A study on the application of electroactive nanomaterials in the peripheral nerve regeneration was conducted by the Emerging Investigator 2021, Dr Yun Qian, at Shanghai Jiao Tong University affiliated Sixth People's Hospital.

Electroactive nanomaterials in the peripheral nerve regeneration

Electroconductive and piezoelectric nanomaterials are two major electroactive nanomaterials that conduct or generate electrical signals. After peripheral nerve injury, these electroactive nanomaterials effectively promote the regeneration and reconstruction of peripheral nerves. Electroactive nanomaterials can modulate various cellular behaviours. In addition, they rebalance the nerve microenvironment in terms of the new vessel formation, oxidative stress stabilization and immune response.

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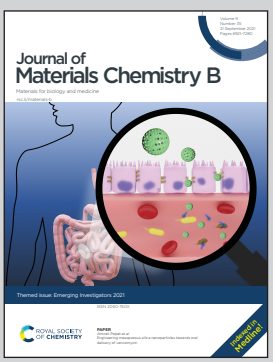

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Electroactive nanomaterials in the peripheral nerve regeneration

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Severe peripheral nerve injuries are threatening the life quality of human beings. Current clinical treatments contain some limitations and therefore extensive research and efforts are geared towards tissue engineering approaches and development. The biophysical and biochemical characteristics of nanomaterials are highly focused on as critical elements in the design and fabrication of regenerative scaffolds. Recent studies indicate that the electrical properties and nanostructure of biomaterials can significantly affect the progress of nerve repair. More importantly, these studies also demonstrate the fact that electroactive nanomaterials have substantial implications for regulating the viability and fate of primary supporting cells in nerve regeneration. In this review, we summarize the current knowledge of electroconductive and piezoelectric nanomaterials. We exemplify typical cellular responses through cell-material interfaces, and the nanomaterial-induced microenvironment rebalance in terms of several key factors, immune responses, angiogenesis and oxidative stress. This work highlights the mechanism and application of electroactive nanomaterials to the development of regenerative scaffolds for peripheral nerve tissue engineering.

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1. Introduction

Peripheral nerve injuries have been one of the most difficult clinical problems to handle for several decades. They usually result from traffic accidents, industrial incidents, falls, and war injuries and lead to extreme numbness, neuropathic pain, muscle strength decrease and even loss, and nutritional problems for regional tissues. The recovery of peripheral nerve injuries requires both the structural and functional reconstruction of injured nerves. However, the intrinsic regenerative capacity of nerves is so inadequate in severe peripheral nerve injuries that it is difficult to achieve the regrowth and reinnervation of proximal axons.[1] Autologous nerve transplantation is the gold standard for treating severe nerve defects in clinical application. Nevertheless, surgeries for large nerve gaps using autograft transplantation may bring side effects and contain certain limitations, such as donor site infection, lack of sources and neuroma formation.[2] Therefore, it is urgent and vital to seek for potential alternatives to improve clinical therapy.

Peripheral nerves consist of bundled axons that are enclosed with micro-vessels by connective tissues, including endoneurium, perineurium and epineurium.[3] The cable-like nerve fiber is located in a special fluid microenvironment and is wrapped directly by glycocalyx and a mesh of collagen.[4] With the rapid development of tissue engineering and regenerative medicine, it becomes increasingly important and promising to apply biomaterials for peripheral nerve regeneration. In this context, a biomaterial scaffold provides a possible option in the future clinical treatment of large nerve gaps. The nerve tissue

engineering is identified as the fabrication of nerve supporting scaffolds that offer better alternatives for autologous nerve grafts. The appropriate design of biomimetic scaffolds should characterize the inherent properties of peripheral nerves, such as stiffness, nanotopography, adhesiveness, and chemical functionality.[5] Therefore, there are consistent efforts to develop appropriate supporting scaffolds for nerve repair using different advanced techniques.

The fabrication techniques of scaffolds can affect physicochemical properties, such as morphologies, dimensions and topographies of biomaterials and thus influence the neurogenesis process.[6] The additive manufacturing can be utilized to fabricate structure-tailored scaffolds or substrates.[7] The electrospinning technique dictates the orientation of cell alignment through tethered fibers.[6,8,9] The self-assembly technique takes advantage of the spontaneous organization of bioactive materials through non-covalent bonds.[10–12] The multilayered fabrication technique provides cell–material interactions and therefore is beneficial to cell development due to controlled drug release from the scaffold.[13] The multichannel nerve conduits can mimic the native structure of highly aligned axonal bundles and fascicles.[14] The surface patterning technique can be used to produce a micro-patterned structure which directs cellular behaviors in an efficient way.[15] Engineered nanoparticles can take effects within specific intracellular compartments and impact the intracellular mechanics.[16–18] These techniques help to improve scaffold functionality with sophisticated cell-material interfaces and appropriate nerve regeneration milieu.

Apart from the exquisite structure, an ideal supporting scaffold for peripheral nerve repair must possess electroactive properties, similar to native nerves. Neuronal cells are electroactive and therefore the bioelectrical signal transmission is crucial to the functional restoration of peripheral nerves. The complex electrical transmission function of nerves is well maintained in a homeostatic microenvironment.[19] It is important to comprehend the physiological nature of peripheral nerves in order to guarantee the structural and functional biomimetics of nervous tissues. The electroactive nanomaterials can transmit electricity or generate it upon external stimulation. The electricity may be further used to activate different intracellular signaling pathways that are vital in cell viability and function.[20,21] Therefore, the biological functionality of nerve supporting scaffolds can be improved by surface coating or incorporation of electroactive materials. Functional nerve supporting scaffolds are required to show excellent electroconductive or electro-transformative properties and contribute to the intraneural angiogenesis, inflammation modulation and antioxidant balance in the regenerative microenvironment.[22–25]

In this review, we first introduce current electroactive nanomaterials and their primary biochemical and biophysical properties. Then, the impact on the cellular activity of these nanomaterials is also discussed based on the cell-material interaction for neurons and their supporting cells. We further summarize the recent utilization and development of electroactive nanomaterials and also propose the nanomaterial-based reconstruction of the microenvironment in peripheral nerve regeneration. Hopefully, it will contribute to the understanding and advances of electroactive nanomaterials for peripheral nerve regeneration.

2. Classification of electroactive nanomaterials

Electroactive nanomaterials are a series of smart materials with exceptional electrical and mechanical properties. They are usually categorized into two major groups, namely, electroconductive and nonconductive nanomaterials. The most frequently-used nonconductive electroactive nanomaterial in peripheral nerve tissue engineering is the piezoelectric nanomaterial. Therefore, we outline the primary electroconductive and piezoelectric nanomaterials and evaluate their primary properties and functions for regenerating and repairing peripheral nerves (Table 1).

