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Engineering Tissues of the Central Nervous System: Interfacing Conductive Biomaterials with Neural Stem/Progenitor Cells

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Abstract

Conductive biomaterials provide an important control for engineering neural tissues, where electrical stimulation can potentially direct neural stem/progenitor cell (NS/PC) maturation into functional neuronal networks. We anticipate that stem cell-based therapies to repair damaged central nervous system (CNS) tissues and *ex vivo*, "tissue chip" models of the CNS and its pathologies will each benefit from development of biocompatible, biodegradable, and conductive biomaterials. Here, we review technological advances in conductive biomaterials over the past two decades that may facilitate development of engineered tissues with integrated physiological and electrical functionalities. First, we briefly introduce NS/PCs of the CNS. Then, the significance of incorporating microenvironmental cues, to which NS/PCs are naturally programmed to respond, into biomaterial scaffolds is discussed with a focus on electrical cues. Next, practical design considerations for conductive biomaterials are discussed followed by a review of studies evaluating how conductive biomaterials can be engineered to control NS/PC behavior by mimicking specific functionalities in the CNS microenvironment. Finally, steps researchers can take to move NS/PC-interfacing, conductive materials closer to clinical translation are discussed.

Graphical Abstract

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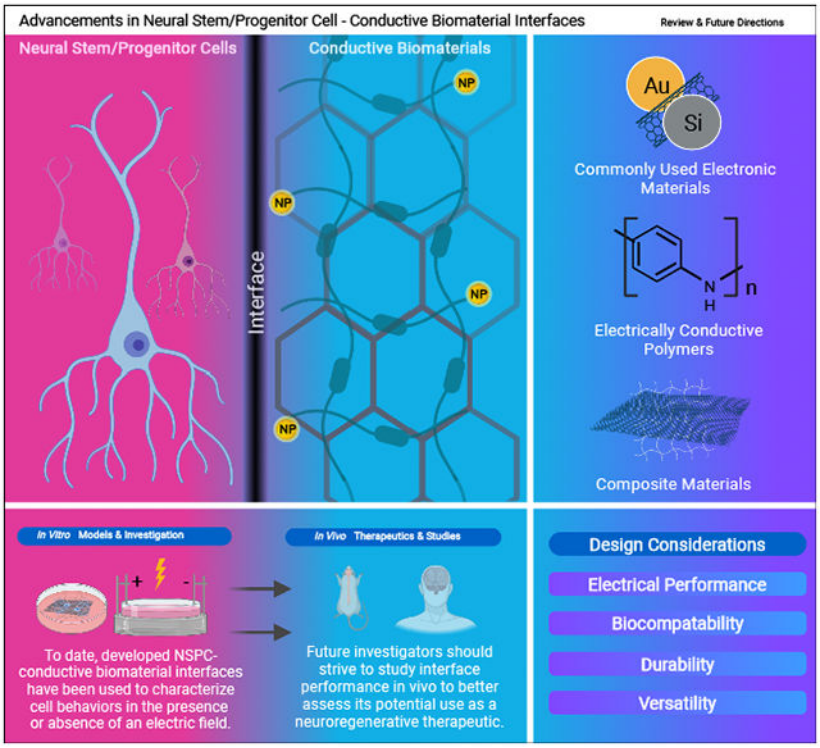

Keywords

Neural stem/progenitor cells; conductive biomaterials; neural engineering; central nervous system degeneration; regenerative medicine; cell-material interface

1. Introduction

Neural tissue engineering is an interdisciplinary field that integrates concepts from materials science, neuroscience, and engineering to develop platforms that can replace or regenerate neural tissues *in vivo* and serve as experimental models of neural tissue, including its pathological states, *ex vivo*.[1-3] Biomaterials can be interfaced with neural stem and progenitor cells (NS/PCs), either in a culture dish or in the body, to provide the raw materials and structure to generate new, functional tissues.[1] Central nervous system (CNS) tissues, consisting of the brain and spinal cord, have been particularly challenging to engineer, at least in part because of our limited understanding of the raw materials needed for their generation and what goes awry in various pathologies.[4] Development of CNS tissue models that incorporate NS/PCs and adopt the biological activities of native tissues promises to improve our understanding of NS/PC physiology and lead to effective, regenerative therapies.

Electrical activity is a defining property of neural tissues that can be leveraged to promote regeneration and improve fidelity of tissue models *ex vivo*. Use of conductive materials is a popular approach to incorporate electrical activity into tissue-engineered constructs.[5-7] This review describes previous work that has evaluated how well NS/PCs interface with

conventional electronic materials, and more recent attempts to improve the biocompatibility by integrating biologically active components, which can directly interact with cells, into conductive materials. As few studies have evaluated the potential of conductive materials to promote NS/PC-mediated regeneration in animal models or clinical settings, the majority of work described in this review was performed *in vitro*. With continued technological advances in biomaterials and knowledge gains in NS/PC biology, we expect conductive biomaterials to provide unique and valuable opportunities for improving NS/PC functions and guiding NS/PC behavior through the addition of electrical stimulation. These advances are expected to benefit the development of cell-based therapies, for which conductive biomaterials can be used as tools for *in vitro* biomanufacturing or *in vivo* scaffolds that can direct the differentiation of transplanted cells, and *ex vivo* models of human pathologies. Beyond these applications, availability of conductive biomaterials with tunable biological functions will likely benefit development of therapeutics for electrically active tissues other than the CNS, such as in the heart.

In the first part of this review, we discuss the biological contexts in which biomaterials are interfaced with NS/PCs, focusing on the many roles of electrical cues in the tissue microenvironment. The remainder of this manuscript comprehensively reviews scientific literature describing the development of conductive biomaterials for interfacing with NS/PCs. While the focus of this review is on interfacing human NS/PCs with conductive biomaterials, many foundational studies of conductive biomaterials for use in the CNS did not consider NS/PCs and, instead, demonstrated interfacing with cell lines or fetal rodent cells. Discussions of such studies are included here when results are relevant for NS/PC interfacing.

2. Neural Stem/Progenitor Cells

NS/PCs have the potential to differentiate into most of the mature cells of the CNS, including neurons,[8] ependymal cells,[9] astrocytes,[10] and oligodendrocytes.[11] While the terms neural stem cells (NSCs) and neural progenitor cells (NPCs) are often used interchangeably in the scientific literature, researchers generally agree that NSCs can continuously self-renew and are multipotent, while NPCs have a finite timeline for self-renewal and are often fated to a specific lineage.[12] NPCs are often classified as either progenitors (typically less mature) or precursors (typically more mature).[13] NPCs are typically restricted to glial (e.g., oligodendrocyte progenitor cells (OPCs) and astrocyte progenitor cells (APCs)) or neuronal (e.g., motor neuron progenitor cells and interneuron progenitor cells) fates. In this review, we use the term NS/PCs to encompass cells falling into any of these categories. In addition, herein we use the term “neural cell” to include any cell derived from neural tissues. In contrast, we use the term “neuronal cell” to refer to both immature and mature neurons.

In adults, well-characterized NS/PC populations can be found in distinct regions of the CNS, including the subventricular zone (SVZ) and subgranular zone of the dentate gyrus (SGZ) in the brain (Figure 1).[14] Several studies suggest similar populations of multipotent cells are resident within the adult mammalian spinal cord.[15-17] These regions represent niche microenvironments that provide unique signals to NS/PCs, acting to maintain quiescence

and initiate proliferation and differentiation as appropriate; for example, in response to tissue damage.[18] However, the native ability of the NS/PCs to repair damaged tissue is limited in adult humans,[6] necessitating tissue-engineered interventions to promote repair. Notably, while direct evidence of neurogenesis and gliogenesis has been found in many species, including non-human primates, which cells represent NS/PCs and the mechanisms by which they act in humans remain unclear.[19]

3. Applications of Interfacing NS/PCs with Conductive Materials

Conductive biomaterials have emerged as a promising tool to treat symptoms of neurodegenerative diseases and promote CNS regeneration.[20] Conductive scaffolds implanted into damaged regions of CNS tissues have the potential to amplify the effects of endogenous or applied electric fields (EFs) and improve tissue regeneration.[21] Electrical stimulation at the cellular level can occur from endogenous EFs or application of a current or voltage across paired electrodes to induce an exogenous EF, either of which then causes a change in transmembrane voltage and, in neurons, possible synaptic firing. Conductive scaffolds could be used to both facilitate and monitor synaptic engraftment of transplanted NS/PCs into the host circuitry. In the future, one can imagine development of a fully closed-loop system, where external stimulation is applied through a conductive biomaterial implant in response to some change in the endogenous EF or neuronal activity as detected by the same implant, that could be used over a patient's lifetime to address a chronic condition, such as epilepsy. Additionally, conductive scaffolds will be instrumental for advancing cell-based therapies, where they can be used for biomanufacturing and transplantation of NS/PC-based tissue grafts.[21,22] Conductive scaffolds may enable the development of *ex vivo* models of human disease that are highly physiologically relevant and can be used to better understand pathological mechanisms and develop new therapies.

Improved integration of conductive biomaterials with CNS tissues, through parameters including electrode geometry and mechanical properties, will be needed to increase the lifetime over which an implant is electrically viable.[23-25] For context, implants typically lose function within 5 years, depending on the particular device used.[24,26-30] One study of two individuals reported that implants lost 85% of function after 3 years.[26] However, more recently, microarrays of conductive nanofibers, smaller and more flexible than typical electrodes, have been reported to last at least 6 years in non-human primates.[23,25]

Conductive scaffolds can promote NS/PC differentiation and neurite outgrowth even in the absence of an externally applied EF, indicating that scaffolds can help coordinate communication through electrical synapses.[21,22,31] We can start to understand this effect by considering the conductivity of the matrix, cells, and fluids that make up the native CNS. The conductivity of cerebrospinal fluid (CSF) has been measured at around 0.017 S cm^{-1},[32] which is comparable to that of 0.9% saline.[33] Conductivity measurements for CNS tissues (matrix and cells) range from 0.002 – 0.007 S cm^{-1}; however, individual studies consistently report lower conductivities for white matter than for gray matter.[32,33] Applied current will take the path of least resistance, passing through the CSF *in vivo* or the culture medium *in vitro*. Thus, a biomaterial with greater conductivity than CSF or medium can relay applied current directly to adhered cells, which may more efficiently translate into

neural cells.[34] Biocompatible scaffolds with higher conductivities than CSF may enable researchers to directly probe the effects of EFs on NS/PCs and translate these findings to inform therapeutic applications.

3.1. *In Vivo* Applications

Acellular conductive scaffolds can be designed to modulate the *in vivo* microenvironment and promote infiltration of host cells into implants.[35] Ideally, the scaffold microenvironment will induce host cells to adopt functional phenotypes and organize into new tissues. Provision of electrical cues in such scaffolds has the potential to improve maturation of endogenous NS/PCs and their assembly into functional neuronal networks.[36] Likewise, externally applied EFs have the potential to direct migration of transplanted and endogenous NS/PCs *in vivo* as a therapeutic strategy after CNS injury.[37,38] For example, an EF may be used to guide endogenous NS/PCs into an implanted scaffold, which would then provide a conductive substrate for cell adhesion,[39] survival,[6] and directed differentiation.[40] Similarly, conductive scaffolds could provide directional cues for regenerating neurites,[41] potentially guiding new axons to re-create functional synaptic connections lost to injury or disease. Finally, the combined effects of such electrical and topographical guidance cues may affect directed axonal outgrowth in a synergistic manner.[42,43]

NS/PC transplantation is an attractive strategy for regenerating CNS tissues. Despite widespread efforts to translate this approach into clinical practice, which have been reviewed previously in a number of articles previously including Zhu, et al. (2018)[44] and Kourgiantaki, et al. (2020),[45] historically transplanted NS/PCs have displayed low survival rates, inefficient differentiation, and poor host engraftment. Together, these limitations have precluded any strategies evaluated to date from transitioning to Phase III clinical trials. Biomaterial carriers can protect NS/PCs during and after transplantation, likely by providing lubrication during injection, a cell-adhesive substrate, and some shielding from local inflammation.[46-53] There is evidence that transplantation within a conductive scaffold can further improve survival rates[54,55] and enhance maturation,[6,40] although the mechanisms behind these effects remain unclear. Ideally, external EF application to NS/PCs transplanted in conductive biomaterial carriers could guide transplant- and host-derived neurons to create new, functional connections.

3.2. *In Vitro* Applications

In general, survival of cultured, mature CNS cells, which typically have extensive and delicate processes necessary for their functions, is compromised by passaging, which involves detaching extensive and delicate cellular processes from a substrate. Furthermore, these cells must survive transplantation and be capable of regenerating these processes *in vivo*. On the other hand, less mature cells can differentiate *in vivo* and create new processes that interface with host circuitry. However, these cells tend to differentiate into undesirable cell types, such as astrocytes, and may cause allodynia.[56] One strategy for overcoming these limitations is to develop a biomaterial scaffold that supports expansion and directed differentiation of NS/PCs *in vitro* and subsequent implantation of adhered mature cells into the CNS. This approach would make it possible to biomanufacture defined neuronal circuits that could be implanted and integrated into compromised CNS tissues to

restore lost functions. Electrical stimulation may provide an additional control mechanism for biomanufacturing NS/PC therapies to complement, amplify, and/or fine-tune existing methods. Furthermore, conductive scaffolds could be used to monitor their spontaneous electrical activity in cultured NS/PCs, which may provide an important quality control measure of cell phenotype prior to transplantation.

