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HIGHLIGHTS

Restoring nervous system structure and function using tissue engineered living scaffolds

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Abstract

Neural tissue engineering is premised on the integration of engineered living tissue with the host nervous system to directly restore lost function or to augment regenerative capacity following nervous system injury or neurodegenerative disease. Disconnection of axon pathways – the long-distance fibers connecting specialized regions of the central nervous system or relaying peripheral signals – is a common feature of many neurological disorders and injury. However, functional axonal regeneration rarely occurs due to extreme distances to targets, absence of directed guidance, and the presence of inhibitory factors in the central nervous system, resulting in devastating effects on cognitive and sensorimotor function. To address this need, we are pursuing multiple strategies using tissue engineered "living scaffolds", which are preformed three-dimensional constructs consisting of living neural cells in a defined, often anisotropic architecture. Living scaffolds are designed to restore function by serving as a living labeled pathway for targeted axonal regeneration – mimicking key developmental mechanisms– or by restoring lost neural circuitry via direct replacement of neurons and axonal tracts. We are currently utilizing preformed living scaffolds consisting of neuronal clusters spanned by long axonal tracts as regenerative bridges to facilitate long-distance axonal regeneration and for targeted neurosurgical reconstruction of local circuits in the brain. Although there are formidable challenges in preclinical and clinical advancement, these living tissue engineered constructs represent a promising strategy to facilitate nervous system repair and functional recovery.

***Key Words:** living scaffolds; neural tissue engineering; cell transplant; biomaterials; regeneration; neurotrauma; neurodegeneration; axon pathfinding; cell migration*

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Repairing the Nervous System Centers on Reestablishment of Connectivity

The nervous system is a complex interconnected network linked by axons - specialized neural fibers that form the basis for communication between functionally distinct regions of the central nervous system (CNS) and relay sensorimotor signals in the peripheral nervous system (PNS). Remarkably, there are over 160,000 km (100,000 miles) of axons in the adult nervous system (Kandel, 2013). The long-distance axonal connections in the nervous system are formed during embryonic development when source neurons and target are in close proximity (Varier and Kaiser, 2011). Through *in utero* and prenatal development, and indeed into adolescence, the length of these axonal pathways is progressively increased as the brain grows and the spinal cord and peripheral nerves are lengthened due to bone and other connective tissue growth. Eventually, the lengths of crucial axonal pathways in the brain end up on the order of several centimeters, in the spinal cord on the order of tens of centimeters, and in the PNS up to one meter long.

Nervous system injury and disease comprise a diverse group of disorders that include traumatic brain injury (TBI), stroke, spinal cord injury (SCI), peripheral nerve injury (PNI), as well as neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). A common feature across virtually all of these disorders is the loss of long-distance axonal connections. When these axonal pathways become dysfunctional or degenerated, the adult CNS has virtually no capacity and the PNS has only limited capacity to re-grow these tracts (Fitch and Silver, 2008; Pfister et al., 2011). These failings owe to extreme distances to appropriate targets, insufficient or lack of directed guidance, and in the case of the CNS, an inhibitory environment (Fitch and Silver, 2008; Pfister et al., 2011). Thus, loss or dysfunction of axonal pathways results in debilitation that is often severe, chronic, and life altering. To date, strategies to restore axonal connections have had minimal success. These strategies have utilized biomaterial scaffolds, molecular signals, and/or transplanted cells, but have been unable to overcome the degree of axon targeting and extreme regenerative distances required to functionally reestablish lost neural connections (Eberli and Atala, 2006; Chan and Leong, 2008; Kim and de Vellis, 2009).

