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REVIEW ARTICLE

Conductive Polymers and Hydrogels for Neural Tissue Engineering

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Abstract | Conventional approaches for the rescue and repair of the damaged neural tissue generally remain ineffective and do not provide functional recovery due to the difficulties in mimicking the complex anatomical functioning of the nervous system. Mimicking the natural microenvironment of the glial, neuronal, and stromal cells of the nervous system through the use of functional biomaterials-based platforms, and further combining these platforms with stem cell-based therapies has been considered as a promising alternative strategy for the efficient regeneration and functional recovery of the damaged neural tissue. The functionalities of biomaterial-based platforms provide 3D matrices with desired pore sizes, porosities, elasticities, and wettability along with various chemical, biological, and topographical cues that favor cellular attachment, growth, proliferation, directed alignment, and differentiation as well as proper nutrient flow for neural tissue regeneration. In addition, considering the inherent presence of electrical fields and synapses in the nervous system, application of electrical stimuli through conductive biomaterials-based platforms in the form of films, hydrogels, fibers, composites, and flexible electronic interfaces has also been used to enhance the nerve regeneration process. These platforms providing electrical stimuli have been particularly used for controlling neurite extension, directed migration of neuronal and glial cells, and differentiation of stem cells. In this review, we will summarize the recent advances in conductive biomaterials-based platforms and the use of electrical stimuli to control cellular behavior to enable neural regeneration.

Keywords: *Conductive biomaterials, Electrical stimuli, Neural regeneration*

1 Introduction

Our nervous system, consisting of two main components: the central nervous system (CNS) and the peripheral nervous system (PNS), has a vital and complex role in conveying signals for physiological processes in limbs and organs as well as controlling sensory and motor functions. Damage or injury to the nervous system may result in serious dysfunction of limbs or organs causing lifelong disabilities and reduced quality of life accompanied by major economic and social burdens. According to reports, it is expected that people will continue to experience increasing rates of spinal cord injuries, traumatic brain injuries, peripheral nerve injuries, and neurodegenerative disorders along with many other nervous system problems around the globe, causing significant economic burden ranging from $3 to $800 billion in the US alone.[1–5] Among these, peripheral nerve injuries (PNIs) affect ~ 3% of trauma patients (mostly young adults of working age and U.S. veterans) in the U.S alone.[6–8]

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According to estimates, over 200,000 peripheral nerve repair procedures are performed in the U.S,[6–8] causing ~ $150 billion in medical expenditures spent annually.[9] It is also expected that the U.S. transected peripheral nerve repair market will exceed $1.6 billion by 2022.[6–8, 10]

The PNS, consisting of neuronal, glial, and stromal cells, has the intrinsic capacity for spontaneous regeneration and regrowth of axons to a certain extent. Besides the neurons, glial and stromal cells play a key role in maintaining the proper function of the peripheral nerves. Injury-induced Schwann cell (SCs) reprogramming, which involves upregulation of trophic factors and cytokines, myelin clearance by activation of myelin autophagy in SCs and macrophage recruitment, and formation of Bungner bands are some of the cellular functions orchestrating spontaneous regeneration and regrowth of axons.[11–16] Despite the key responses of SCs to injury, the regenerative capacity of PNS remains insufficient, particularly for large PNIs (> 1 cm), without any additional surgical/therapeutic interference.[8] Although it is possible to enable functional recovery using autologous nerve grafts, this strategy has limitations such as multiple surgery requirement, biological complexity, donor site morbidity, and lack of graft tissue.[17] Alternatively, cell-based therapies offer promising outcomes for PNIs.[18] Implantation of SCs using different platforms (scaffolds, conduits, films, etc.) has shown enhanced axonal regeneration across nerve gaps in many studies;[19–23] however, lack of availability and slow in vitro growth of SCs restrict clinical translation.[7, 8, 24] Stem cells, isolated and derived from various connective tissue sources, offer promising translational strategies for PNIs without any ethical concerns due to their accessibility, plasticity, and multipotency.[25–28] Most studies use autologous transplantation of stem cells for PNIs.[18] Although they have reported improved axonal regeneration through synergetic interactions of stem cells with host SCs and enhanced paracrine activity,[29–34] the multipotency of stem cells and lack of direct control in complex in vivo environment and the design of a platform mimicking extracellular matrix (ECM) raise concerns regarding the implanted cells' fate, especially over the duration of the nerve regeneration and limit the clinical use of MSCs.[35, 36] Because of these reasons, designing a cell-laden, implantable platform with desired 3D microstructural/mechanical properties that mimics the complex ECM microenvironment is critical in neural tissue regeneration.[35, 36] Hence, a multifunctional platform possessing biological, chemical, and physical cues and mimicking cellular microenvironment significantly affects and determines successful control of cellular behaviors such as growth, proliferation, directed migration, differentiation, and in turn, neural tissue regeneration.[18, 37–41]

The inherent ability of neuronal cells to send electrical signals along axons[42–45] and the positive effects of electric fields on paracrine activity,[46] cellular alignment and migration[47, 48], and recovery from peripheral nerve injuries[49–51] are known. It is also previously reported that electrical stimulation is an effective cue in stimulating not only the neuronal cells, but also various other cell types' behavior such as proliferation or differentiation.[52] Therefore, besides the biological, chemical, and physical cues, the designed platforms should also have conductivity to enable electrical stimulation for the enhancement of neural tissue regeneration.

In this review, we first discuss electrically conductive biomaterials-based platforms such as films, hydrogels, fibers, composites, and flexible electronic interfaces. Then, we summarize the application of electrical stimulation through these platforms to control cellular behavior such as neurite extension, directed migration and differentiation, and neural tissue regeneration. Finally, we will conclude this work with future perspectives.

2 Conductive Polymers (CPs)

Polymers with loosely held electrons in their backbones are generally classified as conductive polymers (CPs) possessing common polymeric properties along with electrical features similar to metals and semiconductors. The source of electrical conductivity comes from the atoms at the backbone with weak ϖ bond enabling delocalization and free movement of electrons, which in turn results in formation of electrical current through mobile charges.[53] Most CPs are synthesized through doping process (oxidation or reduction), in which a charge is transferred from dopant molecules to polymer chains via charge carriers (polarons and bipolarons).[52] The efficiency of the doping process and properties of the formed CPs depend on many different factors, including polaron, chain and conjugation lengths, charge transfer efficiency, and type of the dopants and molecular size. Typical CPs are polyacetylene (PA), polythiophene (PT), poly(3,4-ethylenedioxythiophene) (PEDOT), polypyrrole (PPy), poly(p-phenylene) (PPP), and polyaniline (PANI).[54]

PA, which is also called acetylene black, can be synthesized by Ziegler–Natta catalysis, radiation methods, or by the controlled combustion of acetylene under air.[55] This material shows excellent biocompatibility, electrical conductivity, and a large specific surface area, which favor biochemical[56] and electrochemical sensor applications.[57] PT with high conductivity (10^3 S cm^{-1})[58] and transparency[59] can be synthesized by different electrochemical and chemical methods.[60] The most popular derivative of PT, highly conductive and stable, PEDOT, is mostly used in electroanalytical applications.[52, 61, 62] PPy, which can easily be synthesized by various chemical methods, has high conductivity, stability, and biocompatibility. The conductivity and biocompatibility of PPy may further be improved through hybridization with other materials, including myocytes, biotin, alginate, and silk fibroin.[52, 55, 61–64] Because of these features, PPy is eligible for many biomedical applications, including neural tissue engineering.[52, 55, 61–64] PPP with thermal, optical, electrical, and chemical properties can be synthesized by electrochemical and chemical methods.[52, 62, 64] PPP-based thin films can be conventionally used in light-emitting diodes, photodetectors, and other optoelectronic devices as well as dental applications and cellular alignment.[64] PANI, which is a nontoxic and stable polymer with high electrical conductivity, has simple and cost-effective chemical and electrochemical synthesis routes. The conductivity of PANI can be controlled through determination of oxidation state,[65] increasing crystallinity and conjugation length of the polymer,[66] or the polymer chain structure (i.e. 1D: nanofibres, nanorods, and nanotubes, 2D: ribbons, nanobelts, and nanoplates and 3D: microspheres, nanospheres, and granules). The properties of PANI facilitate its application in biosensing, medicine, and tissue engineering.[52, 55, 62–64]

CPs are attractive biomaterials for tissue engineering applications due to their physical and chemical properties, along with their ability to convey electrical signals to cells and provide favorable platforms controlling and promoting specific cellular responses such as cell adhesion, growth, and proliferation, combination of which enhance tissue regeneration.[52, 61–63] Although CPs show good in vitro and in vivo biocompatibility and support the in vitro adhesion, proliferation, and differentiation for different cell types, their non-degradability limits their in vivo application.[67] However, these properties of CPs can be controlled based on the dopant type and selected synthesis method and conditions in such a way that CPs can retain their electroactivity, while gaining biodegradability.[62, 67] Another limitation of using CPs is their stiff, brittle, and insoluble nature, which makes them mechanically poor and difficult to manipulate and process. However, blending CPs with other degradable synthetic or natural polymers (such as PLA, PLGA, PCL, chitosan, and silk fibroin) is a widely used strategy to fabricate conductive and biodegradable platforms for tissue engineering applications.[62, 67]

Overall, the chemical, physical, and electrical properties of CPs can be controlled and manipulated by polymer chemistry and synthesis methods. Hence, various CP-based platforms in the form of films, hydrogels, fibers, composites, and flexible electronic interfaces can be obtained by physically compositing or forming co-networks with other polymers for neural tissue engineering applications.

3 Carbon-Based Conductive Materials

Carbon-based conductive materials, such as graphene and carbon nanotubes (CNTs), are another group of biomaterials that can be incorporated into non-conducting polymers to provide structural reinforcement and impart novel properties such as electrical conductivity, enhancing cell attachment, directed growth, proliferation, and differentiation.[63, 68–70]

Graphene-based materials can be obtained through mechanical exfoliation, chemical vapor deposition, and liquid-phase exfoliation. Graphene oxide (GO) is formed through sp2 and sp3 hybridization of carbon atoms and has the ability to be easily dispersed in water and interact with different inorganic and organic materials enabling various conductive ink formulations.[71, 72] However, the conductivity of GO-based materials is limited due to the presence of oxides, which can be improved by reducing GO through thermal or laser processing leading to reduced GO (rGO) with enhanced physical and electrical properties.[73]

These materials offer several advantages, including exceptional electrical and thermal conductivity, mechanical strength, chemical stability, non-toxicity, and biocompatibility, which make them well suited for tissue scaffolds capable of mediating cell growth, proliferation, and differentiation.[74–83] The intimate cell-graphene-based material interaction is most likely due to the ϖ–ϖ interactions of aromatic amino acids in the cell membrane that orient proteins with the graphene layer.[78, 84, 85] The hydrophobic nature of graphene can also potentially facilitate the immobilization

of extracellular matrix (ECM) proteins[78, 86] and facilitate physicochemical interactions to further enhance cellular attachment and proliferation.[78, 79] Therefore, graphene-based materials can be used in the fabrication of conductive and biocompatible platforms; however, non-degradable nature of graphene could be a limitation for in vivo applications.

CNTs, with cylindrical shape, nano-scale dimensions, and high aspect ratio, can be fabricated by laser cutting, arc discharge, or chemical vapor deposition. The high aspect ratio, low density, and electrical and physical properties of CNTs favor their use in biomedical applications.[87, 88] The structure of CNTs has the potential to induce oxidative stress in the cells, when applied in the form of suspension causing toxic response.[89, 90] However, the toxic effect of CNTs could be eliminated through the surface functionalization or immobilization of CNTs to a platform.[52] Nevertheless, graphene and CNTs are promising materials for producing conductive and biocompatible platforms for neural tissue engineering.

