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Abstract

In the past decades, great efforts have been developed for neurobiologists and neurologists to restore nervous system functions. Recently much attention has been paid to electrical stimulation (ES) of the nervous system as a potential way to repair it. Various conductive biocompatible materials with good electrical conductivity, biocompatibility, and long-term ES or electrical stability have been developed as the substrates for ES. In this review, we summarized different types of materials developed in the purpose for ES of nervous system, including conducting polymers, carbon nanomaterials and composites from conducting polymer/carbon nanomaterials. The present review will give our perspective on the future research directions for further investigation on development of ES particularly on the nerve system.

Keywords

materials, nerve, cell, stimulation, biocompatible, electroactive

Disciplines

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Electroactive Biomaterials for Nerve Cell Stimulation

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Abstract

In the past decades, though surgery techniques have been improved greatly, the clinical results of nerve repair still remain unsatisfactory due to the complexity of the nervous system anatomy and function. Thus, great efforts have been developed for neurobiologists and neurologists to restore nervous system function. Recently many attentions have been paid on electrical stimulation on the nervous systems as a potential way to repair nervous system. Various conductive biomaterials with good electrically conductivity, biocompatibility, ideally biodegradability, and long–term electrical stimulation or electrical stability have been developed as the substrates for electrical stimulation. In this review, we summarized different types of biomaterials developed in the purpose for applications as electrodes in the electrical stimulation of nervous systems, including conducting polymers, carbon nanomaterials and composites from conducting polymer/carbon nanomaterials. The present research will give our perspective on the future research directions with the purpose for further investigation on developments of the.electical stimulation particularlly on the nerve systems.

Keywords: Nerve Cell Stimulation, Biomaterials, Conducting Polymers, carbon nanomaterials, Nano-Composites.

1. INSTRUCTION

Nerve tissue engineering (NTE) is one of the most promising methods to restore central nerve systems in human health care. Nowadays, neural diseases are considered as complicated and significant clinical problems in the world with increasing age and polulation. Demand on various neural implants is necessary. It is however, a big challenge for nerve repaire when compared to other tissue repairs (such as bone repair) due to the complexity of the nervous system anatomy and function. Comparing with peripheral nervous system (PNS, such as spinal and autonomic nerves), the central nervous system (CNS, such as the brain and spinal cord) cannot be regenerated by itself after trauma or disease, because it lacks Schwann cells to promote axonal growth and the thick glial scar tissue which may result in an unfavorable environment inhibiting neural regeneration [1–4]. Therefore, restoring its function became a challenge for neurobiologists and neurologists. In general, there are mainly two strategies to repair the nervous system. One is the use of biomaterials as cell carriers for cell replacement therapies, including the use of biomaterials as scaffolds to replace natural ECM and to support axonal growth. The aother way is the use of biomaterials as drug delivery devices. Traditionally, tissue transplantation or peripheral nerve grafting are mainly used to repair damaged or diseased regions at the CNS (such as using autografts, allografts, xenografts, and silicon probes for the continuous diagnosis and treatment of neural tissue and other biomaterial nerve graft devices). A variety of problems, however, can not be ignored since this technique could't satisfy the high performance demands, such as the lack of donor nerves [4–6], the risk of transmitting diseases and the foreign body response and so on [7–9].

Electrical stimulation of the nervous system has been considered as a good approach to ameliorate conditions such as epilepsy, Parkinson disease, depression, hearing loss and chronic pain. It is known that bioelectricity present in the human body plays an integral role in maintaining normal biological functions such as signaling of the nervous system, muscle contraction, and wound healing [10]. Upon exposure to electric fields, one side of the cell becomes hyperpolarized while the opposite side is depolarized [11]. Fundamental physiological processes can be catalyzd by electric potential differences across biological membranes. This synergistic effect between electrical stimulation and neurotrophin delivery can increase the number of Trk receptors expressed on the cell surface, facilitating a larger effect of neurotrophins [12,13]. The representation of electrical stimulation was shown in Figure 1 [14]. Additionally, cellular activities such as cell migration [15], cell adhesion [17], DNA synthesis [17, 18] and protein secretion can be modified by electrical stimulation [19]. These characteristics make electrical stimulation become attractive in therapies for various neurological diseases and significant in tissue engineering since regulating cellular activities in an artificial scaffold is of great importance with respect to controlling the regeneration of damaged tissues. Presently, electrical stimulation has been successfully utilized in some clinic cases such as deep brain stimulators [15, 16] and cochlear implants [17–19], which was used to reduce symptoms of Parkinson's disease and restore auditory function, respectively. Medical applications of neural electrodes, e.g. the treatment of retinitis pigmentosa [20], epilepsy [21], depression [22] and chronic pain [23], have also been reported. The therapy efficiency is highly depended on the quality of the neuron–electrode interface. A universal interface with high selectivity, sensitivity, good charge transfer and long–term chemical and recording stability is a great challenge for nerve regeneration.