To address this need, we are pioneering novel neural tissue engineering strategies to create "living scaffolds", which are preformed three-dimensional (3-D) constructs consisting of neural cells and biomaterial matrices in a defined cytoarchitecture (Cullen et al., 2007a, b, c, 2011a, b, 2012; Huang et al., 2009; Struzyna et al., 2014). In particular, we focus on creating anisotropic living scaffolds consisting of long, aligned axonal tracts extending from discrete neuronal population(s). To enable precise control of neuronal phenotypic composition, axonal architecture, and functional attributes, these constructs are generated *in vitro* prior to delivery *in vivo*, where they act to facilitate restoration of nervous system structure and function. These constructs are precisely engineered to recapitulate features of damaged or lost neuroanatomy in order to fulfill one or more of the following interrelated objectives: (1) *neuroregeneration*: provide a living labeled pathway to orchestrate long-distance axonal pathfinding and/or cell migration; (2) *neuron and axon tract replacement*: exploit local plasticity to physically "wire in" and form a new functional relay; and/or (3) *biological neuromodulation*: affect the neurophysiology of specific neural circuitry based on feedback from other regions. This article will discuss the state-of-the-art of tissue engineered living scaffolds for nervous system restoration, the promise of these constructs to replace axonal tracts and/or repair neural circuitry, as well as the challenges to translating this exciting regenerative medicine technology into clinical use.

Attributes of Tissue Engineered Living Scaffolds for Nervous System Restoration

Successful neural tissue engineering strategies involve the integration of engineered living tissue with the host nervous system to directly restore lost function or to augment the capacity for endogenous nervous system regeneration. Representing a subset of tissue engineering strategies, living scaffolds are unique in that they possess a preformed, often anisotropic architecture consisting of living neural cells within a 3-D biomaterial matrix (Huang et al., 2009; Cullen et al., 2011a, 2012; Struzyna et al., 2014). A unique attribute of living scaffolds is that they can be designed to mimic robust developmental mechanisms, particularly in the case of guiding axon growth and cell migration. In particular, cells within living scaffolds can simultaneously and temporally affect numerous pathways by secreting potentially thousands of synergistically acting factors. In addition, living scaffolds can respond to host feedback to modulate signaling, such as electrophysiological parameters to affect network behavior (*e.g.*, based on desired circuit function) and/or secrete factors to diffuse throughout the local microenvironment (*e.g.*, based on the state and progression of the regenerative process). Moreover, there are numerous examples of these various mechanisms being conserved from *in vitro* to *in vivo* environments (Weick et al., 2010; Pina-Crespo et al., 2012; Bryson et al., 2014). In contrast, acellular biomaterial strategies frequently lack these attributes, as their effects are generally short-lived and involve only a few factors (Thorne and Frey, 2001; Eberli and Atala, 2006; Rosenstein et al., 2010). Although controlled release of soluble factors is a common objective, contemporary acellular scaffolds lack the ability to effectively alter secretion based on the progression and state of the regenerative process or *via* feedback from host (Thorne and Frey, 2001; Eberli and Atala, 2006; Rosenstein et al., 2010). Despite robust *in vitro* results affecting neurobiological phenomena such as axonal extension or cell differentiation using biomaterial strategies, the mechanism of action found *in vitro* often does not translate to the *in vivo* setting. In the nervous system, biological signaling from endogenous cells may override the regenerative effects of current acellular biomaterial strategies, or the biomaterial is quickly remodeled by cell types not present in the *in vitro* system. Moreover, grafts containing seeded cells are generally more favorable than acellular grafts in the promotion of regeneration (Park et al., 2002; Teng et al., 2002; Fouad et al., 2005). Our work and that of others have shown that living scaffold based strategies can be highly effective to facilitate restoration in preclinical models of nervous system injury and disease.

Restorative Mechanisms of Tissue Engineered Living Scaffolds

Living scaffolds with preformed 3-D architecture and the ability to dynamically present biological cues possess considerable advantages over more traditional cell replacement and/or acellular biomaterial approaches. The living cells incorporated into the scaffold are uniquely able to actively drive axon regeneration and circuit restoration rather than simply being passive substrates. Incorporated cell types may include primary, stem, differentiated, genetically engineered, autologous, allogeneic, or heterologous cells (Eberli and Atala, 2006; Korecka et al., 2007). Tissue engineered constructs also possess a defined architecture that not only facilitates integration of the transplanted cells/processes with native tissue, but also maintains their desired organization. We are exploring specific tissue engineering design parameters to create living scaffolds to serve as regenerative pathways for axonal guidance, to physically "wire in" to directly replace lost neurons and reconstruct circuits, and/or to modulate existing neural circuitry.