Conductive biomaterials also can be used as *ex vivo* models of healthy and diseased CNS tissues, enabling study of these tissues in a controlled environment outside of a complete organism (e.g., “tissue chips”). These models will provide new insights into fundamental processes in cell- and tissue-level physiology as well as a platform for evaluating the therapeutic potential of various pharmacological agents. Real-time monitoring of local electrical activity of cells within a conductive scaffold can show how implanted cells evolve with tissue development, disease progression, or in response to a particular drug treatment.[57,58] In a recent review by Khan, et al. (2019), various types of conductive biomaterials are described that provide accurate stimulation and monitoring of cultured cells, demonstrating the utility of conductive biomaterials for understanding the electrical dynamics of neural tissues.[58]

4. Neural Stem/Progenitor Cell Microenvironment

In order to direct NS/PC differentiation using engineered biomaterials, we must consider how specific, endogenous microenvironmental cues dictate NS/PC fate during developmental and regenerative processes. In the adult CNS, these specialized microenvironments act to maintain a pool of NS/PCs that may be utilized for tissue repair. In this section, we briefly summarize influential features of the NS/PC microenvironment, including cell-cell contacts (Figure 1, Figure 2), cell-secreted soluble factors, extracellular matrix (ECM) (Figure 3), and endogenous EFs. While cellular and physiological mechanisms governing NS/PC maintenance and differentiation are not yet fully understood, these microenvironmental features are hypothesized to act together to drive development and support tissue function.[59,60] Biomaterials have been developed to mimic these microenvironmental features, enabling researchers to leverage the same biological pathways used in native tissues to control neural cell behavior.

4.1. Cell-Cell Contacts

Cell-cell contacts are fundamental to dictating NS/PC behavior during CNS development (Figure 2), as recently reviewed by Agustín-Durán et al. (2021).[62] For example, direct interactions among cultured NS/PCs have been found to upregulate neurotropic factor production.[63] Gap junctions among NS/PCs themselves are particularly important, as they conduct ionic currents between coupled cells that can generate EFs across CNS tissues,[64] and are thought to lay the groundwork for subsequent development of chemical synapses between mature neurons.[64,65] Formation of gap junctions between transplanted NS/PCs and host cells in the CNS, including mature neurons,[66] astrocytes,[67] and microglia,[68] may be necessary to maintain stem cell niches and repair damaged tissues.

Several other transmembrane proteins help regulate NS/PC proliferation, differentiation, and migration through cell-cell contacts. Adherens junctions, composed of cadherin proteins,

provide mechanical connections among NS/PCs and neighboring cells that influence proliferation and maturation.[69] For example, N-cadherins are critical in radial glial cell-mediated migration of NS/PCs during rodent neurogenesis.[70] NS/PCs also interact with surrounding cells via ephrin ligands and Eph receptors that mediate NS/PC migration, proliferation, and neurogenesis.[71,72] Interactions of the transmembrane protein Notch and its corresponding receptors (e.g., jagged[73]) maintain self-renewal and multipotency of NS/PCs.[74] Differentiating NS/PCs express ligands that interact with Notch on neighboring, immature NS/PCs to maintain stemness in a process known as “lateral inhibition” (Figure 2).[75]

4.2. Secreted Soluble Factors

NS/PC behaviors are regulated by a number of cell-secreted, soluble factors in their microenvironment, including growth factors and cytokines (Figure 3). These factors may be incorporated into implanted scaffolds for added control of NS/PC fate.[76,77] While we will briefly discuss the role of these factors in NS/PC function, please see Ruddy and Morshead 2018[78] for a more comprehensive review. Morphogens, of which bone morphogenic proteins (BMPs), Wnt and sonic hedgehog (SHH) are among the best characterized, regulate NS/PC fate in both embryonic development and adult niches. BMPs play a variety of roles across all stages of CNS development, including fate specification during embryonic development[79] and regulation of proliferation and neurogenesis in adult NS/PC niches.[80] The Wnt family comprises several proteins produced by NS/PCs and astrocytes that are key to NS/PC maintenance, proliferation and fate specification throughout the developmental timeline and in adult niches.[81] SHH is best characterized for its role in embryonic development when spatial concentration gradients along the floor plate and notochord dictate NS/PC fates.[82] Many other soluble growth factors, including fibroblast growth factor-2 (FGF-2) and epithelial growth factor (EGF), have been identified as regulators of NS/PC survival, maintenance and proliferation.[83] Cytokines, typically secreted by astrocytes and microglia, are important in both developing CNS and adult NS/PC niches.[84,85] In particular, interleukin-1β (IL-1β) and interleukin-6 (IL-6) drive neurogenesis, while tumor necrosis factor-α (TNFα) and interferon-γ (IFNγ) drive oligodendrogenesis.[86]

A growing body of evidence suggests that the benefits of NS/PC transplants are at least in part attributable to the effects of NS/PC-produced extracellular vesicles.[87,88] Extracellular vesicles, which include exosomes and matrix-bound vesicles, carry material typically considered to be intracellular, including miRNA, DNA, proteins, and even lipids, and have crucial roles in synaptic function, neurodevelopment, and neuroimmunity (Figure 3).[89] EFs can affect extracellular vesicles; for example, EF strength can modulate extracellular vesicle release by cultured rat astrocytes.[90]

4.3. Extracellular Matrix

The CNS ECM is an intricate, three-dimensional (3D) mesh of fibrillar proteins, proteoglycans, and glycosaminoglycans bathed in electroactive CSF that can regulate NS/PC survival, quiescence, proliferation, migration and fate specification (Figure 3).[91,92] The ECM evolves as the CNS develops, resulting in distinct ECM compositions in niche microenvironments of mature tissues. A recent study characterized the ECM proteome

unique to neurogenic niches (SVZ and olfactory bulb in mice) and demonstrated that astrocytes, neurons, and NS/PCs each produced abundant ECM.[93] Laminin binding to integrin β1 receptors, perhaps the most well-characterized of NS/PC-ECM interactions to date, contributes to proliferation during embryonic development and quiescence within the adult SVZ niche.[91,94] Sulfated proteoglycans can directly interact with and sequester soluble factors to indirectly amplify the effects of morphogenic signals.[95] In particular, heparan sulfates readily sequester cationic species, including many growth factors, through ionic interactions that then act on NS/PCs.[96]

From another vantage point, the ECM is a heterogeneous, 3D scaffolding to which NS/PCs adhere. Cells respond to physical cues in their microenvironment, including geometric architecture, mechanical properties, and nano-to-microscale topographical features.[91,97] In contrast to a two-dimensional (2D) setting, 3D cell culture in defined microenvironments offers key advantages, including, but not limited to, improving preservation of *in vivo* morphologies,[98,99] increasing densities of cell-cell contacts,[100] higher rates of proliferation,[101] and greater maturation.[102] Several studies have found that softer matrices, typically <500 Pa shear elastic modulus, promote NS/PC neurogenesis and neurite extension, while stiffer matrices bias differentiation towards gliogenesis.[103-105] In addition, the geometrical landscape of the microenvironment, which includes porosity/tortuosity, defined ridges, and surface roughness, affects cell behavior. Nanoscale and microscale geometrical features affect cell adhesion, morphology, migration and maturation.[106,107] Topography can also mediate contact guidance, a process by which neurites or axons follow a defined feature like a groove, channel or fiber.[108-110] The combined effects of electrical and topographical guidance cues may have synergistic effects on directed axonal outgrowth.[111] Topography in 3D scaffolds is often embodied by the pore structure, in which cells are confined. Spatial confinement, as experienced by cells within 3D, porous biomaterials, can affect several cell types involved in wound healing, including stem cells.[112] In 3D matrices, porosity also affects diffusion of nutrients, oxygen, paracrine signals and waste products.

5. Effects of Electrical Cues on Neural Stem/Progenitor Cells

While the majority of studies of the NS/PC microenvironment have focused on the contributions of cell-cell interactions, soluble factors, and ECM to NS/PC behavior, a number of studies have suggested that electrical signals are likely equally influential.[113-116] Tissues produce endogenous EFs that guide development, wound healing, and regeneration.[117] In the brain, typical endogenous EFs have been measured with extracellular voltages less than 0.5 mV and field strengths less than 5 mV mm^{-1}.[118,119] Endogenous and applied EFs affect multiple NS/PC behaviors, including proliferation,[22] differentiation,[22] and directed migration.[59] EFs also can affect differentiated cells in the CNS microenvironment in ways that can influence NS/PC differentiation and proliferation. For example, astrocytes align with an externally applied EF and adopt a morphology conducive to guiding neurite outgrowth.[120] Microglia are also sensitive to EFs, where stimulation has been reported to induce inflammatory activation.[121]

While the following sections will discuss studies evaluating effects of applied EFs on NS/PCs, we have not found any cases where the experimental design exactly matched

EF strengths to reported endogenous EF strengths.[118,119] In practice, effective levels of electrical stimulation are not universal and a unique stimulation threshold must be determined empirically for each individual animal or cell culture subject evaluated.[122] However, there are still upper limits to the levels at which electrical stimulation can be safely applied to biological organisms, as discussed in more detail in the Section 6.1.

As discussed in Section 4.1, electrical connections (e.g., gap junctions) among NS/PCs in the developing embryo are thought to pattern chemical synapse development among newly differentiated neurons.[64,65] Applied direct current (DC), alternating current (AC), and biphasic stimulation have each been reported to promote proliferation and differentiation of NS/PCs.[123,124] While the majority of previous studies used DC stimulation, Lim, et al. (2013) reported that NS/PC differentiation into both neurons and glia increased with increasing frequency of applied AC stimulation.[125] While much remains unknown about how applied EFs affect NS/PCs, researchers are actively working to understand the governing principles in an effort to translate these findings into effective therapies.

5.1. NS/PC Responses to Electrical Signals

Cells regulate electrochemical gradients and produce transient electrical signals through ion channels, pumps, and exchangers embedded in the plasma membrane.[126-128] Ion channels, activated by changes in membrane potential, binding of specific ligands, or mechanical stretch, are heterogeneously expressed by NS/PCs in the developing CNS and complement neurotransmitter-based signaling.[129] If the current regulated by these transmembrane proteins reaches a threshold value for a particular neuron, an action potential is generated and synaptic transmission is initiated. However, even transmembrane currents below this threshold, such as those generated across two cells via gap junctions, can be measured, at least *in vitro*, using a dual electrode setup.[130-131] In general, adult NS/PCs are more hyperpolarized than embryonic NS/PCs[129] and, unlike mature neurons, neither generates action potentials in response to injected depolarizing currents.[132,133] However, subpopulations of adult, neonatal, and embryonic NS/PCs in culture do display small inward Na^+ currents, indicative of a potential role for Na^+ channels in the NS/PC response to EF.[133-135]

Evidence suggests that ion pumps and exchangers, including Na^+/K^+-ATPase pumps and Na^+/H^+ exchangers, help establish EFs *in vivo* to drive galvanotaxis, the process of EF-induced cell migration (Figure 4).[128,136] Induction of Ca^{2+} influx by neurotransmitter binding to ionotropic receptors, in particular N-methyl-D-aspartate (NMDA) has been implicated in NS/PC galvanotaxis.[137] Glutamatergic, GABAergic, and cholinergic receptors may also contribute to NS/PC differentiation and migration.[138,139]

5.2. Mechanisms of Galvanotaxis

Galvanotaxis is essential during development[140,141] and regeneration of the CNS.[142] For example, endogenous EFs produced by polarized distribution of ion pumps on cell membranes are thought to drive NS/PC migration from the SVZ to the olfactory bulb, a well-characterized example of adult neurogenesis in rodents.[143] NPCs are thought to undergo galvanotaxis towards the cathode,[123,144] while differentiated neurons do not

migrate in response to EF.[136,137] In contrast, OPCs[145] and astrocytes[146] undergo galvanotaxis towards the anode. The phenotypically distinct, biophysical properties of the cell membrane appear to be a key determinant of a cell's response to an EF; for example, DC dielectrophoresis has been used to efficiently sort NS/PC populations into neurogenic and astrocytic progenitors based on their membrane capacitance.[147,148] While the direction of galvanotaxis appears to be cell-type dependent, other features of the microenvironment including ECM adhesion, may affect directional migration.[146,149-151] For example, Ahmed, et al. (2020) found that human NS/PCs seeded onto Matrigel migrated cathodally while those seeded on fibronectin migrated anodally.[149]

EF-induced polarization of membrane-bound receptors, including ion channels, integrins, receptor tyrosine kinases (RTKs), and their immediate downstream binding partners, including key signaling proteins in the Rho/Rac, PI3K/AKT, and MAPK/ERK pathways, are widely thought to drive cell responses (Figure 4).[152-154] Pharmacological inhibition of EGF Receptor (EGFR),[37] ROCK,[144] or PI3K[153] has confirmed that each is required for NS/PC galvanotaxis. As an example of how NS/PC galvanotaxis may occur, Li, et al. (2008) showed that NMDA receptors aggregated with intracellular Tiam1 in response to EF (250 mV mm^{-1} for 60 minutes), leading to activation of downstream Rac1 and PAK1, which have broad effects on cell migration, proliferation, and fate.[137]

Most likely, multiple molecules must polarize in response to an EF to induce changes in cell behavior. For example, in Rajnicek, et al. (2006) embryonic spinal neurons under an applied EF, Rac/Cdc42 complexes polarize towards the cathode, driving growth cone extension, while Rho polarizes towards the anode, inducing local cytoskeletal collapse.[155] Together these actions mediate net movement of the cell towards the cathode. In another example, PI3K facilitates galvanotaxis of NS/PCs, which is effectively concentrated at the leading edge.[153] However, PI3K does not respond directly to an applied EF. Instead, PTEN polarizes to the trailing edge of the cell where it locally inhibits PI3K.[156,157] While scientists have begun to get a handle on the mechanisms dictating galvanotaxis, these underlying EF-induced proliferation and differentiation remain less understood. For more detailed discussions of the cellular mechanisms driving EF effects, please refer to Thrivikraman, et al. (2018)[158] and Chen, et al. (2019).[113]

6. Biomaterial Design Considerations

The remainder of this review discusses conductive materials for neural cell interfacing within the context of key design considerations: *electrical performance*, *biocompatibility*, *durability* and *versatility*. General criteria for each of these design parameters are defined in this section. As a single conductive material is unlikely to achieve optimal values of each of these design parameters, compromises must be made. Perhaps the most difficult challenge arises from the tradeoff between electrical performance and mechanical properties, where conventional electronic materials (e.g., metals) are substantially stiffer than neural tissue, [159] and thus elicit an increased inflammatory response and poor tissue integration when implanted.[160] In Section 9, we detail recent efforts to overcome this challenge by developing new, composite biomaterials that combine those known to be compatible with

neural tissues (e.g., soft hydrogels) with those more conventionally used as electrodes (e.g., metals and carbon-based materials) (Figure 5).

