error bars represent the standard error of the mean. Reproduced with permission from [97].

6.1.3 Topographically modified MEA for structured explant and myotube cultures

The next step in our reductionist system is showing that the components above can be integrated on a single device. A prototype device was designed incorporating topographical

modifications that direct myotube formation with a substrate embedded MEA. Specifically, two regions of trenches used to direct myotube formation to specific electrode sites are connected to a central field (Fig. 12 A). Two trench regions, oriented horizontally and vertically, consist of four grooves with a single electrode contact at the bottom. The central field contains five recording electrodes to record from multiple points. A large pad is included as an internal reference electrode (not shown). The electrodes are patterned to interface with a Multichannel Systems MEA recording headstage through external contact pads located around the periphery of the chip (Fig. 12 B). The electrode contact and lead pattern is produced by a lift-off technique [98] using standard optical lithography followed by sputtering of a 200/700 Å thick chromium/gold (Cr/Au) conducting electrode layer. A layer of SU-8 PR is then spin-coated onto the electrode-patterned surface and exposed and developed using a topographical feature mask to generate topographical trenches and a central confinement region, also selectively exposing the electrode contact pads located at the bottom of both while leaving the electrode leads electrically insulated from the culture environment. A PDMS ring is affixed to the surface creating a culture chamber around four recording fields, enabling multiple simultaneous experiments (Fig. 12 B). The fabrication process generates devices capable of recording myotubes and neuronal explant EAPs, which withstands repeated cycles through the sterilization-usage-regeneration processes involved in cell culture.

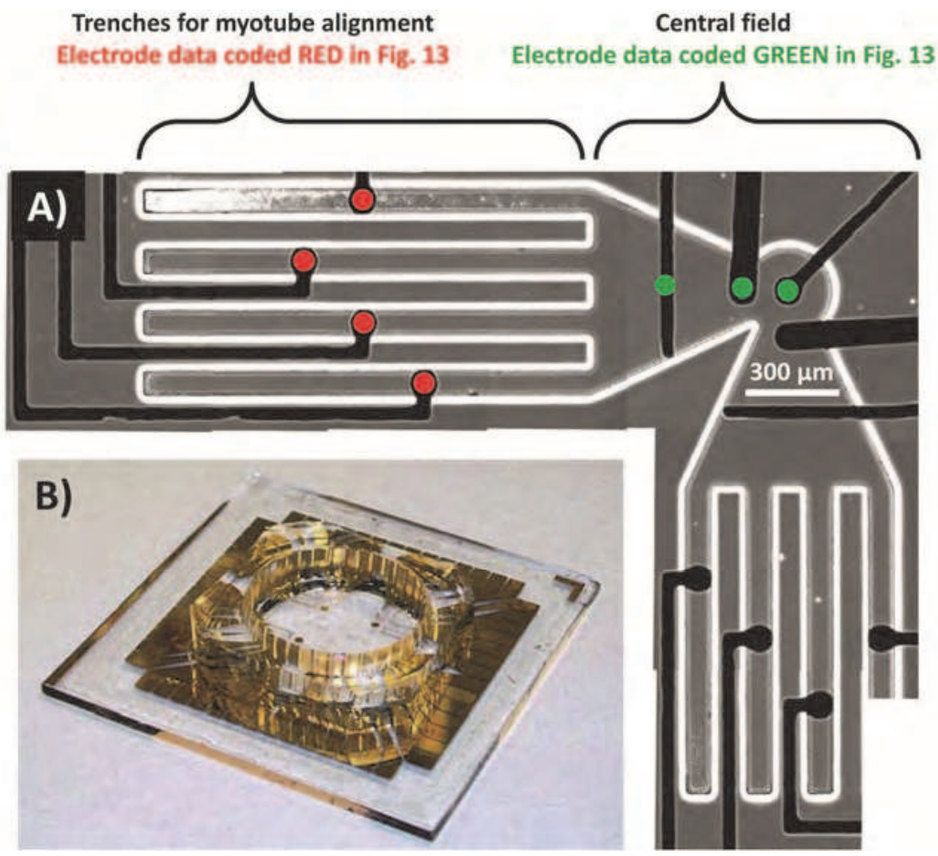

Fig. 12. Myo-MEA prototype. A) Microscopic view of 1 of the 4 recording fields. B) Full prototype including culture chamber.