Neuroregeneration

Successful nervous system regeneration requires a precisely orchestrated reestablishment of neural connections and reformation of cellular structure. To address this need, tissue engineered living scaffolds provide a living labeled pathway to choreograph long-distance axonal pathfinding and/or neural cell migration (Struzyna et al., 2014). Directed axon growth and cell migration along pre-existing pathways established by other cells is common in nervous system development, and is necessary to form proper axonal connectivity and cellular localization (Raper et al., 1983; Jacobs and Goodman, 1989; Sepp et al., 2001; Raper and Mason, 2010). Living scaffolds may exploit the mechanisms of cellular/axonal pathfinding seen during development, and serve as chaperones to support, guide, and aid regenerating cells and/or processes. Growth and migration along living neural cells is driven by juxtacrine signaling, involving the concurrent and often synergistic presentation of myriad cell-mediated haptotactic, chemotactic, and neurotrophic cues (Fine et al., 2002; Xu et al., 2004; Smeal et al., 2005; Apel et al., 2010; Madduri et al., 2010; Yan et al., 2012). Utilizing the presentation and modulation of these cues, living scaffolds may be able to actively drive and direct regeneration to maintain an environment optimal for cell migration and axon guidance, sprouting, and myelination to restore complex 3-D tissue structures.

Direct circuit replacement

Living scaffolds may be utilized for direct reconstruction of lost circuitry by replacing neurons and axon tracts. In this modality, living scaffolds are designed to exploit local plasticity to integrate

with preserved host circuitry, thus physically "wiring in" to form a new functional relay across damaged or lost axonal tracts. This strategy is premised on the plasticity of endogenous as well as tissue engineered neural networks, whereby neurons intrinsically have the ability to sense and respond to local activity (Colicos and Syed, 2006; Shein-Idelson et al., 2011; Ganguly and Poo, 2013). It has been shown that transplanted neurons are capable of receiving synaptic input from local networks as well as propagating action potentials (Wernig et al., 2004; Weick et al., 2010). Preformed living scaffolds consisting of long axonal tracts build on these promising studies and suggest that tissue engineered constructs could be transplanted to directly reconnect circuitry across lost and/or damaged axonal tracts. Once the appropriate synapses are established, preformed living scaffolds could act as functional relays to transmit signals between populations of previously disconnected host cells.

Neuromodulation

The third modality of living scaffold approaches that we are pursuing is "biological neuromodulation" of existing host circuitry based on feedback from other regions. Biologically based circuit modulation would be useful for a range of applications, such as PD, depression, obesity, drug addiction, and pain disorders. Currently, the vast majority of neuromodulation strategies employ magnetic or electrical methods, such as deep brain stimulation (DBS). Alternatively, biological neuromodulation is a radical approach that may allow for more specific control of neural circuits and challenge the status quo of "hardware-based" neuromodulation. The theoretical advantages to using living scaffold strategies over DBS in this capacity are that they can be smaller, permanent, and completely self-contained (requiring no power). Moreover, modulatory living scaffolds are capable of responding to temporal/local host conditions by relaying feedback from one region to another region. These designer constructs for biological neuromodulation could effectively increase or decrease the gain of specific components of a neural circuit, towards the goal of mitigating cognitive, sensory, or motor deficits.

Our Strategies

Our research group is currently pioneering two separate living scaffold strategies to achieve nervous system restoration. The first approach is based on the use of tissue engineered nerve grafts (TENGs) consisting of stretch-grown axonal tracts for regeneration across major nerve lesions in the PNS. A second strategy involves the use of micro-tissue engineered neural networks (micro-TENNs), which are miniature injectable constructs that recapitulate the neuroanatomy and function of discrete neuronal populations spanned by long axonal tracts. Micro-TENNs are designed to rebuild disrupted axonal pathways and modulate existing neural circuitry using network feedback. While we will discuss TENGs in their function as scaffolds for neuroregeneration and micro-TENNs as constructs for direct circuit replacement and neuromodulation, it is noteworthy that either approach could be utilized for any of the three aforementioned mechanisms of living scaffolds.