6.1. Electrical Performance

Electrical performance describes how efficiently a material transfers electrical stimulation to the intended target, which in the context of this review is living cells. Optimal electrical performance parameters vary depending on the intended application. Currently, no measures of electrical performance for cell interfacing have been established as the standard in scientific literature. Instead, published reports typically provide one or two of the following measurements: conductance, conductivity, resistance, and/or impedance.

Conductivity is an intrinsic property of a material that describes how well it transfers current. In contrast, conductance describes the amount of measured current resulting from an applied electrical potential difference, and thus is dependent on the cross-sectional area and length of the specific material being tested. Resistivity and resistance are the reciprocals of conductivity and conductance, respectively. In order for current to be routed through a conductive material, the material must have a greater conductivity than surrounding media. For a conductive biomaterial implanted into the CNS, this means that a conductivity greater or equal to that of CSF (0.017 S cm^{-1}) would be required.[32] Table 1 provides a reference for conductivity values of commonly used conventional electronic materials, carbon-based materials, and biomaterials.

No standard set of measurements or methods by which to characterize a material's electrical properties is consistently used in scientific reports, making direct comparisons of different materials challenging across studies. Furthermore, typically experimental measurements are acquired under ideal conditions, and thus may overestimate electrical performance in practice.[161] Alternatively, electrical performance can be assessed indirectly by observing how application of EFs through a material affects interfaced cells or tissue. Examples of such indirect measures include evidence of evoked action potentials (e.g., neurotransmitter release), increasing current flow through gap junctions, and changes in cell behavior (e.g., increased alignment with the scaffold, neurite extension, galvanotaxis or directed cell differentiation).

Important considerations for electrical performance of a material include the kinetic processes and types of reactions that occur at the material-tissue interface. When an electrode is implanted into a physiological microenvironment, its surface accumulates a double layer of ionic species.[162] When voltage is applied across a material-tissue interface, the resulting EF adjusts to an equilibrium potential where electrical drift within the material balances chemical diffusion of ionic species in the tissue. Thus, the maximum current generated depends on the bulk transport of ions through the tissue.

Furthermore, charge transfer from material to tissues often results in oxidation and reduction reactions, resulting in a faradaic current.[163] An ideal material for biological applications would maintain the equilibrium potential of the electrical double layer when a voltage is applied, ensuring that resulting oxidation and reduction reactions are readily reversible and do not substantially alter the chemical environment of the tissue.[164] However,

when voltage is increased to the charge injection limit of a particular electrode material, irreversible reactions occur that deposit reaction by-products into an interfaced biological microenvironment. These irreversible reactions are generally undesirable, as they may damage interfaced cells and tissues, induce inflammation, and impair electrode function.[165] Thus, applied voltages are typically maintained within the so-called “water window” (~−0.6 V to 0.8 V), which has been established as a safe range for use in tissues to avoid hydrolysis and irreversible electrochemical reactions.[164,166]

6.2. Biocompatibility

The term biocompatibility is used in this review to describe both a material’s ability to support cell survival and function (e.g., cytocompatibility) and how well the body tolerates the material once implanted. One of the most influential events affecting material biocompatibility is protein adsorption, as these proteins determine the response of interacting immune cells. An applied EF can dramatically affect protein adsorption, so much so that EFs have been used to control adsorption patterns.[167-169] Protein adsorption and material biocompatibility are heavily influenced by the chemistry, porosity, and stiffness of conductive biomaterials.[31,170-173]

Chemistry, porosity, and stiffness also determine cell-material interactions. For example, size scale and interconnectivity of pores within biomaterial scaffolds are key to their ability to integrate functionally with host tissues.[171,174] The size and geometry of the biomaterial and its features (e.g., pores, surface roughness, etc.) influence virtually all cell behaviors, including inflammatory cell activation[175] and NS/PC differentiation.[176] For example, biomaterial implants with an interconnected network of uniform pores on the order of tens of microns, which are large enough to permit host cell infiltration yet small enough to still provide some guidance via confinement, promote formation of a seamless tissue interface with minimal scarring in the rodent spinal cord.[177] Alternatively, single axons have been reported to align well with features on the order of 1 μm[178,179] and bundles of spinal cord axons regenerate robustly through guidance tubes on the order of 100’s of μm.[180-182]

Mechanical mismatch between the material and surrounding tissues can severely compromise long-term biocompatibility and has been a particularly difficult challenge when developing implantable electronic devices, given that highly conductive materials are typically relatively hard.[183] Mechanical mismatch has been linked to tissue damage and subsequent electrode failure.[184] While CNS tissues exhibit shear elastic moduli in the range of 50-1000 Pa, depending on the specific region measured, conventional implanted electrodes typically exceed this range and are stiffer by orders of magnitude.[23,25,185-189]

Degradation of implanted materials *in situ* is often considered an advantage, offering increased biocompatibility and potential to be remodeled by the host into new, functional tissues. However, by-products of biomaterial degradation can elicit an immune response.[190] The by-products may also be too large for renal clearance from the body (> 6 nm, proteins 20-60 kDa), leaving them to accumulate in circulation and tissues. However, degradation into, or release of, in the case of nanoparticle composite materials, nano-sized, non-biological by-products may cause significant toxicity.[191] The inflammatory response can also influence degradation, where a strong response lowers local tissue pH to accelerate the

breakdown of many common biomaterial implants.[192] Meanwhile, corrosion of metallic implants can also influence the local pH.[193] Unfortunately, predicting degradation kinetics of a biomaterial when implanted *in vivo* is not straightforward, as it is not currently possible to adequately recapitulate the ionic and enzymatic microenvironment of an implant site *ex vivo*.[170]

6.3. Durability

The term durability is used in this review to describe electrical performance of a material over time and is heavily influenced by biocompatibility. Mechanical integrity of conductive biomaterials, which is highly affected by the degradation rate of the material, is often the limiting factor of their functional lifetime.[193] Another important contributor to durability is the inflammatory response, which can essentially "wall off" the implant through protein adsorption, adhesion of phagocytic cells, protein deposition, disrupting the material-tissue interface, and compromising electrode performance.[194,195] At some point, the applied EF required for therapeutic effect exceeds safety limits and the device fails — a phenomenon that has been well-documented with clinical use of electrodes for deep brain stimulation in Parkinson's disease patients.[196]

6.4. Versatility

The term versatility is used in this review to describe how easily a material can be adapted or modified for a wide range of biomedical applications. Modular control over microenvironmental cues, as discussed in Section 4, and other features, such as controlled delivery of therapeutic factors incorporated into the biomaterial, can provide a means to precisely direct cell behavior. Versatility also includes the potential for a material to be fabricated with structures beyond flat electrodes (e.g., 3D scaffolds for cells) and dynamically change shape as needed (e.g., during injection into the CNS).

The following sections describe materials commonly used and in development for neural interfacing with regard to these parameters. In particular, we highlight recent works in the emerging area of interfacing NS/PCs with conductive materials. The majority of studies using more conventional electronic materials have investigated interfacing with mature neural tissues or cancer-derived cell lines (e.g., PC12 cells), rather than NS/PCs, and thus we summarize concepts about the suitability for these materials for use in the CNS.

7. Commonly Used Electronic Materials

A number of materials, including metals, silicon, carbon-based structures, and insulating polymers, have long been used for interfacing with neural cells *in vitro* and *in vivo* due to their ease of use and good electrical properties.[197-199] However, ideal electrical performance is often at the expense of biocompatibility and durability, depending on material composition and geometry.[200] Neural cells do not readily adhere to conventional electronic materials in the absence of modification.[201] Thus, these materials typically are modified on their surface or integrated with more conventional biomaterials to improve biocompatibility. While the latter approach is the focus of Section 9, Section 7 first provides an overview of scientific literature describing the interactions of NS/PCs with conventional

and carbon-based electronic materials. Table 2 provides summarizes of studies referenced in Sections 7 and 8. While much progress has been made in developing electrodes based on conventional electronic materials for interfacing with intact brain tissues, relatively few studies have focused on interfacing these materials with NS/PCs.[200] Thus, this section will discuss studies investigating material interfaces with living brain tissue and non-NS/PC neural cells, insofar as results indicate their suitability for use with NS/PCs, in this section.

Several design criteria must be considered when applying conventional and carbon-based electronic materials as implants for electrical stimulation and/or recording of neural cells, including electrode size, impedance, thermal noise content and durability.[202] Smaller electrodes correspond to better spatial resolution, as they can be targeted to more specific biological areas and/or be incorporated into larger arrays to characterize activity within a defined spatial area. Evidence suggests that reducing the size of conductive elements can provide better short-term biocompatibility.[203] However, the smaller the electrode, the higher the impedance and thus higher thermal noise, which can compromise current transfer at the material-tissue interface.[202] Furthermore, degradation rates, and thus durability, of conducting and insulating components also depend on current density at the electrode surface.[202] Since a smaller electrode will need to pass a larger current to achieve the same level of efficacy as a larger electrode, a short-term increase in biocompatibility due to smaller size might come at the expense of long-term durability.[204] Even when overcome in the short-term, often long-term use *in vivo* remains compromised by chronic inflammation and implant encapsulation.[205]

7.1. Metals

Conventionally, conductive biomaterials have been metallic, for example composed of silver (6.3×10^5 S cm^{-1}),[159] gold (4.1×10^5 S cm^{-1}),[159] platinum (9.4×10^4 S cm^{-1}),[159] iridium (2.1×10^5 S cm^{-1}),[206] and/or palladium (1.0×10^5 S cm^{-1}) (Table 1).[206] Electrodes used clinically for deep brain stimulation therapy in Parkinson's disease patients have been made of materials including nickel-cobalt[207] or platinum-iridium[208] alloys. However, impedance, and therefore stimulation efficacy, decrease with long-term implantation, likely due to fibrotic encapsulation and loss of tissue contacts.[209,210] Metallic microwires (on the order of 10-200 μm in diameter) incorporated into compact microarrays have been used to record neuronal activity in the cortex in awake animals, providing decent spatial and temporal resolution and the flexibility to be interfaced with various anatomical features.[211,212] Several small-scale clinical studies have demonstrated the use of microwire arrays in humans,[213-215] for example as brain-machine interfaces to provide patient control of prosthetic limbs.[216]

Despite these remarkable strides, long-term biocompatibility and durability of implanted electrodes still present significant obstacles.[194] Loss of electrical performance in CNS tissues is often observed after encapsulation of the device by dense scar tissue consisting of adsorbed proteins, immunoreactive cells, and newly secreted ECM.[217,218] The reactive scar, or fibrous encapsulation, essentially walls off the device from interfacing directly with the tissue of interest, creating a physical barrier, such that recording quality is affected and higher levels of electrical stimulation are required to elicit the same therapeutic

response.[164,218,219] In addition, adsorbed proteins can either slow or accelerate corrosion of metal electrodes, depending on the metal used and the specific proteins adsorbed.[220] Corrosion and applied electrical stimulation near the charge injection limit cause the release of potentially cytotoxic chemical species, thereby compromising biocompatibility and durability.[163] Several electrode characteristics, including size, probe shape, cross-sectional area, and surface roughness, have been adapted to create better control over the charge injection limit to reduce irreversible reactions.[165]

Some effects of fibrous encapsulation may be mitigated by strengthening the interface between metal electrodes and the neurons with which they form electrical connections. Mechanical mismatch between stiff metal electrodes and soft tissues of the CNS has been identified one cause of excessive inflammation and electrode failure.[221] Beyond matching tissue mechanical properties, strategies like increasing the nanoscale roughness of metal electrode surfaces[222-224] and functionalizing the surface to conjugate ECM-derived adhesive sites[225,226] can improve the tissue-biomaterial interface by promoting adhesion of neurons while discouraging adhesion of astrocytes.

Beyond improving cell adhesion, nanostructures increase the electrode surface area to increase the charge injection limit. For example, a nanostructured platinum coating for platinum/iridium microwires can improve biocompatibility and durability, even under high charge injection stimulation *in vivo* in mice.[222] However, it has been reported that nanostructures increase adhesion and activation of glia, which may increase the inflammatory response.[227] Activation of cultured astrocytes in response to electrical stimulation has been reported to depend on the mode of simulation (e.g., anodic vs cathodic, monophasic vs biphasic).[228,229] Astrocytes are an attractive target for direct electrical stimulation due to their extensive connections with neurons via gap junctions and role regulating synaptic transmission[229,230]. For example, electrical stimulation of astrocytes has been reported to trigger release of leukemia inhibitory factor (LIF), a paracrine signal for oligodendrocytes promoting myelination.[67] Furthermore, electrical stimulation of astrocytes has been suggested as a possible mechanism underlying the clinical benefits of deep-brain stimulation.[231] Overall, while metals have excellent electrical performance and versatility to be integrated into many different designs for electrodes and cell interactions, their biocompatibility and durability still have much room for improvement, especially for use in long-term implants.

7.2. Silicon

Silicon has been used as a basis for several brain-interfacing electronics.[197,232,233] Unlike the conductive metals in the previous section, silicon offers unique versatility as a semiconductor with conductivity around 10 S cm^{-1} (Table 1). While silicon has a faster degradation rate than metals,[207] its degradation products exhibit negligible cytotoxicity *in vitro*.[235] Compared to platinum, silicon substrates have also been shown to reduce astrocyte proliferation.[236] *In vivo*, silicon-based electrodes have similar biocompatibility and durability to their metal counterparts, indicated by damage from mechanical mismatch with CNS tissues and induced inflammatory responses.[217,237] Working to overcome issues with inflammation and silicon device encapsulation, researchers have modified silicon

surfaces to provide nanostructure or neuron-adhesive sites.[238,239] For example, silicon surfaces modified with L1-CAM protein preferentially promoted adhesion of neurons when compared to astrocytes.[238] More recently, culture of mouse hippocampal NS/PCs on arrays of vertically aligned silicon nanowires was reported to increase both proliferation rates and differentiation towards neurons when compared to silicon wafer substrates.[240] Another group made electrode microarrays of vertically aligned, silicon-based nanowires which they interfaced with human fetal NSCs derived from brain tissue at gestational age 13 weeks.[241] The nanowires were used to pierce individual cells and deliver intracellular electrical stimulation for 10 min/day with ±10 mV biphasic electrical pulses at a frequency of 1 Hz. Stimulation increased activity of voltage-dependent ion channels and led to neuronal maturation. Overall, while silicon faces challenges with long-term durability and biocompatibility *in vivo*, its electrical performance and versatility to be fabricated in many forms still make silicon an attractive material for tissue interfacing.