6.1.4 Explant and myotube monoculture behavior on the myo-MEA

Rat myotubes have been cultured on the myo-MEA to characterize their activity. Myoblasts are seeded on the whole chip surface at 300,000 cells/cm² and allowed to differentiate into myotubes. Prior to seeding, the myo-MEA surface is treated overnight with laminin. Myotube formation is guided to the electrode contact sites by the trenches (Fig. 13 A), and contractility is directed by the topographical cues (Fig. 13 B). Because the entire chip surface was seeded, myotube EAP activity can be observed in the trench regions (red points) and the central regions (green points) (Fig. 13 C). Repeating electrode activation motifs, in which the same electrode activation pattern is repeated at multiple time points, are generated by single myotubes that span multiple electrodes. Total depolarization activity detected a combination of repeating vertical banding patterns (myotubes spanning multiple trenches and the central region) and units that fire in isolation (myotubes confined to a single trench). Obvious repeating activation motifs have been identified by hand (Fig. 13 C – colored rectangles).

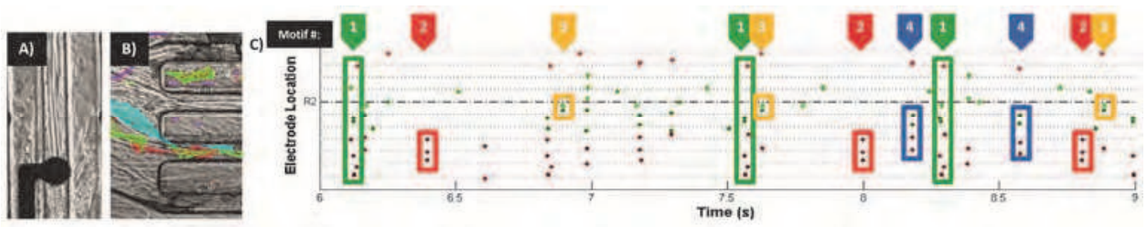

Fig. 13. Myotube formation (A) and contractility (B) guided by trenches. C) Myotube EAPs recorded from one of four fields of a myo-MEA as shown in Fig. 12 A, with example repeating activation motifs identified and labeled by hand (colored rectangles with labeled tags). Units are color-coded based on electrode location (central region = green, trench regions = red). Adjacent points represent adjacent electrodes within the myo-MEA.

Isolation of these activation motifs will ultimately allow us to identify how many independent myotubes are active on an electrode surface and their spatial distribution based on their EAPs. Within the context of the next-generation hybrid-biosensors and neural interfaces, this is an indicator of the number of independent signals the interface can record, and will enable us to optimize the myo-MEA geometry.

7. Clinical adaptations & future directions

The myo-MEA is a neural interface design meant to build on the success of EMG-based and PNS-based approaches to recording motor intent, taking advantage of the larger extracellular voltage changes caused by the depolarization of muscle cells relative to axons and the high degree of cellular specificity available using MEAs with a cultured probe approach (Fig. 14) [12, 81, 92]. The interface described herein (the myo-MEA) employs myotubes integrated with a topographically modified MEA to act as biological signal amplifiers for regenerated PNS motor axons. Using myotubes in this capacity represents a shift from current designs, which aim to record directly from neuronal sources (PNS axons) on a microscale, or from muscle tissue on a macroscale. In the proposed design, the myotubes are coupled to motoneuron axons through the NMJ, contracting in response to the EAPs they transmit. The myo-MEA records the activity of the myotubes as a proxy for recording from the axonal EAP directly. Because the myotube creates higher depolarization

potentials, which are more easily detectable than those of a motor axon, this effectively amplifies the signal traveling from the motoneuron axon in the way a loud speaker amplifies the voice of someone speaking into a microphone.

