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When Bio Meets Technology: Biohybrid Neural Interfaces

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The development of electronics capable of interfacing with the nervous system is a rapidly advancing field with applications in basic science and clinical translation. Devices containing arrays of electrodes can be used in the study of cells grown in culture or can be implanted into damaged or dysfunctional tissue to restore normal function. While devices are typically designed and used exclusively for one of these two purposes, there have been increasing efforts in developing implantable electrode arrays capable of housing cultured cells, referred to as biohybrid implants. Once implanted, the cells within these implants integrate into the tissue, serving as a mediator of the electrode–tissue interface. This biological component offers unique advantages to these implant designs, providing better tissue integration and potentially long-term stability. Herein, an overview of current research into biohybrid devices, as well as the historical background that led to their development are provided, based on the host anatomical location for which they are designed (CNS, PNS, or special senses). Finally, a summary of the key challenges of this technology and potential future research directions are presented.

1. Introduction

The nervous system plays a key role in the regulation of body function, voluntary movement, consciousness, and cognitive function. As a result, dysfunction of the nervous system—whether by damage or disease—can have devastating consequences in the life of the patient and their families. Despite the clinical relevance of neurological dysfunction, current treatment options remain limited.

Neural interfaces and cell transplantation have both been two powerful strategies for the restoration of neurological function. Both strategies target dysfunctional regions of the nervous system and attempt to restore function by either electrically stimulating or recording from healthy neural circuitry (implantable neural interfaces) or providing new cells to replace the damaged tissue (cell transplantation). These two approaches have however been traditionally considered independently. While electrode arrays interfacing with cells in vitro is a well-studied concept, the extension of this into biohybrid interfaces—implantable neural interfaces containing cells which integrate into the host tissue—remains a largely unexplored concept despite offering novel opportunities for treatment.

1.1. History of Cells on Electrode Arrays

The first studies of electrophysiological activity of cells in vitro were carried out using intracellular recording techniques.[1] These relied on micropipettes filled with physiological solution, which could be manually approached onto cells to pierce or patch into their intracellular environment. Although intracellular recording techniques offer a great degree of insight into the electrical behavior of an individual cell, the need for bulky micromanipulators to handle the micropipette probes greatly limits the number of cells which can be simultaneously studied.[2]

While extracellular electrical recordings are less informative than their intracellular counterparts, the greater flexibility in their design and implementation allowed them to gain traction within the scientific community. The first generation of electrodes designed for extracellular recordings were developed in the 1950s,[3] consisting of sharp pipettes similar to those used in intracellular recordings designed to pierce into nervous tissue, and loaded with a metallic microwire protruding from their tip to facilitate conduction of the weak recorded extracellular electrical signals. The micropipette design nonetheless meant that these were similarly limited in number of electrodes that could be simultaneously used. This changed with the development of multi electrode arrays (MEAs).

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The first MEA designs were strongly inspired by the semiconductor industry, consisting of thin metal films deposited onto flat glass substrates to form arrays of electrodes. The flat designs and rigid materials made them highly suitable for in vitro applications, allowing cells to be seeded and grown directly on top of the electrode array. This application was, as far as it is known, first tested by Thomas and colleagues in 1972[4] with chick embryonic heart cells using an array of 30 electrodes. Both invertebrate[5] and mammalian[6] neurons were later similarly cultured and studied on MEAs, introducing these devices as key tools in the field of neuroscience.[7]

MEA designs for in vitro applications have since progressed significantly. Current MEAs can pack over 20 000 electrodes, at a density high enough to track membrane depolarization events within subcellular compartments.[8] Transistor technology has also been implemented into MEAs. By locally amplifying signals at the site of recordings, transistor arrays applied in vitro can achieve higher signal-to-noise ratios compared to their traditional designs.[9] The last few years has also seen the development of MEA designs incorporating penetrating electrode architectures, intended to pierce or be engulfed into the cytoplasm of cells, blurring the boundary between extracellular and intracellular recordings.[10]

MEA technology has developed hand-in-hand with advances in cell culture techniques. Recent years have seen a rise in neural organotypic tissue culture—entire sections of primary freshly dissected tissue with preserved neuron connectivity. These have been used in combination with MEAs to study the function of hippocampal circuitry,[11] as well as cortical connectivity.[12] More recently, techniques for the cultures of organoids—small organs derived from differentiating stem cells in vitro, and developing a circuitry reminiscent of their in vivo counterparts—have begun to emerge as an in vitro model of neural circuitry[13] capable of being studied using MEAs.[14]

MEAs offer a promising strategy to establish connections with electrically active cells such as neurons and study their activity under the controlled conditions of a dish.

1.2. Cell Transplantation in Regenerative Medicine

Stem cells are defined by their potential to self-renew indefinitely by division and to differentiate into a variety of different cell types. Adult organisms possess few of these stem cell populations dwelling within their tissues, which normally give rise to new skin, blood, or gut tissue.[15] In 1981, however, populations of these stem cells were identified in developing mouse embryos. These embryonic stem cells (ESCs) could be isolated, expanded in vitro, and guided to differentiate into a wide range of cell types.[16] This discovery led not only to great advances in our understanding of embryo development, but also led to the appearance of an entire new approach to regenerative medicine: transplantation of stem cells into a host organism to restore function.

Transplanted stem cells can be used in two different ways to restore function. First, in cases where a particular cell type population has been severely depleted or damaged, stem cells can be programmed to differentiate into said cell type of interest and implanted. In this way, the cell transplant integrates into the host to become new tissue. Alternatively, cells can also be implanted to provide a favorable environment for host cells, so that these host cells themselves may proliferate to repopulate

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a lesion. For example, cell types such as olfactory ensheathing glia are known for their ability to promote neurogenesis, and transplants of these have been successful in enhancing recovery in conditions such as cerebral palsy[17] and spinal cord injury.[18]

The discovery that adult cells can be reprogrammed to become stem cells (termed induced pluripotent stem cells, or iPSCs) by Takahashi and Yamanaka[19] generated renewed interest in the field of cell transplantation. iPSCs cannot only be derived from almost any cell type, providing a more reliable and less ethically questionable source of stem cells compared to ESCs, but can also be later reimplanted back into the same patient they were derived from (autologous transplants). This opened new avenues for cell transplantation therapies as an approach to personalized medicine.

Cell transplants have been particularly attractive for the treatment of neurological disorders. Unlike other tissues in the body, the nervous system has an inherently low capacity to regenerate following injury due to the limited ability of neurons to proliferate or extend new axons. Although lost neurons cannot be replaced from intrinsic populations, neurons or their progenitors can be derived from stem cells under in vitro conditions and implanted into the affected region of the nervous system. These cells are then free to integrate into the existing neural tissue and restore lost function.

This strategy has been used in the treatment of a wide range of neurological disorders. Parkinson's disease, characterized by the loss of dopaminergic neurons terminating in the striatum, has been treated by the transplantation of dopaminergic neurons derived from ESCs, and is currently undergoing human clinical trials.[20] Similar clinical trials are also underway for the treatment of Huntington's disease (transplants into the striatum),[17] and spinal cord injury (neural progenitors transplanted into the lesion).[21]

One of the main challenges faced when developing cell transplantation therapies is the risk of immune rejection. As cells are uniquely tagged based on their genetic makeup, the host immune system is capable of recognizing and attacking transplanted cells if these come from a different organism, resulting in transplant failure. Immunosuppressive drugs such as cyclosporin are therefore often used to maximize the chances of successful treatment; but doing so leaves the patient vulnerable to postsurgical infections.[22] Autologous iPSC transplants may be a good long-term solution to this issue. As these are derived from the patient and therefore are genetically identical, they can generally avoid immune rejection.[23,24]

The history of cell transplantation in and out of the clinic is extensive and an in-depth description is beyond the scope of this review. The topic has been covered by other authors.[25,26]

1.3. Biohybrid Implants—at the Interface of Two Ideas

These two different research avenues—culture and electrical probing of cells on MEAs in vitro and cell transplantation for tissue regeneration in vivo—can yet be combined into one concept: implantable electrical–cellular hybrid devices. Such systems were first described by Stieglitz et al. in their 2002 seminal paper,[27] who coined the term "biohybrid" to describe this class of neural implants. Though their characteristics can vary depending on the context for which they are developed, biohybrid implants in essence consist of implantable electrode arrays containing live cells grown in culture. Once implanted, these cells integrate into the host tissue (either allowing the host tissue to grow into them, or themselves growing into the host tissue), forming a bridge between electronics and host. The cells of the implant therefore serve as mediators of the electrode–tissue connection (**Figure 1**).

Biohybrid systems offer a number of advantages over traditional implantable electrodes. While neural implants hold a unique potential for the treatment of neurological disabilities, they must remain implanted in the body for years or even decades to be effective therapy tools. Over this prolonged chronic implantation period implants must face several challenges which can compromise the electrode–tissue interface.