7.3. Carbon-Based Materials

While the potential toxicity of carbon-based materials as biomedical implants,[242-244] including diamond, carbon nanotubes (CNTs), carbon nanofibers (CNFs), and various forms of graphene, are of concern, their tunable electrical properties and structural flexibility make them an attractive conductive material for interfacing with neural cells.[245] Carbon-based materials such as diamond, CNTs, CNFs, and graphene have conductivities of 1×10^{-2} – 1×10^{-15},[246] 1×10^{3} – 1×10^{7},[246] 1-5 $\times 10^{4}$,[247,248] and 2×10^{3} –1×10^{5} S cm^{-1},[246] respectively (Table 1). Diamond, acting as an insulator, has been used as a coating for conventional metal and silicon electrodes to increase charge injection capacity, effectively combating the gain in impedance that accompanies electrode miniaturization.[249] Such diamond-coated electrodes have been functionalized with cell adhesive macromolecules, such as laminin, to improve neuronal attachment.[201,250] Alternatively, CNTs, CNFs, and graphene are good electrical conductors and each is under extensive investigation for neural tissue interfacing. For an in-depth review of carbon-based material interactions with neural cells please refer to Rauti, et al. (2019).[246]

CNTs are composed of submicron-diameter, carbon-lattice cylinders with low chemical reactivity, high electrical conductivity, high mechanical strength, nanoscale topography, and the potential for surface functionalization.[251,252] They have shown promise as platforms for electrophysiology when fashioned into electrode films and arrays.[253-255] While a dose-dependent toxicity of CNTs is recognized,[243,244] researchers have reported good biocompatibility at low doses both *in vitro*[251] and *in vivo*.[244] CNTs are amenable to chemical modification, typically after surface oxidation.[256] Finally, CNTs can be incorporated into other materials, such as poly(lactic-co-glycolic acid) (PLGA) or poly-ε-caprolactone (PCL), to create nanofibrous scaffolds that can improve cell and neurite guidance along fibers.[257-259]

While CNTs are hollow, CNFs have a stacked planar or conical structure with chemically active ends which is advantageous for their use as conductive elements in polymeric composites and enables their vertical assembly into microarrays, which have similar geometries to metallic microwire arrays.[260] Microarrays of vertically aligned CNFs grown

with nanoscale precision directly onto prefabricated electronic circuits or silicon wafers have been fabricated with tunable topographical, electrical, biochemical and mechanical properties.[261-263] CNFs exhibit a Young's modulus comparable to silicon and significantly lower than CNTs, a property which may impart better biocompatibility when implanted into the brain.[264] Zhu, et al. (2018) described an alternative approach to generating fibrous, carbon-based scaffolds in which they thermally annealed electrospun mats of polyacrylonitrile.[22] This carbonization process resulted in 3D scaffolds with a graphite layer coating electrospun fibers. Culture on these scaffolds induced mouse NE-4C NS/PCs (ATCC) to differentiate into neurons. Applied electrical stimulation further enhanced neuronal differentiation.

Graphene is a single layer of carbon atoms arranged in a 2D hexagonal lattice with sp^2 hybridization. A number of available methods for processing graphene into nanoparticles provides tunability of mechanical, electrical and chemical properties. Nanoparticles of reduced graphene oxide appear to be the most biocompatible processed form, with a large surface area amenable to cell adhesion.[265,266] Moreover, a recent study demonstrated that fibronectin adsorbed to graphene-based materials adopts predictable conformations and control over the fate of interacting cells.[267] Reduced graphene oxide substrates support viable cultures of human NS/PCs, while promoting their differentiation and maturation.[268,269] When cultured in 3D graphene foams, mouse NS/PCs displayed increased proliferation and differentiation when compared to 2D controls.[7] Furthermore, seeded NS/PCs in this study responded to electrical stimulation through the graphene scaffold, rather than the surrounding culture media. More recently, 3D graphene foams were used to investigate the effects of material stiffness on NS/PCs isolated from mouse hippocampus (P1).[39] NS/PC viability was equivalent on 30 kPa and 64 kPa substrates. However, cell proliferation and astrocyte differentiation (through GFAP expression) increased for cultures on the stiffer substrate when compared to cultures on the softer substrate. In line with previous studies,[270-272] neuronal differentiation was upregulated in cultures on the softer substrate. The micron-scale porosity in graphene foams is a positive attribute for *in vivo* implantation, as this size scale of pores in other materials have been shown to support rapid cell and blood vessel infiltration, and thus effective integration with host spinal cord tissues.[273,274] Overall, versality in architecture and chemical presentation make graphene-based materials attractive candidates for neural tissue engineering and interfacing.

Biocompatibility of graphene-based implants has remained a controversial topic, with some studies reporting good biocompatibility[275,276] and others reporting cytotoxicity,[277,278] acute inflammatory responses,[277,278] and tissue fibrosis.[278,280] The varied outcomes have been linked to material differences such as surface chemistry, formulation, geometry, thickness, and degree of oxidation, each of which can dramatically affect cell-material interactions.[267] For example, when graphene is oxidized and/or functionalized to increase hydrophilicity and used as a substrate, rather than in a suspension of aggregates, biocompatibility and cytocompatibility improve significantly.[265,281-284] Studies have demonstrated that nanoparticles from graphene oxide sheets can be cleared from the bloodstream by urinary excretion without compromising kidney function and effectively degraded into nontoxic by-products.[285] Despite these promising results, some fraction of graphene-derived nanoparticles appear to accumulate in large organs, including the lungs

and liver, which may result in chronic inflammation.[276,285] Given the lack of clarity on biocompatibility, there is a strong need for more comprehensive studies to determine the chemical and physical forms of graphene most appropriate for interfacing with NS/PCs *in vitro* and in various CNS sites *in vivo*. For in-depth reviews of graphene-based materials, please reference Amani, et al. (2018)[286] and Zhang, et al. (2020).[287]

8. Electrically Conductive Polymers (CPs)

Electrically conductive polymers (CPs) are organic polymers with the inherent ability to conduct through conjugated π systems, where loosely bound electrons carrying current can move to unoccupied *p* orbitals. CPs have shown great promise as biomaterials for interfacing with NS/PCs and have been widely investigated as key components of therapeutics for peripheral nerve, spinal cord and brain regeneration.[1] The most common examples of CPs as biomaterials include poly(pyrrole) (PPy), polythiophene derivatives such as poly(3,4-ethylenedioxythiophene) (PEDOT), and polyaniline (PANI). Compared to metals, CPs and some carbon-based materials are relatively porous, providing a greater surface area for electronic exchange and thus better potential for miniaturization.[288] Generally, while unmodified CPs are not acutely cytotoxic, functionalization with biomolecules to modulate cell interactions can significantly improve the health of interfaced neuronal cells *in vitro*.[76] Topographical, biochemical, and electrical cues have been engineered into a single CP to create a relatively cytocompatible substrate that can direct neural cell behavior.[289-291] A number of strategies for additive manufacturing have been developed that have made CPs highly versatile, including photolithography, transfer-printing onto flexible substrates, direct writing, and electrospinning.[292]

Despite significant advancements in the development of neural interfacing CPs, their utility has remained limited due to their relatively poor electrical performance, when compared to metals and carbon-based electronics (Table 1), and their lack of biodegradability, and versatility in processing, when compared to more conventional biomaterials. Most CPs are not biodegradable unless blended with another polymer that is susceptible to hydrolytic or enzymatic degradation in the body.[293] However, polymer blends may have reduced conductivity and cytotoxic or inflammatory degradation products. Furthermore, synthesis of truly biodegradable CPs continues to present a challenge.[294] Similarly, while bioactive functionalization is needed for cytocompatibility, most functionalization strategies involve partial disruption of the CP's conjugated electronic structure and thus can reduce conductivity.[295]

8.1. Polypyrrole (PPy)

PPy is a biocompatible CP that exhibits good conductivity ($1 \times 10^{-12} – 1 \times 10^{3}$ S cm^{-1}) (Table 1), flexibility for altering surface chemistry, and *in vitro* biocompatibility with mammalian cells.[43,296,297] PPy is an effective material for coating electrodes and passing current to neural cells with low cytotoxicity.[298] In a pioneering work, Schmidt, et al. (1997) demonstrated that oxidized PPy can be used as a substrate to enhance rat PC12 cell differentiation, as evidenced by neurite growth in the presence of electrical stimulation.[43] This study laid groundwork for pursuing PPy as a conductive substrate for neural tissue

engineering. More recently, George, et al. (2017) reported that electrical stimulation of human NS/PCs through a PPy substrate increased their production of vascular endothelial growth factor-A (VEGF-A), which in turn improved the therapeutic effects of pre-stimulated NS/PCs transplants in a rodent stroke model.[55]

Over the past several decades, techniques developed with the goal of using PPy as an electroactive, neuronal substrate include chemical and topographical surface modifications and PPy-polymer blends to achieve specific, desirable properties. PPy is often doped, for example with alkyl benzensulfonates,[297,299] polystyrene sulfonate (PSS),[300] and tosylate.[300] Relatively small dopants have been found to better benefit conductivity without compromising yield strength of doped PPy films. This is likely because smaller dopants minimally disrupt the spacing between PPy chains, across which electrons must "jump" for conductance.[299] Less disruption between PPy chains also creates a more crystalline, stronger material. PPy doped with tosylate was reported to exhibit an order of magnitude better conductivity than PPy doped with PSS.[300] Electrical stimulation of human NS/PCs on PPy substrates doped with anionic dodecylbenzenesulfonate (DBS) has been reported to induce differentiation predominately towards neurons rather than glia, a desirable outcome for biomanufacturing cell-based therapies for neurodegeneration and avoiding implant-related scar formation.[297]

Beyond improving conductive properties, doping can also be used as a means to control how PPy materials interface with cells. Surface charge, roughness and hydrophobicity/hydrophilicity can be controlled through dopant selection.[300] Furthermore, PPy can be doped with biologically active molecules that can help mediate cell adhesion and interfacing. The net negative charge, relatively delocalized electronic structures, and bioactivities of sulfates that occur naturally in the ECM, like chondroitin sulfate and heparin sulfate, are attractive dopants to increase PPy conductivity without compromising biocompatibility.[301,302] Other ECM-related biomolecules, such as adhesive peptides from laminin, have been doped into PPy to control both conductivity and cell attachment.[303] For example, heparin, a polysaccharide side chain of ECM proteoglycans, was doped into PPy and used as a coating to increase the hydrophilicity of gold electrodes.[304] Biotin, a small biomolecule commonly used for its high affinity to the avidin enzyme, has also been doped into PPy to provide a facile way to incorporate bioactive molecules[305] or metal nanoparticles[306] to increase conductivity.

An alternative strategy for modifying PPy with bioactive molecules is through direct modification of the polymer backbone, including amines,[306] carboxyls,[307,308] and activated esters.[111] For example, RGD peptides conjugated to carboxyls on modified PPy substrates improve cell adhesion.[314] Modifications to the surface of PPy films have also been used to introduce bioactive molecules. The simplest method is adsorption to a PPy substrate.[310] To immobilize biomolecules onto PPy films in a defined orientation, Nickels and Schmidt (2013) identified an amino sequence with a unique affinity for PPy using phage display technology.[311] Gomez and Schmidt (2007) developed an azido-based, photochemical method for spatially patterning proteins, such as nerve growth factor (NGF), onto PPy substrates to enhance neurite extension.[42] Biodegradable forms of PPy have been developed using β-substituted pyrrole monomers that contain ionizable and/or hydrolyzable

side groups.[312] Pyrrole can also be fabricated as a co-polymer with thiophene[296] or other hydrolyzable biomaterials, such as PCL,[313] to impart biodegradability.

PPy can be micropatterned to provide topographical cues to interfaced cells. For example, PPy films micropatterned with 1–2 μm grooves using electron-beam lithography were found to speed up axon polarization and direct axon extension in rodent embryonic hippocampal neurons.[314] Similarly, nanofibers containing PPy have been used to provide contact guidance and electrical cues, which together were reported to enhance neurite extension better than either cue alone.[291,315] Conventionally, CPs have been made conventionally as thin, brittle films electrochemically deposited onto a secondary substrate, such as a polydimethylsiloxane (PDMS), which enables the films to be rolled into tubes for nerve guidance scaffolds.[316] In a recent study, tubular scaffolds of DBS-doped PPy promoted increased expression of neurotrophic factors from seeded human induced pluripotent stem cell (iPSC)-derived NPCs under an applied EF.[317] Despite this advantage over electrical stimulation through 2D films, cell viability was reduced on 3D scaffolds. More recently, tubular guidance scaffolds based on PPy have been fabricated with more complex geometries, such as single- and multi-channeled tubes, and shown to support sciatic nerve regeneration in rats.[318]

PPy-polymer blends have been developed to form scaffolds, including 3D foams and fibers, with more complex architectures. PPy-silk blends have been made into 2D and 3D scaffolds[319] that can be further modified with microscale, cell-instructive topographies.[320] PPy has been incorporated into electrospun polylactic acid (PLA) nanofibers by surface coating after fabrication[41] and as embedded nanoparticles[315] to impart conductivity. In general, PPy's hydrophilicity, relative to other CPs, may improve its ability to be blended with more conventional biomaterials.[319]

8.2. Polyaniline (PANI)

PANI-based materials often achieve conductivities on the order of $1\text{-}10^1$ S cm^{-1} (Table 1); however, around an order of magnitude greater conductivity has been achieved by doping electrospun fibers of homogenous PANI doped with sulfuric or hydrochloric acid.[321] PANI has been approached cautiously as a biomaterial due to concerns over potential toxicity from by-products of the manufacturing process, including low molecular-weight residues or fabrication by-products.[322] One report confirmed this concern, demonstrating that purified PANI had reduced cytotoxicity, when compared to PANI hydrochloride and unpurified PANI.[323] Adding to evidence of biocompatibility, nanoparticles from PANI, in most low and high molecular weight forms evaluated, were found to be cleared in mice with minimal observable effects on the kidney and liver; however, minor liver lesions were identified with administration of low molecular weight (~4,000 Da) PANI nanofibers at the highest dose evaluated.[324]

Due to its highly brittle nature, PANI must be blended with other polymers or materials as a dopant to enable processing into biomaterial scaffolds.[325] Blending can also facilitate biodegradation, biocompatibility, and moldability of PANI-doped materials. For example, peptide-conjugated PANI can be bound to nanoribbons to introduce such features while promoting adhesion, proliferation, and neurite outgrowth of rat neural cortical cells.[326]

Blending of PANI with PCL has resulted in biodegradable scaffolds with relatively high conductivity (4.2 S cm^{-1}).[327] Applied electrical stimulation to PC12 cells seeded onto this PANI/PCL scaffold increased cell proliferation and expression of neurotrophic factors; however, it is not clear how these measures compare to PC12 cells on standard culture substrates. Furthermore, PC12 cells derived from rat pheochromocytomas outside of the CNS are unlikely to behave as human NS/PCs.[328] Blended nanofibers of PANI and PCL have been fabricated with lower conductivities (0.08 S cm^{-1}), but supported cultures of human-derived, ReNcell-VM immortalized NSCs (EMD Millipore) as well as pure PCL nanofibers.[329] PANI is a promising NS/PC interface material due to its moderate conductivity and versatility for the development of composites; however, further investigation is needed to define the concentration and quantity that is safe for use *in vivo*.