2.1 Electroconductive nanomaterials

The electroconductive nanomaterials are versatile biomaterials that can restore electrical signaling of the neural pathway in traumatic neuropathy.[26] On the one hand, electrically conductive biomaterials affect cell membrane-associated functions which regulate the nerve electrical activities. On the other hand, internal and external electrical stimulation improves the axonal elongation, neurite outgrowth and remyelination.[27] One of the major drawbacks of electroconductive nanomaterial-based scaffolds is the requirement for additional external electrical supply. Therefore, the proper use of electroconductive materials necessitates the implantation of external electrodes. It has considerably limited the *in vivo* application of these electroconductive materials for clinical work. Metal nanomaterials, carbon-based materials and electroconductive polymer nanofibers are three major types of conductive nanomaterials in the fabrication of biomimetic nerve supporting scaffolds. Pure metal, such as gold, silver and copper, nanomaterials are ideal candidates because they readily conduct electricity by allowing free electrons to move between the atoms.[28] The gold nanomaterial is one of the most electroconductive and stable materials. The size, shape, surface characteristics and aggregation status of gold nanomaterials can significantly affect theit electrical properties.[29] When the size of gold is on the nanoscale, gold exhibits different properties from those of bulk gold, including its optical and electrical properties. The addition of gold nanoparticles into other substrate materials can allow the fabrication of hybrid nanocomposites that enjoy enhanced electrical conductivity and minimized metal toxicity.[30,31] In this context, we successfully fabricated gold nanocomposite-loaded nerve supporting scaffolds by mixing gold nanoparticles with polycaprolactone (PCL) suspension. The suspension was then sprayed onto the rolling tube model to produce gold/PCL composite scaffolds with multilayered deposition. The neural differentiation of bone marrow stem cells (BMSCs) was greatly enhanced as indicated by the enhanced expression of S100 and nestin. The axonal regrowth was also significantly enhanced as

Table 1 General classification and characteristics of electroactive nanomaterials in the current application of peripheral nerve regeneration

Categories	Electroconductive nanomaterials				Piezoelectric nanomaterials		
Subcategories	Pure metals	Carbon-based materials	Electroconductive polymers	Black phosphorus	Crystalline	Piezoceramics	Piezoelectric polymers
Examples	Gold Silver Copper	Graphene and its derivatives (GO, rGO); carbon nanotubes (SWCNT, MWCNT)	PPy PANi Aniline pentamer	—	Black phosphorus	Barium titanate; boron nitride; zinc oxide Lead zirconate titanate	PVDF PVDF–TrFE; PLLA
Electroactive properties	Conduct internal or external electrical currents (both electronic or ionic)				Generate electrical currents and electrical potentials in response to mechanical stress (mechano-electrical transduction)		
Cellular responses	Membrane biophysics Increased neuronal excitability and neuronal firing Enhanced axonal elongation and neurite outgrowth Promotion of SC viability, proliferation, migration, myelination and the secretion of neurotrophic factors Increased neural differentiation of bone marrow mesenchymal stem cells				Membrane channel activation Increased cell adhesion, protuberance extension, axonal elongation and higher expression of neural markers and nerve growth factors in SCs Induction of neuronal differentiation of PC12 cells Enhanced calcium transients, cell proliferation and neuronal differentiation in SH-SY5Y cells Increased neural differentiation of stem cells		
Microenvironment restoration	*Angiogenesis*: The generation of intracellular ROS and reactive nitrogen species Activated AKT-eNOS-vascular endothelial growth factor (VEGF) signaling cascade Initiation of phagocytosis in macrophages which secrete proangiogenic cytokines Interaction with endothelial cell receptors and secretion of proangiogenic cytokines Increased the expression of CD31 to enhance microvessel density and improved endothelial cell migration *Anti-inflammation*: Reducing the infiltration of macrophages and secretion of pro-inflammatory cytokines Suppressing the activation and migration of macrophages Promoting the polarization of seeded macrophages from M1 to M2 *Oxidative stress regulation*: Scavenging the accumulated ROS Clearance of the free oxygen radicals through covalent bonding with carbon atoms and neutralization of electron transfer processes				*Angiogenesis*: Production of intracellular ROS in endothelial cells Increased endothelial cell migration which promoted the maturation of new blood vessels *Inflammation regulation*: Reducing tumor necrosis factor α expression levels and consequently alleviating cell apoptosis *Oxidative stress*: —		

GO: graphene oxide; rGO: reduced graphene oxide; SWCNTs: single-walled carbon nanotubes; MWCNTs: multi-walled carbon nanotubes; PANi: polyaniline; PPy: polypyrrole; PVDF: polyvinylidene fluoride; PVDF–TrFE: poly(vinylidenefluoride-*co*-trifluoroethylene); PLLA: poly-L-lactic acid; ROS: reactive oxygen species; eNOS: endothelial nitric oxide synthase.

evidenced by the enhancement of NF-200 and Tuj1 expression. Besides, gold-loaded scaffolds promoted the expression of CD31, which demonstrated the angiogenic potential of gold nanocomposites. This scaffold showed excellent reparative effects on 15 mm sciatic nerve defects in rats.[32]

The renowned carbon-based materials in the nerve tissue engineering include carbon nanotubes, graphene and graphene derivatives (*e.g.* graphene oxide and reduced graphene oxide). Nanotubes and nanofibers are considered very promising structures for the fabrication of carbon-based nanomaterials. Their relatively large length-to-diameter aspect ratio and surface roughness allow for the tight interactions with neuron membranes. In this context, bi-directional electronic current flow at the cell-material interface may lead to redistribution of charges along the membrane surface and increasing neuronal excitability. Graphene and graphene derivatives are produced by exfoliating graphite into a sheet. The strong carbon–carbon bonds, hexagonal structures and free electrons provide graphene and its derivatives with excellent electrical properties. With a unique one-dimensional structure and highly anisotropic electroconductivity, carbon nanotubes (CNTs) prove to be excellent substrates or reinforcing materials for nerve regeneration. Single-walled CNTs (SWCNTs) are smaller in size and possess a higher aspect ratio and mechanical strength. In the fabrication of SWCNT composite scaffolds, SWCNTs were dispersed in aqueous substrate solutions in the form of suspended particles. Brain derived cells seeded on SWCNTs could assemble while retaining their normal morphology.

When incorporated into natural polymers, SWNT nanofibrous membranes formed a supportive architecture, which provided anchorage to the cells. As a consequence, SWCNTs accelerated the growth and proliferation of brain derived cells.[33] SWCNTs are characterized with one layer of hybridized carbon atoms and thus any chemical modification can decrease its electroconductivity greatly. Therefore, it is difficult to fabricate nanocomposite scaffolds by blending SWCNTs with other nanomaterials.[34] In this context, multi-walled CNTs (MWCNTs) attract more interest in application as they remain highly conductive after chemical modification and being mixed with other materials.[35]

Electroconductive polymers are attractive synthetic materials for nerve regeneration due to their simple synthesis and modification. The well-known conductive polymers, such as polyaniline (PANi), polypyrrole (PPy) and aniline pentamer, are usually incorporated into biodegradable and biocompatible materials such as PCL, chitosan and poly(lactic-*co*-glycolic acid) (PLGA). These composite scaffolds are characterized with improved biocompatibility, mechanical properties and electroconductivity. The nerve reparative potential of PPy/silk fibroin was tested by culturing Schwann cells. After 3 days of culture, the morphology, proliferation, adhesion and migration of Schwann cells were examined. Schwann cells on PPy/SF scaffolds displayed excellent distribution with elongated fibropodia. The proliferation and migration of cells were significantly enhanced as evidenced by increased EdU and S100β staining on the nanofiber surface. To produce such composite scaffolds, SF was first printed into aligned fibers and then coated with electroconductive PPy by saturating these SF fibers into PPy solution. The composite fibrous scaffolds were then subjected to an electrospinning procedure, in which electrospun nanofibers of SF were deposited onto the surface of scaffolds.[36]