One of the most important of these challenges is the foreign body reaction (FBR). Upon implantation of any material, the body recognizes it as foreign and mounts an inflammatory response in an attempt to degrade it.[28,29] The inflammatory response itself can not only damage both the implant and the surrounding tissue through the generation of factors such as reactive oxygen species, but over the course of weeks will lead to the formation of a fibrotic layer of tissue encapsulating the implant and physically separating it from the tissue into which it is implanted. These inflammatory and fibrotic processes, which occur irrespective of the properties of the implanted materials, lead to the slow but often inevitable degradation of the electrode–tissue interface.[29]

Biohybrid implants address the issue of FBR through the use of a biologically active intermediate layer between tissue and electronics. Biohybrid interfaces are more accurately formed of two separate interfaces: electrodes-to-cells and cells-to-tissue. If designed properly, this double-interface design can be used to greatly minimize or avoid entirely interface degradation due to FBR, as the host tissue is only in contact with the cellular interface. A stable cell–tissue interface relies on an adequate selection of the cells to be implanted, as improper choices may lead to an immune response similar to that seen in graft rejection.[22–24] Lessons learned from the long clinical history of cell transplants can, however, be applied to great effect for this purpose—with techniques such as the use of iPSC cultures offering great potential for the development of immune-compatible biohybrid interfaces.

Another advantage of biohybrid implants over other designs relates to their ability to address neurological symptoms not related to well anatomically defined structures, thanks to their ability to integrate into the host tissue. Many traditional neural probes are designed to treat symptoms resulting from a dysfunction localized to a specific area of the body. Examples of this are cochlear implants for hearing restoration (cochlea), deep brain stimulation electrodes for treatment of Parkinson's disease and other movement deficits (striatum), and artificial cardiac pacemakers (heart). Biohybrid implants, on the other hand, are designed to integrate into the host tissue, growing out from the site of implantation into their target structures. This allows biohybrid devices to establish connections with multiple structures and does not limit their effect to the area surrounding the implant itself. The designs presented by Stieglitz and colleagues,[27] for example, relied on neuronal

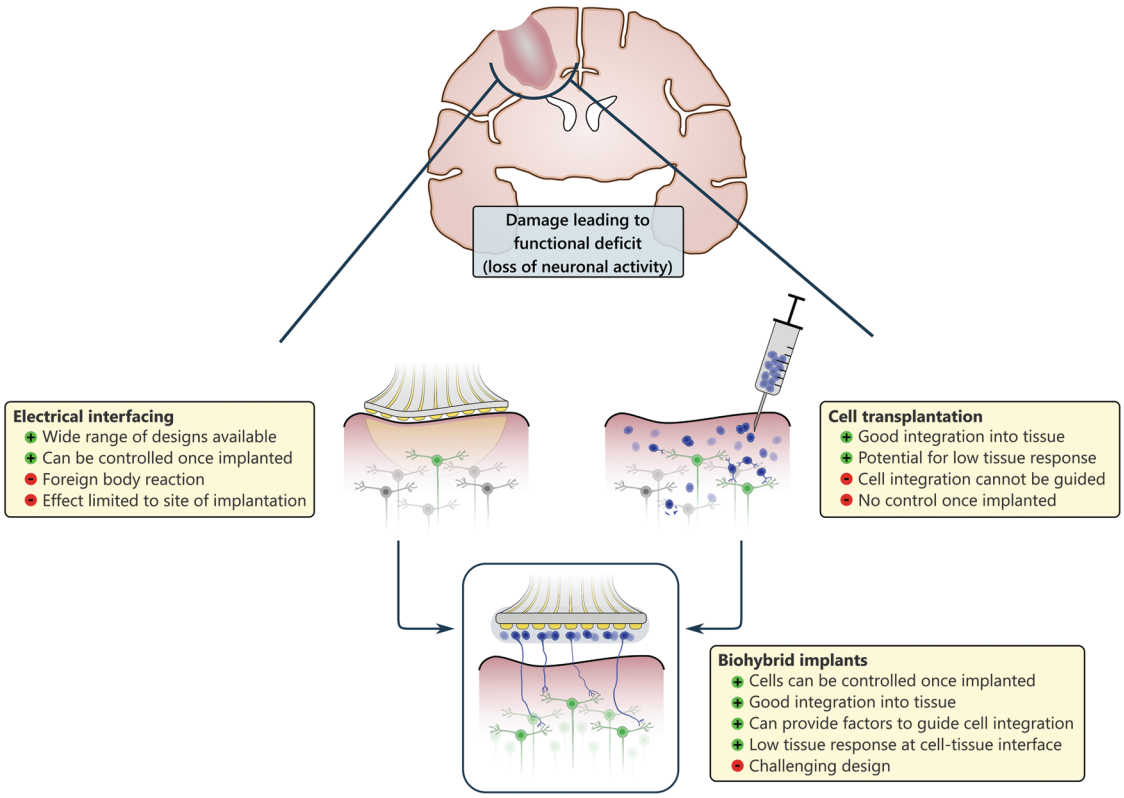


Figure 1. Biohybrid implants as a combination of electrical interfacing and cell transplantation to restore lost function. Implantable interfaces allow control of healthy neuronal circuitry through electrical stimulation and recording, but their effect remains limited to the area surrounding the implant. Transplanted cells integrate well into the host circuitry, but no control over their function can be exerted following transplantation. Biohybrid implants, though challenging to design, combine many advantages of neural interfacing and cell transplantation.

cultures within the implant extending into a damaged peripheral nerve, reaching into multiple denervated muscles and connecting these to an electrode array. Recent advances in neural implant designs indeed highlight the need for implants which better imitate and integrate with neural tissue.[30] By providing a platform onto which cells are seeded, biohybrid devices can also incorporate molecular cues (growth factors, guidance molecules, etc) to stimulate and guide outgrowth of cells. Whether fixed to the implant surface or delivered via microfluidic channels, these molecular cues offer an advantage over traditional cell transplantation by facilitating integration of cells to the intended targets.

Additionally, many traditional neural implant designs see the quality of signals recorded quickly deteriorate when tested under chronic implantation conditions. Although this is to a degree caused by FBR, another major contributor to this interface instability is motion.[31,32] The constant movement of body tissues in chronic implantation scenarios (consequence of natural locomotion, ventilation, blood flow, etc.), combined with mechanical mismatch between tissues and implanted materials, leads to relative motion of the two. This motion makes it difficult to record from the same subset of cells over long timespans, and causes continuous damage to the surrounding tissue, further degrading the electrode–tissue interface. As a strategy to address this issue, there is a growing interest in the development of soft and/or flexible neural probes,[31,33] that better mechanically integrate into the host tissue. The double interface design present in biohybrid devices offers an alternative approach to this same problem. Since the rigid electrodes do not have to be positioned in immediate proximity to the fragile tissue of interest (with cells instead growing out into it), the host tissue of interest remains unperturbed by any micromotion of the device.

One final advantage that biohybrid implants can offer stems once again from its two-interface design. Although fabrication and preparation of biohybrid implants requires an additional layer of work, as cultures of cells need to be seeded on these prior to implantation, the electrode–cell interface offers an excellent opportunity to design the connectivity of the implant under controlled conditions. One of the primary challenges faced by all neural interface designs is the need to decode and interpret the data recorded. The uncertainty introduced by the implantation procedure and the unknown distribution of the host neural network means that it is often unclear what is the biological interpretation underlying the data recorded by each electrode. Extensive efforts are as a result put into developing strategies to aid in decoding this based on the recordings obtained following implantation.[34]

Biohybrid interfaces offer a strategy to address this by setting up the interface between biology (cell cultures in the implant) and electronics under the controlled environment offered by a dish. Seeding of cells onto the MEA implant surface can be,

for example, accurately controlled by patterning or substrate functionalization;[35] and the development of the electrode–cell interface can be monitored using live imaging techniques. This can additionally facilitate design and fabrication of the electronics part of the device. Since electrode arrays do not have to imitate the architecture of the host tissue (with the cells instead performing the integration into the tissue), fabrication efforts can instead focus on producing more robust systems capable of performing well under chronic implantation scenarios.

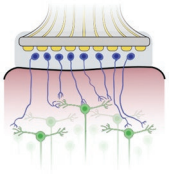
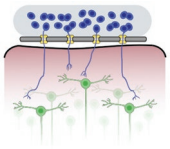
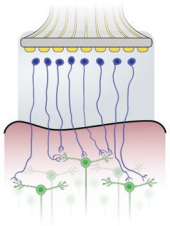
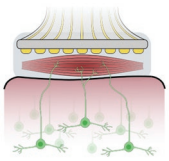
Biohybrid implants, lying at the interface of biology and electronics, constitute a unique tool with great potential in both the clinic and basic research. Despite the multiple challenges in their design and fabrication due to the need to integrate expertise in microfabrication, cell culture, and implantation surgery, amongst others, biohybrid implants offer numerous advantages over traditional implantable electrode array designs. This niche held by biohybrid implants can translate into unique therapeutic opportunities.

Multiple research laboratories have applied the concept of biohybrid implants to various body tissues: ranging from CNS to muscle tissue (**Table 1**). Incorporating this distinction by host tissue location (CNS, PNS, and special senses) followed by a summary of the key challenges and future perspectives, we hereby provide a comprehensive description of the current state of research on neural biohybrid implants.

2. Central Nervous System

The CNS is comprised of a complete network of both neurons and glia. Neurons, in the vast majority of cases, are responsible for receiving electrical signals via dendrites and these signals are transmitted along axon fibers.[36] Glia (oligodendrocytes, astrocytes, and microglia) are supportive cells in the CNS acting as protection for neurons and insulation for axons. Injury to the CNS can occur in multiple ways including traumatic brain injury or degenerative diseases, this results in degradation and loss of long axonal connections and electrical communications. When the CNS

Table 1. Types of biohybrid neural interface designs.