8.3. Poly(3,4-ethylenedioxythiophene) (PEDOT)

Another CP commonly used for biomaterial applications is PEDOT, a polythiophene derivative ($3 – 5 \times 10^2$) typically doped with PSS to achieve conductivities in the range of 0.2 – 4000 S cm^{-1} (Table 1). Many synthetic and biological dopants have been explored, but dopants must be carefully selected as they can affect the phenotype of cells interfaced with a material, possibly causing cytotoxicity.[330,331] Similar to other CPs, PEDOT is most commonly used as a thin film, but can be produced in various ways, including as nanofilms, nanofibers, and inks.[330] Since their first use in the human brain in 2015,[332] PEDOT-based coatings (e.g., Amplicoat®, Heraeus Medical Components) have been used in invasive devices approved for medical use by the United States Food & Drug Administration. As a coating for conventional metal microelectrodes, PEDOT lowers impedance and raises charge injection capacity, properties that enable production of smaller electrodes desired for cochlear and brain implants.[330,333] Moreover, PEDOT-based coatings can improve electrode durability, when compared to other CP-based coatings or bare metallic electrodes, in biological media[334] and in the brain.[335] While PEDOT itself is not biodegradable, efforts have been made to render biodegradable derivatives, for example by synthesizing new co-polymers of thiophene with hydrolyzable polymers.[336]

As with PPy, PEDOT has been doped with biomolecules, such as heparin,[337] laminin-derived peptides,[338] and NGF[295] to improve neuronal cell adhesion and stimulate neurite outgrowth. PEDOT has also been doped with graphene oxide, films of which are reported to improve conductivity and enhance survival, proliferation and maturation of primary NS/PCs from E18 rat cortices.[338] Furthermore, PEDOT:graphene oxide films can be functionalized using the carboxylic acids on graphene oxide. When functionalized with platelet-derived growth factor (PDGF), modified PEDOT:graphene oxide films promoted differentiation of rat cortical NS/PCs into $O4^+$ oligodendrocyte precursors.[338]

Topographical cues have been added to PEDOT:PSS as anisotropic wrinkles on films.[339] When seeded with a human neuroblastoma cell line, films with topography provided directional guidance to neurites. In another study, embryonic stem cell-derived, human NS/PCs were seeded in ionically active, polysaccharide hydrogels laden on an array of PEDOT:PSS pillars, 3D printed using a direct-write method.[340] When electrical stimulation was applied, differentiation and maturation into functional neural networks was enhanced,

indicating that PEDOT:PSS is at least not cytotoxic and that electrical stimulation can benefit tissue-engineered neuronal constructs.[116] Similarly, a separate study found that ReNcell VM human NPCs seeded onto crosslinked PEDOT:PSS films were healthy and underwent maximum neuronal differentiation when stimulated with pulsed DC of 1 V cm^{-1} over 12 days in culture.[341]

In their pure forms, CPs lack remarkable biocompatibility and durability and are significantly less conductive than metal and carbon-based materials. However, use of CPs in conjunction with conventional metal, carbon-based, and polymeric materials is an attractive strategy to improve electrical performance, durability, biocompatibility, and versatility, for example by enabling use of smaller sites for electrical contacts.

9. Composite Materials for Interfacing with Neural Cells

Combining conductive materials with others well-established to be biocompatible is an attractive strategy for creating new, composite materials with good electrical performance, biocompatibility, and versatility. Many polymeric biomaterials have been developed that can interface with neural cells and direct their functions through various biochemical and physical cues engineered into 2D and 3D biomaterial scaffolds.[1] A large body of work has demonstrated the immense potential of various biomaterial scaffolds as *ex vivo* tissue models and *in vivo* therapeutics. Adding conductivity to biomaterials with any of the wide range of functions, including drug delivery, wound healing, and NS/PC transplantation vehicle, will enable development of scaffolds that are highly interactive with the body. For example, conductive biomaterials could be used to engineer closed-loop therapeutics, where the biomaterial responds to, and perhaps even records, changes in the body by releasing a drug on-demand.[330,341]

Generally, biomaterials can be categorized into those based on naturally occurring polymers and those based on synthetic polymers. Naturally occurring polymers are typically biodegradable and can be modified easily to create hydrogel biomaterials that have similar water content and mechanical properties as CNS tissues. Polysaccharides, including chitosan, cellulose, alginate, and hyaluronic acid, and proteins, including silk and melanin, have been modified with conductive elements to create neural cell interfacing biomaterials (Table 1).[342-348] Synthetic polymers used commonly as biomaterials, including PLA, PLGA, and PCL, have the advantages of biocompatibility, hydrolytic degradation in the body, capacity for tunable release of therapeutic molecules, and structural versatility.[193]

The remainder of Section 9 highlights examples where composites of conventional, carbon-based, and CP electronic materials and conventional biomaterial polymers have been fabricated to create new biomaterials that are both highly conductive and highly biocompatible, as summarized in Figure 5 and detailed in Table 3. Furthermore, we discuss how such composite materials can be made truly bioactive and biodegradable, by leveraging numerous previously developed technologies using biomaterial scaffolds, to direct NS/PC behavior. Many reports describing conductive, composite biomaterials investigated cytocompatibility in cultures of neural-related cells, like PC12 cells and mesenchymal stem cells (MSCs), rather than true NS/PCs. Thus, this review includes

discussion of some conductive biomaterials that have yet to be directly evaluated *in vivo* or with NS/PCs but do have excellent potential for such.

9.1. Coatings, Films, and Nanofibrous Mats

As discussed in the previous sections, conductive films and coatings have been developed to facilitate favorable interactions of electrodes with neural tissues and can be used to coat biomaterials. For example, coating of regenerated silk fibroin with a conductive thiophene derivative, hydroxymethyl-3,4-ethylenedioxythiophene (EDOT-OH), yielded maximum conductivity around 6 x 10^{-3} S cm^{-1} and supported cultures of PC12 cells.[350] Thin 2D substrates, or films, enable researchers to easily assess material composite formulations and the effects of surface modifications on interfaced cells. In another example, a composite film of PLGA and graphene oxide was found to have good cytocompatibility when seeded with mouse E14 cortical NSCs.[40] Applied electrical stimulation (100 mV, 1 hour daily) increased NSC proliferation, differentiation, and neurite extension. To create a conductive, yet flexible, material, films of polyurethane (PU), a conductive elastomer, and PEDOT:PSS were synthesized.[351] Increasing PEDOT:PSS concentration led to increased values of conductivity of the PEDOT:PSS-PU composite, maximally at 7.1 S cm^{-1}, and increased material flexibility. PEDOT:PSS-PU composites supported viability, even under applied electrical stimulation, of ReNcell VM human NPCs; however, effects on the functional phenotype of these cells were not evaluated. Gupta, et al. (2019) compared the effects of incorporating multi-walled CNTs or graphene nanoplatelets into chitosan films.[352] They reported that while hippocampal mouse neurons (HT-22 cell line) were elongated and neuron-like when seeded onto chitosan/CNT films, they adopted rounded morphology with minimal spreading on chitosan/graphene scaffolds.

Beyond flat substrates, nanofibrous mats present a semi-3D substrate (~10s to 100s of microns) where cells experience guidance cues as they travel along fibers and between fibers in relatively thick mats. Such nanofibrous mats, created from biomaterial polymers by electrospinning, have been coated with conductive materials to impart electrical activity. For example, electrospun fiber mats of a PLA and PCL blend were coated with a mixture of chitosan, to facilitate cell adhesion, and PPy, to impart conductivity.[353] Electrical stimulation (100 mV, 2 hrs every other day) and chitosan together maximized neurite outgrowth from seeded PC12 cells. Similarly, electrospun fiber mats of PCL and cellulose acetate were coated with a mixture of chitosan and sulfonated PANI, which added conductivity.[354] Both coating alone and subsequent electrical stimulation (100 mV, 1 hr daily) increased neuronal differentiation of seeded human MSCs. In a final example, electrospun PLGA nanofibrous mats were coated with graphene oxide. A hydrophobic small molecule, methylene blue, was adsorbed to the graphene oxide coating to enable its controlled release in physiological media.[355] Below 0.5 wt% graphene oxide, it supported survival, growth, and differentiation of NPCs isolated from mouse cortex (E14), even when under oxidative stress in culture.

As opposed to coating nanofibrous mats to add conductivity, they can be electrospun directly from a mixture of polymer and conductive element.[356-358] In general, CNT incorporation led to increased surface roughness,[356,358] which likely improves cell adhesion, and

increased modulus, which may be detrimental for applications in very soft tissues such as the CNS. It is common practice for CNT particles to be surface oxidized or further functionalized to increase aqueous solubility in biopolymer mixtures and hydrophilicity to improve biocompatibility. For example, oxidized multi-walled CNTs were suspended in a solution of PU and silk fibroin and the entire mixture was electrospun into a nanofibrous mat with good conductivity.[357] When seeded with PC12 or S42 (a mouse-derived Schwann cell line) cells, inclusion of CNTs increased proliferation rates. Moreover, fibers induced elongated morphologies in both cell types and increased neurite outgrowth in PC12 cells. While biochemical surface modifications can improve initial cell adhesion and viability, applied EF and topography can accelerate cell differentiation into mature phenotypes. For example, when seeded with rat MSCs, electrical stimulation through TPU/multi-walled CNTs increased differentiation towards neurons.[358]

Other reports have fabricated conductive nanofibrous mats from mixtures of silk fibroin and reduced graphene oxide[359] or PCL and gelatin[360] and reported similar benefits on neural cell lines, but not NS/PCs specifically. Another study reported that addition of 2% graphene powder to PCL to create electrospun nanofibers enhanced differentiation of mouse E12 NSCs (StemCell Technologies™) towards dopaminergic neurons in culture.[361] To generate retinal neurons, trabecular meshwork MSCs were seeded onto a nanofiber mesh blended from PCL, PPy, and multi-walled CNTs.[362] Applied electrical stimulation-induced expression of rhodopsin and peripherin, distinct markers of retinal neurons. Nanofibrous mats electrospun from PLGA and graphene oxide were functionalized with brain-derived neurotrophic factor (BDNF) and/or insulin-like growth factor (IGF) and implanted into a small incision at T9-T10 in a rat model of spinal cord injury.[363] Rats receiving scaffolds containing graphene oxide, BDNF, and IGF recovered more hindlimb functionalities when compared to those receiving scaffolds with only three or fewer of these components.

Conductive, electrospun fibers can be aligned to provide directional guidance to interfaced cells, which may amplify effects of electrical stimulation.[42] Aligned nanofiber mats composed of graphene, alginate, and polyvinyl alcohol were highly compatible with seeded PC12 cells, which exhibited alignment and increased proliferation with an applied EF.[364] Coating of aligned, PLA nanofiber mats with graphene nanosheets yielded conductive scaffolds with increased surface roughness and hydrophilicity, properties which increased directional alignment and proliferation of PC12 and rat Schwann cells.[77] Overall, conductive coatings and nanofiber mats can provide conductivity and aligned topography, which together can improve viability and maturation of NS/PCs, in particular in the presence of an applied EF.

9.2. Porous, 3D, and Tubular Scaffolds

Polymers and fibrous proteins, like collagen I, can be fabricated as 3D scaffolds containing micron-scale pores that allow for rapid colonization by cells and diffusional exchange of nutrients and wastes — desirable events when working to grow a tissue *ex vivo* or increase integration of a biomaterial implant with host tissues. Composites of conductive and biodegradable materials could be engineered to degrade by hydrolysis, cell-produced enzymes, or in response to active changes in microenvironment, such as pH. Degradability

enables development of tissue constructs that can be completely remodeled by seeded or host cells, where the biomaterial acts as a template for this new tissue. Porous biomaterial scaffolds have also been widely developed as delivery vehicles for sustained, local release of drug and biomolecule therapeutics. Addition of conductivity to such scaffolds may enable development of closed-loop systems with “smart” functionalities. For example, a change in local pH, such as with inflammation, may induce biomaterial degradation, which in turn could cause the release of a therapeutic and a measurable change in biomaterial conductivity.