Black phosphorus (BP) and its analogues are exceptional materials which cannot be categorized into any beforementioned groups due to their tunable band gaps. Based on such flexibility in the band structure, the electroconductivity of BP may be susceptible to different manufacturing techniques and external interference. Kim *et al.* reported the improvement of electrical conductance in BP by tuning the band gaps to resemble the natural state of graphene.[37] Despite its excellent electroconductivity, the application of BP in the treatment of PNIs is rare. Our previous work was the first to report a BP/PCL scaffold which induced neurogenesis after peripheral nerve injury. The most suitable nano-scaled structure for BP is a nanoplate which is generally 100 nm in diameter and contains 1 to 10 layers. We mixed BP plates in a biocompatible matrix (*e.g.* PCL) to make a mixture solution which was then sprayed onto the conduit mold to form a nanoscaffold. In our research, BP boosted calcium-dependent axonal regrowth and remyelination under conditions of mild oxidative stress. The activation of the Ca^{2+} signaling pathway up-regulated the level of brain-derived neurotrophic factors in the nerves. Besides, in a biological environment, BP could be oxidized into phosphates or PxOy which further restored immune homeostasis and angiogenesis.[38]

2.2 Piezoelectric nanomaterials

The piezoelectric nanomaterials are non-conductive smart materials that reversibly undergo dimensional changes or structural deformations under mechanical stimulation.[39] More importantly, piezoelectric nanomaterials can generate electrical currents and electrical potentials in response to mechanical stress (Fig. 1).[40] Then, the mechanical stress may be transformed into electrical signals and thus spares the use of external electrical stimulation.[41] In this context, piezoelectric nanomaterials are an ideal candidate for the development of wireless reparative nerve supporting scaffolds.[42] Piezoelectric nanomaterials with higher piezoelectric coefficients have better electromechanical performance.[43] The current application of piezoelectric biomaterials can be classified into the following categories: crystalline, ceramics and piezoelectric polymers.

Crystalline piezoelectric nanomaterials include monoelemental or compound piezoelectric materials. Although monoelemental materials lack ionic polarization, BP may generate piezoelectricity based on their non-centrosymmetric structure. In addition to the electroconductive potential, BP also has excellent piezoelectric properties. Ma *et al.* reported piezoelectricity in both phosphorene and multilayer BP.[44] The peculiar electrical properties of BP, as being both electroconductive and piezoelectric, render it extraordinary performance in the nerve tissue engineering. Piezoceramic nanomaterials are presently one of the most widely used piezoelectric nanomaterials. They are ferroelectric and possess polycrystalline structures. Such centrosymmetric structures can be categorized into piezoelectric non-centrosymmetric structures by temperature control adjustment.[45] Barium titanate, boron nitride, zinc oxide (ZnO) and lead zirconate titanate are the earliest studied piezoelectric ceramics that can be used as electroactive nanoscaffolds and augment nerve electrical activities.[46] Interestingly, a wireless implantable neural stimulator was fabricated to treat sciatic nerve injury in a rat model. In the production of this neural stimulator, piezoceramic PZT, integrated circuits, storage

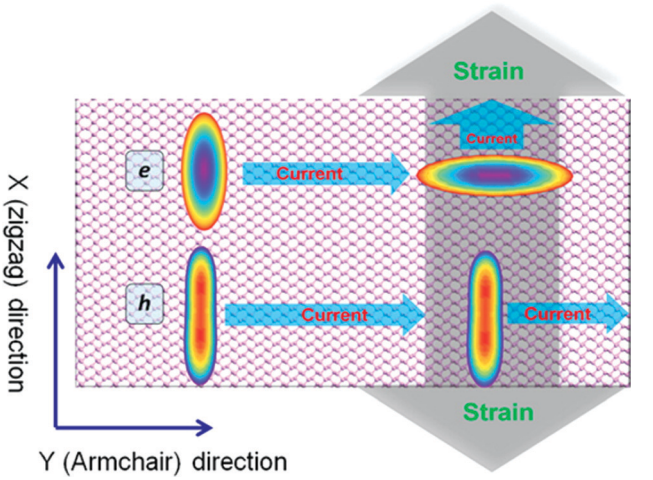

Fig. 1 Schematic representation of electrical currents generated in single-layer black phosophorus by means of mechanical stress. Reproduced with permission. Copyright©2014, American Chemical Society.

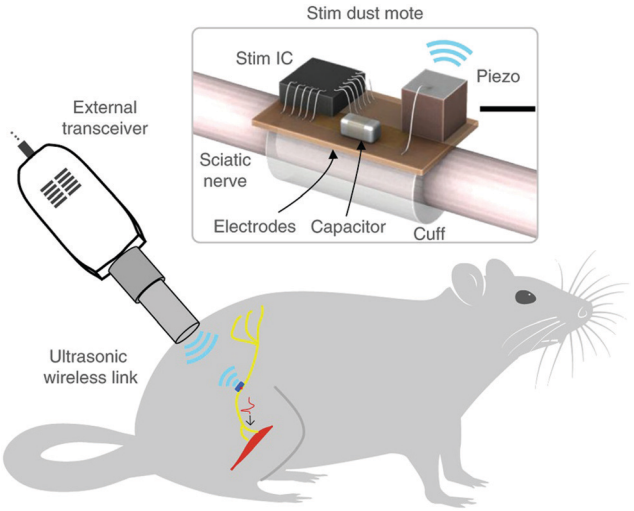

Fig. 2 Schematic representation of the wireless neural stimulator system is shown here. Piezoceramic transducer is placed on top of the system to receive ultrasonic signals and thus generates electricity. Reproduced with permission. Copyright©2020, Springer Nature.

capacitors and interface electrodes were used. Such a nerve cuff could send out repeatable electrical stimulation for therapeutic interventions without an externally applied electricity stimulator. The piezoceramic nanomaterial (PZT) inside the device functioned as a transducer that converted the energy of ultrasound into electrical signals in the nerve conduit (Fig. 2). The stimulation parameters of the neural stimulator controlled the physiological and biological responses (*e.g. in vivo* electromyogram response) of rats.[47]

Piezoelectric polymer nanofibers have better biocompatibility than the before-mentioned piezoelectric materials because of their relatively insignificant toxicity to cells. Polyvinylidene fluoride (PVDF), poly(vinylidenefluoride-*co*-trifluoroethylene) (PVDF–TrFE) and poly-L-lactic acid (PLLA) are representatives of these piezoelectric polymer nanofibers.[48] The fabrication procedure of piezoelectric polymeric nanoscaffolds is relatively simple. Piezoelectric polymer pellets are dissolved in organic agents to make piezoelectric solutions. Afterwards, the polymeric solutions are loaded in an electrospinning apparatus to obtain piezoelectric polymeric nanofibers. The piezoelectricity in these nanofibers arises from the strong molecular dipoles within the polymer nanofiber structure and the dipole density changes under mechanical stress.[39]

3. Electroactive nanomaterials and cellular responses

The interaction between cells and electroactive nanomaterials occurs at the material interface *via* physical and chemical signal recognition and propagation (Fig. 3).

Biochemical cues (*e.g.* nerve growth factors) are modified in the material interface and can be detected by specific receptors on the cell membrane. Biophysical cues, in contrast, are decided by the inherent properties of nanomaterials and regulate cellular behaviors cooperatively. Mechanosensitive proteins (Piezo1 and Piezo2) on the human cell membrane can sense the conversion of mechanical signals (*e.g.* stiffness and elasticity) into cellular biological signals.[49,50] However, there is no report on the specific electrical signal receptors and associated signaling pathways. The mechanisms by which cells perceive and respond to electrical signals remain to be elucidated.

Nerves employ axons to transmit electrical signals between central nervous systems and organs or tissues. The electrical signal transmission of nerves relies on the ionic currents on the neuron surface. In this context, Teng *et al.* constructed conductive MXene nanosheets sealed with dielectric polymers to create ionic nanofluidics.[51] They applied ionic alternating current to generate electronic signals and mimicked the nervous signal transmission (Fig. 4). Therefore, electroconductive nanomaterials have promising potential to be used for the fabrication of nerve prosthetics.