Biohybrid neural interface types	Example papers	Development stage/Target tissue	Key challenges
Cells-on electrode 	Purcell et al.[38] Neural stem cell seeded probes Azemi et al.[44] NPC seeded neural probes De Faveri et al.[45] Coated microelectrodes Wise et al.[78] Neurotrophin secreting interfaces	In vivo (rat) CNS/PNS In vivo (mouse) CNS In vivo (rat) CNS In vivo (cat) Cochlea	• Achieving good cell adhesion to electrode to avoid cell migration and reduce the distance between electrode and neurons
Cell-through electrode 	Stieglitz et al.[27] Regenerative Sieve Electrode	Device fabrication PNS	• Achieving appropriate guidance of implanted cells through microsieve holes • Survival of cells within the device once implanted
Long distance cell electrode 	Cullen et al.[46] Living electrode Tang-Schomer et al.[57] Microfluidic neuron–electrode brain interface	In vivo (rat) CNS/PNS In vivo (mouse) CNS	• The efficiency of axonal integration with the host once implanted • The distance that axons can grow through tracts to reach the target tissue • If living electrodes fail it is difficult to remove them once implanted as they integrate well with host tissue compared to electrodes
Host cell electrodes 	Urbanchek et al.[64] Regenerative Peripheral Nerve Interface (with cultured myoblasts) Irwin et al.[66] Regenerative Peripheral Nerve Interfaces (with muscle tissue)	In vivo (rat) PNS In vivo (macaque) PNS	• The amount of signal transmitted through the muscle graft RPNI • The degree of signal amplification through the muscle tissue

is injured, an inhibitory environment is generated resulting in the lack of capacity to repair and regenerate.[37] Hence, functional axonal restoration is rarely possible due to lack of guidance, local inhibitory signals, and large distances from targets.

Here we discuss tissue engineering approaches using biohybrid electronic devices for functional neurological restoration in the CNS. The biohybrid strategies discussed include technologies that incorporate both cells and electronics. By bridging the gap at the site of injury with a biohybrid approach, cells could act as biological amplifiers, transducing signals from electrical to biological allowing reestablishment of lost connections. There are two key strategies that have been described in literature targeting the CNS using this biohybrid approach, those with cells on electrodes and those with cells growing through electrodes.

2.1. Cell-Seeded Probes

A conceptual neural stem cell-seeded probe design was presented by Purcell et al.[38] to evaluate the capability of functional neurological restoration in patients with motor and sensory deficits resulting from injury. The aim of this concept was to facilitate integration between implanted devices and the host tissue, to improve recording quality and stability during chronic implantation. Probes were Parylene C based, with the dimensions 2.6 mm (length), 200 µm (wide), 40 µm (thick) containing a hollow well to encase a scaffold capable of hosting cells (**Figure 2**). E14 cortical neural stem cells (NSCs) (Stem Cells Technologies) were cultured as neurospheres and a mixture of heterogenous stem cells and progenitors.[39] Scaffolds encapsulated these neural stem cells within an alginate hydrogel and were implanted into Sprague Dawley rats that were not immunosuppressed. Four probes were implanted into the somatosensory cortex of individual rat brains, two cell seeded probes and the control probes, an unseeded probe and an alginate coated probe. Histological analysis was performed at 1 d, 1 week, 6 weeks', and 3 months' time points.

The neuronal density was higher surrounding the NSC-seeded probes compared to the unseeded probes at both 1 d and 1 week post implantation. Probes seeded with NSCs were associated with a reduced initial tissue response after implantation (at 1 d and 1 month time points). This may have been due to the bystander effect, resulting from cell therapy. Transplanted cells are thought to secrete specific factors that allow for correction of biochemical imbalances that may lead to cytotoxicity and further injury.[40] There was evidence of secretion of neuroprotective factors that support neuronal survival. Purcell et al. in an earlier study showed that brain-derived neurotrophic factor and glial-derived neurotrophic factor are released from alginate-encapsulated NSCs in vitro during a 3 week culture.[41]

At the 1 d postimplantation time point, despite the scaffold being intact, there were a limited number of NSCs detected, with more than 90% of cell viability lost in the grafts. This could have been due to either an initially low cell seeding density or impact during insertion. A possible strategy to reduce

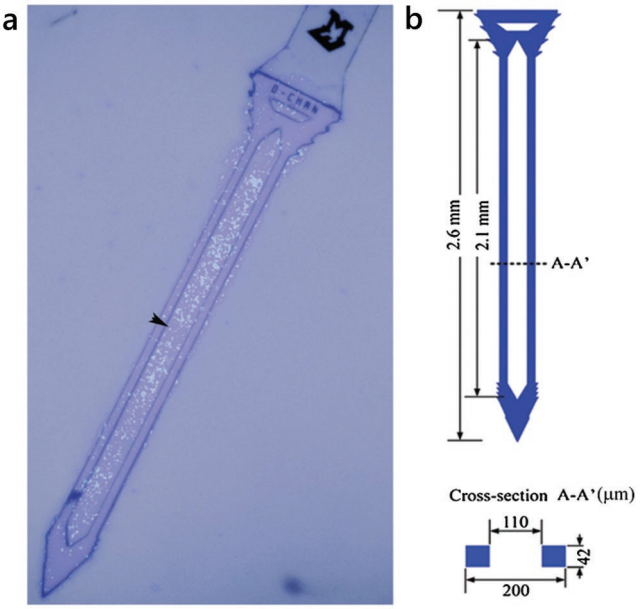


Figure 2. Neural stem cell seeded probe presented by Purcell et al. a) A parylene based device containing a hollow well that hosts NSCs within an alginate hydrogel. NSCs on the device are Hoechst stained. b) A schematic of the neural stem cell seeded probe design including its dimensions. Reproduced with permission.[38] Copyright 2009, IOP Publishing.

the initial cell loss could be to improve cell adhesion to the scaffold. Conversely, at the 6 week and 3 month post implantation time points, there was a reduction in neuronal density for the NSC-seeded probes and an increased tissue response compared to controls. This may be due to the alginate gel dissolving and fragmenting overtime (shown by histological analysis) and the components affecting the viability of the implanted NSCs causing a lower neuronal cell density as a result.[42]

This was the first report in literature analyzing the change in neuronal density surrounding a prosthesis over time. The recording performance relative to neuronal density was unknown at the time of publication. It was useful to understand why the recording capabilities of implanted probes decrease over time relative to neuronal density. The timing of the neuronal cell density changes corresponds with recording performance in silicon probes alone in literature. Ludwig et al. showed that the loss of recording units after the initial insertion injury but recording quality is recovered when the neuronal cell density increases after 1 week post implantation.[43] Additionally, this was the first report in literature where doublecortin (DCX) positive neurons were located around neural implants associated with seeded probes (1 week after implantation). DCX is a microtubule associated protein found in neuronal precursors that migrate, indicating regenerative capacity of the grafted cells or the injured brain or both.[42]

A good extension of this study would be to investigate mechanisms and functionalizing recording sites. Acute electrophysiology experiments are needed to analyze the effect on implanted cells and chronic electrophysiology tests to assess for long-term biocompatibility of the NSC-seeded probes.

The concept of cell-seeded probes has been demonstrated by others. Azemi et al.[44] aimed to improve the interface of neural implants by seeding neural probes with neural progenitor cells (NPCs). By adding a layer of cells to the surface of laminin-coated probe this may help mediate the tissue interface mismatch. The growth, differentiation, and postimplantation survival of these NPCs was analyzed. Green fluorescent protein (GFP) labeled NPCs were cultured in vitro for 14 d and implanted into murine cortex. The tissue response observed at two different time points, on day 1 and 7 post implantation. There was improved attachment of cells on laminin-coated probes. On days 1 and 7 post implantation viable NPCs that still expressed GFP were found on the neural interface of the implant. NPCs adhered to a laminin-coated silicon-based probe, grew and differentiated. This tissue friendly surface reduced the implant-induced brain injury. The NPCs cell layer improved the astrocyte reaction around implant site by releasing neurotropic factors, consequently reducing the foreign body reaction. Results confirmed that cells might help reduce the glial scar at two different time points, yet further in vivo work is needed for chronic recording performance with cells.

An alternative approach for cell-seeded probes was introduced by De Faveri et al.[45] They presented a biohybrid solution to improve the performance of intracortical implants long term.[46] This method was developed to help control the biological response and promote integration of implanted electrodes using a compact layer of cells within a reabsorbable fibrin hydrogel. Electrodes were coated with reprogrammed neural (hippocampal neurons) and glial cells (astrocytes). Neurons were isolated from E18 rat embryos and glial cells isolated from the cerebral cortex of P0 rat pups. Wire probes were dip coated in a human fibrin hydrogel and electrode tips were coated with poly-d-lysine, creating a positively charged layer to enhance cell adhesion.