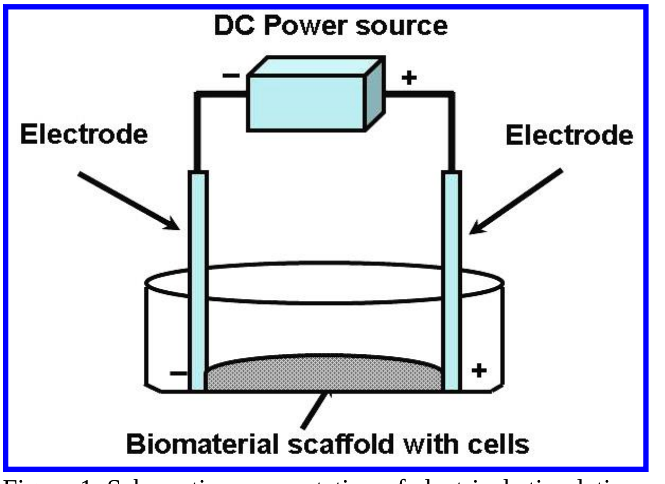

Figure 1. Schematic representation of electrical stimulation. Adapted from Ref. [14].

Neural electrodes, normally in electrode arrays, are the key elements in long–term implantable neural prostheses. The electrically conducting materials may benefit neural repair in the form of scaffolds implanted within lesion cavities to provide mechanical support and spatially arranged molecular cues for regenerating neurons [24]. Electrical stimulation via the scaffold would activate the molecular machinery necessary for axon elongation either by inducing nerve action potentials [25] or multicellular healing responses [26]. These materials should be electrically conductive, biocompatible, and ideally biodegradable, long–term electrical stimulation or electrical stability. Nowadays, most neural electrodes are mainly from considerably stable metals e.g. platinum, gold, iridium, titanium and stainless steel. Pt is one of the most popular candidates for neural electrodes due to its unique properties [27]. The maximum safe charge injection (Q_{inj}) limit for Pt electrodes, however is only about 0.15 mC/cm^2, which limits its futher application in neural microelectrodes [28]. Futhermore, metallic electrodes are often suffering from poor performance in long–term stimulation and recording due to poor contact with tissue or scar formation. Numerous surface modification techniques was thus developed to improve the electrode performance. For example, Though Iridium oxide (IrOx) was generally used as the coating material for neural electrodes [29–32], application of IrOx was still limited by its poor adhesion to underlying substrates and degradability under chronic aggressive stimulations due to its low structural and chemical stability [33, 34]. This may cause tissue damage and aggravate inflammatory responses. Uilisation of these microelectrodes in the long–term in vivo are still need to be investigated further.

In the past few decades, development of conducting polymers (CP) and carbon nanomaterials (CNTs) have attracted for great attention in many application areas such as energy storage, drug delivery, and bio/chem–sensors, considering their unique electrical and chemical properties [35–37]. Recently, employments of CP and CNTs in the the area of implantable neural electrodes have been attracted for increasing attention. Great efforts have been paid in preparation of novel biomaterials with high electrically conductivity, good biocompatibility, and ideal biodegradability, and long–term electrical stimulation or electrical stability. In this review, we will summarize recent efforts on developments of CP and CNTs as neural electrodes, providing new directions and useful information for clinical application.

2. Conducting Polymers

Electrically conductive polymers, known as synthetic metals, are widely investigated and studied in various applied chemistry and physics fields since they could simultaneously possess physical and chemical properties of organic polymers and the electrical characteristics of metals [38]. Recently, conducting polymers (CPs) attract considerable interests for numerous biomedical applications in tissue engineering, such as attachment, proliferation, migration, and differentiation modulated through electrical stimulation [39]. Normally, CPs possess a conjugated backbone with a high degree of p–orbital overlap, which can be readily oxidized or reduced to become either positively charged (oxidative or p–type) or negatively charged (reductive or n–type) through a "doping" process, respectively [40]. CPs can be electrochemically deposited on neural electrodes with well-defined and controlled thickness. Different bioactive molecules can also be subsequently incorporated into the polymer matrix as a dopant or via physical penetration to promote neuronal growth and adhesion to the electrode surface [41, 42]. Up to now, various biodegradable synthetic polymers [43, 44], peptide copolymers [45], and natural proteins [46–48] have been synthesized for various biomedical applications, such as tissue engineering [49–52], wound dressing [53], drug delivery [54], and vascular grafts [55]. Various type of conductive polymers including polypyrrole (PPy), polyacetylene, polythiophene, polyaniline (PANi), poly(3,4–ethylenedioxythiophene) (PEDOT), and poly(para–phenylene vinylene) have been fabricated and investigated.