4 Conductive Polymer-Based Platforms and Electrical Stimulation

The presence of endogenous electrical fields during the embryonic development[91–93] and the inherent ability of neuronal cells to send electrical signals along axons[42–45] are well known in the literature. Based on this fact, there have been various attempts to control cellular alignment and migration,[47, 48] differentiation,[79, 94, 95] paracrine activity,[46] neurite extension, and recovery from nerve injuries[49–51] through the application of electrical stimuli.[46, 96–99] Electrical stimuli can be applied through different platforms such as films, hydrogels, scaffolds/conduits, fibers, composites, and flexible electronic interfaces (Table 1). In the following sections, we will summarize the use of different platforms to control cellular behavior via applied electrical stimuli with a specific emphasis on neural regeneration (Table 1).

4.1 Films

Recording the electrical and neural signals through implantable electrodes and probes is important to provide direct measurement of cellular function or communication between the brain/nervous system and machines. CPs are considered as potential coating materials, creating thin films on the probe surfaces due to their high surface area and conductivity, which can enable effective ion exchange between recording sites and the surrounding tissue.[127, 128] It was reported that platinum electrodes coated with PANI via in situ polymerization enhanced the aggregation of retinal fragments and demonstrated long-term stability along with significant promise for the reduced inflammation and scar formation.[129] In another study, microfabricated electrode arrays were coated by PEDOT: PSS providing high charge injection accompanied by safe and efficient stimulation of central auditory system.[130]

CPs can also be used to fabricate stand-alone films as implantable platforms to electrically stimulate cells. It was reported that PC12 cells seeded on PPy films responded to the applied electrical stimuli (100 mV) by showing significant neurite extension (18.14 μm)[100] due to electrical stimuli-induced protein adsorption from serum-containing medium.[131] Besides self-standing films, the CPs can also be used as coating materials on biodegradable polymer-based films/membranes. In a study, PC12 cells demonstrated neuronal differentiation and neurite outgrowth as a result of the electrical stimuli applied though PPy-coated poly(D,L-lactide-co-ε-caprolactone) membranes. These platforms also demonstrated significant axonal regeneration on rat sciatic nerve model.[101]

As an alternative to coatings or composites with other polymers, CPs-based copolymers can also be developed. It was shown that electrically stimulated PC12 cells significantly extended neurites on conductive films made up of PLA and carboxyl-capped aniline pentamer-based copolymer.[102] The copolymer-based conductive films can further be modified with biologically active components. N-hydroxyl succinimidyl ester pyrrole; copolymer-based films were further modified with NGF through surface immobilization. The synergistic effect of NGF and electrical stimulation resulted in neurite extension of PC12 cells.[103]

The electrically conductive platforms can also be used in combination with Schwann cells, which are the key players of the peripheral nervous system contributing to myelination and axonal regeneration. Schwann cells, seeded on polypyrrole–chitosan based composite films, demonstrated enhanced cell viability and paracrine activity (secretion of NGF and brain-derived neurotrophic factor (BDNF) (Fig. 1a), along with upregulated gene expression (Fig. 1b) upon applied constant potential gradient (100 mV/mm).[104]

Overall, conductive polymer-based films demonstrated potential to control cell behavior; however, lack of a natural 3D microstructure is

Table 1. Summary of the used platforms to apply electrical stimuli for neural regeneration

Platform type	Polymer material	Electrical stimuli	Cellular response	References
Film	PPy	100 mV	Neurite extension in PC12 cells (18.14 μm)	100
	PPy coated poly(D,L-lactide-co-ε-caprolactone) membranes	No external stimuli	Neuronal differentiation and neurite outgrowth of PC12 cells. Axonal regeneration on rat sciatic nerve model	101
	PLA and carboxyl-capped aniline pentamer-based copolymer	No external stimuli	PC12 cells significantly extended the neurites	102
	NGF-modified n-hydroxyl succinimidyl ester pyrrole copolymer	No external stimuli	Neurite extension of PC12 cells	103
	Polypyrrole–chitosan based composite	100 mV/mm	Seeded Schwann cells demonstrated enhanced cell viability, paracrine activity, and upregulated gene expression	104
Hydrogel	PANI and polyethyleneglycol diacrylate (PEGDA)-based microporous hydrogels (conductivity: 1.1×10^{-3} mS cm^{-1})	No external stimuli	Improved biological response of PC12 and human mesenchymal stem cells (hMSCs)	105
	PPy and cellulose-based nanoporous hydrogels (conductivity: 80 mS cm^{-1})	No external stimuli	PC12 cells showed enhanced viability, adhesion, proliferation, and induced neurite extension	106
	PPy/alginate hydrogels	No external stimuli	Improved adhesion and growth of hMSCs as well as induced expression of neural differentiation markers (i.e., Tuj1 and MAP2). In vivo biocompatibility	107
	PPy, and carboxymethyl chitosan-based hydrogel (conductivity: 2.41 mS cm^{-1})	No external stimuli	Improved cell adhesion and proliferation for PC12 and bone marrow-derived MSCs as well as high in vivo biocompatibility	108
	GO and chitosan-based hydrogel	No external stimuli	Enhanced the nerve cell growth by 20%	109
	Polyacrylamide, GO, gelatin, and sodium alginate-based composite hydrogel	No external stimuli	Improved adhesion and proliferation of Schwann cells along with the expression of myelination markers (Sox10, GAP43, and myelin basic protein)	110
	Single-walled carbon nanotubes (swCNTs) and collagen-based hydrogel	50 mV/mm DC	Improved neurite outgrowth sevenfold	111
	swCNTs and/or PPy incorporated hyaluronic acid (HA) hydrogels	No external stimuli	Supported neuronal differentiation of hNSPCs. Improved electrophysiological cellular function through the upregulation of calcium channel expression, activation of depolarization, and increase in calcium influx	112
	Multi-walled CNTs (mwCNTs) incorporated polyacrylamide/polyethylene glycol hydrogels	1 h at 30 V m^{-1} DC	Significant alignment and directional extension in neurite outgrowth of PC12 cells	113
	GO/CNTs incorporated oligo(poly(ethylene glycol) fumarate) hydrogel (conductivity: ~5.75×10^{-2} mS cm^{-1})	No external stimuli	Good biocompatibility accompanied by enhanced proliferation, spreading, and neurite extension of PC12 cells	114

Table 1. (continued)

Platform type	Polymer material	Electrical stimuli	Cellular response	References
Scaffold	PEDOT/chitosan/gelatin-based scaffolds	No external stimuli	Promoted PC12 cell adhesion, proliferation, and neurite growth by upregulating protein and gene expression levels	115
Conduits	PANI	10 mV/cm to 2 V/cm	Enhanced hMSCs growth and proliferation leading to formation of neural-like cells	116
	PANI/Poly-L-Lactic acid (PLLA) blend	100 mV/mm for a period of 60 min	Facilitated neurite extension of 24 ± 4 μm in rat nerve cells	117
	PPy/poly(DL-lactic acid) (PDLLA)-based conduits (conductivity: 5.65 to 15.56 mS cm^{-1})	100 mV electrical stimuli for 2 h	PC12 cells increased neurite extension upon the applied electrical stimuli. Conduits provided functional recovery in in vivo treatment of 1 cm long rat sciatic nerve transection model	98
Fibers	PPy-coated electrospun poly(lactic-co-glycolic acid) (PLGA) nanofibers	10 mV/cm	Random nanofibers provided a favorable microenvironment for the growth and differentiation of PC12 cells and hippocampal neurons. High number of neurites with longer neurite size upon electrical stimuli in aligned fibers	118
	PPy-coated electrospun poly(ε-caprolactone) (PCL) and poly(L-lactide) (PLA)-based nanofibers	Constant voltage of 10 V for 4 h per day during the period of culture	Dorsal root ganglia (DRG) cells attached, growth and extended neurites in the direction of nanofiber alignment in the presence of electrical stimuli	119
	PCL/PPy nanofibers (conductivity: 1.9 S cm^{-1})	No external stimuli	Supported PC12 cell viability and neurite extension	120
	PANI–PCL–gelatin-based nanofiber scaffold	DC voltage of 100 mV/mm for 1 h	Enhanced PC12 cell proliferation and neurite outgrowth (~30 μm) upon electrical stimulation	121
	PANI and tobacco mosaic virus (TMV)-based nanofibers	No external stimuli	Synergetic effect of electrical and topographical cues promoted the directed neurite outgrowth of PC12 cells	122
	Poly(lactic-co-glycolic acid) (PLGA) and multi-walled carbon nanotubes (MWCNTs)	Voltage of 40 mV	Aligned nanofibers provided directed cellular alignment and neurite outgrowth for PC12 and DRG cells upon electrical stimuli. Synergetic effect of nanofiber alignment and electrical stimuli also enhanced the cell attachment, proliferation and myelination of Schwann cells	123
	PPy-incorporated collagen fibers	biphasic waves of 5.2 V, square wave form electrical pulses of 1.2 V with pulse duration of 5 ms was delivered at a frequency of 200 Hz	hMSCs upregulated the expression of neural markers, such as noggin, MAP2, neurofilament, β tubulin III, and nestin, indicating their differentiation into neuronal-like phenotypes upon electrical stimuli	124
	Polyacrylonitrile and conductive carbon-based nanofibrous scaffolds	AC voltage of 5 V for 4 h during 7 days	Neural stem cells demonstrated neuronal differentiation and maturation through the upregulation of neuronal gene expression levels and MAP2 protein upon electrical stimulation	125

Table 1. (continued)

Platform type	Polymer material	Electrical stimuli	Cellular response	References
	Graphene–PEDOT hybrid microfibers	Step-driven TENG with outputs of 250 V and 30 μA. 3000 pulses/day triggered by human walking with a frequency of about 120 times/min	Differentiate MSCs into neural-like phenotypes through a self-powered electrical stimulation	76
Flexible electronic interfaces	CVD-grown graphene substrates	ELF-EMF; 50 Hz, 1 mT	Enhance neuronal differentiation of hMSCs	79
	Inkjet-printed and laser annealed graphene-based interdigitated circuits on polyimide substrates	100 mV at 50 Hz for 10 min per day during 15 days	MSCs were successfully differentiated into Schwann cells	95
	Graphene circuits transferred onto biodegradable porous polymers	100 mV at 50 Hz for 10 min per day during 10 days	MSCs were successfully differentiated into Schwann cells	126
	3D-printed graphene circuits embedded in gelatin scaffolds	100 mV at 50 Hz for 10 min per day during 10 days	MSCs were successfully differentiated into Schwann cells	94