Two implantable wires were tested within the study; quartz insulated metal electrodes (95% platinum, 5% tungsten) with 20 µm diameter coated with 30 µm of quartz to analyze adhesion of the hydrogel during insertion to electrode surface. Second, to assess the time frame of hydrogel reabsorption and measure the tissue reaction post implantation lead wires (350 µm and 3 mm diameter) were used. These implantable wires were dip coated in fibrin hydrogel, coated in poly-d-lysine to enhance cell adhesion (by creating a positively charged layer) and dipped into a vial of 300 000 cell/500 µL. Implantation of the devices into Sprague Dawley rats was performed by the same subject to minimize variability. Animals were sacrificed at days 3, 7, 30 post implantation, brain slices were cut and stained for immunofluorescent analysis.

Acute neural recordings were performed to indicate the in vivo electrical performance of the fibrin-coated microelectrodes. The fibrin hydrogel allowed good quality recordings and did not alter the electrochemical properties of the microelectrode. Swelling of the hydrogel is thought to allow water and ion adsorption from the saline solution maintaining optimal connectivity. However, when the fibrin-coated wires were implanted in vivo this swelling lead to an increase in the distance between the electrode and the target tissue. Though De Faveri et al.[45] reported that under their conditions fibrin hydrogels caused less swelling than other hydrogels, desired coating thickness could be adjusted.

Immunofluorescent analysis determined that in vivo reabsorption of hydrogel occurred as fluorescent intensity decreased over time post implantation. The hydrogel was fully resorbed into tissue after 7 d post implantation. Bare uncoated electrodes were compared to fibril-coated electrodes to analyze the host tissue response. A total of 7 d after implantation a strong astrocyte reaction was determined. However, the fibrin hydrogel elicited a weaker response after 7 d in comparison to uncoated devices and this response continued to decrease up to 30 d post implantation. This approach reduced amount of host reactive astrocytes compared to a bare wire.

Primary neurons and glial cells created a compact monolayer on the surface of the curved electrode. Although cells were partially stripped during surgical insertion to the brain, hence the fibrin hydrogel layer was incorporated to increase the mechanical compliance of the microelectrodes implanted. Here the cell coating and hydrogel did not significantly affect the impedance of electrodes compared to the uncoated devices (without cells and hydrogels).

De Faveri et al.'s prototype shows promise for improving the biocompatibility of microelectrodes by means of mimicking the properties of the host tissue. This method is a way to potentially improve electrical integration at the neural interface. Additional advantages of this approach are that the aim is to draw all materials from the recipient patient, to reduce the likelihood of an immune reaction. Fibrinogen, a fibrin precursor can be withdrawn from patient's blood and the neurons and glial cells can be reprogrammed from patient's own fibroblasts.

Others have trapped neurons within hydrogels and conductive polymers. Goding et al.[47] presented a conceptual design that combined both cells and electronics to form a bionic device. Two hydrogels, one biosynthetic (degradable) and one conductive (CH), were combined and analyzed to assess their ability to encase cells on the surface of electrodes (**Figure 3**). This tissue engineered approach of the electrode uses a "natural mode of stimulation" by means of a seamless interface.[48] The layer of encapsulated cells offered a soft neural interface between the target tissue and the platinum electrodes.[49] Rat glial and neuroprogenitor cells were encapsulated on top of a CH in a degradable hydrogel for protection from solvents and high DC voltage that are needed for CH fabrication and high capacitance charges at the site of the platinum electrodes. The properties of the degradable hydrogel are important. The degradation rate of the hydrogel should match the rate of ECM regeneration during chronic implantation experiments.[50] As the nonconductive hydrogel degrades the stiffness of the entire biohybrid drive decreased to within the range of neural tissue, this allows for support for transplanted cells; allowing optimal biochemical and mechanical cues to survive and differentiate producing functional neural networks.[51] The viability of encapsulated cells was assessed, and glial cell had a high viability within the hydrogel, however, neuroprogenitor cells did not have a high survival rate and this was possibly due to the harvesting techniques.[47] Additionally, cells encapsulated within the hydrogel bilayer produced ECM proteins. This biohybrid approach shows promise for future proof of concept studies in vivo. A possible extension of this work might aim to

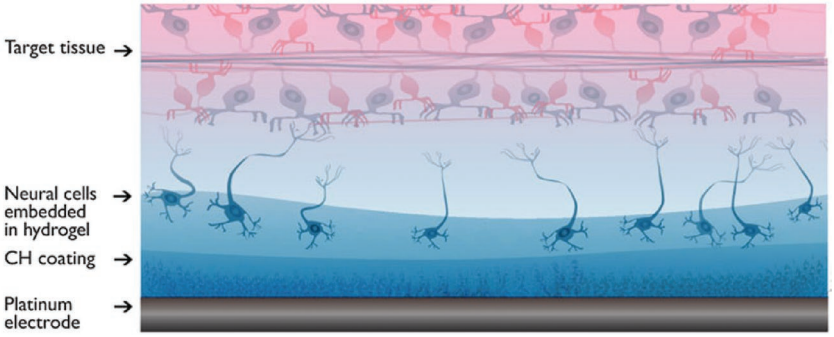


Figure 3. A conceptual design combining both cells and electronics to form a bionic device presented by Goding et al. The schematic shows the seamless synaptic integration between the embedded neural stem cells and the target tissue. Reproduced with permission.[53] Copyright 2017, Cambridge University Press.

quantify the ECM production and compare it to the hydrogel degradation rate.

In addition, by functionalizing conductive hydrogels with gelatin and sericin biomolecule, the survival and proliferation of olfactory ensheathing cells (OECs) at the electrode interfaces of implants was improved.[52] These OECs are glial cells in the olfactory nervous system that have an ability to move through glial scar tissue and support the growth of neural processes. Eight biochemical variations of poly (vinyl alcohol) and heparin with PEDOT hydrogels were compared against platinum electrodes for OEC spreading and the percentage composition of gelatin and sericin were varied ranged between 1 and 3 wt%. Cell attachment studies were performed and 1 wt% gelatin in the hydrogel increased OEC attachment compared to nonfunctionalized conductive hydrogel. These biofunctionalized conducting hydrogels could be an ideal electrode coating for tissue engineered neural interface and customizable to specific cell types that are being transplanted.

Here we have discussed two biohybrid approaches, those with cells seeded on electrodes and those with cells hosted in hydrogels. This approach has allowed for a softer neuron–electrode interface with soft scaffold reducing the risk of iatrogenic nervous injury. Cell seeded probes reduced the tissue response at site of injury, but cell viability decreased, and cells were lost post implantation. Hence further improvements are needed to improve the cell–device adhesion with coatings or encasing hydrogels.[53] Incorporation of a hydrogel layer may increase the mechanical compliance of the microelectrodes implanted[45] and degradable hydrogels encasing cells may allow for ECM regeneration at site of lesion. There have been promising approaches to date for the field of bioelectronics using a biohybrid approach to achieve repair of lost CNS communications, although further in vivo chronic studies are needed to assess biocompatibility and survival of transplanted cells over time.

2.2. "Living Electrode" Technology

The concept of "living electrodes" was introduced by Cullen et al.[46] in vitro, incorporating living neurons and optogenetic based microelectrode technology. This was the first demonstration of unidirectional, long distance growth of axons with a high survival rate in a contained 3D microenvironment.

The design included a columnar structure that acted as tracts for axonal growth (**Figure 4**). These microcolumnar structures were fabricated from an agarose-collagen hydrogel, forming a tube-like structure allowing for support and directional growth of axons.[46] The agarose component of the hydrogel allowed for a stiff casing of the conduit for implantation with the soft collagen on the inner side suppling bioactive ligands, to support the survival and growth of implanted neuronal/axonal cultures.

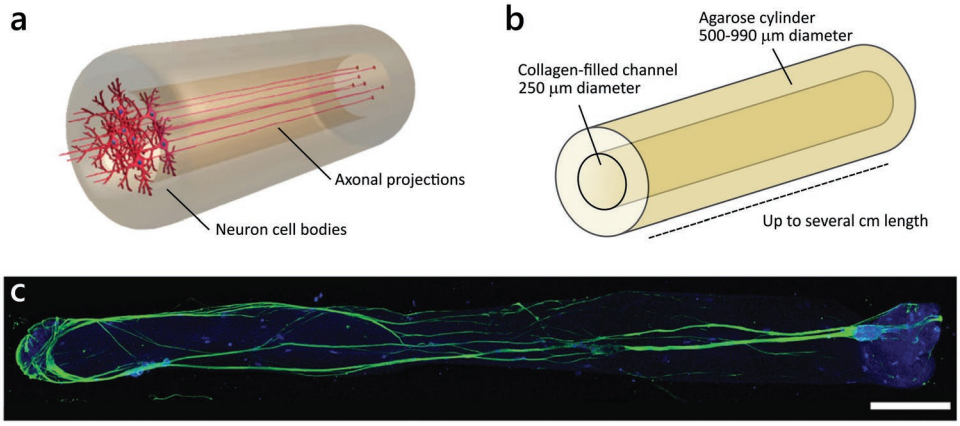


Figure 4. Living electrode concept presented by Cullen et al. a) 3D scaffold comprised of microcolumnar structures fabricated from an agarose-collagen hydrogel, forming a tube-like structure that allows support and directional growth of axons. b) A schematic of the conceptual design with associated dimensions. c) A confocal image of a bidirectional microTENN containing two populations of neurons. These populations span across a long axonal tract within a hydrogel column. Immunohistochemical staining was used to identify the axons (b-tubulin II;green) and cell nuclei (Hoechst;blue). 250 μm scale bar. (c) Reproduced with permission.[37] Copyright 2015, Wolters Kluwer Medknow.