Electrical signals or stimulation can manipulate the behaviors of excitable cells through the alteration of membrane biophysics.[52,53] Cell behaviors like attachment, proliferation, migration, differentiation and apoptosis are all associated with changes of cell membrane potential.[54–61] Electroconductive nanomaterials carry electronic currents while bioelectricity employs ionic currents in electrical signal transmission.[62,63] Although, there is inherent incompatibility between electron conductivities and ionic conductivities, electronic currents can be transduced into ionic currents.[64–66] The interaction between electroconductive nanomaterials and neurons increases neuronal excitability and neuronal firing through the regulation of the membrane ion currents.[67,68] An increased frequency of synaptic events was detected in neurons cultured on electroconductive nanotubes.[69] Niccolo *et al.* reported that the electroconductive single-layered graphene could increase postsynaptic currents and shift the neuronal firing phenotype from adapting to tonically firing.[70] Similarly, Cellot *et al.* found that the improved neuronal network connectivity in the presence of electroconductive carbon nanotubes could lead to an increased post synaptic current frequency.[71] Apart from the neuronal electrophysiological behaviors, electroconductive nanomaterials with electrical stimulation also promote the axonal regeneration, remyelination and neurite outgrowth.[72,73] Fabbro *et al.* fabricated graphene nanomaterial-based substrates which supported neuronal development in terms of the passive properties of the neuronal membrane and synaptic activities.[74] The application of electroconductive nanomaterials that promote the synaptic activities influences the nervous system and subsequent downstream organs and effectors.[75] Interestingly, electroconductive scaffolds not only interact with electroactive cells, but also affect the cells from insulating tissues, such as Schwann cells (SCs) of myelin sheath. The regenerative efficacy of nerve guide conduits is evaluated in terms of their capability to promote SC adhesion, proliferation, myelination and function.[76] The neurotrophin secretion and axon myelination are two most critical functions of SCs. Zhao *et al.* revealed that PPy/silk fibroin electroconductive nanoscaffolds with electrical

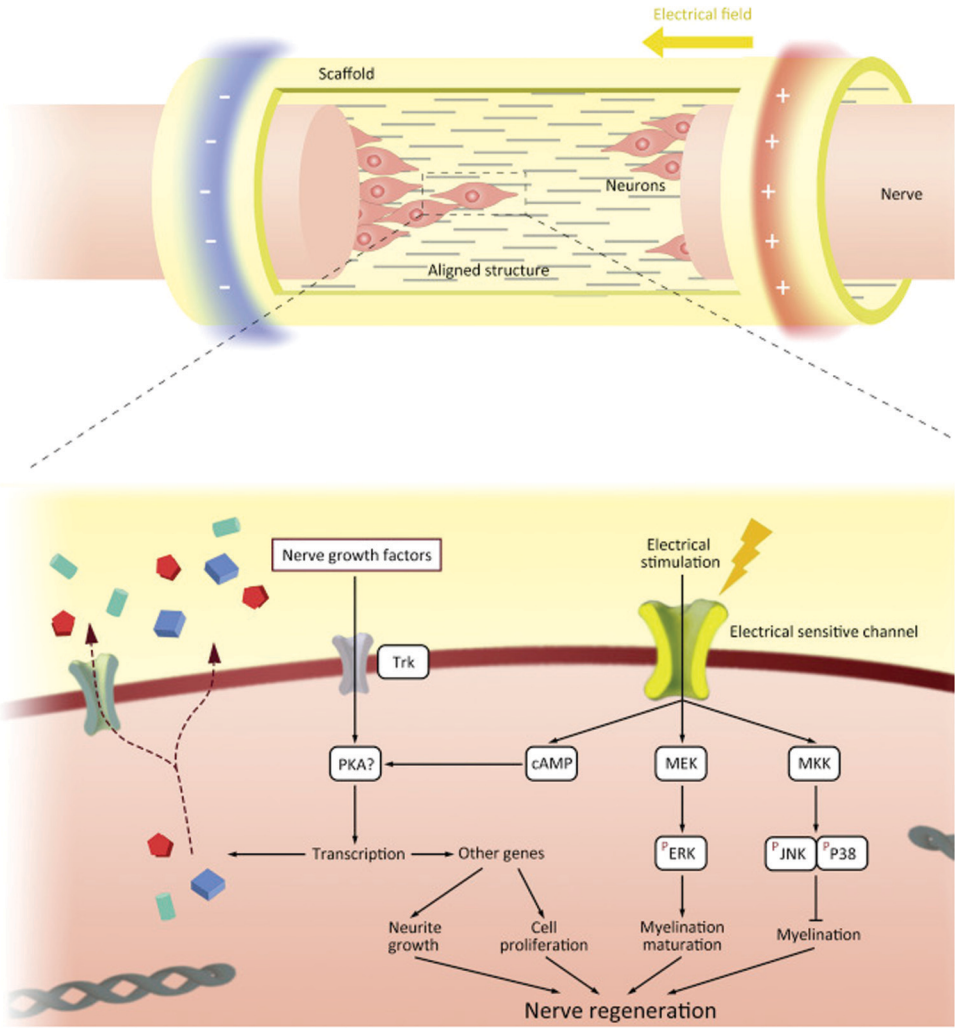

Fig. 3 The mechanism of how electroconductive materials trigger the nerve regeneration through intracellular signaling. Reproduced with permission. Copyright©2020, Elsevier Ltd.

stimulation stimulated the neurite outgrowth and axonal elongation extensively.[77] Furthermore, such electroconductive scaffolds enhanced the proliferation and viability of SCs and facilitated the secretion of neurotrophic factors at the same time.[77] In our previous study, rat SCs were cultured on graphene oxide nanoparticle-loaded PCL scaffolds. We confirmed the advantage of these conductive biomaterials in terms of SC proliferation and viability.[78] Although conductive polymers like PPy are characterized with excellent electroconductivity and mechanical properties, they are limited by poor biocompatibility and biodegradability. A novel electrically conductive biodegradable polyurethane scaffold enhanced the myelin gene expression and neurotrophin secretion of SCs.[79] Wang *et al.* discovered that the migratory abilities of SCs were upregulated in the presence of electroconductive reduced graphene oxide (rGO) and electrical stimulation.[80] In their study, the combined treatment of electrical stimulation and electroactive scaffold promoted the differentiation of PC12 cells, a commonly used neuronal cell model.[80] The electrical microenvironment is an important component of the biomimetic stem cell niche and induces the directional differentiation of stem cells. A graphene-crosslinked collagen conduit in combination with electrical stimuli promoted the neural differentiation of bone marrow mesenchymal stem cells (BMSCs) which showed the enhanced expression of MAP-2 kinase and β-tubulin III.[81] In our previous study, we also identified the potential of a conductive gold nanoparticle-based PCL scaffold to promote the neural differentiation of BMSCs.[32]

The electromechanical properties of materials have gradually become a major research focus due to the growing interest in piezoelectricity.[82] Piezoelectric nanoscaffolds form surface charges under mechanical stress and interfere with the behavior of seeded cells.[83] In peripheral nerve regeneration, cells seeded on such scaffolds can exploit electrical signals by mechano-electrical transduction. PVDF, polyvinyl chloride, and PVDF–TrFE are frequently-used polymers that have high processability and piezoelectric effects. Electrospun piezoelectric PVDF–TrFE nanofibers could support SC growth and further promote neurite extension and myelination.[84] Our previous study utilized ultrasound stimulation to induce the