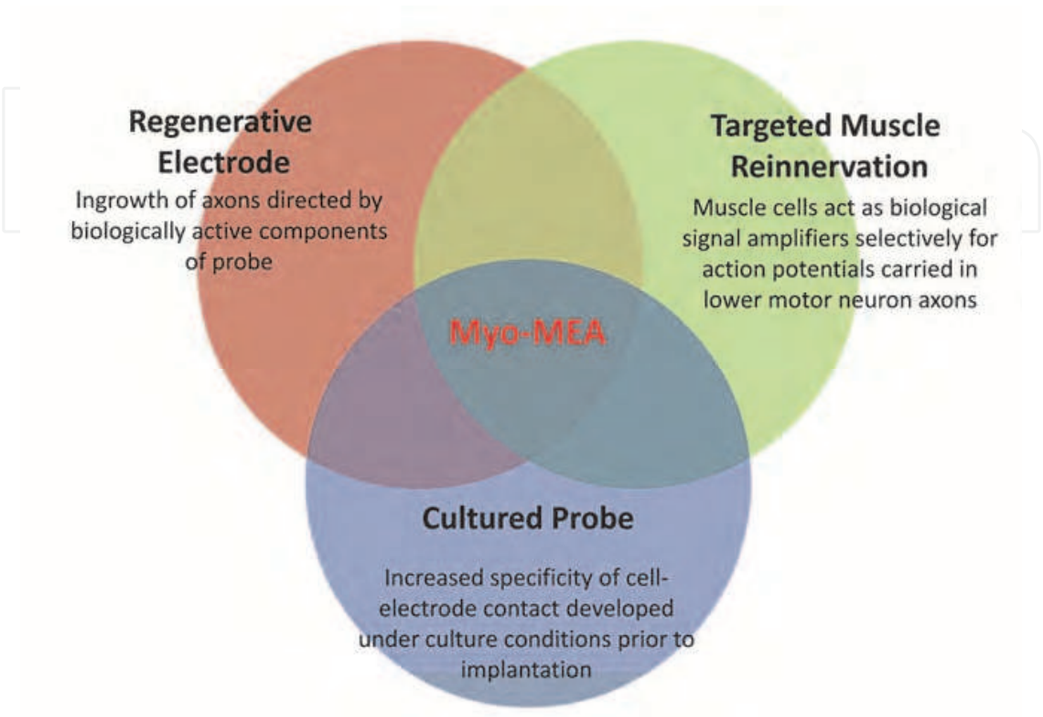

Fig. 14. Ven diagram of conceptual overlap of multiple approaches to neural interfacing and neuroprosthetics contributing to the myo-MEA design process

In the ultimate clinical adaptation of the idea (Fig. 15), we will culture myotubes on an electrode array in a modification of the traditional cultured probe concept [33], specifically employing microscale grooves to accomplish two goals: 1) to direct the formation of myotubes to specific electrode sites and 2) to preserve myotube independence from one another. Once the myotubes have formed and settled to electrode sites (Fig. 15 C), the array will be rolled into a cylinder [67, 68], trapping the myotubes in the resulting channels and putting the device in a conformation ready for implantation as a three dimensional peripheral nerve endcap [66] (Fig. 15 D). After attachment to the severed end of a peripheral nerve, motor axons would be encouraged to grow into the construct by the indwelling myotubes where they would synapse, forming functional neuromuscular junctions [70]. Action potentials carried along these regenerated motor axons would arrive at the myotubes, causing excitation and contraction and generating a microscopic EMG signal for each activated cell. When an individual neuron fires, each associated myotube depolarizes, causing voltage changes at a unique set of electrode contacts. Each neuron has a unique set of associated myotubes due to the synaptic pruning processes occurring at the NMJ, so each neuron will have a distinctive signature of activated electrodes. These EMG signals will therefore contain an encoded version of the motor intention carried along the peripheral nerve, which may be decoded in a similar fashion to the decoding involved in the TMR technique [56].