The conduits had dimensions of 250 µm (inner diameter) and 500 µm (outer diameter) and were several centimeters in length.

Living dorsal root ganglia neurons were injected into one end of the conduit. The implantation end of the conduit aimed to promote neuronal survival and the other encouraging unidirectional growth of axons through the conduit. Over one-week, confocal microscopy revealed that neurons had survived and remained at the seeding site, axons had sprouted from these neurons along the inside of the conduit and grown up to 5 mm in length. This concept showed promise in repairing damaged projections of axons with the conduit acting as a bridge of living axons.

Building on their previous technology, Struzyna et al.[54] developed microtissue engineered neural networks (microTENNs) which are essentially a form of "living scaffold."[46,54,55] These microTENNS comprise of neural bodies at one end of an agarose-collagen hydrogel microcolumn structure and their axonal projections growing unidirectionally toward the target tissue these multiple microstructures form a neural network that could act as a replacement for CNS reconstruction.[37] These microTENNS are fabricated in a similar fashion to the living electrodes by pouring a hydrogel into cylindrical moulds that have a needle in the center. Once the hydrogel is cured, the central needle is removed, and hollow microstructures are formed, an ECM solution aiding the survival of axons is used to line these lumen. With this approach different neural subtypes from the cerebral cortex are used to create the axon-based living electrodes, these have varying functions and abilities to integrate with the target tissue and generate synaptic integration.[54] Advantages of this approach are that the guided axons can act as a bridge for synaptic integration across a lesion and act as a guide for native axons to grow through.

This biohybrid approach could be used as a cell-based probe to record activity in the CNS utilizing either electronic or optical based technologies. These microTENNS can act as a biological intermediate between implanted devices and the host nervous system. Utilizing the unidirectional growth of axons in the microstructures, neuromodulation and neural recording could be performed for brain–machine interfaces.[56]

A modified approach to the living electrode was suggested by Tang-Schomer et al.[57] They presented an integrated microfluidic neuron–electrode brain interface, on a transparent, flexible, silk film (**Figure 5**).[57] Silk-based films are useful for the application of brain implants because they are compatible with technologies used to micropattern electrodes. Silk is a purified biopolymer derived from the cocoons of silk worms and can be tailored to have mechanical properties close to that of native tissue.[58] The mechanical and topographical surface properties of the silk film were optimized for the survival and alignment of primary rat cortical neurons on electrodes.

Primary rat cortical cell neurons were taken from embryos at E18 Sprague Dawley rats. The device design comprised of compartments of neuron cultures and patterned electrode arrays. The mechanical stiffness of silk films was altered by varying the annealing temperature and film thickness parameters during the silk-film processing. The surface topography of silk films was controlled to evaluate the effect of any directional cues on glial cell alignment in vitro.

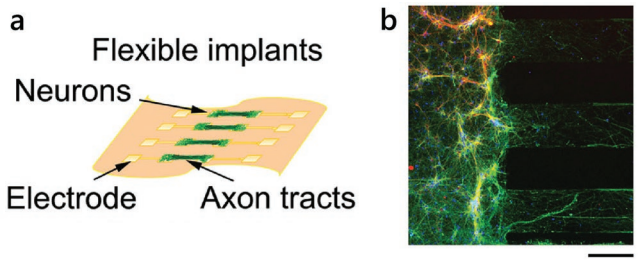


Figure 5. Microfluidic patterned neural brain interface on silk film presented by Tang Schomer et al. a) A schematic of a flexible silk film-based implant. This in vitro model allows for directional growth of axons through tracts located on a silk-film based patterned electrode. b) Cells and axons compartmentalized within culture shown by costaining of β3TB (green), GFAP (red), and DAPI nuclei staining (red). Scale bar, 100 µm. Reproduced with permission.[57] Copyright 2014, Wiley-VCH.

Microfluidic culture systems were incorporated on the prototype, consisting of millimeter long tracts for axon growth to connect separate compartments of cell culture. Each cell culture population was located on a silk-film based patterned electrode. Axons tracts had a depth and width of 100 µm by 50 µm. To determine the functionality of the neuron–electrode interface, neuronal cell cultures were examined under electrical stimulation. Neurons were individually numbered and then randomly selected and analyzed for their calcium levels. Varying frequencies were applied (square waves of 120 mV (peak to peak) at 20, 200, 2000 Hz) for 6 min per condition and every minute, time lapse fluorescence images were taken. Stimulation of these electrodes evoked calcium responses in neurons in vitro, the levels of calcium correlated with stimulation conditions. However, it was noted that there were heterogenous responses between individual neurons, this phenomenon might reflect the complexity of circuitry in the brain and the signal propagation dynamics.

The aim was to examine whether the in vitro model developed could be implanted into the brain with long-term stability and biocompatibility. Tang-Schomer et al. were the first to determine the feasibility of undissolvable silk films to support chronic brain implants. A total of 5 mm diameter silk films (without electrodes) were implanted into the cortex of 3 month-old mice and compared against flat films to examine the effect of brain cells. Minimal inflammatory response was elicited 14 d post implantation. Glial cells aligned at the interface of the electrodes via the microgrooves revealing that the filmed implant integrates well with distinct host brain cells. However, no electrophysiology tests were performed during the in vivo experiments. A good extension of this work would be to perform acute and chronic stimulation on the film-based neuron–electrode interface.

Here we have discussed the living electrode/scaffold biohybrid strategy. This approach has allowed for directional growth of axons that act as electrodes. These living electrodes can integrate with the target tissue and produce synaptic integration.[36] Modifications of this concept have been presented through flexible silk scaffolds that incorporate microfluidics culture in vitro models.[57] Studies in this section have shown promise but have not included chronic in vivo implantation studies to assess

the functionality of the device and level of tissue response after implantation.

3. Peripheral Nervous System

A traumatic lesion or degenerative neuropathy to a peripheral nerve can lead to axon degeneration and destruction of the neuromuscular junction (NMJ).[59] Unlike the CNS, the peripheral nervous system (PNS) has the capacity for regeneration, however, lesions with nerve gaps over 3 cm have a lack of functional recovery due to the distance of axonal regrowth required to reach the target. Consequently, loss of communication and electrical connections occurs from one part of the nervous system to another, effecting motor control and sensory processes.[36] When trying to preserve or regenerate this NMJ it is important to have a stable interface with the peripheral nerve and electronics. A biohybrid approach incorporating cell therapeutic strategies with electronics could help. This section includes biohybrid approaches incorporating regenerative peripheral nerve interface (RPNIs) technologies that harbor cells or tissue grafts to allow for integration between transplants and host tissue for PNS regeneration.

3.1. Regenerative Sieve Electrode (RSE)

The first regenerative biohybrid neural interface approach was introduced by Stieglitz et al.[27] The "neuron microprobe" was a biohybrid system concept that interfaced with peripheral nerve using a high channel sieve electrode, containing transplanted biological cells (**Figure 6**). This approach highlighted the importance of sustaining skeletal muscle function by preserving the NMJ. The prototype was designed for restoration and control of skeletal muscle using functional electrical stimulation. This biohybrid concept aimed to couple implanted neurons and ring electrodes that allow growth of axons from implanted neurons to act as mediators to the target muscle.

It was emphasized that the most important technical requirements for this biohybrid design were the adaption of the distal nerve stump, an ability to host cells and contain microelectrodes that act as transducers for cell monitoring and stimulation.[60] Hence, a sieve electrode design was presented, based on a rat sciatic nerve model with diameter of 1.5 mm. This polyimide-based sieve device contained 19 ring electrodes and a counter electrode over 286 holes. Additionally, the design incorporated three bending flaps for suture fixation to the epineurium of the peripheral nerve of rats. For the microprobe design it was mentioned that a high proportion of holes on the substrate were favorable for axon sprouting but it had to be considered that as the hole number increases, mechanical strength decreases.[27]

Sterilization of devices was performed via autoclaving and this method decreased the bending angle of the interconnecting wire and flaps for adhesion but scanning electron microscopy revealed no cracks or delamination. Preliminary results of electrical characterization for chronic device performance of this biohybrid concept were tested on three devices after thermal bending of polyimide. After sterilization techniques and thermal annealing, the impedance of the ring electrodes increased from 2.7 kΩ a to 155 kΩ at 1 kHz.[27] Although Stieglitz et al.[27] did not chronically implant their devices into animal models with cells, within their research consortium chronic implantation of these devices have been performed. Device requirements for chronic implantation include the ability to remain stable in a physiological environment, with no occurrence of degeneration.[61] Components of the design must not be toxic to cells as the device material needs to act as a diffusion barrier. The conducting materials used must have a low corrosion rate as this too can inhibit regeneration and transplanted cell survival.[62] Six months post implantation this polyimide device (without cells) integrated well into the existing nerve architecture and microfascicles passed through