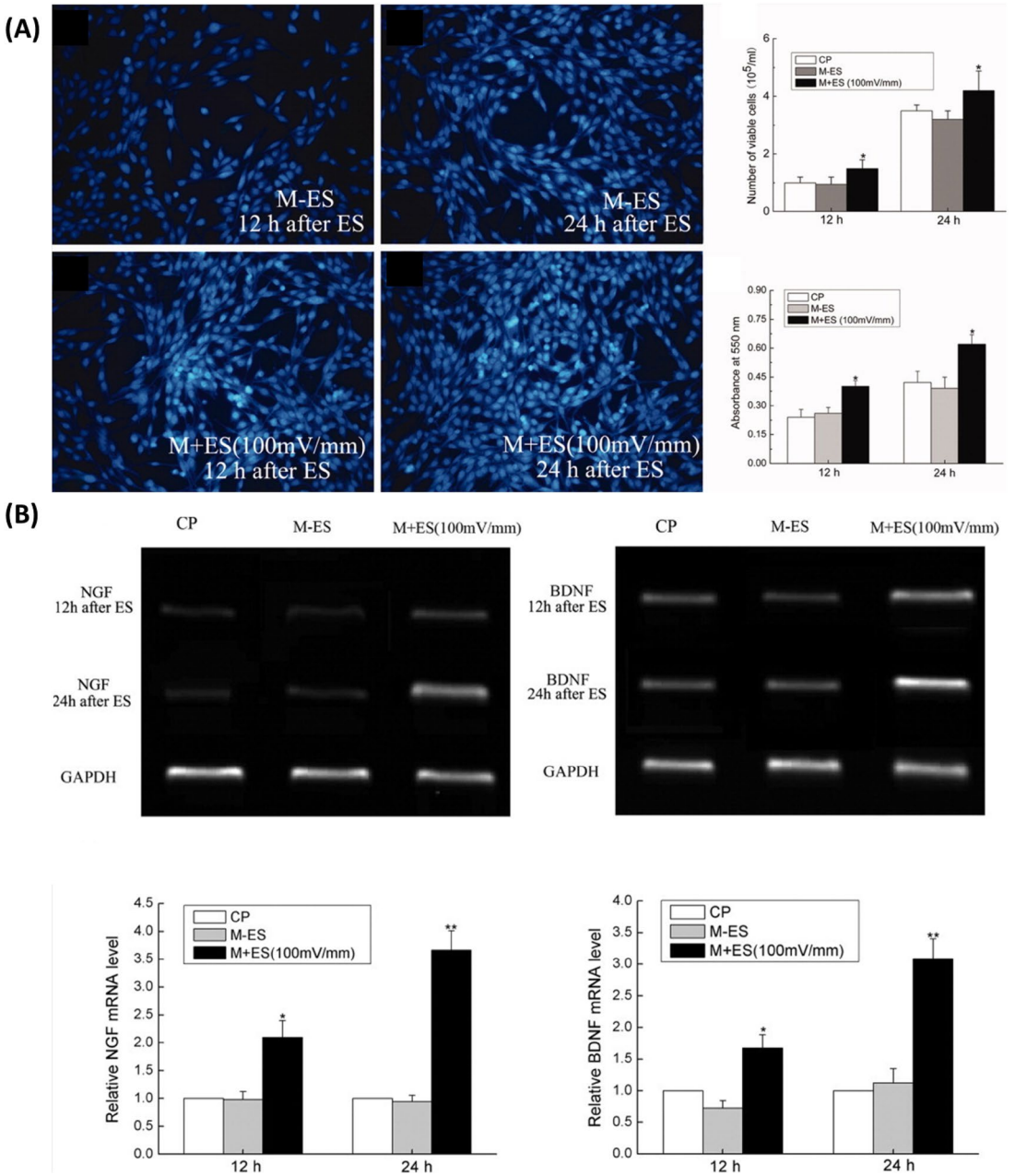


Figure 1 **a** Representative images of DAPI staining of Schwann cells. Magnification: × 200. The cell number count and MTT values in each group (*$p < 0.05$, one-way ANOVA). **b** mRNA levels of NGF and BDNF with or without electrical stimuli (ES) 12 and 24 h after ES. (*$p < 0.05$ and **$p < 0.01$, one-way ANOVA) Reproduced with permission from Huang et al.[104] Copyright 2009 Wiley Periodicals, Inc.

limiting complete mimicry of the ECM. For this reason, 3D hydrogels or scaffolds could be a better alternative.

4.2 Hydrogels and Scaffolds/Conduits

Besides electrical conductivity, the platforms should also have certain properties such as 3D porous structure, optimal mechanical properties, and topographical/physical cues to ensure complete mimicry of naturel ECM of the cells.[132] CPs-based hydrogels, scaffolds, or conduits with proper 3D microstructural and mechanical properties are considered as promising platforms enabling electrical stimuli-based cell manipulation within a 3D microstructure to promote neuronal proliferation and differentiation.

Hydrogels have a hydrated, flexible, and soft nature that makes them ideal for soft tissue applications. The added property of electrical conductivity through polymerization or cross-linking with CPs, further facilitates their use in neural tissue engineering.[61, 132] For example, Guarino et al. (2013) developed macroporous hydrogel platforms with a conductivity of 1.1×10^{-3} mS cm^{-1} through in situ precipitation of PANI in polyethyleneglycol diacrylate (PEGDA) matrix, which was then followed by UV cross-linking and sodium chloride particle leaching, leading to formation of microporous structure (136–158 μm pore size). These platforms provided improved biological response of PC12 and human mesenchymal stem cells (hMSCs) due to their favorable 3D microstructure and electrical conductivity.[105] Similarly, Shi et al. (2014) developed conductive PPy and cellulose-based nanoporous hydrogels with a conductivity of 80 mS cm^{-1}, through in situ polymerization of PPy monomers within nanoporous cellulose gel matrix. Their results indicated that PC12 cells showed enhanced viability, adhesion, proliferation, and induced neurite extension on the developed conductive hydrogel platforms.[106] In another study, Yang et al. (2016) developed PPy/alginate hydrogels with enhanced mechanical and electrical properties via chemical polymerization of PPy within ionically cross-linked alginate hydrogel network as multifunctional neural tissue engineering platforms. Their in vitro studies showed that the developed PPy/alginate hydrogel platforms improved the adhesion and growth of hMSCs as well as inducing the expression of neural differentiation markers (i.e., Tuj1 and MAP2) (Fig. 2a). In addition, the in vivo studies demonstrated that the platforms are sufficiently biocompatible, suggesting the promise of further examining the influence of electrical and mechanical signals on stem cells and/or neural cells using these platforms (Fig. 2b).[107]

Using a different approach, Bu et al. (2018) synthesized sodium alginate, PPy, and carboxymethyl chitosan-based hydrogel with a conductivity of 2.41 mS cm^{-1} as a supporting platform to be used in the conduit systems for peripheral nerve regeneration. They used a different synthesis approach, where calcium cross-linked alginate/carboxymethyl chitosan hydrogels with controlled release properties were further coated with conductive PPy particles, to easily manipulate the hydrogel properties, such as swelling, gelation, elasticity, porosity, and electrical conductivity. These platforms provided improved cell adhesion and proliferation properties for PC12 and bone marrow-derived MSCs as well as high in vivo biocompatibility.[108]

Conductive carbon-based materials, such as GO or CNTs, can also be used in hydrogel formulations as an alternative to CPs. Considering this, Jafarkhani et al. (2018) mixed conductive GO with chitosan powder, and further reacted with lactic acid to synthesize conductive hydrogels possessing different mechanical and structural properties. Their results suggested that the optimum mechanical and structural properties enhanced the nerve cell growth by 20%, demonstrating the potential benefit of these platforms.[109] Similarly, Zhao et al. (2018) developed polyacrylamide, GO, gelatin, and sodium alginate-based composite hydrogel for peripheral nerve regeneration applications. They reported that the microstructural and mechanical properties of hydrogels can be controlled by the amount of GO, which in turn determines the adhesion and proliferation of Schwann cells along with the expression of myelination markers (Sox10, GAP43, and myelin basic protein).[110]

As an alternative to GO, CNTs can also be used as potential conductive carbon-based material. In a study by Koppes et al. (2016), the researchers used single-walled carbon nanotubes (swCNTs) to optimize the electrical properties of collagen-based hydrogels. They reported that the electrical conductivity of hydrogels was increased without any negative effect on hydrogel matrix properties (such as microstructure or elasticity) as the swCNTs among increased, which in turn improved neurite outgrowth by sevenfold through the applied electrical stimuli compared to all other control groups.[111] In a different study, Shin et al. compared the influence of swCNTs and/or PPy-incorporated hyaluronic acid (HA) hydrogels on human neural stem/progenitor cells (hNSPCs) differentiation.

The hydrogels (with swCNTs and/or PPy) provided dynamic and electrically conductive 3D microenvironments that are supportive for neuronal differentiation of hNSPCs. The platforms improved electrophysiological cellular function through the upregulation of calcium channel expression, activation of depolarization, and increase in calcium influx, hence demonstrating the future promise of these platforms to improve neuronal regeneration.[112] As an alternative to swCNTs, Imaninezhad et al. (2018) developed multi-walled CNTs (mwCNTs) incorporating polyacrylamide/polyethylene glycol hydrogels, which provided significant alignment and directional extension in neurite outgrowth of PC12 cells in response to electrical stimulation.[113] In

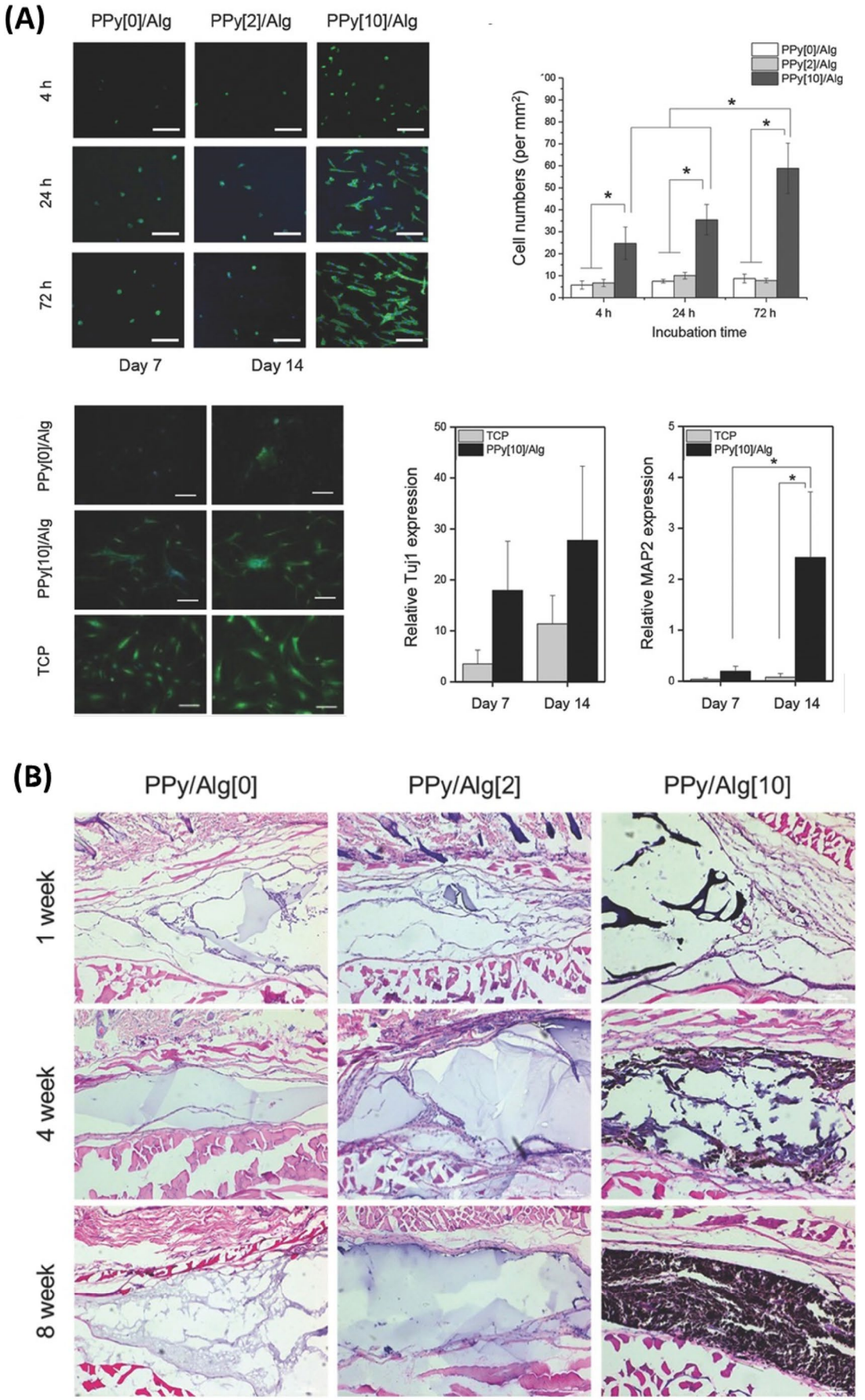

(A)
PPy[0]/Alg
PPy[2]/Alg
PPy[10]/Alg
4 h
24 h
72 h
Day 7
Day 14
Cell numbers (per mm²)
Incubation time
TCP
Relative Tuj1 expression
Relative MAP2 expression
(B)
PPy/Alg[0]
PPy/Alg[2]
PPy/Alg[10]
1 week
4 week
8 week