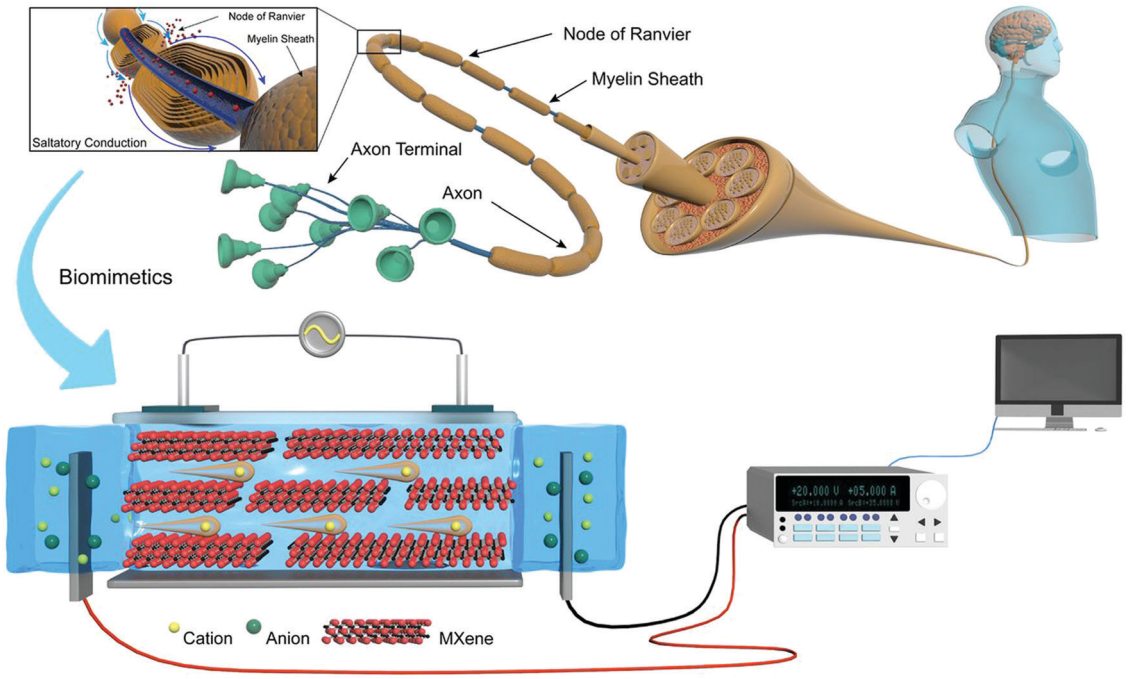

Fig. 4 Biomimetic ionic nanofluidic device that mimics axonal electrical signal transmission ability. Reproduced with permission. Copyright©2020. PNAS.

piezoelectricity of ZnO nanoparticles.[85] SCs seeded on piezoelectric ZnO/PCL conduits exhibited increased cell adhesion, protuberance extension and higher expression of neural markers and nerve growth factors.[85] Consistent with our previous study, Lee *et al.* proved that SC-seeded PVDF–TrFE promoted axon regeneration in the spinal cord repair *in vivo*.[86] Different from these electroconductive materials, piezoelectric materials do not regulate the neuronal behaviors through the conduction of exogenous electrical signals.[87] Hoop *et al.* discovered that the piezoelectric PVDF scaffolds had the ability to induce the neuronal differentiation of PC12 cells, comparable to the induction effect of nerve growth factors.[88] They considered that the piezoelectric PVDF scaffold activated calcium channels on the cell membrane and thus induced the generation of neurites through a cyclic adenosine monophosphate dependent pathway.[88] Barium titanate nanoparticles (BTNPs) are highly biocompatible piezoelectric materials that can be efficiently internalized into cells.[89] Marino *et al.* reported that the piezoelectric BTNP scaffold induced neuron-like differentiation of SH-SY5Y cells (human neuroblastoma-derived cells) in conjunction with the ultrasound stimulation.[90] In response to the stimulation of BTNPs, SH-SY5Y cells enhanced calcium or sodium influxes and activated voltage-gated membrane channels.[90] The additional dispersion of ceramic BTNPs into PVDF–TrFE extensively enhanced the piezoelectricity of the copolymers.[42] SH-SY5Y cells seeded on ultrasound-stimulated ceramic/copolymer composite scaffolds showed better performance than those on the copolymer alone, in terms of calcium transients, cell proliferation and neuronal differentiation.[42] Interestingly, BTNPs increased the neuronal network activity under the excitation of ultrasound waves.[91] In addition, piezoelectric effects can modulate the differentiation direction of stem cells.[92] Stem cells seeded on piezoelectric scaffolds may differentiate along the neural lineage and are promising alternatives to mature neural cells. Human neural stem/progenitor cells were differentiated into β-III tubulin-positive neuron-like cells in the presence of a piezoelectric PVDF–TrFE scaffold and exhibited the greatest average neurite length on annealed fibers.[93] Nanoscaled piezoelectric PVDF films also directed the neural differentiation of BMSCs efficiently.[94] Cellular motions like migration and attachment might lead to the deformation of the piezoelectric interfaces and therefore allowed the production of local electric field that in turn regulated the stem cell fates (Fig. 5).[94]

4. Electroactive nanomaterials and restoration of neural regenerative microenvironment

The electroactive nanomaterials influence the glial and neuronal cell behaviors to restore the bioelectrical signal conduction of nerves. However, when peripheral nerve injury occurs, the native nerve microenvironment is also disrupted and greatly interferes with the neurogenesis process. Therefore, the rebalance of the microenvironment is an essential criterion to measure the therapeutic effect of nerve supporting scaffolds. Apart from the bioelectrical signal conduction, micro-vessel formation, energy metabolism stabilization and immune response modulation are primary aspects that affect the peripheral nerve regenerative microenvironment.

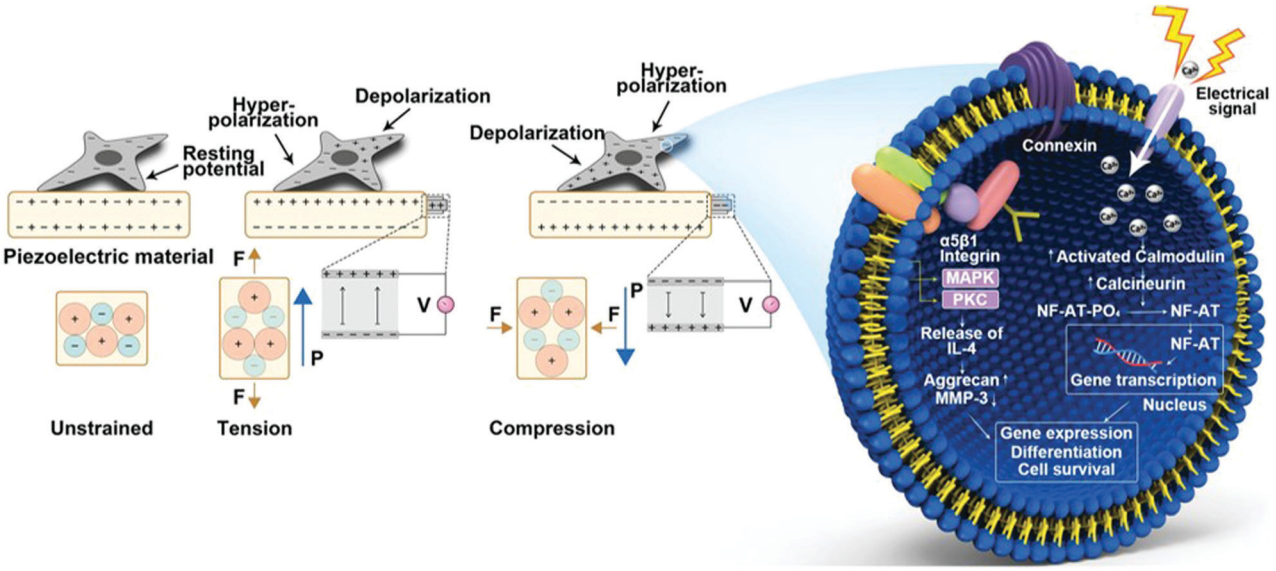

Fig. 5 Piezoelectric materials generate electrical signals through induction of mechanical strains and modulate cellular behaviors. Reproduced with permission. Copyright©2020, Wiley-VCH.