In particular for application to peripheral nerve injuries, researchers have developed guidance tubes or conduits that can be implanted as a bridge across an injury gap.[365,367] Similar guidance conduits have been explored for their ability to facilitate axon regeneration across spinal cord lesions and as carriers for transplanted NS/PCs that direct their maturation.[180,181,367] Multi-layered, tubular structures have been typically made by either stacking multiple films or nanofiber mats that are then rolled together[338] or electrospinning layers directly onto a cylinder.[366,367] Qian, et al. (2018), created layered, tubular scaffolds in which the innermost layer consisted of polydopamine and arginylglycylaspartic acid (RGD), followed by two layers of a PCL/graphene film, and an outermost layer of polydopamine and RGD.[366] Polydopamine and RGD layers facilitated cell adhesion while PCL/graphene layers enabled local electrical activity. Using graphene as the conductive element, these multi-layered conduits achieved around two orders of magnitude higher conductivity (around 6 x 10^{-3} S cm^{-1}) than similar multi-layered conduits based on PPy[317] or PANI.[367] When seeded with Schwann cells prior to implantation, these graphene-based conduits improved peripheral nerve repair. However, it is not clear if addition of the graphene or electrical stimulation, which was not explored in this study, had any effects.[366] Another group created cylindrical conduits that had an outer sheath of randomly aligned PLGA/PANI nanofibers and were filled with aligned nanofibers of PCL/PANI, yielding conductivities around 4 x 10^{-5} S cm^{-1}.[367] However, inclusion of PANI in conduits resulted in worse functional recovery after SCI (T9-T10) in a rat model, likely a consequence of an inflammatory reaction to PANI itself rather than conductivity.

Compared to 2D substrates, scaffolds with 3D geometries can better recapitulate the microenvironment surrounding a cell in CNS tissues. Conductive 3D scaffolds have been made using techniques that include layer-by-layer assembly,[365-367] often combined with electrospinning as with the conduits described above,[365,366] and 3D printing.[368-370] Stacking and annealing 2D, conductive layers have led to the production of relatively thick, 3D cell scaffolds.[22,371] For example, sheets of MnO_2, which are both conductive and biodegradable, were annealed into 3D scaffolds using adsorbed laminin as essentially a cell-adhesive glue.[371] These MnO_2-based scaffolds supported culture and neuronal differentiation of human iPSC-derived NPCs (WT126 clone 8; WT33 clone 1). Furthermore, loading scaffolds with a small molecule Wnt inhibitor effectively promoted neuronal differentiation. In a mouse hemisection model of SCI, MnO_2-based scaffolds reduced subacute inflammation when compared to controls, which would be expected to lead to better functional outcomes; however, behavioral tests were not performed. Overall, porous, 3D scaffolds offer tunable mechanical, biochemical and electrical properties that can be leveraged to direct maturation of NS/PCs and facilitate their integration into host tissues when implanted into the CNS.

9.3. Hydrogels

Hydrogels have been widely used for CNS implantation and 3D culture of NS/PCs. They can be fabricated from highly biocompatible materials with water content and mechanical properties that approximate those in native CNS tissues.[373,374] Hydrogels can be engineered for a variety of bioactive functionalities, most commonly with ECM-derived, integrin-binding peptides and enzymatically susceptible sites to facilitate cell adhesion and migration, respectively. As multi-functional platforms for neural tissue engineering, hydrogels can serve as sophisticated systems for local, controlled delivery of biomolecules and small molecule drugs.[373] While biocompatibility and versatility make hydrogels an attractive biomaterial for interfacing with CNS cells and tissues, they generally lack conductivity (Table 1). To address this limitation, several research groups have incorporated conductive elements, including metal nanoparticles,[374] CNTs,[375] and graphene powders,[376] into hydrogels. Here, we provide a brief overview of these studies with a strong emphasis on those where conductive hydrogels have been investigated for NS/PC interfacing. For a detailed review on conductive hydrogel biomaterials, please see Xu et al. (2020).[377]

Graphene nanoparticles or powders have been incorporated into hydrogels made from various biomaterials, including collagen I,[378] gelatin,[290] alginate,[379] agarose,[380] silk,[381] chitosan,[382] polysaccharides,[383,384] and polyacrylamide,[376] by suspending graphene within the hydrogel solution prior to crosslinking. In both physically and covalently crosslinked hydrogels, increasing concentrations of graphene have been found to correlate with increased mechanical modulus and worsening cytocompatibility.[376,385,386] However, for lower concentrations of homogenously distributed, nano-scale graphene, good compatibility and minimal toxicity have been reported for many cell types, including neural cells.[387] In contrast, larger, suspended aggregates of graphene, as well as CNTs or CNFs, tend to be cytotoxic.[388-390] To increase cytocompatibility, cationic chitosan or polyethyleneimine (PEI) can be condensed with anionic graphene oxide to produce particles of controlled sizes.[385,389]

Researchers have leveraged the versatility of hydrogels, which can be designed to provide multiple types of bioactive cues simultaneously. For example, using an applied magnetic field, Lin, et al. (2020) deposited Fe_3O_4-graphene nanosheets onto silk-fibroin hydrogels, of which the surfaces had been micropatterned with a corrugated topography.[290] The resulting substrates could provide both topographical and electrical cues to cultured PC12 cells, inducing oriented neurite outgrowth. As NS/PCs cannot efficiently differentiate when cultured in a non-degradable, 3D hydrogel, biodegradability is needed.[391] For this reason, hydrogel biomaterials are often made with protease-degradable sites or from naturally biodegradable materials. For example, chitosan-graphene nanoparticles were disbursed within collagen I hydrogels, creating an enzymatically degradable, highly cytocompatible scaffold for NS/PCs.[386] Biodegradable, conductive hydrogels have also been fabricated from hyaluronic acid. For example, single-walled CNTs and/or PPy were dispersed into hyaluronic acid functionalized with catechol to enable covalent crosslinking into relatively soft hydrogels (1-4 kPa elastic modulus).[392] While most strategies to create conductive hydrogels have used CNTs functionalized to improve solubility, the catechol

group on hyaluronic acid appears to facilitate CNT dispersion and negate the need for functionalization. Addition of CNTs or PPy reduced viability of human iPSC-derived NS/PCs cultured in 3D hydrogels to approximately the same extent at all concentrations evaluated.[392] In contrast, culturing NS/PCs with PPy generally increased neuronal differentiation and decreased astrocyte differentiation, while culture with CNTs generally increased oligodendrocyte differentiation.

While generally less conductive than carbon-based composites, CP-based hydrogels exhibit mechanical properties more similar to those of native CNS tissues.[393,394] PPy was conjugated to chondroitin sulfate polysaccharide and physically incorporated into gelatin hydrogels with around a 1 kPa modulus, in the range of native CNS tissues.[394] Mouse hippocampal NS/PCs (E14) cultured with these conductive hydrogels (0.005 S cm^{-1}) differentiated preferentially into neurons over astrocytes. When implanted into a spinal cord contusion injury model in rats, animals receiving 3 wt% of PPy-chondrotin sulfate exhibited better hindlimb functional recovery than those receiving 1 wt%, indicating positive effects on neural tissue repair even in the absence of applied electrical stimulation.

PEDOT:PSS has also been incorporated into hydrogels with relatively high conductivities (on the order of 10-20 S cm^{-1}).[395,396] PEDOT was deposited on the porous surface of a 3D chitosan/gelatin scaffold, on which NS/PCs isolated from rat hippocampus (E13-15) were then cultured.[397] When cultured with mitogens, NS/PCs proliferated more when PEDOT was included in scaffolds. With mitogen withdrawal and exposure to a differentiation medium, NS/PCs matured more, into both neurons and astrocytes, on scaffolds containing PEDOT. While this study was performed without an applied EF, application of an EF to composite hydrogels of PEDOT:PSS, PU, and liquid crystal graphene oxide was reported to support viable cultures of human NSCs (ReNcell CX, EMD Millipore) and promote neuritogenesis.[395]

Advancements in 3D printing and additive manufacturing now provide flexibility to fabricate conductive bioinks into hydrogel-based scaffolds with highly varied architectures.[368] For example, graphene-containing bioinks based on PU-PCL and PPy-PCL co-polymers have been used to print viable cultures of embedded neural cells.[370,398,399] Laponite, a synthetic nanoclay consisting of silicate nanoplatelets, has been explored by a number of groups as a bioink additive for its ability to impart conductivity and shear thinning properties desirable for 3D printing.[399-401] For example, laponite was dispersed in heparin hydrogels that were then evaluated in a spinal cord crush model (T9) in rats.[399] When also loaded with FGF-4 for release *in vivo*, laponite/heparin hydrogels improved tissue pathology and recovery of hindlimb function. In another report, laponite was doped into polyacrylamide and 3D printed into structures onto which PEDOT was subsequently polymerized.[401] The PEDOT-modified hydrogels were highly conductive (0.26 S cm^{-1}) and could be further functionalized with peptides and polysaccharides to encourage cultured NS/PC adhesion and survival. For a detailed review on 3D printing of conductive biomaterials, please refer to Athukorala, et al. (2021).[368]

Hydrogels excellent candidates for interfacing with NS/PCs given their tissue-like physical properties and versatility, which enables them to be functionalized with bioactive moieties,

mediate drug and biomolecule delivery, and be molded into a variety of architectures. However, improvements in conductivity and durability, given their susceptibility to biodegradation, are still needed.

10. Perspectives & Future Directions

CNS tissues are electroactive microenvironments in which currents flow between the CSF and cells though gap junctions and chemical synapses. Leveraging this inherent electroactivity, researchers have worked to develop conductive biomaterials that can interface with NS/PCs and direct their maturation. These technological advances are expected to lead to 1) *ex vivo* models of neural tissues with improved physiological mimicry and 2) new, effective therapies that can regenerate and/or rewire neuronal circuitry to restore function in cases of CNS injury or degeneration. For example, one can imagine transplanting NS/PCs within a conductive biomaterial scaffold into the injured spinal cord and then applying an external EF (e.g., through epidural electrical stimulation),[402] which can synergize with the scaffold's biomolecular and topographical cues to direct neuronal differentiation and engraftment into functional circuitry in host tissues.

Generally, tradeoffs between biocompatibility and conductivity have presented a significant challenge, especially when conductivities above that of CSF are desired. However, advanced composite materials that incorporate conductive elements into established biomaterials may be able to provide biochemical and physical cues to better support NS/PC survival and function, while retaining conductivity. In the future, it will be valuable to expand studies directly comparing the effects of incorporating individual conductive elements with the same biomaterial base.[392] Similarly, inconsistent reporting of electrical performance, either by using unique instrumentation for measurements or arbitrary selection of units reported, such as conductivity, impedance, or conductance, make it difficult to compare the performance of different conductive scaffolds. Consistent reporting of normalized, universal measurements would likely resolve this issue.

Hydrogel composites are good candidates to mitigate the potential cytotoxicity of conductive materials, including metals, carbon-based nanomaterials, and CPs. As opposed to concentrated coatings or larger suspended aggregates, homogenous dispersion of nano-scale conductive materials with a hydrogel appears to at least partially mitigate toxic effects.[385,389,394] Furthermore, hydrogels can be easily formulated as injectable scaffolds that form *in situ*, which is an advantage for therapies in the CNS where it is often crucial to avoid damage to the intact tissue when accessing anatomically deep sites.[403] Future work should focus on developing conductive materials that are injectable and avoid mechanical mismatch with the CNS implantation site. Moreover, researchers should continue to work towards developing materials that provide biochemical, electrical, and physical cues simultaneously. For example, a multi-functional scaffold as a nerve guidance conduit may consist of an outer layer of soft hydrogel and an inner layer of aligned, electrospun nanofibers dispersed with a conductive filler.

Despite numerous reports of new conductive biomaterials for a variety of applications, relatively few studies have evaluated cultures of primary animal[317,371,392] or

human[116,241,329,341,395] neural cells with conductive biomaterials and/or the effects of applied stimulation. As many studies appear to be done by materials scientists instead of neuroscientists, cell lines that are easy to obtain and culture, for example PC12 and SH-SY5Y cells derived from cancers, are commonly used. However, these immortalized neural cells are not very representative of neural cells in intact tissues.[328,404] Usage of primary, rodent-derived NS/PCs likely yields more physiologically relevant data; however, development regulation and timing is significantly different than for human NS/PCs.[405] Ideally, future studies will involve collaborations between materials scientists and developmental neuroscientists to investigate how human NS/PCs, derived from fetal or iPSC sources, interact with and respond to conductive materials and applied EFs.

Additionally, the majority of previous studies made minimal assessments of cell phenotype beyond viability, proliferation, and perhaps some expression of general markers for neuronal and glial lineages rather than assessing regional-specific phenotypes. However, in reality these cells may not be fully mature or synaptically functional. For example, in Parkinson's disease, NS/PCs must be differentiated into dopaminergic neurons to have a therapeutic effect. Alternatively, for treatment of spinal cord injury, cholinergic motor neurons wrapped in oligodendroglial sheaths and/or glutaminergic and GABAergic interneurons are necessary. Thus, assessments of mature subtypes and their synaptic functions (e.g., using electrophysiology) are needed in future studies.

Currently, relatively little is known about the mechanisms underlying the effects of applied EFs, like galvanotaxis, proliferation, and differentiation. A better understanding of these mechanisms will facilitate development of conductive biomaterials and protocols for electrical stimulation that efficiently and reliably control these processes. Use of human NS/PCs will be particularly important for these mechanistic studies to yield clinically translatable findings. Furthermore, the majority of studies investigating NS/PC interactions with conductive materials to date have used 2D culture systems. However, moving to 3D cultures, to better mimic how cells experience native tissues, will be advantageous as biological phenomena identified will be more likely to exist *in vivo*.

Very few studies to date have investigated effects of applied electrical stimulation, including various stimulation parameters, on cells interacting with conductive scaffolds, *in vitro* or *in vivo*. Thus, how conductive scaffolds can be used to amplify electrical stimulation to interfaced cells, and perhaps direct axonal wiring into new neuronal circuits, remains unclear. Furthermore, given that stimulation thresholds must often be determined empirically, in practice conductive biomaterials must be designed to accommodate a range of stimulation schemes (e.g., 0.01 to 1V).[122] Similarly, few reports have investigated the effects of conductive scaffolds *in vivo*, either in healthy CNS or specific pathological models, and thus the scientific literature to date provides a relatively limited data relating to biocompatibility and durability. Arguably, *in vivo* studies in animal models can more accurately reveal the material's biocompatibility, perhaps the most important attribute of clinical translation, as organ toxicity, degradation rate, renal clearance, and immunoreactivity, as well as interactions of these responses, can all be observed. Eventual clinical implementation will depend on a movement towards using human NS/PCs and performing *in vivo* validation studies of any findings.