Figure 2 **a** In vitro hMSC culture onto PPy/Alg hydrogels for 4, 24, and 72 h. Scale bars: 200 μm. Cell numbers on the samples. Relative Tuj1 and MAP2 gene expression levels of the cells cultured on the PPy[10]/Alg and TCP for 14 days. Individual bars indicate the average ± the standard error of the mean. An asterisk (*) denotes a statistical significance between two groups ($p < 0.05$). **b** HE staining of histological sections subcutaneously implanted with various PPy/Alg gels for 1, 4, and 8 weeks. Scale bars represent 100 μm Reproduced with permission from Yang et al.[107] Copyright 2016 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

another study Liu et al. (2017) demonstrated the potential of combined use of GO and CNTs through cross-linkable bonds. They covalently embedded the cross-linked GO/CNTs into oligo(poly(ethylene glycol) fumarate) hydrogel to provide surface charge and electrical conductivity of $\sim 5.75 \times 10^{-2}$ mS cm^{-1}. The composite hydrogel platforms showed good biocompatibility accompanied by enhanced proliferation and spreading of PC12 cells. The electrical stimuli applied through the hydrogel platforms in the form of conduits promoted the neurite extension of PC12 cells depicting potential for neural tissue engineering.[114]

Scaffolds can be considered in the form of a template with defined 3D geometrical shapes and internal configuration, filled with conductive material-based hydrogels, and further freeze-dried to obtain 3D porous microstructure.[132] For example, Wang et al. (2017) developed PEDOT/chitosan/gelatin-based scaffolds through in situ interfacial polymerization of PEDOT nanoparticles on porous chitosan/gelatin scaffolds. The electrical conductivity, hydrophilicity, mechanical properties, and thermal stability of the scaffolds were improved through the incorporation of PEDOT, which at on the other hand, reduces the water absorption and biodegradability of scaffolds. Nevertheless, their results indicated that these platforms significantly promoted PC12 cell adhesion, proliferation, and neurite growth by upregulating protein and gene expression levels through their conductive nature.[115] In another study, Gupta et al. (2019) investigated the influence of morphological differences between graphene nanoplatelets (GNP) and multi-walled carbon nanotubes (MWCNT) on neural cell regeneration. They developed GNP- and MWCNT-incorporated chitosan scaffolds with different electrical conductivity and mechanical properties. They reported that the cellular responses, such as protein adsorption, cell adhesion, cytotoxicity, and alignment, change with respect to the material properties.[133] The same group developed MWCNT-aligned chitosan scaffolds to control the directional neuronal growth and cellular alignment via electrical cues. Their results indicated that this platform can successfully control the alignment in 50–60% of neurons.[134]

Conduits are considered a special form of hydrogels and scaffolds with cylindrical tube geometry, and particularly used for peripheral nerve regeneration applications. For example, electrical stimulation via PANI-based conduits, providing electrical field of 10 mV/cm to 2 V/cm, demonstrated enhanced hMSCs growth and proliferation through the alteration of cytoskeletal arrangement producing long filopodial extensions and leading to formation of neural-like cells.[116] Similarly, electrical stimuli applied through a blend of PANI/Poly-L-Lactic acid (PLLA) conduit resulted in neurite extension of 24 ± 4 μm in rat nerve cells.[117] In another work, Xu et al. (2014) developed PPy/poly(DL-lactic acid) (PDLLA)-based conduits with the conductivity range of 5.65 to 15.56 mS cm^{-1} via oxidative polymerization. The PC12 cells seeded on PPy/PDLLA conduits showed increased neurite extension upon the applied 100 mV electrical stimuli for 2 h (Fig. 3a). The same conduits were used for the in vivo treatment of 1 cm long rat sciatic nerve transection model and the results indicated that the used conduits provided functional recovery (Fig. 3b) similar to that of autologous nerve grafts, a gold standard for the peripheral nerve injury treatment.[98]

As a brief summary, the 3D microstructured platforms with multiple functionalities in the form of hydrogels, scaffolds, and conduits hold significant promise to pave the way for the development of new strategies to enhance neural tissue regeneration.

4.3 Electrospun Fibers

Fibers can be considered as another type of 3D scaffolds possessing inherent characteristics to mimic the ECM microenvironment for tissue engineering applications. Although most of the fibers used are non-conductive, electrical conductivity can be provided through CPs coating or direct fabrication of fibers using CPs. Electrospinning is the most common process enabling fiber fabrication using a wide range of polymers, including CPs, at nano- and micrometer-scale,

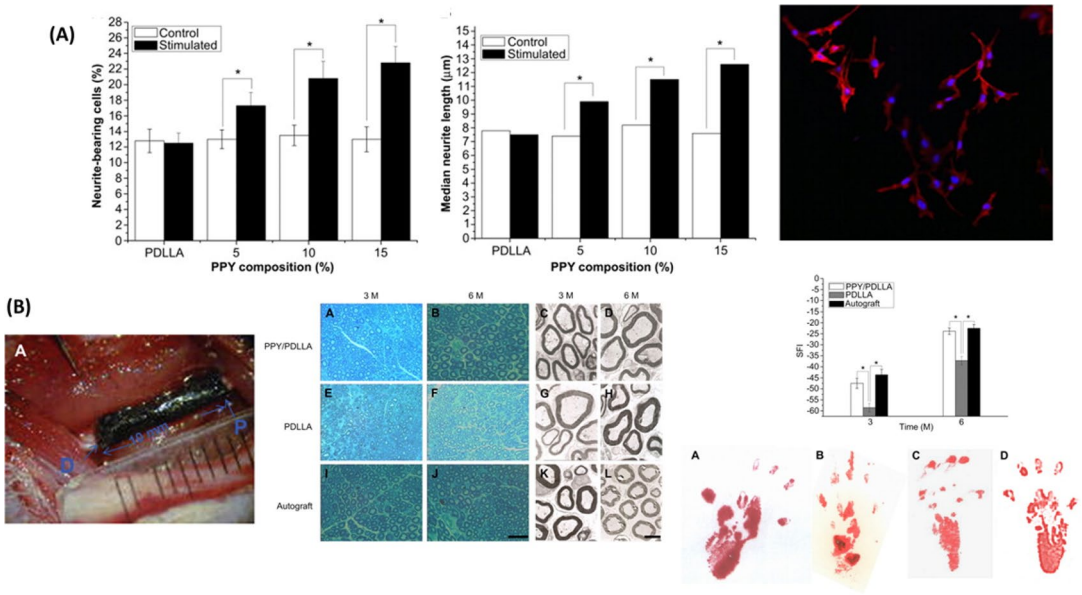


Figure 3 **a** Percentage of neurite-bearing PC12 cells and median neurite length on PPY/PDLLA composite films with varying PPY composition ($n=4$, $^*p<0.05$). **b** Intraoperative photographs of the PPY/PDLLA nerve conduits. "P" signifies the proximal end and "D" signifies the distal end. Histology images stained with methylene blue and transmission electron microscopy (TEM) micrographs. Recovery of sciatic nerve function. Sciatic function index (SFI) as a function of implantation time (top). Footprint stamps in walking track analysis after 6 months of implantation (bottom). (A) PPY/PDLLA. (B) PDLLA. (C) Autograft. (D) Normal left leg Reproduced with permission from Xu et al.[98] Copyright 2013 Elsevier Ltd.

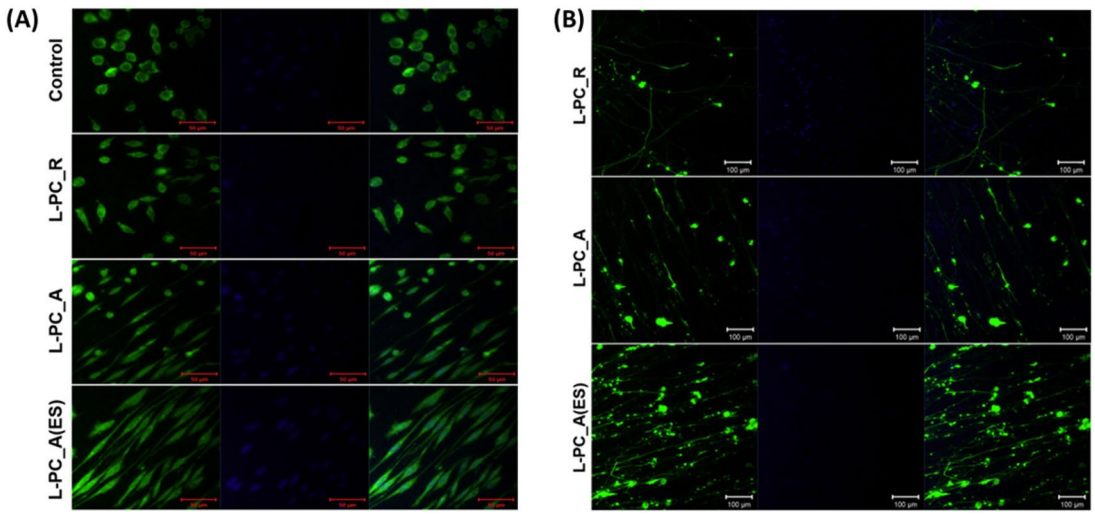


Figure 4 **a** The expression of NF200 on poly-L-lysine-coated random and aligned nanofibers. **b** The neurite extension of DRG neurons on poly-L-lysine-coated random and aligned nanofibers Reproduced with permission from Wang et al.[123] Copyright 2018 Elsevier B.V.

supporting adhesion, and guiding extension of neurons for nerve regeneration.[132, 135] For instance, Lee et al. (2009) developed electrically conductive random or aligned fiber structures through the coating of PPy on electrospun poly(lactic-co-glycolic acid) (PLGA) nanofibers. Their random nanofibers provided a favorable microenvironment for the growth and differentiation of PC12 cells and hippocampal neurons, while application of electrical stimuli (10 mV/cm) through the nanofibers resulted in formation of high number of neurites with longer neurite