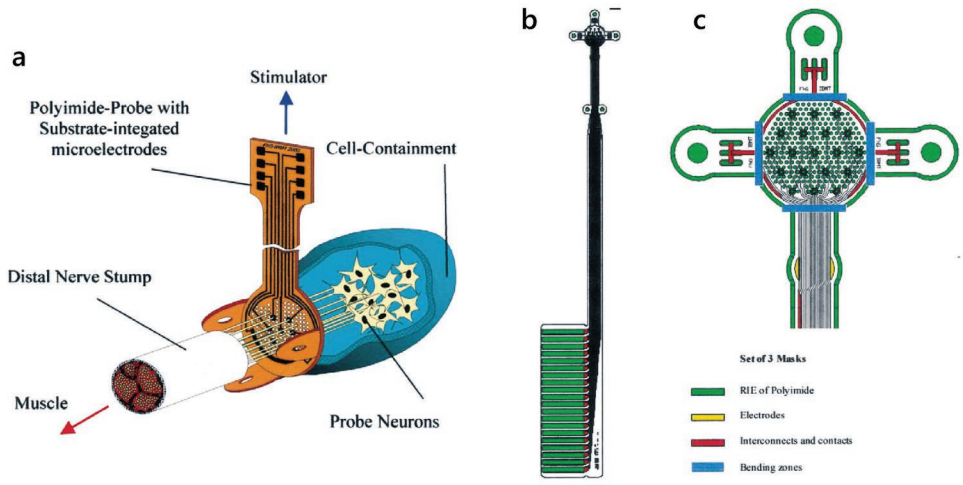


Figure 6. Biohybrid device for peripheral nerve regeneration presented by Stieglitz et al. a) A conceptual design of the neuron microprobe approach combining a polyamide structure incorporating microelectrodes that interface with transplanted cells at a distal nerve stump. b) A set of three masks that allow fabrication of the biohybrid design presented. RIE Polyamide mask (Green), Electrodes (Yellow), Interconnects, and contacts (Red) Bending zones (Blue) (scale bar: 1 mm). c) A magnified version of the sieve areas of the biohybrid device. Reproduced with permission.[27] Copyright 2002, Elsevier.

the microsieve holes across the implant.[63] A good follow up of this work would be to investigate the incorporation of cells and electronics by recording and stimulating neurites sprouting through holes in the sieve for peripheral nerve regeneration.

3.2. Regenerative Peripheral Nerve Interfaces with Muscle Cells

Since the first introduction of the biohybrid neural interface system, adaptions to this approach have been developed. RPNI present a potential strategy to interface divided peripheral nerves with prosthetic limbs. Urbanchek et al.[64] encased myoblasts within a biologically stable scaffold (RPNI) that had an ability to provide signal amplification through mature myotubes and prevent neuroma formation (**Figure 7**). Three scaffold materials were compared: silicone mesh (SM), acellular muscle (AM), and acellular muscle combined with chemically polymerized poly(3,4-ethylendioxythiopene) (PEDOT) conducting polymer (AM+PEDOT). These RPNIs had enough permeability and stability to host myocytes for chronic implantation. Soleus muscle myoblasts were isolated from rats and cultured for 13–17 d. A total of 3 million myoblasts were implanted onto each scaffold (Acellular muscle group (AM) and Acellular muscle and PEDOT (AM+PEDOT)) when myotubes showed signs of contraction in vitro.[64]

PEDOT was used to chemically polymerize the inner lining of the implant with the aim of improving the conductivity. A 2 cm section of the distal peroneal nerve was removed. RPNI were implanted into peroneal nerve of rats and recovery was allowed for 2 months. The average implant time was 93 d (59–111 d). Electrophysiology tests were performed at the endpoint of the experiment to assess the variability and ability of this RPNI to transduce neural signals into muscle signals. A 26-gauge stainless steel needle was inserted into the central portion of the RPNI and the current was increased to elicit compound muscle action potentials (CAMPs).

Contractible myoblasts encased within RPNIs can address some limitations of other peripheral nerve interfaces. First, when interfaced with the peroneal nerve the RPNI could detect physiologic efferent motor action potentials.[65] Electromyographic (EMG) activity was recorded from RPNIs and CMAPs were produced in SM, AM, and AM+PEDOT with high reproducibility. Low amplitude nerve signals resulted in the contraction of myotubes within the RPNI due to reinnervation. This revealed the RPNI was capable of transducing nerve signals into muscle signals. Additionally, myoblasts could differentiate and mature into functioning desmin-positive muscle fibers and were reinnervated and revascularized. This directional growth of regenerating axons into the myoblast based RPNI helped prevent neuroma at the end of the dissected peroneal nerve.

A good follow up of this would be to investigate chronically implanted electrodes for long term and high specificity control of neuroprosthetic devices. Additionally, an extension of this work could be to quantitatively validate the observations made. The health and contractile properties of mature muscle fibers in the RPNI need to be examined. Nerve conduction studies are required to assess the quantity of signal that the RPNI can transmit and the amount of signal amplification performed by the muscle fibers. Despite the subjective approach to this study, findings suggest that the incorporation of muscle cells within an RPNI are an exciting approach that should be investigated further.

3.3. Regenerative Peripheral Nerve Interfaces with Muscle Tissue Grafts

To overcome the limitations of current technologies, Irwin et al.[66] developed an RPNI from small, partial, autologous

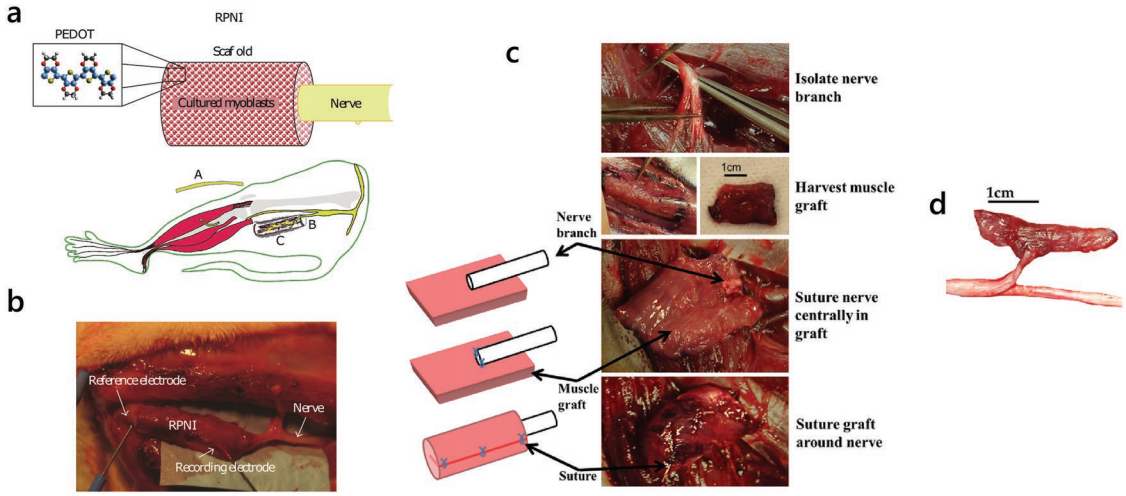


Figure 7. Regenerative Peripheral Nerve Interfaces (RPNI). a) A schematic presented by Urbanchek et al. of an RPNI that is fabricated using a scaffold made from either a silicon mesh, acellular muscle, or acellular muscle with PEDOT. (Below) A section of the distal common peroneal nerve is removed (A) and the residual nerve (B) the entire RPNI construct (including myoblasts) is wrapped around a peripheral nerve for 2 months. b) An RPNI 4 months after implantation in situ. The compound muscle action potentials (CMAPs) were recorded and stimulated using an electrode placed on the peroneal nerve. Reproduced under the terms of the Creative Commons Attribution License.[64] Copyright 2016 Melanie G. Urbanchek et al., published by Hindawi Publishing Corporation. c) Irwin et al. presented an RPNI with a muscle graft. The implantation procedure of the RPNI can be seen here from top to bottom. d) A newly implanted RPNI with a muscle graft. The peroneal nerve is sutured into the middle of the RPNI. Scale bar, 1 cm. Reproduced with permission.[66] Copyright 2016, IOP Publishing.

muscle grafts that is reinnervated with a peripheral nerve branch transection. For the first time the stability, lifetime, and signal strength of an RPNI was shown as a promising approach for restoring movement in nonhuman primates. Signals from a prosthetic hand were controlled using chronic recording through muscle graft RPNI. Once reinnervated the RPNI biologically amplifies motor signal commands in the descending nerve with high SNR and specific EMG signals.

Muscle grafts for the RPNI were dissected from healthy autologous muscle tissue with dimension of 1 cm by 3 cm. Nine of these RPNIs were folded over the distal ends of branches of the medial and radial nerves of two rhesus macaques and secured to the epineurium. The branches of nerve ended on long finger flexor and extensor muscles, ideal for prosthetic hand control.

Both macaques were trained to perform finger movements. Bipolar EMG electrodes (silicone backed) were implanted onto the surface of the RPNI and led up to a headcap subcutaneously. However, animals destroyed the leads within the headcap consequently meaning there were no working electrodes. A revision surgery revealed that the stiffness of the silicone had resulted in scaring and prevented regeneration of the RPNI, hence electrodes were removed from both animals. Instead, whilst animals were performing tasks, fine wire electrodes were implanted to the RPNIs for acute recording.