The PNS: tissue engineered nerve grafts as bridges to drive neuroregeneration

We are applying novel TENGs to facilitate ultra-long distance regeneration in the PNS, which presents a formidable challenge. The loss of peripheral axons – either due to trauma, surgery, or a degenerative neuropathology – can have devastating effects on motor control and sensory processing. Although the PNS possesses an intrinsic capacity for regeneration, axonal degeneration in proximal regions (*i.e.*, closer to the spinal cord) or cases involving nerve gaps greater than 3–5 cm (~1–2 inches) generally results in poor functional recovery owing to extraordinary distances for axons to regenerate to appropriate targets. The PNS has a notable absence of neuronal somata – outside the immediate vicinity of the spinal cord – and thus functionally consists solely of long axonal projections. Upon nerve injury, there is generally a complete and rapid degeneration of axons from the site of injury to distal target (*e.g.*, hand), leaving only the distal nerve structure (*i.e.*, ECM, support cells). Thus, axon deficits following injury can be tens of centimeters in length. To restore connectivity and function, the body must regenerate axons from the site of injury all the way to the appropriate distal target. However, the window for repair is temporary and the rate of axonal regeneration is slow, generally averaging 1mm/day (~1 inch/month); with even slower rates across the site of injury (Burnett and Zager, 2004). The distal nerve structure serves as the path for axon regeneration once across a lesion; however this path degrades and disappears over time, which generally blunts the extent of functional recovery.

TENGs are lab-grown nervous tissue, comprised of long, integrated axonal tracts spanning two populations of neurons. The ability to generate these nerve grafts is based upon seminal discoveries regarding the process of axon growth *via* continuous mechanical tension or "stretch growth" (Smith et al., 2001). Stretch growth is a natural axon growth mechanism that we replicate in custom mechano-bioreactors to grow axons of unprecedented lengths in a short time frame 5–10 cm (2–4 inches) in 14–21 days, with no theoretical limit as to the final axon length (Pfister et al., 2004; Smith, 2009). TENGS are subsequently created by embedding these living axonal tracts in an extracellular matrix to ensure stability before removing them *en masse* for transplantation (Huang et al., 2009). To date, TENGs have been generated from a number of key species including rat embryonic and adult dorsal root ganglia (DRG) neurons, rat embryonic cortical neurons, and human adult DRG neurons (cadaveric and live donors) (Pfister et al., 2004; Huang et al., 2009; Smith, 2009). TENGs are designed to bridge severe peripheral nerve injuries to facilitate axon regeneration to reinnervate target muscles. As depicted in **Figure 1**, the living, aligned axonal tracts in TENGs serve as a living scaffold for regenerating axons by providing structural support, as well as haptotactic, chemotactic, and neurotrophic cues. To demonstrate this phenomenon, we utilized high-resolution confocal microcopy to show that regenerating host axons grew directly along TENG axons *in vivo* (**Figure 1**). In preclinical efficacy studies, we previously utilized rat DRG TENGs to repair sciatic nerve lesions in rats. At 6 weeks post transplantation, we observed TENG survival, preservation of axonal architecture, and anatomic integration with the host nerve tissue. Host axons grew directly along transplanted axon tracts, suggesting that transplanted axon tracts indeed mediated host axonal growth across the lesion (Huang et al., 2009). At 16 weeks post transplantation, the segments of neural tissue bridging the gap appeared grossly normal, with the robust presence of myelinated host axons (Huang et al., 2009).