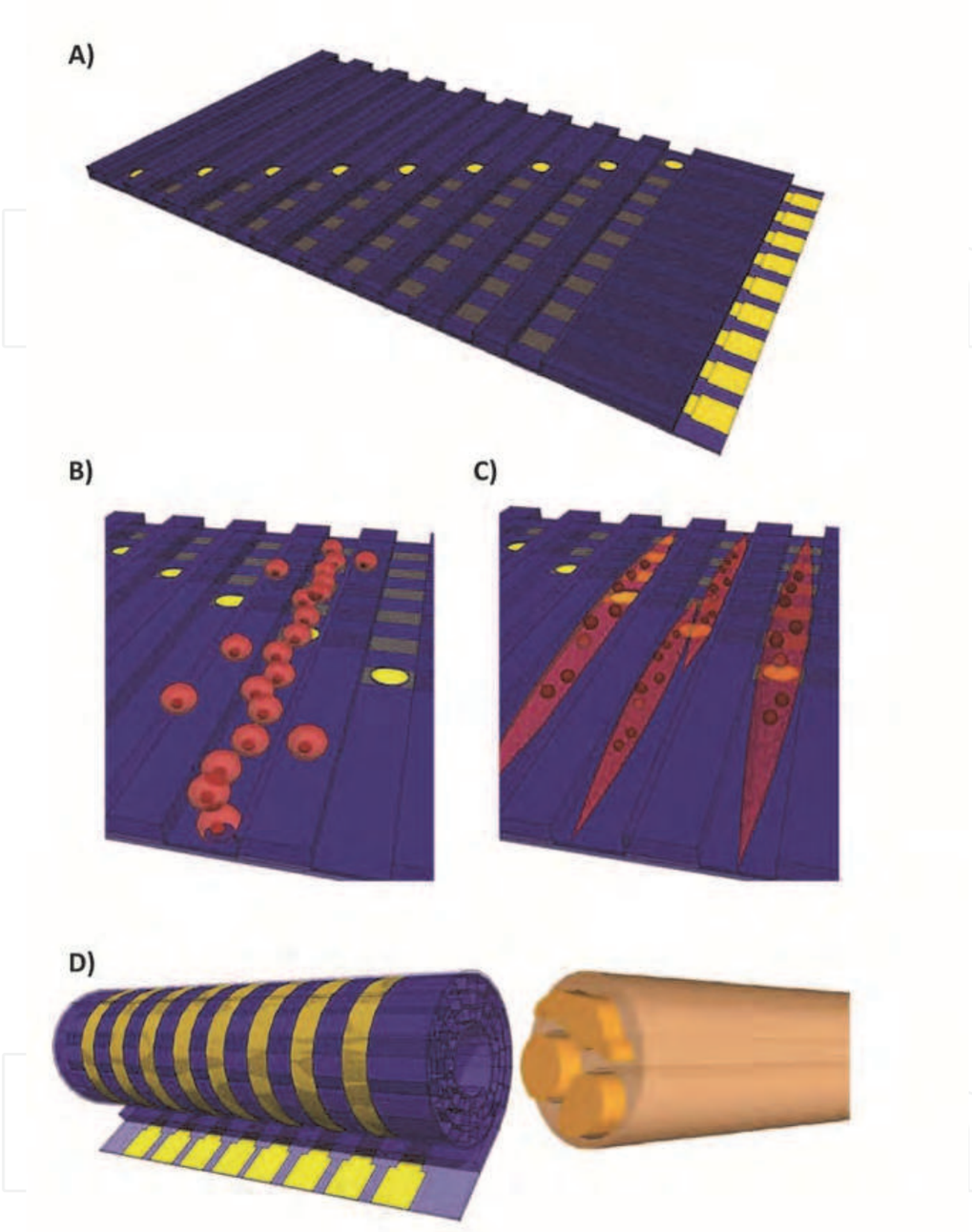

Fig. 15. Conceptual clinical implementation of the myo-MEA. A) A planar MEA is fabricated on a flexible substrate, and topographically modified with grooves. B) The myo-MEA is seeded with myoblasts or satellite cells taken from the patient. C) Myoblasts fuse into myotubes overlying the electrode sites located at the bottom of the grooves. D) The myo-MEA is rolled into a cylinder, trapping the myotubes inside and allowing axonal ingrowth from the open groove ends at the end of the device.

The crucial difference between this proposed device and existing regenerative electrodes is in the origin of the acquired signals. Traditional regenerative electrodes record signals

directly from nerve cell axons, while the myo-MEA records signals generated by myotubes. Similarly, our technique relies on a similar re-wiring of the PNS as is involved in TMR. However, it differs substantially in that TMR relies on signals recorded at the skin's surface and which are therefore generated by muscle tissue, while the myo-MEA's recording sites are in direct contact with the myotubes and therefore reflect activity at a cellular level. It is our hope that by creating a neural interface based on a large array of isolated myotubes innervated by regenerated motor axons, we will be able to record a greater number of independent signals, and therefore, improve the efficiency of the interface. Eventual clinical adaptation of the myo-MEA would therefore bring with it the following benefits: **1)** selective regeneration of motor axons onto myotubes provides signals encoding primarily motor intention and largely excludes the sensory information and cognitive information carried by neurons that would not form NMJs with the myotubes, **2)** robust growth of myotubes and adhesion to the MEA surface relative to PNS axons provides a more stable long-term recording platform, and **3)** the neurotrophic activity of myotubes provides cues directing axonal ingrowth. The myo-MEA is uniquely designed to take advantage of myotube properties and their interaction with the PNS to specifically target neural signals that are highly tuned to motor intention, which have already undergone cerebellar processing. In so doing, the design addresses the specific needs of amputees and severe PNS injury patients in a way that other neural interfaces do not, and therefore, increases the chances of its clinical success in this patient base.

8. Acknowledgements

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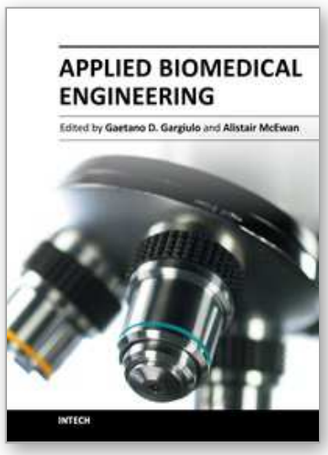

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