20 months postimplantation RPNIs showed no adverse effects in either of the monkeys and normal EMGs were recorded from RPNIs with high SNR like that of healthy intact muscle. Signals could be easily recorded using and decoded into commands for the use in a functioning prosthesis. With RPNI signals recorded during a behavioral task, Irwin et al. could calculate each macaque's finger movements (classified as flexion, extension, or rest) with up to 95% accuracy. RPNI could aid monkeys control their virtual hand. This was made possible by decoding signals in real time and offline. RPNI could aid monkeys control their virtual hand with 280 and 447 finger movements decoded by both monkeys. This approach enabled prosthetic control to one degree of freedom matching what is currently commercially available. Each macaque was able to perform the same behavioral task equally using the finger movement classifications.

RPNIs were able to successfully both reinnervate and regenerate muscle tissue based on histological staining of muscle at implantation and 4 months postimplantation. Hematoxylin and eosin staining showed that the muscle tissues were healthy, well vascularized with no indication of necrosis. Irwin et al. mention that the muscle cells appear rounder and nuclei are centrally located indicating that regeneration was still ongoing. A possible extension of this could have included quantitative analysis of cell morphology and nuclei roundness.

Irwin et al.[66] were able to track the level of reinnervation over time as an electrode was implanted with the muscle graft in the EDCa nerve RPNI. Stimulation of this RPNI led to the production of CMAPs that indicate a healthy neuromuscular interface. As all the RPNIs were able to reinnervate, with some even reintegrating with surrounding muscle tissue it was difficult to identify the RPNI to replace electrodes.

Here RPNIs were implanted into healthy, uninjured monkeys. During surgical procedures it was ensured that the surrounding muscles remained intact and no motor function deficits. This made it very difficult to calculate whether there was any crosstalk from other adjacent muscles. However, authors expected that because the intramuscular electrodes were highly selective any effects were negligible based on their previous work on rats.[65] This needs to be confirmed in future human studies whilst recording activity as the anatomy in humans differs slightly.[67]

Irwin et al.[66] demonstrated for the first time that the stability, lifetime, and signal strength of this RPNI are promising for restoring movement using long-term physiological control of an artificial limb. Vu et al.[67] extended this work by increasing the capability of continuous estimation of finger movements, through the implementation of a Kalman filter. This work opens the opportunity for new myoelectric sites to potentially replace what has been lost after implantation. Future developments could combine increased number of EMG electrodes with wireless stimulation and EMG muscle recordings to restore control and function of a lost limb.

3.4. PNS "Living Electrode" (Tissue Engineered Nerve Grafts)

As previously mentioned in the CNS section of this report Cullen's group pioneered the "living electrode" technology and have adapted this to target neuroregeneration in the PNS.[37] In this instance the concept is termed TENGs (tissue engineered nerve grafts) and these comprise of axon tracts that are stretch-grown to create a regenerating bridge of axons across a PNS lesion.[68] These TENGs are nervous tissue that consist of two cell populations with the capability of growing up to 5–10 cm in length within 14–21 d.[69] The speed of axonal growth using this strategy is advantageous as axonal regeneration in the PNS is notoriously slow at a rate of 1 mm per day and slower across a lesion injury.[59] The TENGS are created by embedding axon tracts into ECM for stability before transplantation.[70] In recent years, TENGs have been generated with rat dorsal root ganglion neurons (DRG), cortical neurons and human DRG (from cadavers and live subjects).[69] Huang et al.[70] performed a preclinical efficiency study to repair lesions in rat sciatic nerves using rat DRG TENGs.[70] Six weeks post implantation the architecture of axons was preserved and integration of the DRG neurons with host tissue was seen. Confocal images of green fluorescent protein positive (GFP+) axons confirmed that transplanted axonal tracts in TENGSs helped host axons grow directly across the lesion. After 16 weeks of implantation the neural tissue with the lesion appeared normal and host axon appeared myelinated.[70]

In this section we have discussed the first introduction of a biohybrid peripheral nerve interface and reviewed recent adaptions that include cells and tissue grafts within RPNIs. In vivo studies including macaque animal models comprised a muscle flap encasing nerve, where the muscle grafts acted as organic amplifiers, which in turn integrated with existing musculature.[66] Additionally, a PEDOT lined RPNI containing transplanted myocytes were able to amplify signals and reduce neuroma formation by giving the axons directional growth.[71] Synaptogenesis was identified at NMJ indicating integration of the RPNIs with existing musculature.[66]

4. Special Senses

4.1. Hearing

It is common clinical practice to restore hearing in deaf patients using cochlea implants to electrically stimulate spiral ganglion neurons (SGNs). Acoustic energy is converted into an electrical signal via an external device.[72] A subcutaneous internal signal receiver then stimulates these SGNs.[73] The silicone-based devices contain platinum electrodes and are inserted into the scala tympani.[74] Insertion trauma resulting from the positioning electrodes inside the cochlea can affect the nerve electrode interface and consequently impact the patient's hearing.[75,76] When tissue within the cochlea becomes damaged an immune reaction occurs, this can lead to FBR and osteogenic changes in the scala tympani.[77] In humans, the amount of fibrous tissue is inversely proportional to outstanding SGNs.[76] Since the onset of deafness, these SGNs gradually degenerate, and they are the target cells for electrical stimulation. Hence there is a need to replenish/regenerate the residual SGN population and reduce insertion trauma. A biohybrid approach coating electrodes in cells could overcome these challenges allowing for a softer interface between neurons and electrodes.

Wise et al.[78] demonstrate that cells can be combined with cochlea implants and used to protect neural cell populations. Lack of neurotrophins (NTs) in the SGN is thought to contribute to their degradation so transplant of NTs with electrical stimulation may help prevent SGN loss and consequently increase performance of cochlea implants. Encapsulated choroid plexus cells were implanted along with eight platinum intracochlear electrodes (0.3 mm wide and 0.45 mm apart) to release NTs to resident SGNs (**Figure 8**). As neuroprotective agents were released by the transplanted cells, which in turn increased the survival of SGN and reduced the formation of scar tissue at the neural interface.

Neonatal cats were deafened and at 8 months of age animals were separated into three groups, those receiving chronic electrical stimulation only, cell transplantation only, or cochlea implant and cell therapy combined. Each cat was treated unilaterally in one ear within each group and one ear served as a control with no device/treatment applied.

Results revealed survival of SGNs and their peripheral processes was not increased in groups with electrical stimulation alone or NTS alone. However, in the upper basal region of the cochlea implantation of NTs only led to an increase in SGN survival and density of their peripheral processes revealing that NTs are of benefit to the SGNs. The group receiving electrical stimulation and NTs led to a significantly greater survival of SGNs and their peripheral processes in more regions of the cochlea. Further investigations are needed to confirm whether resprouting of these peripheral processes were in contact with the electrode array, their location within the cochlear anatomy suggested this.

4.2. Human Studies

The first report of autologous cells being transplanted into the inner ear of human subjects was presented by Roemer et al.[79] The safety study described a biohybrid approach combining bone marrow derived mononuclear cells (BM-MNC) and flexible electrodes to release neuroprotective agents to support existing SGN.[79] BM-MNCs were chosen as they have an ability to repair damaged tissue and participate in the regulation of immune responses, thus may have the potential to reduce implantation trauma and help regenerate and support damaged SGNs.[80]

This study included three human subjects who were classified as "severely hearing-impaired" and had been unsuccessful with cochlear implants in the past. Subjects were selected for bilateral implantation (one biohybrid and standard cochlear implant). Patient 1 was a 43 years old male who experienced progressive hearing deterioration because of hypoxia at birth. His left ear was considered appropriate for implantation of the biohybrid device as deafness was more severe. Additionally, Patient 2 was a 43 years old male who had a long history of

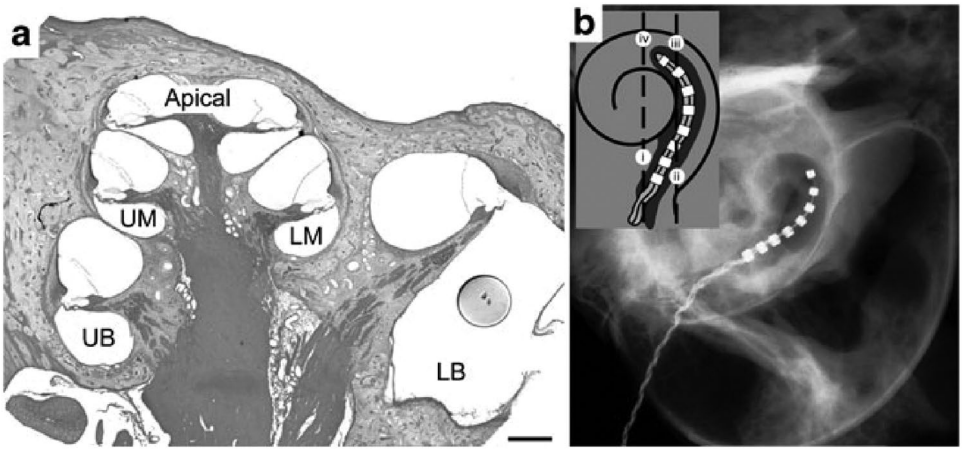


Figure 8. Neurotrophin (NT) secreting cells combined with a cochlear implant presented by Wise et al. a) A micrograph of a cat cochlea showing the regions of the cochlea: lower basal (LB), upper basal (UB), lower middle (LM), upper middle (UM), and Apical regions. In the LB region there is a schematic representation of a capsule containing NT secreting cells to show the dimensions of the capsule in respect to the scala tympani. Scale bar, 500 µm. b) An X-ray showing a cochlear implant. The schematic shows the locations (i–iv) within the cochlea where tissue responses were examined. Reproduced with permission.[78] Copyright 2011, The American Society for Experimental NeuroTherapeutics Inc, published by Springer Nature.

continuous hearing loss. Patient 3 was a 21 years old male who had diminished hearing since childhood.