The CNS: micro-tissue engineered neural networks to restore or modulate neural circuitry

The exquisite capacity of the human brain relies on a multitude

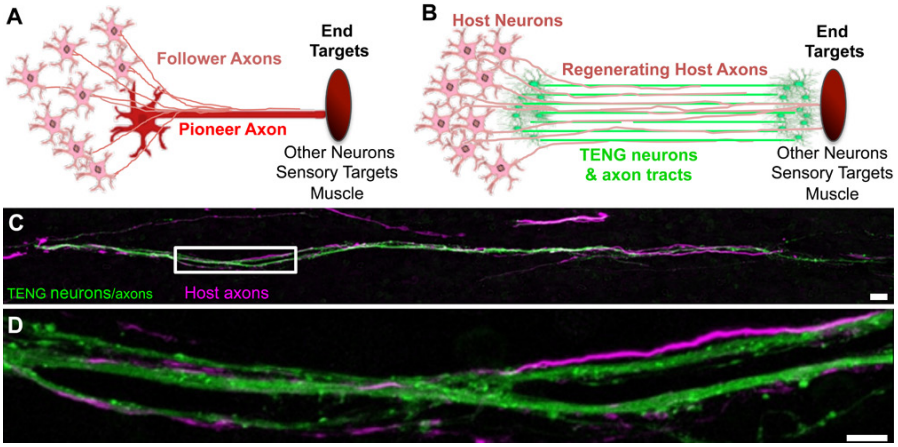

Figure 1 Tissue engineered nerve grafts (TENGs), comprised of long, stretch-grown axon tracts.
TENGs serve as a living scaffold to facilitate nerve repair following peripheral nerve injury. (A) Schematic representation of a prominent axon guidance mechanism seen during development, in which host axons are guided along a pioneer axon that is the first to reach the appropriate end target. (B) Schematic representation of axon-facilitated axon regeneration. Similar to the axon guidance mechanism seen during development, regenerating host axons are guided along TENG axon tracts to the end target. (C) Confocal reconstruction following immunohistochemistry demonstrating regenerating host axons (SMI31; purple) growing along TENG axons (GFP^+; green) *in vivo* to bridge a peripheral nerve lesion. (D) Zoom in of the same region showing host axons (purple) growing directly intertwined with TENG axons (green). Scale bars: 25 μm in C and 6 μm in D.