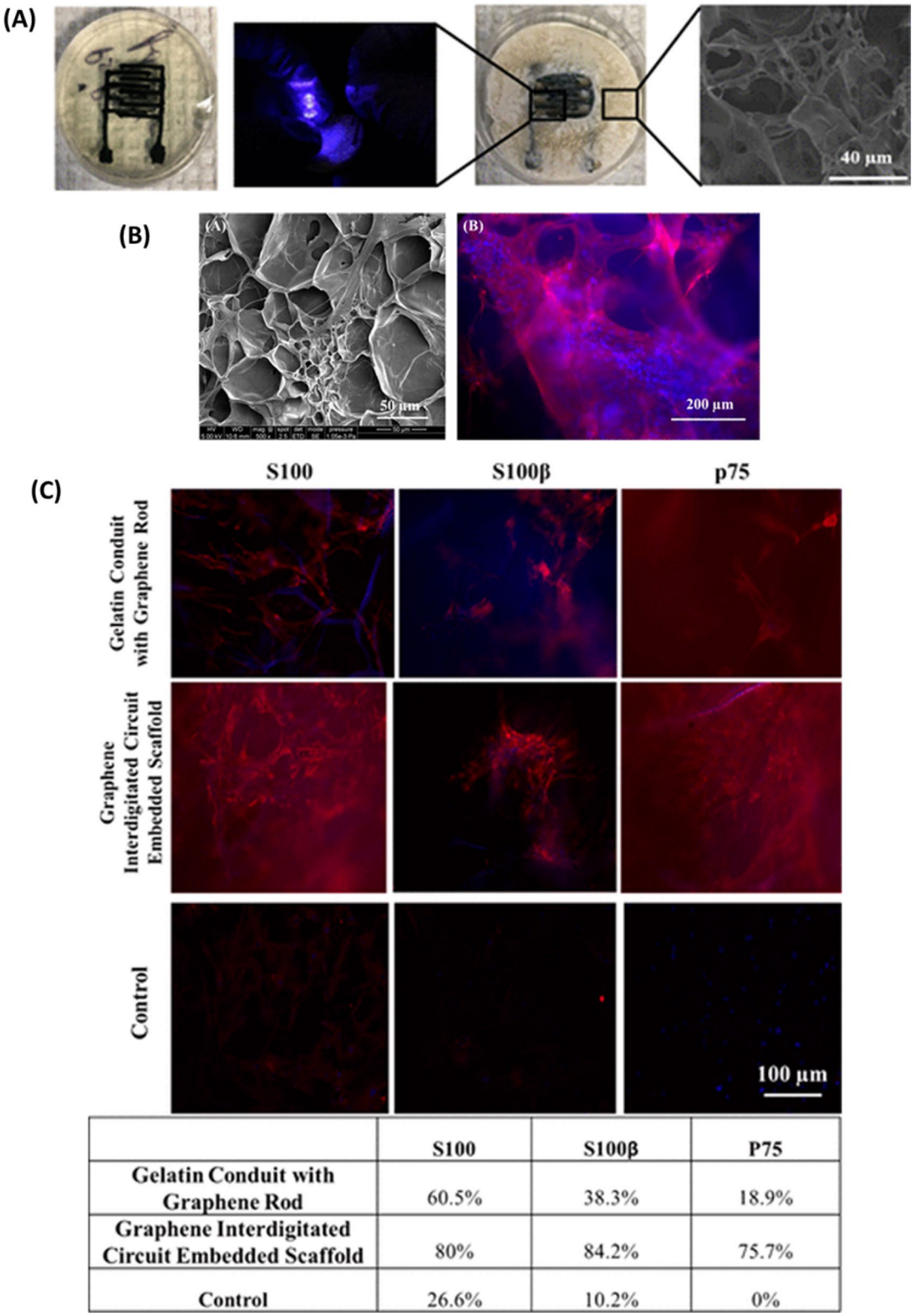
size. They further reported that the same electrical stimuli applied via the aligned nanofibers led to even longer neurites and more neurite-bearing cells than that of the random ones.[118] In a similar study, Xie et al. (2009) developed electrospun poly(ε-caprolactone) (PCL) and poly(L-lactide) (PLA)-based nanofibers, further coated with PPy through in situ polymerization. They observed that dorsal root ganglia (DRG) cells attached to grown and extended neurites in the direction of nanofiber alignment in the presence of electrical stimuli revealing the potential of these platforms for neural tissue engineering applications.[119] In the work of Shafei et al. (2017), a novel approach based on electrospinning and vapor-phase polymerization, was used to fabricate highly conductive (1.9 S cm^{-1}) PCL/PPy nanofibers supporting PC12 cell viability and neurite extension.[120] In similar work, Ghasemi-Mobarakeh et al. (2009) designed conductive nanofiber scaffolds made of PANI–PCL–gelatin, which enhanced PC12 cell proliferation and neurite outgrowth (~ 30 μm) upon electrical stimulation (DC voltage of 100 mV/mm for 1 h).[121] In a different study, Wu et al. (2015) used a different material to fabricate electroactive nanofibers. They applied in situ polymerization of conductive PANI on the surface of non-conductive scaffold material, tobacco mosaic virus (TMV), in the presence of dopant, poly(styrenesulfonate) (PSS). They concluded that the synergetic effect of electrical and topographical cues promoted the directed neurite outgrowth of PC12 cells.[122] In another study, Wang et al. (2018) developed surface modified, electrically conductive, aligned nanofibrous scaffolds composed of poly(lactic-co-glycolic acid) (PLGA) and multi-walled carbon nanotubes (MWCNTs) in the form of conduits for neural regeneration. They observed that the topographically aligned nanofibers provided directed cellular alignment and neurite outgrowth for PC12 (Fig. 4a) and DRG (Fig. 4b) cells in the presence of electrical stimulation (voltage of 40 mV). In addition, the synergetic effect of nanofiber alignment and electrical stimuli also enhanced the cell attachment, proliferation, and myelination of Schwann cells.[123]

Besides the in vitro work on PC12 or DRG cells, the studies on the stem cells also show great potential for neural tissue repair. In that sense, the use of fibers providing topographical, biochemical, and electrical cues to control the stem cell behaviors such as differentiation into desired cell lineages, including neuronal or glial cells, has also been investigated. In the work of Yow et al. (2011), PPy-incorporated collagen fibers with significant mechanical and electrical properties were prepared using interfacial polyelectrolyte complexation. After applying 10 days of electrical stimuli to the hMSCs seeded on the developed fibers in proliferating medium, they observed that hMSCs upregulated the expression of neural markers, such as noggin, MAP2, neurofilament, β tubulin III, and nestin, indicating their differentiation into neuronal-like phenotypes, accompanied by slight reduction in hMSCs viability.[124] In addition to the CPs, carbon-based conductive materials can also be used in combination with biodegradable and biocompatible non-conductive polymers in the form of nanofibers for the differentiation of stem cells. For instance, Zhu et al. (2018) developed polyacrylonitrile and conductive carbon-based nanofibrous scaffolds with high flexibility and conductivity using high temperature annealing method. They reported that the neural stem cells seeded on the scaffolds demonstrated neuronal differentiation and maturation through the upregulation of neuronal gene expression levels and MAP2 protein upon the application of biphasic electrical stimulation (AC voltage of 5 V for 4 h during 7 days).[125] In a different study, Guo et al. (2016) developed graphene–PEDOT hybrid microfibers to differentiate MSCs into neural-like phenotypes through a self-powered electrical stimulation without bio/chemical cues.[76]

Overall, these studies demonstrated the potential use of conductive CPs-based fibers as promising platforms for neural tissue engineering applications.

4.4 Flexible Electronic Interfaces

The graphene is a well-known material due to its excellent mechanical and electric properties, allowing its use in combination with other biomaterials as mechanical support or as conductive fillers to design functional and biocompatible hydrogel or scaffold platforms for tissue engineering applications.[64] Alternatively, conductive graphene has also been widely used in the development of implantable and flexible bioelectronic interfaces to be used in neural tissue engineering.[69] These flexible electronic interfaces with high resolution and low feature size conductive graphene circuits are mostly fabricated via inkjet printing, 3D printing/bioprinting, chemical vapor deposition (CVD), or various other graphene pattern transfer methods and used to control cellular function. For instance, Lee et al. (2015) used CVD-grown graphene substrate to enhance the neuronal differentiation of hMSCs



	S100	S100β	P75
Gelatin Conduit with Graphene Rod	60.5%	38.3%	18.9%
Graphene Interdigitated Circuit Embedded Scaffold	80%	84.2%	75.7%
Control	26.6%	10.2%	0%

Figure 5 **a** 3D printed circuit embedded in gelatin matrix. **b** MSCs attachment and formation of 3D cellular network. **c** Immunolabeling of Schwann cell marker after electrical stimuli Reproduced with permission from Uz et al.[94] Copyright 2019 American Chemical Society.

under low-frequency electromagnetic fields (ELF-EMF; 50 Hz, 1 mT). They demonstrated that successful differentiation was obtained as a result of ELF–EMF induced cell adhesion, upregulation of intracellular calcium influx, and activated focal adhesion kinase signaling pathway, producing extracellular matrix molecules. However, the success of this approach was relying on not only the electrical stimuli, but also the use of many different chemicals and growth factors triggering neuronal differentiation in their cell culture media. These chemicals and growth factors are expensive and not suitable for use in in vivo applications, limiting the clinical translation of this approach.[79] To address this issue, for the first time, we used inkjet printer and laser-annealed graphene-based interdigitated circuits on polyimide substrates as flexible bioelectronic interface to control the stem cell differentiation via sole electrical stimuli, free from any additional chemical induction. Our results indicated that the MSCs were successfully differentiated into Schwann cells by applying voltage of 100 mV at 50 Hz for 10 min per day for 15 days. We reported high immunolabeling of Schwann cell markers along with enhanced paracrine activity.[95] Considering the fact that the used polyimide substrate was not porous and biodegradable, we developed a novel method, enabling effective fabrication of biodegradable and flexible electronic interfaces via simple polymer casting method.[126] In this method, first conductive graphene-based micropatterns (or circuits) were developed on molds via microfluidic filling, inkjet printing or CVD. Then, by applying a polymer casting method, we were able to transfer the graphene patterns (or circuits) to the target substrate, by which we can control the microstructure, mechanical properties and also biodegradability. This is a versatile method that can be used for various synthetic or natural biodegradable polymers. The obtained biodegradable and implantable and flexible bioelectronic interfaces that demonstrated successful differentiation of MSCs into Schwann cells under same electrical stimuli conditions.[126] Although these flexible electronic platforms are porous and biodegradable, they still do not possess actual 3D microstructure creating a favorable microenvironment for the formation of 3D cellular network. Considering this, we created a 3D printed graphene-based circuit and embedded it into a gelatin matrix possessing actual 3D microstructure and desired mechanical properties (Fig. 5a, b). Following the application of electrical stimuli, we observed MSCs to Schwann cell differentiation accompanied by significantly enhanced paracrine activity (Fig. 5c), which was due to the synergistic effort of 3D microstructural/mechanical properties and electrical stimuli.[94]

The application of biodegradable and implantable bioelectronic interfaces for the control of stem cell differentiation and fate commitment is a promising approach for future neural regeneration strategies. This is a new hot topic in the field and more research is needed in this aspect.

5 Potential Cellular Mechanisms

The influence of electrical fields during the embryonic development along with the existing electrical signals throughout our body, particularly in our nervous system, has been known for centuries. However, the exact role of electrical fields and signaling such as time-dependent voltage gradients, ion fluxes or regulation of synapses in developmental processes have only recently been investigated.[46, 47, 50] In the literature, there have been some efforts to connect cellular behaviors, such as proliferation,[136] migration,[137] protein/growth factor secretion,[138] adhesion, and differentiation,[139] with the applied electrical stimuli through the regulation of various signaling pathways such as FAK and p38[75, 76, 79, 140–142] ion channels and ERK pathway,[143–145] MAPK, PI3K, and ROCK,[97, 146] and ROS.[97, 147] Some studies have also suggested that the electrically induced intracellular Ca^{2+} signaling along with the signaling pathways related to ferritin serve as a novel regulatory mechanism controlling the neural differentiation of MSCs.[148] For the CPs, it is claimed that the expulsion of negative ions through the neutralization of CPs or uptake of positive ions such as Na^{+} from the medium has several effects on cellular processes, including protein adsorption and the cell cycle.[64] Despite the promising attempts to elucidate the effect of electrical stimuli on cellular processes, further investigation needs to be performed to understand the physicochemical mechanisms of this phenomenon.