Finally, several advanced conductive biomaterials that have yet to be evaluated for neural applications may address some of these issues encountered thus far. For example, bio-ionic liquids (e.g., based on choline) have been used to functionalize biomaterials, such as gelatin and polyethylene glycol, to yield biocompatible, conductive scaffolds that can promote healing of cardiac tissue in animal models.[406,407] Given their natural origin, bio-ionic liquids likely offer better biocompatibility than other conductive materials. Furthermore, while biomaterial composites with carbon-based materials or CPs are opaque, those with bio-ionic liquids typically remain transparent, which is an advantage for imaging of cells laden throughout 3D scaffolds.

Furthermore, development of multi-functional “smart” biomaterials will be incredibly valuable to neural engineers. For example, PEI-graphene oxide nanocomplexes were crosslinked together with sites that can be cleaved by an enzyme produced by bone marrow MSCs so that cells encountering the hydrogel triggered release of a therapeutic plasmid.[386] These composite hydrogels were 3D printed into microfibers, in which the shells contained alginate-PPy-graphene oxide hydrogels loaded with stromal-derived factor-1α (SDF-1α) and the cores contained crosslinked PEI-graphene oxide nanocomplexes loaded with plasmid DNA encoding for FGF-2. When implanted in a skin wound model in rats, host MSCs were attracted to the injury by expressing SDF-1α plasmid, which promoted differentiation along with the expressed FGF-2 plasmid. One can imagine applying this approach to treat brain or spinal cord injuries, where SDF-1α could attract endogenous NS/PCs and an enzymatically released factor would then promote NS/PC differentiation. Finally, conductive biomaterials could be engineered as closed-loop, CNS therapeutics. For example, differentiation of transplanted NS/PCs into neurons would likely change the conductivity of an interfaced biomaterial scaffold in a measurable way, an event which could trigger a change in applied electrical stimulation that would direct engraftment into host circuitry and synaptic stabilization.

In summary, conductive biomaterials offer the opportunity to leverage synergistic responses of NS/PCs to electrical cues with biochemical and physical cues in a single, engineered microenvironment, enabling development of bioactive scaffolds that can direct formation and/or repair of CNS tissues. However, further studies will be required to better understand the responses of human NS/PCs to conductive materials and applied EFs and whether these responses will translate *in vivo*. We expect future studies will take advantage of the latest technologies in conductive biomaterials and additive manufacturing to create scaffolds with improved biocompatibility, bioactivity, and complex architectures. Together, these advancements will enable development of clinically accurate, micro-physiological models (e.g., “tissue chips”) for drug screening and discovery as well as “smart” therapeutics for CNS pathologies.

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Biography

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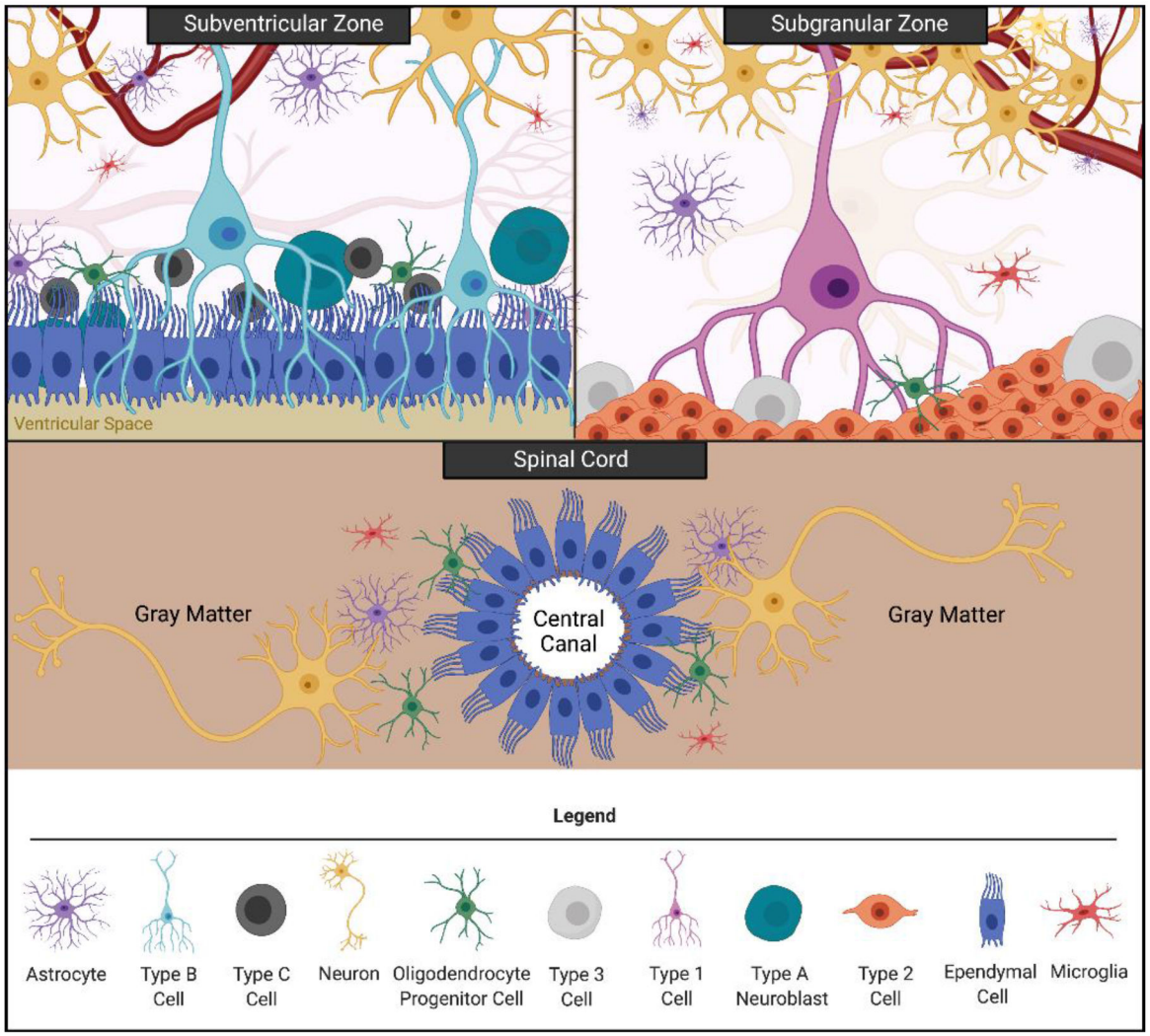

Figure 1. NS/PC niches in adult humans.

Well-characterized populations of NS/PCs reside in the cortical SVZ and SGZ of the dentate gyrus. The regions of the grey commissure most proximal to the central canal and the filum terminale (not shown) may also harbor populations of NS/PCs or NS/PC-like cells jnto adulthood. Each site is populated by mature neurons, glia, and stem-like cells at varying points of differentiation, such as the Type B, Type C, and Type A cells of the SVZ. Thus, interfaced NS/PCs may display varying amount of differentiation, proliferation, or other phenotypes based on the time at which it was isolated or the region from which it was isolated.

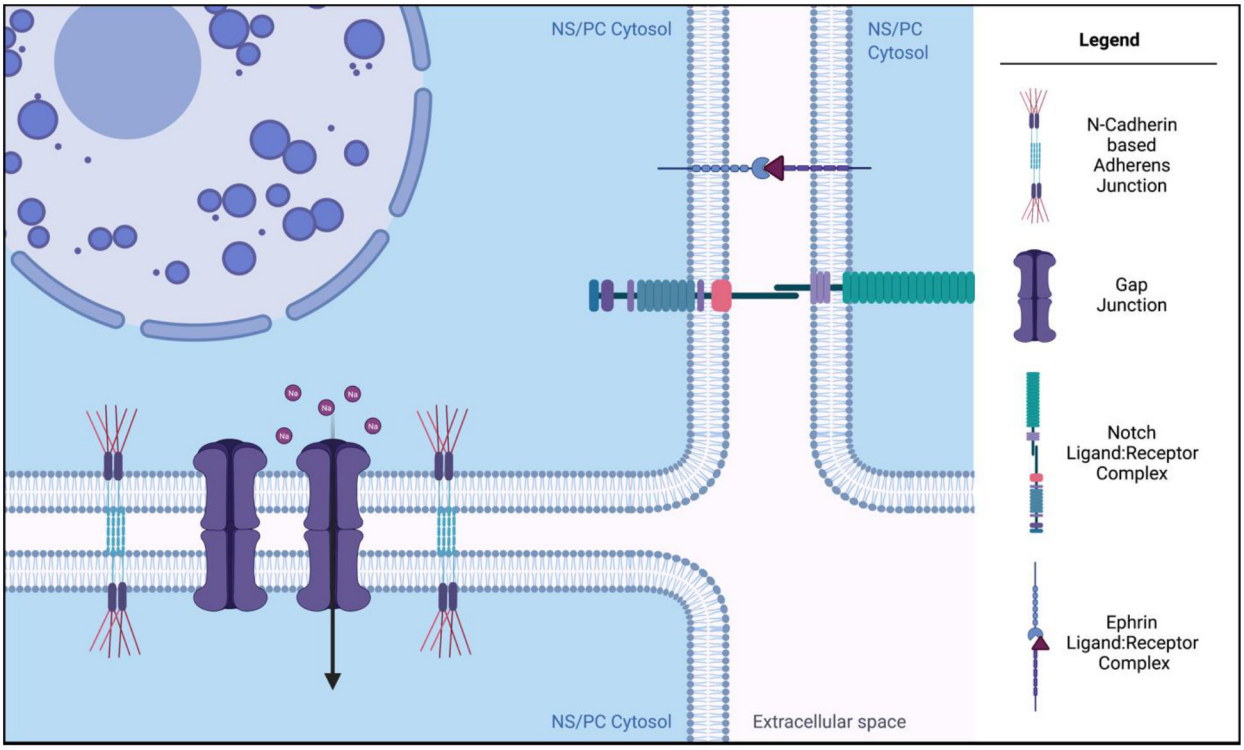

Figure 2. Cell-cell contacts are integral in development of the central nervous system.

NS/PCs interact with each other through N-cadherin-based adherens junctions. NS/PCs also interact with surrounding cells through notch and its receptors and Eph/ephrin. Gap junctions, formed by connexin proteins, facilitate ion transport between NS/PCs, playing an integral role in the propagation of current due to endogenous or exogenously applied fields.

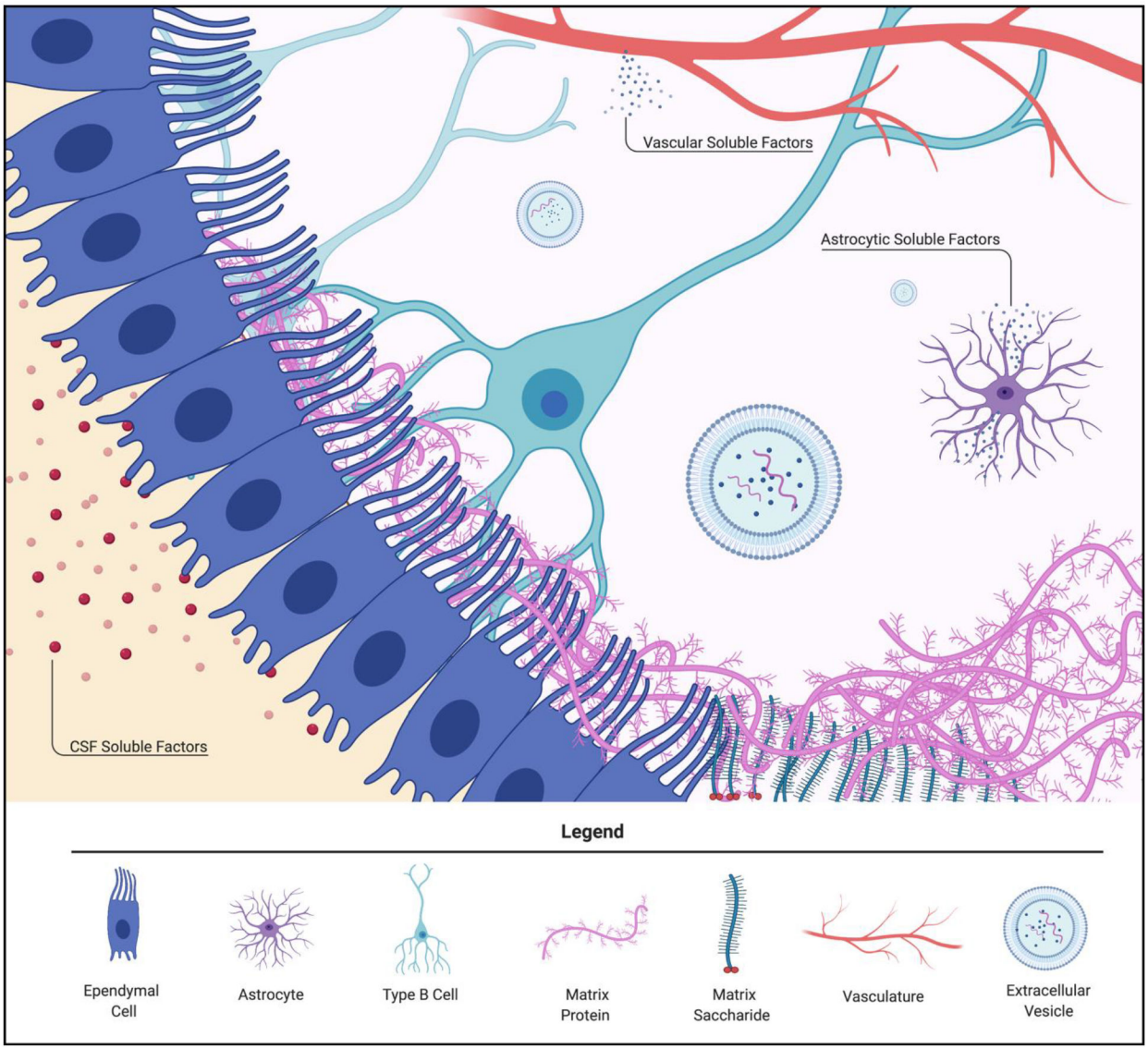

Figure 3. Acellular components of the NS/PC microenvironment include soluble factors and biochemical and physical influences of the ECM.