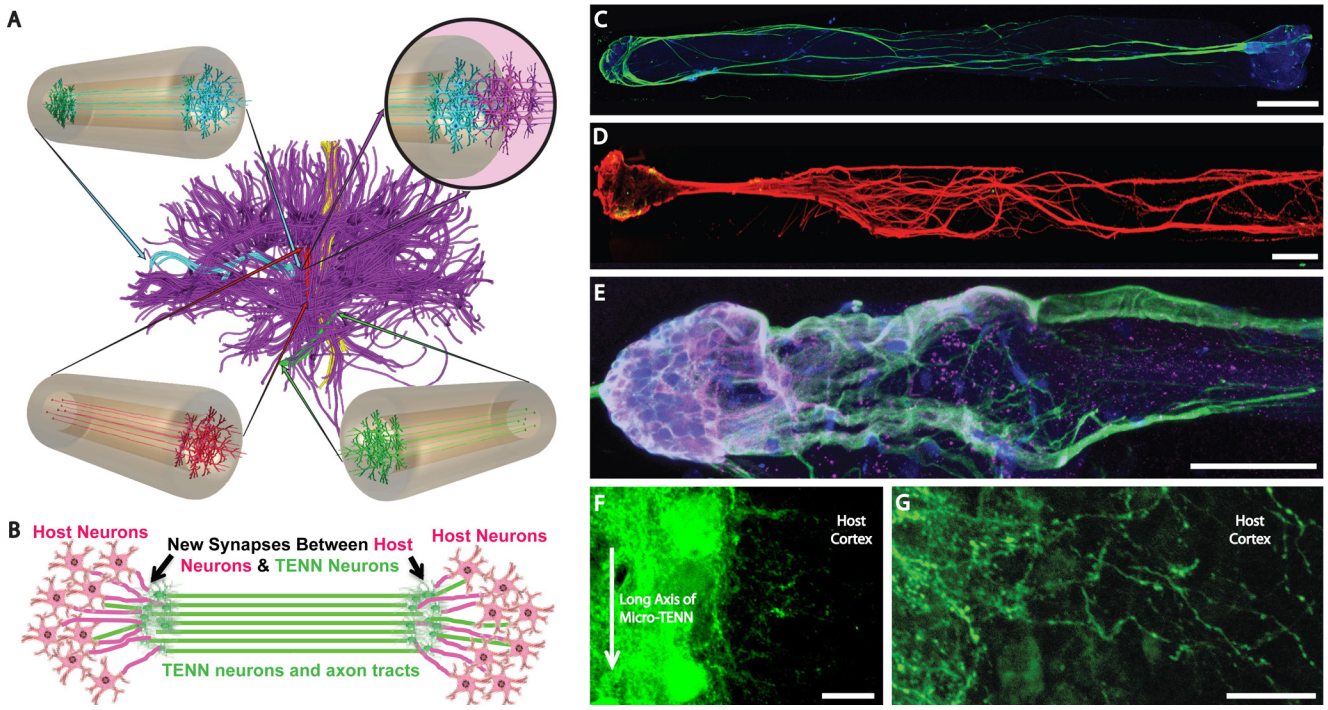

Figure 2 Micro-tissue engineered neural networks (Micro-TENNs), consisting of discrete neuronal population(s) with long axonal tracts within a biocompatible micro-column.
Micro-TENNs are used for the direct reconstruction of long-distance axonal pathways after central nervous system (CNS) degeneration. (A) Diffusion tensor imaging representation of the human brain demonstrating the connectome comprised of long distance axonal tracts connecting functionally distinct regions of the brain. Unidirectional (red, green) micro-TENNs and bi-directional (blue) micro-TENNs can bridge various regions of the brain (blue: corticothalamic pathway, red: nigostriatal pathway, green: entorhinal cortex to hippocampus pathway) and synapse with host axons (purple; top right). (B) Conceptual representation of a micro-TENN forming local synapses with host neurons to form a new functional relay to replace missing or damaged axonal tracts. (C) Confocal reconstruction of a bi-directional micro-TENN, consisting of two populations of neurons spanned by long axonal tracts within a hydrogel micro-column stained *via* immuncytochemistry to denote axons (b-tubulin III; green), and cell nuclei (Hoechst; blue). (D) Confocal reconstruction of a unidirectional micro-TENN, consisting of a single neuron population (MAP2; green) extending axons (Tau; red) longitudinally (adapted from (Cullen et al., 2012)). (E) Confocal reconstruction of a unidirectional micro-TENN, stained *via* immunocytochemistry to denote neuronal somata/dendrites (MAP2; purple), neuronal somata/axons (Tau; green), and cell nuclei (Hoechst; blue). (F) Confocal reconstruction of a transplanted GFP^+ micro-TENN showing lateral outgrowth *in vivo*. (G) Confocal reconstruction showing GFP^+ processes extending from a transplanted micro-TENN into the cortex of a rat. Scale bars: 300 μm in C, 250 μm in D, 100 μm in E, 20 μm in F and G.

of long-distance axonal connections between specialized neuroanatomical structures – referred to as the connectome – which enables profound parallel processing. Dysfunction and disconnection of these axonal pathways, with or without concomitant neuronal degeneration, is a common feature of most CNS disorders. While the field of neuroregenerative medicine offers tremendous promise for neural cell replacement, there is currently no strategy capable of restoring long-distance axonal pathways in the CNS. Micro-TENNs are composed of discrete population(s) of neurons connected by long axonal tracts within miniature tubular hydrogels (roughly three times the diameter of a human hair) (Cullen et al., 2012). As shown in **Figure 2**, micro-TENNs are designed to reconstitute the architecture of white matter pathways; thus, they are a promising technology capable of simultaneously addressing neuronal replacement and physical restoration of axonal connections (**Figure 2**). To date, we have created micro-TENNs using a range of neuronal subtypes, including embryonic rat DRG neurons and cerebral cortical neurons, with both unidirectional and bi-directional architectures (**Figure 2**). Moreover, we have achieved lengths ranging from several millimeters to centimeters while maintaining the micron-scale form factor. Based on the ability to employ defined neuronal phenotype(s), cytoarchitecture, and length, micro-TENNs may serve as an effective substrate for tailored neurosurgical reconstruction of long-distance axonal tracts.