6 Conclusions and Future Perspectives

The conductive polymer or carbon-based platforms hold significant potential to manipulate cellular behavior and promote neural regeneration. However, most of the ongoing research is at pre-clinical level as opposed to clinical trials for the application of conductive platforms for neural tissue engineering. Thus, the properties of these platforms need further improvements to

provide precise control of cellular mechanisms to enable clinical translation. The improvements in microstructural and mechanical properties of these platforms along with the electrical conductivity and biodegradability can enable better mimicry of the ECM, which in turn allows enhanced control of implanted cells and facilitate clinical translation. There are still number of unknown questions regarding the relationship between the electrical properties and cellular functions, particularly from a mechanistic perspective. Therefore, elucidating the regulated genes, proteins, and cellular pathways upon electrical stimuli with a desirable mimicry of ECM environment will pave the way for the development of novel and functional platforms. In addition, most of the conductive materials are not biodegradable. Therefore, the use of conductive platforms that are also biodegradable, in tissue engineering applications, can potentially open the way for minimally invasive implantation, which can prevent additional surgical interventions improving the comfort of patients. Furthermore, development of conductive and biodegradable polymeric platforms that are capable of manipulating cells through remotely applied electrical stimuli will be the next generation approach to provide a minimally invasive strategy for patients, not only for neural tissue engineering, but also for other tissue engineering areas, including skin, muscle, and cardiac and even brain–computer interfaces. In conclusion, the conductive polymer and hydrogel-based platforms are promising and have a lot of potential to address neural tissue engineering problems, but there are still certain gaps in this field that need immediate attention to accelerate clinical translation.

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References

1. Gooch CL, Pracht E, Borenstein AR (2017) The burden of neurological disease in the United States: a summary report and call to action. Ann Neurol 81:479–484. https://doi.org/10.1002/ana.24897
2. Krueger H, Noonan VK, Trenaman LM, Joshi P, Rivers CS (2013) The economic burden of traumatic spinal cord injury in Canada. Chronic Dis Inj Can 33:113–122
3. Feigin VL et al (2017) Global, regional, and national burden of neurological disorders during 1990–2015: a systematic analysis for the global burden of disease study 2015. Lancet Neurol 16:877–897. https://doi.org/10.1016/S1474-4422(17)30299-5
4. Furlan JC, Gulasingam S, Craven BC (2017) The health economics of the spinal cord injury or disease among veterans of war: a systematic review. J Spinal Cord Med 40:649–664. https://doi.org/10.1080/10790268.2017.1368267
5. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S (2019) Traumatic spinal cord injury: an overview of pathophysiology, models and acute injury mechanisms. Front Neurol. https://doi.org/10.3389/fneur.2019.00282
6. Chiono V, Tonda-Turo C (2015) Trends in the design of nerve guidance channels in peripheral nerve tissue engineering. Prog Neurobiol 131:87–104. https://doi.org/10.1016/j.pneurobio.2015.06.001
7. Gu X (2015) Progress and perspectives of neural tissue engineering. Front Med 9:401–411. https://doi.org/10.1007/s11684-015-0415-x
8. Gu X, Ding F, Williams DF (2014) Neural tissue engineering options for peripheral nerve regeneration. Biomaterials 35:6143–6156. https://doi.org/10.1016/j.biomaterials.2014.04.064
9. Grinsell D, Keating CP (2014) Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies. Biomed Res Int 2014:13. https://doi.org/10.1155/2014/698256
10. Uz M, Altınkaya SA (2011) Development of mono and multilayer antimicrobial food packaging materials for controlled release of potassium sorbate. LWT-Food Sci Technol 44:2302–2309
11. Bunge RP (1994) The role of the Schwann cell in trophic support and regeneration. J Neurol 242:S19–S21
12. Ide C (1996) Peripheral nerve regeneration. Neurosci Res 25:101–121
13. Jessen KR, Mirsky R (1999) Schwann cells and their precursors emerge as major regulators of nerve development. Trends Neurosci 22:402–410
14. Mahanthappa NK, Anton ES, Matthew WD (1996) Glial growth factor 2, a soluble neuregulin, directly increases Schwann cell motility and indirectly promotes neurite outgrowth. J Neurosci 16:4673–4683

15. Terenghi G (1999) Peripheral nerve regeneration and neurotrophic factors. J Anat 194:1–14. https://doi.org/10.1046/j.1469-7580.1999.19410001.x
16. Jessen KR, Mirsky R (2016) The repair Schwann cell and its function in regenerating nerves. J Physiol 594:3521–3531. https://doi.org/10.1113/jp270874
17. Gao Y et al (2015) Nerve autografts and tissue-engineered materials for the repair of peripheral nerve injuries: a 5-year bibliometric analysis. Neural Regener Res 10:1003–1008. https://doi.org/10.4103/1673-5374.158369
18. Hondred JA, Stromberg LR, Mosher CL, Claussen JC (2017) High-resolution graphene films for electrochemical sensing via inkjet maskless lithography. ACS Nano. https://doi.org/10.1021/acsnano.7b03554
19. Guenard V, Kleitman N, Morrissey TK, Bunge RP, Aebischer P (1992) Syngeneic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration. J Neurosci 12:3310–3320
20. Mosahebi A, Fuller P, Wiberg M, Terenghi G (2002) Effect of allogeneic Schwann cell transplantation on peripheral nerve regeneration. Exp Neurol 173:213–223. https://doi.org/10.1006/exnr.2001.7846
21. Mosahebi A, Woodward B, Wiberg M, Martin R, Terenghi G (2001) Retroviral labeling of Schwann cells: in vitro characterization and in vivo transplantation to improve peripheral nerve regeneration. Glia 34:8–17
22. Rodriguez FJ, Verdu E, Ceballos D, Navarro X (2000) Nerve guides seeded with autologous schwann cells improve nerve regeneration. Exp Neurol 161:571–584. https://doi.org/10.1006/exnr.1999.7315
23. Rutkowski GE, Miller CA, Jeftinija S, Mallapragada SK (2004) Synergistic effects of micropatterned biodegradable conduits and Schwann cells on sciatic nerve regeneration. J Neural Eng 1:151–157. https://doi.org/10.1088/1741-2560/1/3/004
24. Sandquist EJ et al (2016) Neural engineering. Springer, New York, pp 25–81
25. Dezawa M, Takahashi I, Esaki M, Takano M, Sawada H (2001) Sciatic nerve regeneration in rats induced by transplantation of in vitro differentiated bone-marrow stromal cells. Eur J Neurosci 14:1771–1776
26. Jiang Y et al (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418: 41–49. http://www.nature.com/nature/journal/v418/n6893/suppinfo/nature00870_S1.html. Accessed 22 July 2019
27. Keilhoff G, Goihl A, Stang F, Wolf G, Fansa H (2006) Peripheral nerve tissue engineering: autologous Schwann cells vs. transdifferentiated mesenchymal stem cells. Tissue Eng 12:1451–1465. https://doi.org/10.1089/ten.2006.12.1451
28. Pittenger MF et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
29. Gaertner A et al (2012) Use of poly(DL-lactide-epsilon-caprolactone) membranes and mesenchymal stem cells from the Wharton's jelly of the umbilical cord for promoting nerve regeneration in axonotmesis: in vitro and in vivo analysis. Differentiation 84:355–365. https://doi.org/10.1016/j.diff.2012.10.001
30. Ladak A, Olson J, Tredget EE, Gordon T (2011) Differentiation of mesenchymal stem cells to support peripheral nerve regeneration in a rat model. Exp Neurol 228:242–252. https://doi.org/10.1016/j.expneurol.2011.01.013
31. Lee EJ et al (2012) Regeneration of peripheral nerves by transplanted sphere of human mesenchymal stem cells derived from embryonic stem cells. Biomaterials 33:7039–7046. https://doi.org/10.1016/j.biomaterials.2012.06.047
32. Liu Y et al (2014) Conserved dopamine neurotrophic factor-transduced mesenchymal stem cells promote axon regeneration and functional recovery of injured sciatic nerve. PLoS ONE. https://doi.org/10.1371/journal.pone.0110993
33. Tseng T-C, Hsu S-H (2014) Substrate-mediated nanoparticle/gene delivery to MSC spheroids and their applications in peripheral nerve regeneration. Biomaterials 35:2630–2641. https://doi.org/10.1016/j.biomaterials.2013.12.021
34. Zhuang H et al (2016) Gelatin-methacrylamide gel loaded with microspheres to deliver GDNF in bilayer collagen conduit promoting sciatic nerve growth. Int J Nanomed 11:1383–1394. https://doi.org/10.2147/ijn.s96324
35. Morrison SJ, Spradling AC (2008) Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell 132:598–611. https://doi.org/10.1016/j.cell.2008.01.038
36. Scadden DT (2006) The stem-cell niche as an entity of action. Nature 441:1075–1079
37. Discher DE, Mooney DJ, Zandstra PW (2009) Growth factors, matrices, and forces combine and control stem cells. Science (New York, N.Y.) 324:1673–1677. https://doi.org/10.1126/science.1171643
38. Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126:677–689. https://doi.org/10.1016/j.cell.2006.06.044
39. McBeath R, Pirone DM, Nelson CM, Bhadriraju K, Chen CS (2004) Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. Dev Cell 6:483–495
40. Sun Y, Chen CS, Fu J (2012) Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. Annu Rev Biophys 41:519–542. https://doi.org/10.1146/annurev-biophys-042910-155306
41. Thorpe SD, Buckley CT, Steward AJ, Kelly DJ (2012) European Society of Biomechanics S.M. Perren Award

2012: the external mechanical environment can override the influence of local substrate in determining stem cell fate. J Biomech 45:2483–2492. https://doi.org/10.1016/j.jbiomech.2012.07.024