BM-MNCs were isolated from recipients own bone marrow and mixed with Fibrin adhesive (Tisseel) preimplantation. The autologous cell suspension was mixed with the fibrinogen component of the fibrin adhesive. The cochlea implant was then pulled through this solution with the addition of the thrombin component of the adhesive to entrap a layer of cells on top of the electrodes.

As it is not possible to assess neuroprotective effects of BM-MNC in vivo in humans, an in vitro demonstration of this was performed in a neonatal rat coculture. Compared to both positive and negative controls BM-MNCs lead to the highest SGN survival. The BM-MNCs were found to secrete cytokines, chemokines, and various growth factors known for their anti-inflammatory and neuroprotective characteristics. Authors stated, based on these in vitro results, that it could be expected that the cells would perform in the same way when implanted into human subjects. Previous works on animal models with the addition neurotrophins have shown increased SGN survival.[81] Additionally, progenitor cells can reduce fibrosis and allow regeneration after tissue damage without scar formation in some species.[82]

The insertion forces of the biohybrid cochlear device were similar regular cochlear implants. Five months after implantation electrode impedances and perception of speech were analyzed in both ears of each patient. Similar impedances were found across all devices in all patients. Patient 2's speech perception using biohybrid implant had a higher performance than the implant in the corresponding ear. Patient 1's speech perception was similar in both ears. However, in Patient 3 the standard implant outperformed the biohybrid implant.

Here the biohybrid approaches discussed targeting the special senses focus on coating electrodes with cells. This strategy has shown potential to reduce insertion forces that lead to a tissue response by creating a softer interface between electrodes and target SGNs.[78] The transplanted cells were able to increase SGN survival in the scala tympani by releasing NTs. In terms of increasing the precise activation of the SGN, this is difficult, as the fluids within the cochlea are conductive so an increased density of SGN may not improve the spatial precision but could be tested in the future. Roemer presented for the first time that autologous cells and electronics are safe in humans.[79] However, future directions should focus on increasing patient sample size and varying the age and sex of subjects.

4.3. Vision

Despite the clinical application of both neural interface technology[83] and stem-cell therapies,[84] a biohybrid approach for vision restoration has yet to be described to date.

5. Key Challenges

Whereas the blend of the bioengineering and cell therapy allows many challenges to be overcome, several issues still need to be addressed. In this section we offer an overview of the existing and potential key challenges that need to be addressed before these concepts are translated into a clinical setting.

In recent years, a vast range of cell populations have been transplanted during biohybrid approaches targeting different parts of nervous system, but cell survival posttransplantation remains a constant challenge. When designing a biohybrid device several factors must be considered to ensure that transplanted cell survival is not impeded. Cell toxicity due to exposure to components and DC voltages from fabrication process requires thorough in vitro testing before in vivo studies. Multiple aspects of the materials, such as elasticity, porosity, and stiffness, will need to be carefully considered to optimize cell adhesion and survival. Some researchers have incorporated neurotrophins release to the devices to increase survival of SGN in cochlea post implantation.[78] Microfluidics or ionic pumps,[85] integrated to neural interface devices, could be used to deliver locally neurotrophic factors, chemoattracts and/or immunomodulants.

Different types of coatings, such as hydrogels, could be used to increase the ability of devices to host and support the growth and survival of cells in an electrically active environment. However, also the use of coatings can cause further problems, such as hydrogel swelling leading to displacement of the target tissue from the electrodes.[86] Electrode coatings must be also optimized to improve cell adhesion to electrodes. If the cell niche is not optimized for the transplanted cells, then they are unable to receive the correct biomechanical and chemical cues to proliferate and survive. As shown in the CNS section, surgical implantation has been shown to decrease cell viability as insertion forces cause cells to separate from the device. Therefore, further improvements are needed to improve the cell-device adhesion with coatings or encasing hydrogels.[53] Incorporation of a hydrogel layer may increase the mechanical compliance of the microelectrodes[45] implanted and degradable hydrogels encasing cells may allow for ECM regeneration at site of lesion.

Achieving a good cell adhesion is important to avoid cell migration and reduce the distance between electrode and neurons. This distance influences the strength of the electrode recording and stimulating signals, with an optimal maximum distance between the body of a neuron and an electrode between 50 and 100 μm.[47] Urbanchek suggested that future work should incorporate a biocompatible conducting polymer into the RPNI design. Urbanchek et al. 2016 identified synaptogenesis via identification of NMJ via histology. In all specimens, regenerating axons were identified adjacent to muscle fibers, but were not near PEDOT. Multiple reports of PEDOTs biocompatibility have been made, but further optimization of PEDOT polymerization for optimal muscle reinnervation via axons within in this RPNI are needed.

Biohybrid devices, which host immortalized cell types or stem cells are inherently still suffering from some of the drawbacks of cell therapy. There are two main risks associated with implanting cells. The first is the risk of developing a teratoma, which is linked to the capacity of stem cells to overproliferate. This risk could be counterbalanced by assuring the implanted stem cells lose their stemness overtime, as also requested by regulatory bodies. The second risk is graft rejection, where the host immune systems attack and destroy the exogenous cells.

To moderate this process different level of immunosuppression needs to be considered, either locally or systemically.[26] However, different strategies are under development to provide immune tolerance strategies to pluripotent stem cells derivatives.[24]

6. Future Perspectives

Biohybrid technology offers prospects currently inaccessible to either cell therapy or neural interfaces on their own, due to their intrinsic limitations. In the introduction section, we have discussed advantages provided by having an intercalated biological layer between neural interface electrodes and targeted tissue. This layer has the potential benefit to reduce tissue injury and foreign body response, improve long-term integration with the underlying tissue and increase target selectivity. On the other hand, there are important gains for transplanted cells to have neural interface technology support. There have been numerous reports on the role of electrical stimulation in neuronal regeneration for both the central and peripheral nervous systems.[87] Hence, being able to deliver electrical stimulation to transplanted cells has the potential benefit to promote cell regeneration and axonal sprouting in situ. This approach is recognized in the context of tissue regeneration, where metals, carbon nanotubes, or polymers, have been used to fabricate conducting scaffolds in order to promote wound healing or bone, nerve, and cardiac regeneration.[88]

The potential of biohybrid devices goes beyond the one described in this progress report. While standard neural interface technology can only target structures with an existing neuronal population, biohybrid implants can restore functions by both regenerating and stimulating. There are many examples where this strategy might prove superior to cell therapy alone. The majority of injuries to the nervous system cause not just loss of neurons, but also disruptions of the network they were part of. As fundamental neuronal connections within and outside the nervous system are made during development, there is no "blueprint" which can be followed to re-establish the lost networks following an injury. It is therefore unsurprising that transplanted neurons, which rely on environmental cues to lead their migration, regeneration, and connection to resident cells, have not yet been successful in areas such as stroke, complete spinal cord injury, or peripheral nerve transections. There have been several reports describing how electrical fields could be used to guide cell migration and axon elongation direction.[89] Hence, biohybrid devices could also be used to direct neurons to preselected targets, to restore and control functional neural networks.

While the research effort has so far been focused in developing this technology, there has been little emphasis on the clinical translational strategies. Our group is currently working in integrating iPSC derived cells[90] on electrodes, with the aim to both provide an off the shelf device, easy for the clinicians/surgeons to use, but also able to target the individual need of each patient. By using iPSC derived cells, we can seed them at a high density and therefore improve postimplantation cell viability. This approach reduces the need for patients to undergo multiple surgeries and circumvent the risk of using immortalized cells (e.g., teratoma). We can also control the stage of differentiation and type of transplanted cells to suit the targeted location requirements.

Recent advances in implant manufacturing techniques may also complement well with emerging biohybrid technologies. 3D printing is becoming an increasingly popular technique for implant fabrication, and can be used to incorporate electronics,[91] cells,[92] and structural and biochemical cues[93] into implantable constructs. The precise control and versatility offered by additive manufacturing has the potential to greatly benefit biohybrid technology, reducing the time and complexity needed to combine cells, electronics, and structural materials into an implantable biohybrid device. This may be a particularly attractive option in scenarios where the host tissue may have a complex architecture and good integration between implant and tissue may be difficult to achieve, such as CNS contusion lesions.

Biohybrid implants lie at the interface of biology and electronics and constitute a unique tool with great potential in both the clinic and basic research. Despite the multiple challenges in their design and fabrication due to the need to integrate expertise in microfabrication, cell culture, and implantation surgery, amongst others; biohybrid implants offer numerous advantages over traditional implantable electrode array designs. This niche held by biohybrid implants can translate into unique therapeutic opportunities.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

biohybrid interfaces, cell transplantation, implantable devices, nervous system injury, neural interfaces

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