The miniature dimensions of the constructs permit minimally invasive implantation into the brain. In an initial efficacy study, micro-TENNs expressing green fluorescent protein (GFP) were stereotaxically injected into rats with the goal of connecting thalamic structures with the barrel fields of the cortex. At 1 week and 1 month post-implant, we observed surviving clusters of GFP^+ neurons within the micro-TENN ends in both the thalamus and the cortex. Further along the length of the micro-TENNs, we found radially aligned neurons and neurites from the transplant at the micro-TENN–cortex interface (Struzyna et al., 2013). As shown in **Figure 2**, cortical neurons from the micro-TENNs extended neurites deep into the host cortex (Struzyna et al., 2013). Although this evidence of micro-TENN neuronal survival, maintenance of architecture, and structural integration was promising, ultimately electrophysiological assessment will be required to determine functional connectivity and assimilation into host neural networks. This strategy possesses considerable promise to facilitate repair following a number of disorders, such as reconstructing the nigrostriatal pathway preferentially lost in PD.

Although we have focused on the use of micro-TENNs in circuit reconstruction (*i.e.*, tract replacement), tailored micro-TENNs may also play a role in modulating the activity of existing but dysfunctional circuits, a phenomenon we refer to as "biological neuromodulation". Here, micro-TENNs may be precisely delivered to key locations to influence the strength of specific connections. For instance, inhibitory (*e.g.*, GABAergic) micro-TENNs may be designed to form synapses to modulate pathways that are exerting too much influence and causing detrimental functional effects. Conversely, excitatory (*e.g.*, glutamatergic) micro-TENNs may form synapses to augment weak pathways. Micro-TENNs may also act by bulk release of neurotransmitters at the axonal terminal, either *via* tonic (self pacing/continuous) activity or by responding to inputs from the host to the micro-TENN neuronal somata/dendrites. For pain neuromodulation, tailored micro-TENNs may be useful to modulate inputs to a pain-dampening circuit. Also, inhibitory micro-TENNs may potentially be used to attenuate excitable circuits based on early epileptiform activity. Thus, tailored micro-TENNs may fulfill so-called "biological neuromodulation" by providing excitatory or inhibitory inputs with controlled neurotransmitter release to augment circuit function. Micro-TENNs can uniquely fulfill this role – over standard cell transplants for instance – by acting based on network feedback relayed from one brain region to another.

Challenges and Future Directions

Tissue engineered living scaffolds represent a potentially transformational solution for nervous system restoration, as this approach challenges the current paradigms for neuronal replacement, axonal tract regeneration, and neuromodulation. Our living scaffold strategies are based on creating preformed constructs with differentiated neurons and long axonal tracts, thus challenging transplanting the current dogma in neuroregenerative medicine based on transplanting stem cells for neuronal replacement and axon guidance for tract replacement (Bradbury et al., 2002; Borisoff et al., 2003; Jain et al., 2004; Tsai et al., 2004; Mingorance et al., 2006; Moore et al., 2006; Tang et al., 2007; Filous et al., 2010; Liu et al., 2010; Yip et al., 2010). Similarly, in the field of neuromodulation, the vast majority of applications employ DBS based on placing 1–2 mm wide electrodes into the brain. Our concept of "biological neuromodulation", with completely self-contained living constructs that can affect activity in one area based on input/feedback from another area, is a radical approach that may challenge the status quo of hardware based neuromodulation. Our collective approaches for structured living scaffolds may be simultaneously capable of addressing neuronal replacement, physical restoration of axonal connections, and circuit modulation – whereas to date these challenges have largely been addressed independently.