42. Ariza CA et al (2010) The influence of electric fields on hippocampal neural progenitor cells. Stem Cell Rev 6:585–600. https://doi.org/10.1007/s12015-010-9171-0
43. Ariza CA, Mallapragada SK (2010) Advanced biomaterials. Wiley, Hoboken, pp 613–642
44. Pires F, Ferreira Q, Rodrigues CA, Morgado J, Ferreira FC (1850) Neural stem cell differentiation by electrical stimulation using a cross-linked PEDOT substrate: expanding the use of biocompatible conjugated conductive polymers for neural tissue engineering. Biochem Biophys Acta 1158–1168:2015. https://doi.org/10.1016/j.bbagen.2015.01.020
45. Stewart E et al (2015) Electrical stimulation using conductive polymer polypyrrole promotes differentiation of human neural stem cells: a biocompatible platform for translational neural tissue engineering. Tissue Eng Part C 21:385–393. https://doi.org/10.1089/ten.tec.2014.0338
46. Koyama S, Haruyama T, Kobatake E, Aizawa M (1997) Electrically induced NGF production by astroglial cells. Nat Biotechnol 15:164–166. https://doi.org/10.1038/nbt0297-164
47. Li L et al (2008) Direct-current electrical field guides neuronal stem/progenitor cell migration. Stem cells (Dayton, Ohio) 26:2193–2200. https://doi.org/10.1634/stemcells.2007-1022
48. Yao L, Shanley L, McCaig C, Zhao M (2008) Small applied electric fields guide migration of hippocampal neurons. J Cell Physiol 216:527–535. https://doi.org/10.1002/jcp.21431
49. Anderson M et al (2015) Peripheral nerve regeneration strategies: electrically stimulating polymer based nerve growth conduits. Crit Rev Biomed Eng 43:131–159. https://doi.org/10.1615/CritRevBiomedEng.2015014015
50. Schmidt CE, Shastri VR, Vacanti JP, Langer R (1997) Stimulation of neurite outgrowth using an electrically conducting polymer. Proc Natl Acad Sci USA 94:8948–8953
51. Willand MP, Nguyen MA, Borschel GH, Gordon T (2016) Electrical stimulation to promote peripheral nerve regeneration. Neurorehabilit Neural Repair 30:490–496. https://doi.org/10.1177/1545968315604399
52. Ghasemi-Mobarakeh L et al (2011) Application of conductive polymers, scaffolds and electrical stimulation for nerve tissue engineering. J Tissue Eng Regener Med 5:e17–e35. https://doi.org/10.1002/term.383
53. Le T-H, Kim Y, Yoon H (2017) Electrical and electrochemical properties of conducting polymers. Polymers. https://doi.org/10.3390/polym9040150
54. Nezakati T, Seifalian A, Tan A, Seifalian AM (2018) Conductive polymers: opportunities and challenges in biomedical applications. Chem Rev 118:6766–6843. https://doi.org/10.1021/acs.chemrev.6b00275
55. Tomczykowa M, Plonska-Brzezinska ME (2019) Conducting polymers, hydrogels and their composites: preparation, properties and bioapplications. Polymers. https://doi.org/10.3390/polym11020350
56. Lin L et al (2013) In vivo study on the monoamine neurotransmitters and their metabolites change in the striatum of Parkinsonian rats by liquid chromatography with an acetylene black nanoparticles modified electrode. J Pharm Biomed Anal 72:74–79. https://doi.org/10.1016/j.jpba.2012.09.011
57. Silva TA, Moraes FC, Janegitz BC, Fatibello-Filho O (2017) Electrochemical biosensors based on nanostructured carbon black: a review. J Nanomater 2017:14. https://doi.org/10.1155/2017/4571614
58. Johansson E, Larsson S (2004) Electronic structure and mechanism for conductivity in thiophene oligomers and regioregular polymer. Synth Met 144:183–191. https://doi.org/10.1016/j.synthmet.2004.03.005
59. Lee B, Seshadri V, Sotzing GA (2005) Water dispersible low band gap conductive polymer based on thieno[3,4-b]thiophene. Synth Met 152:177–180. https://doi.org/10.1016/j.synthmet.2005.07.231
60. Roncali J, Garreau R, Delabouglise D, Garnier F, Lemaire M (1988) Recent developments in the synthesis and functionalization of conducting poly(thiophenes). Makromol Chem, Macromol Symp 20–21:601–614. https://doi.org/10.1002/masy.19880200159
61. Balint R, Cassidy NJ, Cartmell SH (2014) Conductive polymers: towards a smart biomaterial for tissue engineering. Acta Biomater 10:2341–2353. https://doi.org/10.1016/j.actbio.2014.02.015
62. Guo B, Ma PX (2018) Conducting polymers for tissue engineering. Biomacromolecules 19:1764–1782. https://doi.org/10.1021/acs.biomac.8b00276
63. Min JH, Patel M, Koh W-G (2018) Incorporation of conductive materials into hydrogels for tissue engineering applications. Polymers 10:1078. https://doi.org/10.3390/polym10101078
64. Palza H, Zapata PA, Angulo-Pineda C (2019) Electroactive smart polymers for biomedical applications. Materials. https://doi.org/10.3390/ma12020277
65. Inzelt G, Csahók E, Kertész V (2001) Preparation and characterisation of polyaniline electrode modified with diamino-methylbenzoate. Electrochim Acta 46:3955–3962. https://doi.org/10.1016/S0013-4686(01)00691-0
66. Yoo JE et al (2007) Improving the electrical conductivity of polymer acid-doped polyaniline by controlling the template molecular weight. J Mater Chem 17:1268–1275. https://doi.org/10.1039/B618521E
67. Guo B, Glavas L, Albertsson A-C (2013) Biodegradable and electrically conducting polymers for biomedical

applications. Prog Polym Sci 38:1263–1286. https://doi.org/10.1016/j.progpolymsci.2013.06.003

68. Harrison BS, Atala A (2007) Carbon nanotube applications for tissue engineering. Biomaterials 28:344–353. https://doi.org/10.1016/j.biomaterials.2006.07.044
69. Uz M et al (2018) Advances in controlling differentiation of adult stem cells for peripheral nerve regeneration. Adv Healthcare Mater 7:1701046. https://doi.org/10.1002/adhm.201701046
70. Gupta P, Rajput M, Singla N, Kumar V, Lahiri D (2016) Electric field and current assisted alignment of CNT inside polymer matrix and its effects on electrical and mechanical properties. Polymer 89:119–127. https://doi.org/10.1016/j.polymer.2016.02.025
71. Gao W, Alemany LB, Ci L, Ajayan PM (2009) New insights into the structure and reduction of graphite oxide. Nat Chem 1: 403. https://doi.org/10.1038/nchem.281, https://www.nature.com/articles/nchem.281#supplementary-information. Accessed 22 July 2019
72. Loh KP, Bao Q, Eda G, Chhowalla M (2010) Graphene oxide as a chemically tunable platform for optical applications. Nat Chem 2:1015. https://doi.org/10.1038/nchem.907
73. Zhou M, Zhai Y, Dong S (2009) Electrochemical sensing and biosensing platform based on chemically reduced graphene oxide. Anal Chem 81:5603–5613. https://doi.org/10.1021/ac900136z
74. Chen GY, Pang DWP, Hwang SM, Tuan HY, Hu YC (2012) A graphene-based platform for induced pluripotent stem cells culture and differentiation. Biomaterials 33:418–427. https://doi.org/10.1016/j.biomaterials.2011.09.071
75. Guo W et al (2016) Construction of a 3D rGO-collagen hybrid scaffold for enhancement of the neural differentiation of mesenchymal stem cells. Nanoscale 8:1897–1904. https://doi.org/10.1039/c5nr06602f
76. Guo W et al (2016) Self-powered electrical stimulation for enhancing neural differentiation of mesenchymal stem cells on graphene–Poly(3,4-ethylenedioxythiophene) hybrid microfibers. ACS Nano 10:5086–5095. https://doi.org/10.1021/acsnano.6b00200
77. Heo C et al (2011) The control of neural cell-to-cell interactions through non-contact electrical field stimulation using graphene electrodes. Biomaterials 32:19–27. https://doi.org/10.1016/j.biomaterials.2010.08.095
78. Lee WC et al (2011) Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. ACS Nano 5:7334–7341. https://doi.org/10.1021/nn202190c
79. Lee Y-J, Jang W, Im H, Sung J-S (2015) Extremely low frequency electromagnetic fields enhance neuronal differentiation of human mesenchymal stem cells on graphene-based substrates. Curr Appl Phys 15:S95–S102. https://doi.org/10.1016/j.cap.2015.04.017
80. Nayak TR et al (2011) Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells. ACS Nano 5:4670–4678. https://doi.org/10.1021/nn200500h
81. Luo Y et al (2015) Enhanced proliferation and osteogenic differentiation of mesenchymal stem cells on graphene oxide-incorporated electrospun poly(lactic-co-glycolic acid) nanofibrous mats. ACS Appl Mater Interfaces 7:6331–6339. https://doi.org/10.1021/acsami.5b00862
82. Hao T et al (2016) Fullerene mediates proliferation and cardiomyogenic differentiation of adipose-derived stem cells via modulation of MAPK pathway and cardiac protein expression. Int J Nanomed 11:269–283. https://doi.org/10.2147/ijn.s95863
83. Lee WC et al (2015) Cell-assembled graphene biocomposite for enhanced chondrogenic differentiation. Small 11:963–969. https://doi.org/10.1002/smll.201401635
84. Kelkar DA, Chattopadhyay A (2006) Membrane interfacial localization of aromatic amino acids and membrane protein function. J Biosci 31:297–302. https://doi.org/10.1007/bf02704101
85. Rajesh C, Majumder C, Mizuseki H, Kawazoe Y (2009) A theoretical study on the interaction of aromatic amino acids with graphene and single walled carbon nanotube. J Chem Phys 130:124911. https://doi.org/10.1063/1.3079096
86. Lee DY, Khatun Z, Lee J-H, Lee Y-K, In I (2011) Blood compatible graphene/heparin conjugate through noncovalent chemistry. Biomacromolecules 12:336–341. https://doi.org/10.1021/bm101031a
87. Samanta SK, Pal A, Bhattacharya S, Rao CNR (2010) Carbon nanotube reinforced supramolecular gels with electrically conducting, viscoelastic and near-infrared sensitive properties. J Mater Chem 20:6881–6890. https://doi.org/10.1039/C0JM00491J
88. Wong KKH et al (2009) The effect of carbon nanotube aspect ratio and loading on the elastic modulus of electrospun poly(vinyl alcohol)-carbon nanotube hybrid fibers. Carbon 47:2571–2578. https://doi.org/10.1016/j.carbon.2009.05.006
89. Shvedova A et al (2003) Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. J Toxicol Environ Health Part A 66:1909–1926. https://doi.org/10.1080/713853956
90. Yan L, Zhao F, Li S, Hu Z, Zhao Y (2011) Low-toxic and safe nanomaterials by surface-chemical design, carbon nanotubes, fullerenes, metallofullerenes, and graphenes. Nanoscale 3:362–382. https://doi.org/10.1039/C0NR00647E
91. Nuccitelli R (2003) Endogenous electric fields in embryos during development, regeneration and wound healing. Radiat Prot Dosimetry 106:375–383. https://doi.org/10.1093/oxfordjournals.rpd.a006375