4.1 Electroactive nanomaterials promote new vessel formation

Micro-vessel formation is crucial to the restoration of peripheral nerve nourishment. Electroactive nanomaterials can induce or regulate blood vessel formation and stimulate the proangiogenic gene expression from different cells for angiogenesis.[95] The angiogenic capacity of some electroactive nanomaterials were reported, including BP, graphene and its derivatives, CNTs, and gold and ZnO nanoparticles. The generation of reactive oxygen species (ROS) is of vital significance for electroactive materials in the stimulation of angiogenesis.[96,97] ZnO nanoflowers induced angiogenesis through the production of intracellular ROS in endothelial cells.[98] The increased ROS subsequently activated pro-angiogenic signaling molecules such as mitogen activated protein kinase (MAPK), Akt, and endothelial nitric oxide synthase (eNOS).[98] In another study, the increased ROS generated by ZnO nanoflowers not only promoted the maturation of new blood vessels but also increased endothelial cell migration.[99] In our previous research, it was also confirmed that the mildly oxidative microenvironment contributed to the pro-angiogenic properties of BP.[38] Similarly, the angiogenic properties of graphene and graphene derivatives were demonstrated and associated with the generation of intracellular ROS and reactive nitrogen species.[100] The activation of endothelial nitric oxide synthase (eNOS) and Akt is a plausible mechanism in the GO/rGO induced angiogenesis.[101] Our previous research fabricated a graphene oxide nanoparticle-based PCL scaffold that activated the AKT-eNOS-vascular endothelial growth factor (VEGF) signaling cascade in the angiogenesis of nerve repair.[13] Xiong *et al.* discovered that human umbilical vein endothelial cells cultured on the rGO composite substrate assembled to form more branched nodes, circles and tubes. These changes indicated angiogenesis at early, interim and later stages respectively (Fig. 6).[102]

Interestingly, the angiogenic capacity of MWCNTs originated from the macrophage-induced degradation. MWCNTs initiated the phagocytosis of macrophages. The engulfing macrophages consequently secreted proangiogenic cytokines, such as matrix metallopeptidase 9 and VEGF.[103] Gold nanoparticles promoted the migration of endothelial cells and the ROS-driven angiogenesis.[104] Furthermore, bioconjugated gold nanoparticles functioned as excellent carriers for the delivery of the proangiogenic medicinal agents.[104] Dorota *et al.* reported that peptide coated gold nanoparticles manipulated angiogenesis by interacting with endothelial cell receptors and promoted secretion of pro- and anti-angiogenic factors.[105] Our previous research showed that gold nanocomposite scaffolds enhanced the microvessel density and improved endothelial cell migration by increasing the expression of transmembrane protein CD31.[13]

4.2 Electroactive nanomaterials stabilize the immune responses

Post-traumatic inflammation plays a key role in the process of nerve injury and regeneration. Macrophages are typically classified into a classically activated macrophage (M1) and an alternatively activated macrophage (M2). The M1 phenotype macrophage has the pro-inflammatory properties and often functions as the cleaner of myelin debris after peripheral nerve injuries. However, excessive inflammation might cause secondary injury to nerves and interfere with the process of axonal regeneration.[106] Therefore, the regulation of pro-inflammatory M1 and anti-inflammatory M2 macrophages is important for the homeostatic balance of immune milieu.[107]

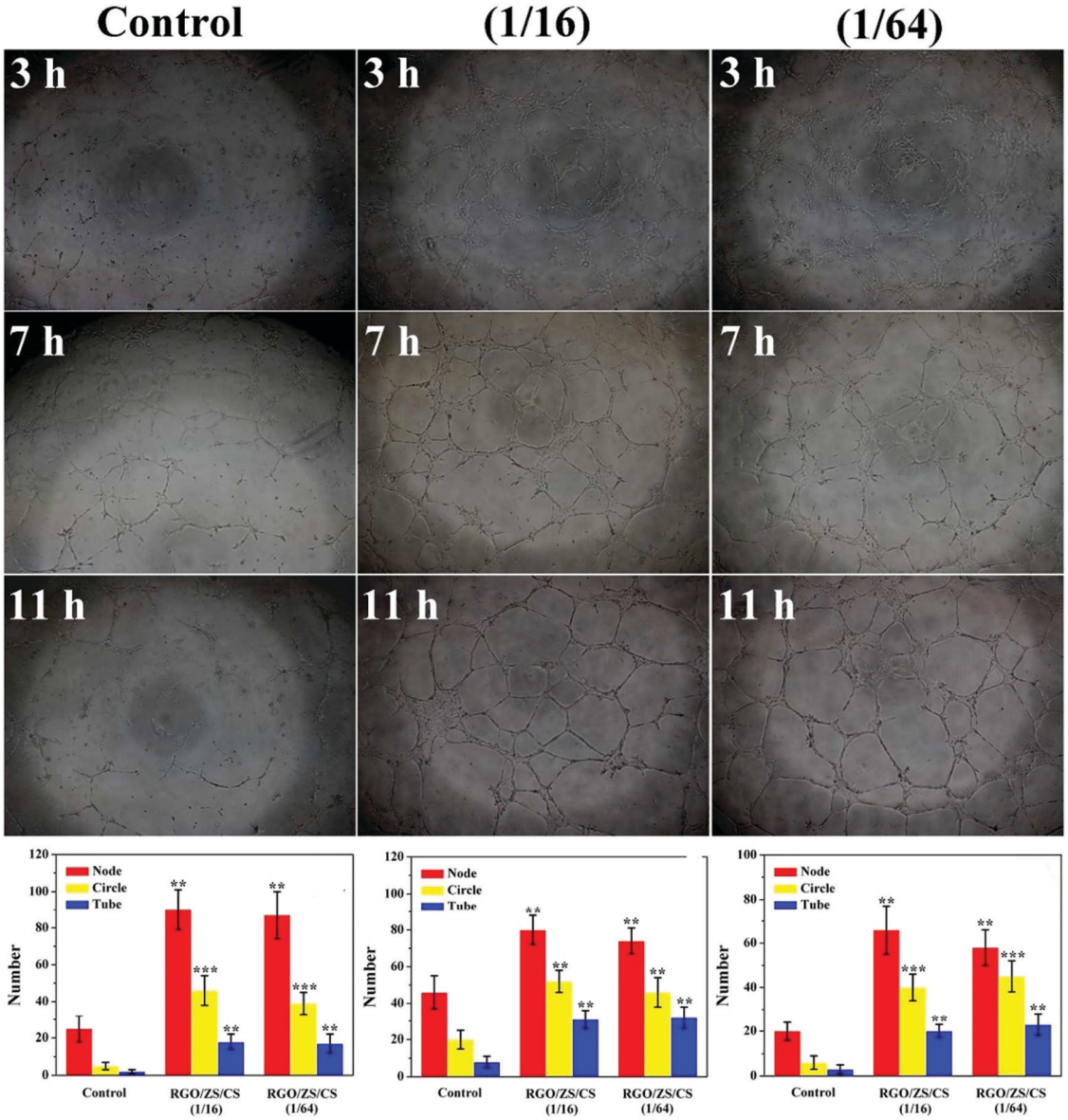

Fig. 6 *In vitro* angiogenesis of human umbilical vein endothelial cells cultured with varying media on ECMatrixTM gel. The rGO/ZS/CS extracts were diluted at 1/16 and 1/64 ratios and cultured for 3 h, 7 h and 12 h. Reproduced with permission. Copyright©2017, American Chemical Society.