While living scaffold strategies provide key advantages for restoring nervous system structure and function, they also present several formidable hurdles. Living cells may elicit an immune response from host tissue leading to inflammation or rejection of the graft (Barker and Widner, 2004). This immune response differs depending on the cell type transplanted. For example, while glial cells elicit a vigorous response and show poor attrition upon transplantation, constructs consisting of pure neurons appear to be well tolerated by the host and show increased survival (Iwata et al., 2006; Huang et al., 2009; Cristofanilli et al., 2011; Dayawansa et al., 2014). A deleterious immune response may also be mitigated through the use of autologous cells from patients. Here, our proposed strategies will eventually converge with personalized stem cell-based approaches. The emergence of multiple sources of autologous stem cells has drastically increased the feasibility of clinical translation. Neurons, oligodendrocytes, astrocytes, and Schwann cells can be differentiated from human embryonic stem cells (hESCs), induced pluripotent stem cells (iPSCs), and adipose-derived stem cells (ASCs) among others (Kim and de Vellis, 2009; Hu et al., 2010; Faroni et al., 2013; Tornero et al., 2013; Wang et al., 2013). Although direct *in vivo* delivery of stem cells may replace lost cells and encourage neural regeneration through the release of trophic factors, the methods by which they stimulate the nervous system remains unclear, and they have the potential to differentiate into undesirable phenotypes and/or result in tumorigenesis (Faroni et al., 2013). In comparison to stem cells, there are several advantages to the use of differentiated neurons within living scaffolds. Protocols have been worked out for the

differentiation of stem cells into specific neuronal sub-types, including cortical projection neurons and interneurons, dopaminergic A9 neurons, and spinal motorneurons. Thus, tissue with specific neuronal compositions can be engineered accordingly. It is also likely that the use of differentiated neurons for transplantation carries less of a risk for tumorigenesis, but more carefully conducted studies are needed to prove this supposition. Finally, differentiated neurons can be genetically modified to enhance regenerative responses. Prior studies suggest that the low survival of transplanted cells can be due to delivery into a degenerating or "hostile" injured environment. Using transfection techniques or viral transduction, the durability and regenerative potential of differentiated neurons could be augmented, for instance through the overexpression of trophic factors (Korecka et al., 2007). This approach could make engineered tissue resistant to the underlying pathophysiology of neurodegenerative disease.

Conclusions

The brain, spinal cord, and PNS have limited capacity for regeneration, making the effects of neurotrauma or neurodegenerative disease particularly devastating and often permanent. Successful regeneration would involve a precisely orchestrated reestablishment of neural connections and reformation of cellular structure, often requiring directed long-distance axonal pathfinding and neural cell migration. The objective of the field of neural tissue engineering is to utilize biomaterial- and cell-based strategies to augment endogenous regeneration and/or to provide direct replacement of neural cells and circuitry. In both the CNS and PNS, there is a clear need for strategies to restore lost axonal pathways. To address these gaps, we are pursuing multiple "living scaffolds"designed to restore nervous system function by providing living labeled pathways for targeted axonal regeneration, direct replacement of lost axonal tracts, and/or "biological neuromodulation" of existing circuitry. TENGs may be ideal for ultra-long distance neuroregeneration by exploiting developmental mechanisms involving the simultaneous presentation of haptotaxic, chemotaxic, and neurotrophic cues; they may serve as a living labeled pathway to guide and support regenerating axons. Micro-TENNs are designed to have a small form factor, and thus are ideal for delivery into sensitive or deep neural substrates. Tailored micro-TENNs recapitulate the neuroanatomy and function of the basic systems-level "building blocks" of the CNS. Thus, this strategy is promising to fulfill specific roles in brain circuitry, from *de novo* reconstruction to "biological neuromodulation". These living scaffolds may be superior to acellular approaches in that they can provide direct and sustained interactions with the host environment and regulate these interactions based upon the state of regenerative processes. Overall, there are several significant challenges to the development and translation of living scaffolds, including advancing tissue engineering techniques for the creation of living cellular constructs in a defined 3-D architecture, establishing transplantation strategies to ensure preservation of construct vitality and architecture, and devising strategies for immunological tolerance at both acute and chronic time frames. As these challenges are overcome, living scaffolds have the potential to transform the field of neuroregenerative medicine by driving the reestablishment of complex neural structures and axonal connections, ultimately facilitating functional recovery following a range of currently untreatable traumatic and neurodegenerative disorders.

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