92. Funk RHW (2015) Endogenous electric fields as guiding cue for cell migration. Front Physiol. https://doi.org/10.3389/fphys.2015.00143
93. Robinson KR, Messerli M, Palmer A (1999) Endogenous electrical fields and embryonic development. In: Bersani F (ed) Electricity and magnetism in biology and medicine. Springer, pp 537–540
94. Uz M, Donta M, Mededovic M, Sakaguchi DS, Mallapragada SK (2019) Development of gelatin and graphene-based nerve regeneration conduits using three-dimensional (3D) printing strategies for electrical transdifferentiation of mesenchymal stem cells. Ind Eng Chem Res 58:7421–7427. https://doi.org/10.1021/acs.iecr.8b05537
95. Das SR et al (2017) Electrical differentiation of mesenchymal stem cells into Schwann-cell-like phenotypes using inkjet-printed graphene circuits. Adv Healthcare Mater. https://doi.org/10.1002/adhm.201601087
96. Koppes AN et al (2014) Electrical stimulation of Schwann cells promotes sustained increases in neurite outgrowth. Tissue Eng Part A 20:494–506. https://doi.org/10.1089/ten.tea.2013.0012
97. Park J-E et al (2013) Electromagnetic fields induce neural differentiation of human bone marrow derived mesenchymal stem cells via ROS mediated EGFR activation. Neurochem Int 62:418–424. https://doi.org/10.1016/j.neuint.2013.02.002
98. Xu H et al (2014) Conductive PPY/PDLLA conduit for peripheral nerve regeneration. Biomaterials 35:225–235. https://doi.org/10.1016/j.biomaterials.2013.10.002
99. Yan Q et al (2011) Electro-acupuncture promotes differentiation of mesenchymal stem cells, regeneration of nerve fibers and partial functional recovery after spinal cord injury. Exp Toxicol Pathol 63:151–156. https://doi.org/10.1016/j.etp.2009.11.002
100. Schmidt CE, Shastri VR, Vacanti JP, Langer R (1997) Stimulation of neurite outgrowth using an electrically conducting polymer. Proc Natl Acad Sci USA 94:8948–8953. https://doi.org/10.1073/pnas.94.17.8948
101. Zhang Z et al (2007) electrically conductive biodegradable polymer composite for nerve regeneration: electricity-stimulated neurite outgrowth and axon regeneration. Artif Organs 31:13–22. https://doi.org/10.1111/j.1525-1594.2007.00335.x
102. Huang L et al (2008) Synthesis of biodegradable and electroactive multiblock polylactide and aniline pentamer copolymer for tissue engineering applications. Biomacromol 9:850–858. https://doi.org/10.1021/bm7011828
103. Lee JY, Lee J-W, Schmidt CE (2009) Neuroactive conducting scaffolds: nerve growth factor conjugation on active ester-functionalized polypyrrole. J R Soc Interface 6:801–810. https://doi.org/10.1098/rsif.2008.0403
104. Huang J et al (2010) Electrical regulation of Schwann cells using conductive polypyrrole/chitosan polymers. J Biomed Mater Res Part A 93A:164–174. https://doi.org/10.1002/jbm.a.32511
105. Guarino V, Alvarez-Perez MA, Borriello A, Napolitano T, Ambrosio L (2013) Conductive PANi/PEGDA macroporous hydrogels for nerve regeneration. Adv Healthcare Mater 2:218–227. https://doi.org/10.1002/adhm.201200152
106. Shi Z et al (2014) In situ synthesis of robust conductive cellulose/polypyrrole composite aerogels and their potential application in nerve regeneration. Angew Chem 126:5484–5488. https://doi.org/10.1002/ange.201402751
107. Yang S et al (2016) Polypyrrole/alginate hybrid hydrogels: electrically conductive and soft biomaterials for human mesenchymal stem cell culture and potential neural tissue engineering applications. Macromol Biosci 16:1653–1661. https://doi.org/10.1002/mabi.201600148
108. Bu Y et al (2018) A conductive sodium alginate and carboxymethyl chitosan hydrogel doped with polypyrrole for peripheral nerve regeneration. RSC Adv 8:10806–10817. https://doi.org/10.1039/c8ra01059e
109. Jafarkhani M, Salehi Z, Nematian T (2018) Preparation and characterization of chitosan/graphene oxide composite hydrogels for nerve tissue engineering. Mater Today: Proc 5:15620–15628. https://doi.org/10.1016/j.matpr.2018.04.171
110. Zhao Y et al (2018) Construction of polyacrylamide/graphene oxide/gelatin/sodium alginate composite hydrogel with bioactivity for promoting Schwann cells growth. J Biomed Mater Res Part A 106:1951–1964. https://doi.org/10.1002/jbm.a.36393
111. Koppes AN et al (2016) Robust neurite extension following exogenous electrical stimulation within single walled carbon nanotube-composite hydrogels. Acta Biomater 39:34–43. https://doi.org/10.1016/j.actbio.2016.05.014
112. Shin J et al (2017) Three-dimensional electroconductive hyaluronic acid hydrogels incorporated with carbon nanotubes and polypyrrole by catechol-mediated dispersion enhance neurogenesis of human neural stem cells. Biomacromolecules 18:3060–3072. https://doi.org/10.1021/acs.biomac.7b00568
113. Imaninezhad M et al (2018) Directed and enhanced neurite outgrowth following exogenous electrical stimulation on carbon nanotube-hydrogel composites. J Neural Eng 15:056034. https://doi.org/10.1088/1741-2552/aad65b
114. Liu XF et al (2017) Functionalized carbon nanotube and graphene oxide embedded electrically conductive hydrogel synergistically stimulates nerve cell differentiation. ACS Appl Mater Interfaces 9:14677–14690. https://doi.org/10.1021/acsami.7b02072
115. Wang S et al (2017) Chitosan/gelatin porous scaffolds assembled with conductive poly(3,4-ethylenedioxythiophene) nanoparticles for neural tissue engineering.

J Mater Chem B 5:4774–4788. https://doi.org/10.1039/C7TB00608J

116. Thrivikraman G, Madras G, Basu B (2014) Intermittent electrical stimuli for guidance of human mesenchymal stem cell lineage commitment towards neural-like cells on electroconductive substrates. Biomaterials 35:6219–6235. https://doi.org/10.1016/j.biomaterials.2014.04.018
117. Prabhakaran MP, Ghasemi-Mobarakeh L, Jin G, Ramakrishna S (2011) Electrospun conducting polymer nanofibers and electrical stimulation of nerve stem cells. J Biosci Bioeng 112:501–507. https://doi.org/10.1016/j.jbiosc.2011.07.010
118. Lee JY, Bashur CA, Goldstein AS, Schmidt CE (2009) Polypyrrole-coated electrospun PLGA nanofibers for neural tissue applications. Biomaterials 30:4325–4335. https://doi.org/10.1016/j.biomaterials.2009.04.042
119. Xie J et al (2009) Conductive core-sheath nanofibers and their potential application in neural tissue engineering. Adv Funct Mater 19:2312–2318. https://doi.org/10.1002/adfm.200801904
120. Shafei S et al (2017) Electroactive nanostructured scaffold produced by controlled deposition of PPy on electrospun PCL fibres. Res Chem Intermed 43:1235–1251. https://doi.org/10.1007/s11164-016-2695-4
121. Ghasemi-Mobarakeh L, Prabhakaran MP, Morshed M, Nasr-Esfahani MH, Ramakrishna S (2009) Electrical stimulation of nerve cells using conductive nanofibrous scaffolds for nerve tissue engineering. Tissue Eng Part A 15:3605–3619. https://doi.org/10.1089/ten.tea.2008.0689
122. Wu Y, Feng S, Zan X, Lin Y, Wang Q (2015) Aligned electroactive TMV nanofibers as enabling scaffold for neural tissue engineering. Biomacromolecules 16:3466–3472. https://doi.org/10.1021/acs.biomac.5b00884
123. Wang J, Tian L, Chen N, Ramakrishna S, Mo X (2018) The cellular response of nerve cells on poly-L-lysine coated PLGA-MWCNTs aligned nanofibers under electrical stimulation. Mater Sci Eng C 91:715–726. https://doi.org/10.1016/j.msec.2018.06.025
124. Yow S-Z, Lim TH, Yim EKF, Lim CT, Leong KW (2011) A 3D electroactive polypyrrole-collagen fibrous scaffold for tissue engineering. Polymers. https://doi.org/10.3390/polym3010527
125. Zhu W et al (2018) Enhanced neural stem cell functions in conductive annealed carbon nanofibrous scaffolds with electrical stimulation. Nanomed Nanotechnol Biol Med 14:2485–2494. https://doi.org/10.1016/j.nano.2017.03.018
126. Uz M, Bulmus V, Altinkaya SA (2016) Effect of PEG grafting density and hydrodynamic volume on gold nanoparticle–cell interactions: an investigation on cell cycle, apoptosis, and DNA damage. Langmuir 32:5997–6009. https://doi.org/10.1021/acs.langmuir.6b01289
127. Choi J-R, Kim S-M, Ryu R-H, Kim S-P, Sohn J-W (2018) Implantable neural probes for brain-machine interfaces—current developments and future prospects. Exp Neurobiol 27:453–471. https://doi.org/10.5607/en.2018.27.6.453
128. Li D-F, Wang W, Wang H-J, Jia X-S, Wang J-Y (2008) Polyaniline films with nanostructure used as neural probe coating surfaces. Appl Surf Sci 255:581–584. https://doi.org/10.1016/j.apsusc.2008.06.150
129. Di L et al (2011) Protein adsorption and peroxidation of rat retinas under stimulation of a neural probe coated with polyaniline. Acta Biomater 7:3738–3745. https://doi.org/10.1016/j.actbio.2011.06.009
130. Guex AA et al (2015) Conducting polymer electrodes for auditory brainstem implants. J Mater Chem B 3:5021–5027. https://doi.org/10.1039/c5tb00099h
131. Kotwal A, Schmidt CE (2001) Electrical stimulation alters protein adsorption and nerve cell interactions with electrically conducting biomaterials. Biomaterials 22:1055–1064. https://doi.org/10.1016/S0142-9612(00)00344-6
132. Alegret N, Dominguez-Alfaro A, Mecerreyes D (2019) 3D scaffolds based on conductive polymers for biomedical applications. Biomacromolecules 20:73–89. https://doi.org/10.1021/acs.biomac.8b01382
133. Gupta P et al (2019) Differential neural cell adhesion and neurite outgrowth on carbon nanotube and graphene reinforced polymeric scaffolds. Mater Sci Eng C 97:539–551. https://doi.org/10.1016/j.msec.2018.12.065
134. Gupta P, Sharan S, Roy P, Lahiri D (2015) Aligned carbon nanotube reinforced polymeric scaffolds with electrical cues for neural tissue regeneration. Carbon 95:715–724. https://doi.org/10.1016/j.carbon.2015.08.107
135. Holzwarth JM, Ma PX (2011) 3D nanofibrous scaffolds for tissue engineering. J Mater Chem 21:10243–10251. https://doi.org/10.1039/C1JM10522A
136. Blank M (2008) Protein and DNA reactions stimulated by electromagnetic fields. Electromagn Biol Med 27:3–23. https://doi.org/10.1080/15368370701878820
137. Feng J-F et al (2012) Brief report: guided migration of neural stem cells derived from human embryonic stem cells by an electric field. Stem Cells (Dayton, Ohio) 30:349–355. https://doi.org/10.1002/stem.779
138. Luo B et al (2014) Electrically induced brain-derived neurotrophic factor release from Schwann cells. J Neurosci Res 92:893–903. https://doi.org/10.1002/jnr.23365
139. Sun S, Titushkin I, Cho M (2006) Regulation of mesenchymal stem cell adhesion and orientation in 3D collagen scaffold by electrical stimulus. Bioelectrochemistry (Amsterdam, Netherlands) 69:133–141. https://doi.org/10.1016/j.bioelechem.2005.11.007
140. Kim T-H, Lee K-B, Choi J-W (2013) 3D graphene oxide-encapsulated gold nanoparticles to detect neural stem cell differentiation. Biomaterials 34:8660–8670. https://doi.org/10.1016/j.biomaterials.2013.07.101
141. Park SY et al (2011) Enhanced differentiation of human neural stem cells into neurons on graphene. Adv Mater 23:H263–H267. https://doi.org/10.1002/adma.201101503

142. Solanki A et al (2013) Axonal alignment and enhanced neuronal differentiation of neural stem cells on graphene-nanoparticle hybrid structures. Adv Mater 25:5477–5482. https://doi.org/10.1002/adma.201302219
143. Henley J, Poo M-M (2004) Guiding neuronal growth cones using Ca^{2+} signals. Trends Cell Biol 14:320–330. https://doi.org/10.1016/j.tcb.2004.04.006
144. Sheng L, Leshchyns'ka I, Sytnyk V (2013) Cell adhesion and intracellular calcium signaling in neurons. Cell Commun Signal 11:1–13. https://doi.org/10.1186/1478-811x-11-94
145. Takeichi M, Okada TS (1972) Roles of magnesium and calcium ions in cell-to-substrate adhesion. Exp Cell Res 74:51–60. https://doi.org/10.1016/0014-4827(72)90480-6
146. Hammerick KE, Longaker MT, Prinz FB (2010) In vitro effects of direct current electric fields on adipose-derived stromal cells. Biochem Biophys Res Commun 397:12–17. https://doi.org/10.1016/j.bbrc.2010.05.003
147. Thrivikraman G, Madras G, Basu B (2014) Intermittent electrical stimuli for guidance of human mesenchymal stem cell lineage commitment towards neural-like cells on electroconductive substrates. Biomaterials 35:6219–6235. https://doi.org/10.1016/j.biomaterials.2014.04.018
148. Kim H-J et al (2013) Extremely low-frequency electromagnetic fields induce neural differentiation in bone marrow derived mesenchymal stem cells. Exp Biol Med 238:923–931. https://doi.org/10.1177/1535370213497173

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