The BP nanoplate-loaded PCL scaffold exhibited certain anti-inflammatory effects by reducing tumor necrosis factor α expression levels and consequently alleviated cell apoptosis and neurite outgrowth in a severe sciatic nerve defect rat model.[100] The iridium oxide–carbon nanotube–poly 3,4-ethylenedioxythiophene (IrOx–CNT–PEDOT) scaffold provided a safe *in vivo* environment for nerve regrowth and exhibited resistance to lipopolysaccharide-induced inflammatory insults.[108] Moreover, the combination of IrOx and electroconductive materials showed better anti-inflammatory results than the single IrOx alone.[108] Gold nanoparticles also had the capacity to ameliorate focal neuroinflammation by releasing gold ions that reduced the infiltration of macrophages and secretion of pro-inflammatory cytokines.[109] Graphene composite scaffolds suppressed the activation and migration of macrophages in the nerve tissue engineering.[110] Agarwal reported that BMSCs cultured on graphene collagen cryogels under an inflammatory microenvironment showed high indoleamine 2,3 dioxygenase activity, which exerted an immunosuppressive effect.[81] Moreover, such a graphene-crosslinked collagen nerve conduit promoted the polarization of seeded macrophages from M1 to M2 (Fig. 7).[81] Interestingly, the topographical features of the graphene interface affected the modulation of neuroinflammation and the 3D graphene foams exhibited better performance than 2D graphene films.[111]

4.3 Electroactive nanomaterials alleviate oxidative stress

Traumatic neuropathy leads to increased metabolic needs such as oxygen consumption and glycolysis, in order to provide adequate energy for peripheral nerve regeneration and

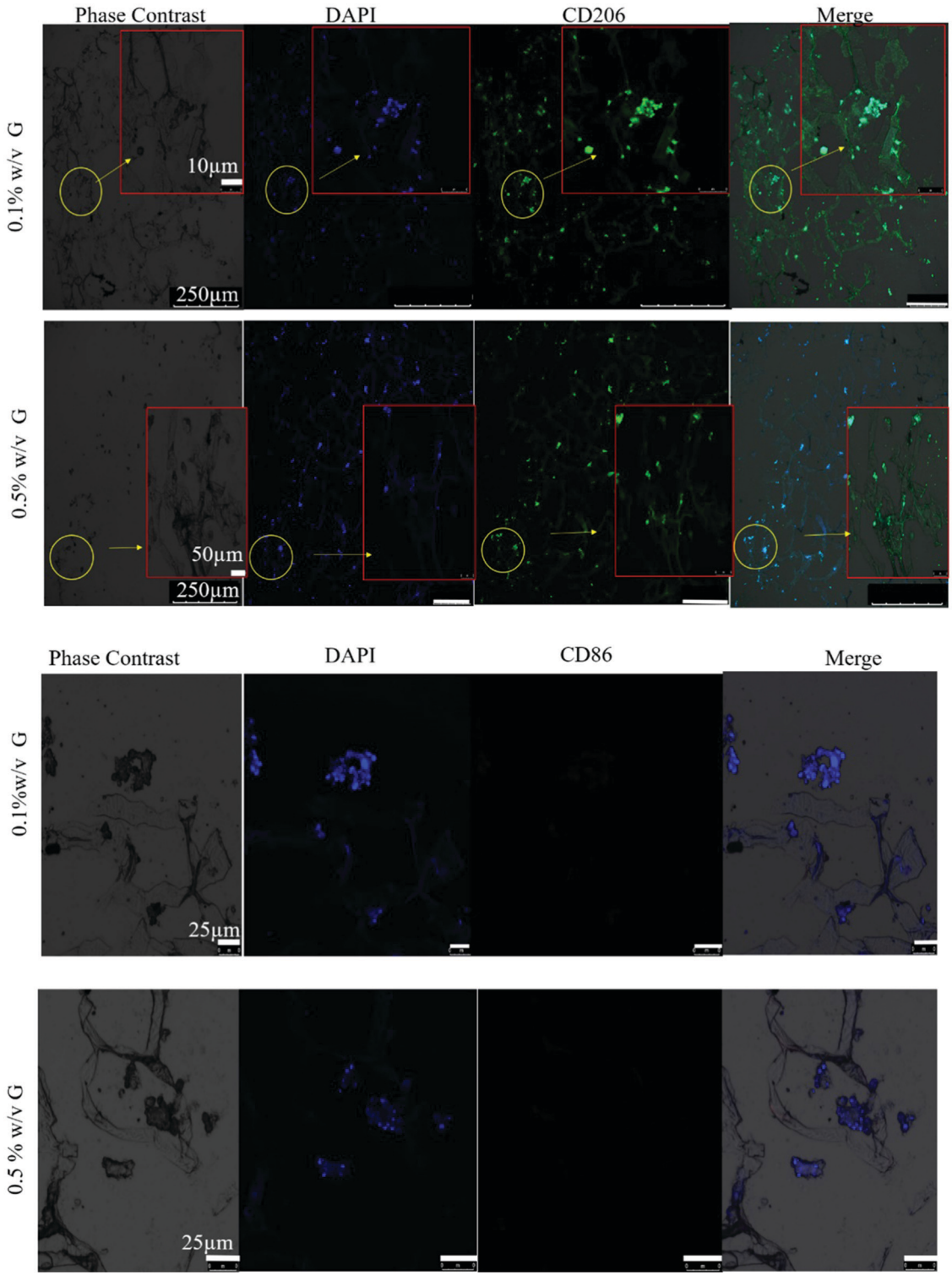

Fig. 7 The expression of M2 marker (CD206) on macrophages was found in both 0.1% and 0.5% w/v graphene crosslinked collagen cryogels on day 7. The expression of the M1 marker (CD68) was found to be very low. This indicates graphene has the potential to modulate M1 to M2 phenotype polarization. Reproduced with permission. Copyright©2020 Elsevier B.V.

angiogenesis. Therefore, energy metabolic disturbance, including bioenergetics and mitochondrial dysfunction should be identified and addressed immediately after nerve injuries. When a nerve injury occurs, the mitochondrial function is impaired and ROS are accumulated in the microenvironment. A mildly oxidative microenvironment could stimulate the angiogenesis process whereas lasting ROS exposure induced Schwann and neuronal cell apoptosis.[112] Mitochondria regulate

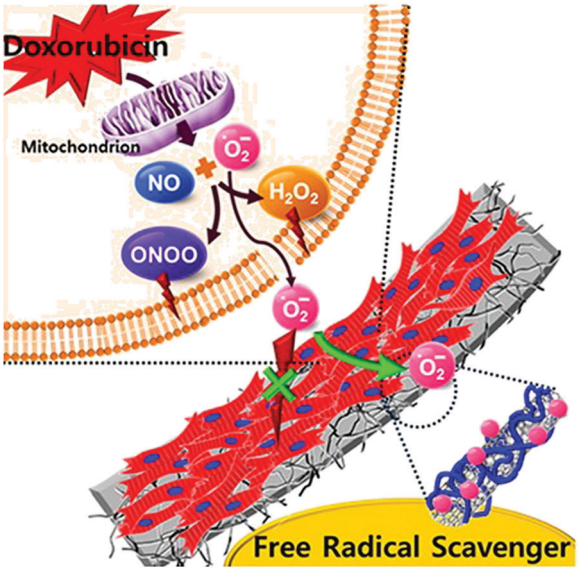

Fig. 8 Schematic representation of how electroconductive scaffolds scavenge free oxygen radicals. Reproduced with permission. Copyright©2013, American Chemical Society.

the cytoplasmic concentration of Ca^{2+}, which is closely associated with neuronal depolarization.[113] Mitochondrial Ca^{2+} uptake controls intracellular Ca^{2+} signaling and consequently regulates cell behaviors.[114] Therefore, the energy metabolism modulation and ROS clearance ability should be taken into consideration in the design of nerve supporting scaffolds.