Soluble factors secreted by local vasculature, ependymal cells that produce CSF, and neighboring glial cells determine NS/PC fate. Proteins and polysaccharides in the ECM interact with NS/PC cell surface receptors to affect proliferation, migration and fate. NS/PCs are also affected by local tissue mechanics (e.g., viscoelasticity) and diffusion of various soluble factors through the porous ECM. Extracellular vesicles packaged with biomolecules (e.g., miRNA) are also secreted by cells and contribute to NS/PC behavior.

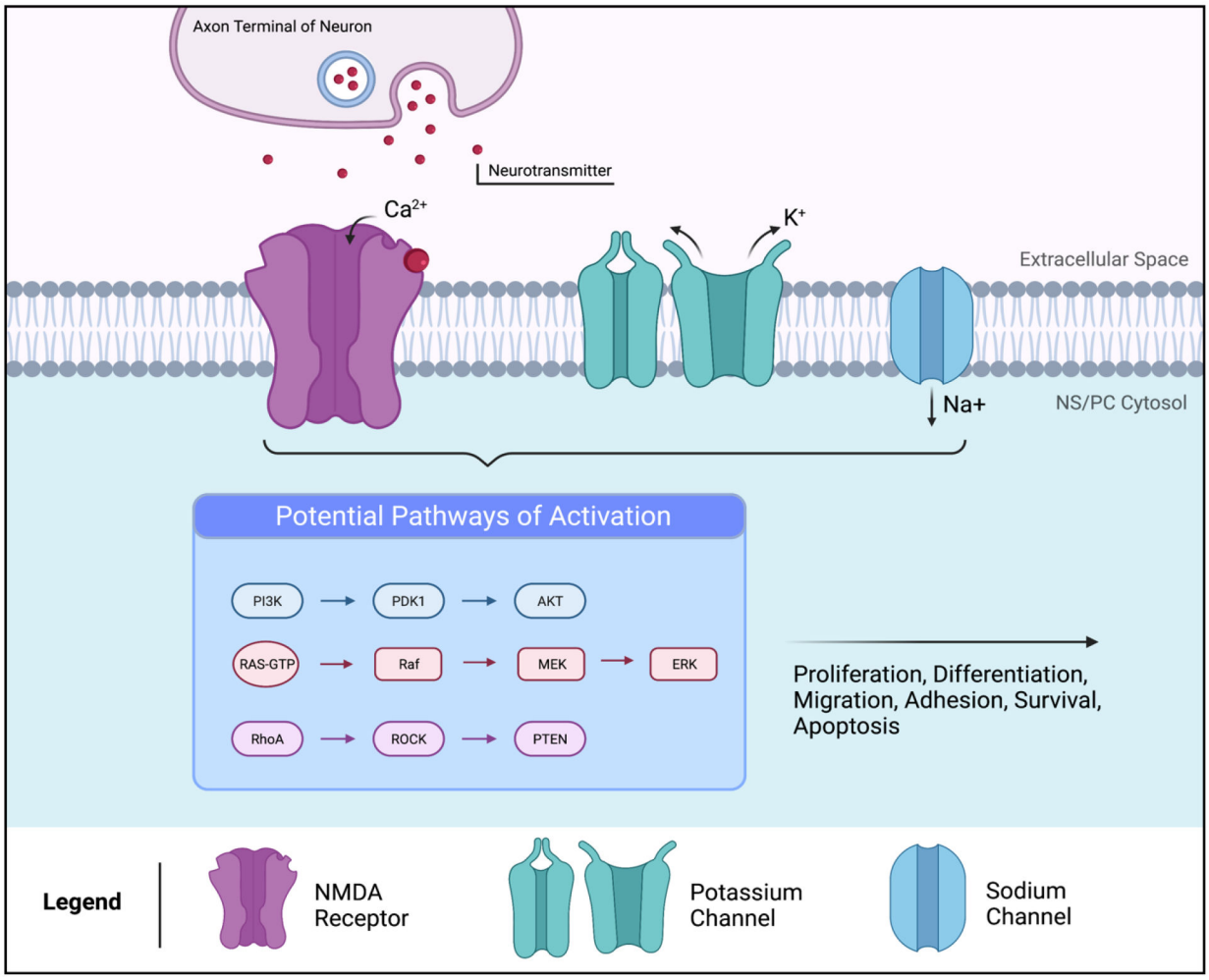

Figure 4. Potential mechanisms of EF effects on NS/PCs.

Signaling proteins mediating cell migration, including PI3K/AKT, RhoA/ROCK, and MAPK/ERK, are thought to polarize within the cell membrane in response to an EF to mediate galvanotaxis. In neural cells, ionotropic receptors, such as NMDA, and other ion channels have been implicated in galvanotaxis and other EF-induced behaviors.

Figure 5. Conductive biomaterials for NS/PC interfacing

include electroactive materials (e.g., metals, silicon, carbon-based materials or electrically conductive polymers) and composite materials in which electroactive materials are integrated with highly biocompatible materials (e.g., natural or synthetic polymers). These composite materials can take on various forms, including 2D films, nanofibrous mats, hydrogels, and 3D microporous or tubular scaffolds.

Table 1.

Reported conductivity measurements of commonly used electronic materials, electrically conductive polymers, and biomaterials.

Material	Forms	Conductivity (S cm^{-1})
Metals	Silver[159]	6×10^{5}
	Gold[159]	4×10^{5}
	Platinum[159]	9×10^{4}
	Iridium[206]	2×10^{5}
	Palladium[206]	1×10^{5}
Silicon	Substrate[206]	1×10^{1}
Carbon-based	Diamond[246]	$1 \times 10^{-15} – 1 \times 10^{-2}$
	Carbon Nanotubes[246]	$1 \times 10^{3} – 1 \times 10^{7}$
	Carbon Nanofibers[247,248]	$1\text{-}5 \times 10^{4}$
	Graphene[159,246]	$2 \times 10^{3} – 1 \times 10^{5}$
Electrically Conductive Polymers	PPy[159,296]	$1 \times 10^{-12} – 1 \times 10^{3}$
	PANI[321]	$1 \times 10^{-1} – 1 \times 10^{1}$
	H_2SO_4-doped PANI[321]	0.42 – 52.9
	HCl-doped PANI[321]	0.60
	PEDOT[159]	$3 \times 10^{2} – 5 \times 10^{2}$
	PEDOT:PSS[331]	$2 \times 10^{-1} – 4 \times 10^{3}$
Proteins	Silk[408]	$1 \times 10^{-8} – 1 \times 10^{-6}$
	Melanin[409]	$1 \times 10^{-8} – 1 \times 10^{-7}$
Polysaccharides	Chitosan[410]	1×10^{-10}
	Cellulose[411]	$1 \times 10^{-11} – 1 \times 10^{-9}$
	Alginate[412]	1×10^{-6}
	Hyaluronic Acid[413]	$1 \times 10^{-13} – 1 \times 10^{-6}$

Table 2.

Referenced studies on interfacing commonly used electronic materials or electrically conductive polymers with NS/PCs and NS/PC-like cells.

Reference	Material	Cell	Applied ES	Key Findings
Garrudo et al. 2019[329]	Electrospun Fibers of PCL and PANI doped with or without camphorsulfonic acid	Human-derived ReN-VM Cells immortalized by transfection with c-myc	N/A	Varying the ratios of PANI and PCL fibers affected viability and proliferation.
George et al. 2017[55]	PPy electroplated scaffold	Human NPCs derived from H9 human embryonic stem cell line	+1 V to −1 V square wave at 1 kHz for 1 h	PPy scaffolds containing NPCs were implanted onto the cortical surface of stroke-injured rats. Rats experienced earlier and sustained improved recovery when compared to rats that received unstimulated NPCs or conductive scaffold alone.
Gomez et al. 2007[314]	Micropatterned PPy films	Embryonic rat hippocampal neurons (E18)	N/A	Axonal polarization and extension were accelerated when cells were interfaced with PPy films micropatterned with 1–2 μm grooves.
Kwon et al. 2021[241]	Electrode microarrays of vertically aligned, silicon-based nanowires	Fetal human NSCs	10 min per day with ±10 mV biphasic electrical pulses at a frequency of 1 Hz	Following intracellular electrical stimulation using the electrodes, the activity of voltage-dependent ion channels was increased which accelerated neuronal differentiation.
Luo et al. 2013[338]	Graphene oxide-doped PEDOT films	Rat cortical NS/PCs (E18)	N/A	Films promoted cell survival, proliferation, and maturation. Functionalized PDGF promoted differentiation of the E18 cells into oligodendrocyte precursors.
Ma et al. 2016[39]	Graphene foam	ICR Mouse (1 day postnatal) hippocampal NSCs	N/A	While high cell viability was noted on both 30 kPa and 64 kPa substrates, proliferation and astrocyte differentiation increased for cultures on the stiffer substrate.
N. Li et al. 2013[7]	3D graphene foam, 2D graphene film	ICR Mouse (1 day postnatal) hippocampal NSCs	Monophasic cathodic pulses of 20-30 μA current	NSCs on 3D foams and 2D films could differentiate into neurons or glia. However, 3D graphene foams enhanced proliferation as well as neuronal and astrocytic differentiation. Stimulation resulted in increased calcium influx in differentiated neurons.
Song et al. 2019[317]	DBS-doped 2D PPy films and 3D PPy tubular scaffolds	Human iPSC-derived NPC	40 V m^{-1} for 1 h	Applied stimulation increased the expression of neurotrophic factors from cells seeded in 3D scaffold. However, there was higher cell viability on 2D films.
Sordini et al. 2021[341]	Cross-linked PEDOT:PSS films	ReNcell-VM human NSCs	1 V cm^{-1} for 12 days	Applied stimulation enhanced neuronal differentiation with high cell viability.
Stewart et al. 2015[297]	PPy:pTS, PPY: DBS and PPY:chondroitin sulfate films	ReN-CX human NSCs	± 0.25 mA cm^{-2} biphasic wave of 100 μs pulses	PPy:DBS interfaced with cells resulted in the most optimal support for neuronal differentiation. Subsequent stimulation on this material increased neuron-to-glial cell ratio and promoted expansive neural networks.
Tomaskovic-Crook et al. 2019[116]	Biogels laden on an array of PEDOT:PSS pillars	Human NSCs (Millipore: SCC008)	0.25 mA cm^{-2} biphasic waveform of 100 μs pulses and 20 μs interphase	PEDOT:PSS was cytocompatible. Applied stimulation further enhanced formation of neural networks and neuronal differentiation.
Yan et al. 2017[240]	Vertically-aligned silicon nanowires and silicon wafers	ICR Mouse (1 day postnatal) hippocampal NPCs	N/A	After 7 days, NS/PCs cultures on nanowires, as opposed to wafers, had increased proliferation expression of neuronal markers. This finding demonstrates that NS/PC differentiation depends on material geometry.

Reference	Material	Cell	Applied ES	Key Findings
Zhu et al. 2018[22]	Carbon scaffold thermally annealed to electrospun mats of polyacrylonitrile	Mouse NE-4C NSCs (ATCC)	Current of 100 μA and pulse rates of 100 Hz with 100 μs duration for 24 h	Stimulation increased the amount of neural differentiation and neurite lengths over unstimulated controls.

Table 3.

Referenced studies on interfacing composites with NS/PCs and NS/PC-like cells.

Reference	Material	Cell	Applied ES	Key Findings
Cuttaz et al. 2019[351]	PU-PEDOT:PSS films	ReNcell-VM human NPCs	N/A	Composites over a range of PEDOT:PSS concentrations supported cell viability and promoted varying levels of neuronal and glial differentiation and neurite elongation.
Fu et al. 2019[40]	PLGA-graphene oxide films	Mouse cortical NSCs (E14)	100 mV for 1 h per day	Alone, the substrate demonstrated good cytocompatibility. Applied stimulation increased cell proliferation, neuronal differentiation, and neurite extension.
Ginestra et al. 2019[361]	Electrospun fibers of PCL and graphene nanoparticles	Mouse NSCs (E12)	N/A	The addition of 2% graphene nanoparticles to PCL fibers enhanced cell differentiation towards dopaminergic neurons.
Javadi et al. 2018[395]	Hydrogel composite of PU, PEDOT:PSS, and liquid crystal graphene oxide	ReN-CX human NSCs	+/−0.25 mA cm^{-2} biphasic waveform of 100 μs with 20 μs interphase at 250 Hz	Stimulation applied to NSCs seeded on composite hydrogels increased neurite count and average length.
L. Wang et al. 2019[355]	PLGA nanofibrous mat coated with graphene oxide	Mouse cortex NPCs (E14)	N/A	High cell viability and growth was supported below 0.5 wt% graphene oxide. NPCs preferably differentiated into astrocytes. Methylene blue adsorption to fibers enabled controlled release.
Luo et al. 2021[394]	Gelatin hydrogels with infused PPy-conjugated chondroitin sulfate	Mouse hippocampal NS/PCs (E14)	N/A	Cultured NS/PCs differentiated into either mature neurons or astrocytes. When implanted in rats with spinal cord injury, the hydrogel with 3 wt% PPy-chondroitin sulfate promoted improved hindlimb functional recovery better than groups with 1 wt%.
Wang et al. 2018[397]	Chitosan/Gelatin 3D scaffold with deposited PEDOT	Rat hippocampal NSCs (E13-E15)	N/A	Cell proliferation increased when cultured with mitogens and PEDOT was added to the scaffold. Exposure to differentiation medium led to increased glial and neuronal differentiation on scaffolds with PEDOT.
Shin et al. 2017[392]	Hyaluronic Acid Hydrogels with CNT or PPy	Human fetal NSCs and human iPSC-derived NPCs	N/A	The incorporation of PPy into the hydrogel generally increased neuronal and decreased astrocyte differentiation, while CNT increased oligodendrocyte differentiation.
Yang et al. 2018[371]	3D scaffold formed by laminin-annealed MnO_2 sheets	Human iPSC (WT126 clone 8; WT33 clone 1)-derived NPCs	N/A	Neuronal differentiation on the scaffold was enhanced with controlled release of a small molecule Wnt inhibitor. Implanting NSC-seeded scaffolds in a mouse hemisection model of SCI reduced subacute inflammation when compared to controls.