The cellular response caused by electroactive nanomaterials remains controversial in terms of oxidative stress. Graphene and its derivatives could induce the generation of intracellular ROS, which led to direct cellular toxicity.[115] Graphene may cause toxic response to neural cells in a dose-dependent manner.[116] Pan *et al.* demonstrated that the cytotoxicity of gold nanoparticles was induced by endogenous ROS production and mitochondrial damage.[117] Similarly, Carlson *et al.* discovered that silver nanoparticles disturbed cellular energy metabolism mainly through oxidative stress.[118] In their study, macrophage exposure exhibited abnormal size and adherence characteristics when exposed to silver nanoparticles at high doses.[118] These electroconductive metallic nanoparticles could be inhaled by mammalian cells and exhibit oxidative stress in a size-dependent manner. Although the intracellular uptake of nanomaterials may cause cytotoxicity by the production of ROS, electroactive nanomaterials can exhibit antioxidant function under specific conditions or modification. Carbon nanotubes possess intrinsic ability to scavenge ROS and are considered as promising redox regulators.[119] Shin *et al.* engineered a CNT-incorporated photo-cross-linkable gelatin methacrylate hydrogel that protected seeded cells from damages caused by free oxygen radicals.[120] They considered that free oxygen radicals could be cleared by CNT scaffolds through covalent bonding with carbon atoms and neutralization of electron transfer processes (Fig. 8).[120] Lee *et al.* discovered that amine-modified CNTs decreased the ROS-induced apoptosis of neurons and ameliorated the ischemic damage on rat nervous tissues.[121] In their study, the electroconductive CNT showed promise in improving the tolerance of neurons to ischemic injury.[121] A schematic diagram was depicted to better describe the restoration of the neural regeneration microenvironment with different electroactive nanomaterials (Fig. 9). These studies encourage further investigation of electroactive nanomaterials in the regulation of oxidative stress and mitochondrial functions.

5. Final remarks and future perspectives

The combination of nanotechnology and biomaterials represents a breakthrough for the regeneration of peripheral nerves. Although the exact role and underlying mechanism of nanomaterials in peripheral nerve repair have not been defined yet,

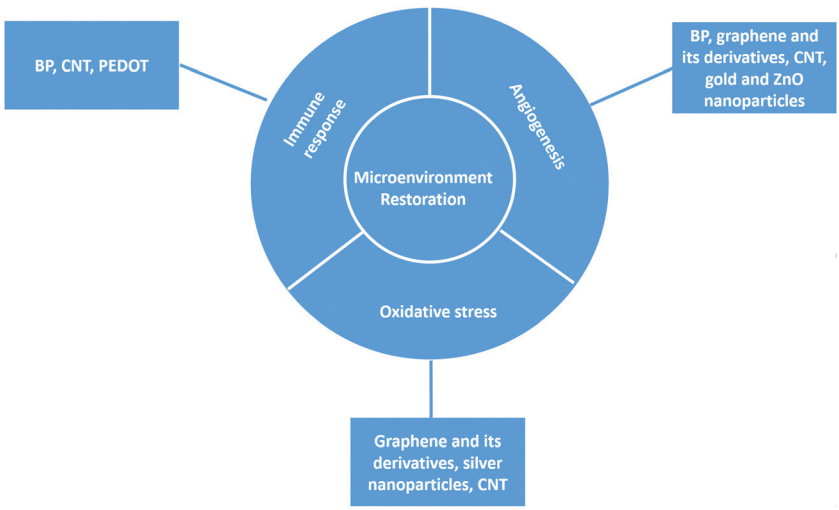

Fig. 9 Schematic representation of the restoration of the neural regeneration microenvironment with different electroactive nanomaterials.

substantial evidence supports the positive role of nanomaterials in the treatment of PNIs. Compared with traditional biomaterials, nanomaterials have greater versatility and flexibility to tailor for personalized peripheral nerve regeneration: (1) deliver bioactive molecules (*e.g.* growth factors) in a localized manner; (2) regulate cellular behavior with a degree of precision never achieved before; (3) modulate the reconstruction of the microenvironment in an effective manner; and (4) nanofibers possess great resemblance to the structure of nerve fibers and the roughness of the ECM, thus excellently mimicking the topography of nerve tissue.[122]

Developments in the past few years have emphasized the substantial implication of cell-material interactions in regenerative medicine. A large surface-to-volume ratio of nanomaterials permits the firm binding of cells and active ingredients on the materials. Therefore in our perspective, electroactive nanoparticles with higher porosity and smaller diameter would offer highly efficient platforms for the reciprocal modelling and synergistic effect of cells and materials. In addition to the zero-dimensional nanoparticles, nanofibers are one dimensional materials with a large surface area-to-mass ratio and mimic the native extracellular matrix. Nanofibers not only promote cell adhesion and spreading, but also increase cellular migration and motility which are important properties for the regenerative potential of SCs.[123] Aligned nanofibers mimic the topography of axon organization and adjust the alignment of SCs. Compared with randomly oriented nanofibers, aligned nanofibers provided better support for SCs and neuritis.[124,125] Therefore, we expect the future fabrication of highly anisotropic electroactive nanofibers in the peripheral nerve regeneration.

Key challenges of moving electroactive nanomaterials from lab benches to the clinics are biosafety requirement and the obstacles to provide well-controlled therapeutic effects. Although the positive therapeutic effects of electroactive nanomaterials have been confirmed, it remains undefined which material properties, structure and surface features will generate the best regenerative outcome. Therefore, in order to fabricate clinically approved electroactive nanoscaffolds, more research on the biodegradation products, long term *in vivo* effects and the underlying regenerative mechanism of electroactive nanomaterials is needed.

In summary, this review systemically introduces the major electroconductive and piezoelectric nanomaterials in the field of nerve tissue engineering.

We analyze the potential interaction between cellular biological activity and nanostructured electroactive materials. The electroactive nanomaterials can conduct or produce electrical signals and therefore modulate the cellular behaviors of glial cells, neurons, and mesenchymal stem cells that can be differentiated into the neural lineage. In addition, electroactive nanomaterial composite scaffolds can regulate the pro-neurogenic regenerative microenvironment in terms of nutritional vessel formation, immune reaction regulation and energy metabolism balance. Herein, we review the present knowledge about the neurogenic effects of electroactive nanomaterials based on previous research and summarize the primary exploration of our own work.

Hopefully, this review will improve the understanding of biomimetics and nanomanufacturing of nanomaterials in peripheral nervous systems and provide inspiration for superior design and application of electroactive nanomaterials for clinical scenarios.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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