Polypyrrole (PPy) and its derivatives are the most widely utilized CP in tissue engineering due to its good electrical conductivity, biocompatibility, high electrical stability, and ease of synthesis [56]. The reduction and oxidation of PPy is a reversible redox process, as shown in Figure 2 [57]. It has already been studied for many industrial applications [58–61] particularly in biomedical field [62, 63]. For example, PPy coated polyester fabrics have found to be good biocompatible both in vitro and in vivo, suggesting its potential applications in bio-medics. Conductive PPy film has shown to support the proliferation of nerve cells [64, 65], chromaffin cells [66], and endothelial cells [67]. Schmidt and coworkers ~~who~~ first found out that electrical stimulation of PPy films could enhance NGF induced neuronal differentiation of PC 12 cells, which was probably mediated by the fibronectin adsorption boosted by an electrical field [68]. Subsequently, Schmidt's group functionalized the surface of chlorine–doped PPy to anchor peptide molecules that could significantly promote nerve regeneration, blood vessel growth, and other biological processes [69]. Lakard et al. cultured olfactory cells on PPy to investigate cell adhesion and proliferation [70]. George et al. examined biocompatibility of PPy and found neurons and glial cells enveloped the PPy implant [71]. Several other scaffolds containing PPy have been used for various applications of tissue engineering [72, 73]. For an instance, conductive nanofibrous scaffolds from PPy coated poly (styrene–b–isobutylene–b–styrene) nanofibrous have shown a good proliferation of PC12 **(Figure 3)** by Liu et al. [74].

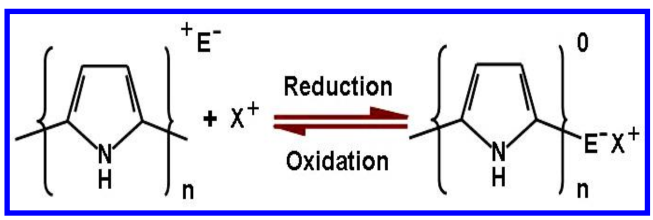

Figure 2. Reversible conversion between the reduction and oxidation states of PPy. Adapted from Ref. [57].

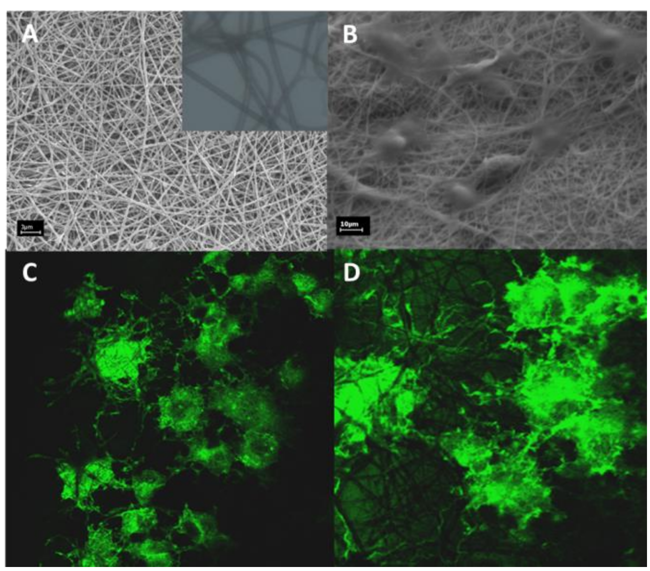

Figure 3. SEM images of (a) PPy/SIBS nanofibres platform, (b) PC12 on PPy/SIBS mat, and (c&d) fluorescence microscope image of phalloidin stained PC12 cells grown on SIBS/PPy nanofibres.

However, the applicability as a biomaterial of PPy was limited by the poor mechanical properties, lack of biodegradability, and difficulties in processing it into complex three–dimensional structures [75]. Therefore, great efforts were paid to combine PPy with other materials that possess the desired material properties to obtain hybrid composites. Various PPy composites have been studied by introduction different types of synthetic polymers with good biocompatibility and mechanical properties, bioresorable degradation products and adjustable degradation rate. These polymers include poly(methyl methacrylate) [76, 77], polyvinylchloride [78], polystyrene [79], polyurethane [80] and poly(a–hydroxy acid) [81–85]. Durgam et al. synthesize a block copolymer composed of PPy and PCL [86], which demonstrated good conductivity, biodegradability, and the ability to support PC12 cell proliferation. While Bao et al. reported that electrical stimulation on the electrospun PLGA/PPy nanofiber mat to PC 12 cells resulted in an increase in the number and length of neurite extensions [87]. Huang et al. measured electrical stimulation on the biodegradable chitosan–PPy composite to Schwann cells to electrical stimulation (ES) and found the enhanced cell proliferation and increased neurotrophin secretion [88]. Moroder et al. synthesized polycaprolactone fumarate–polypyrrole (PCLF–PPy) scaffolds with excellent mechanical properties, which were found to be significantly able to increase the percentage of neurite bearing cells via controlled electrical stimulation [89].

Another popular CP, polyaniline (PANI) and its variants have also been studied in wide range of research areas due to their unique properties such as the various oxidative state, ease of synthesis, low cost, conductivity and environmental stability [90, 91]. PANI has also shown good biocompatibility in vivo with the ability to support cell growth, suggesting potential interests in tissue engineering applications [95–99]. Mattioli–Belmonte et al. demonstrated for the first time that PANi is biocompatible both in vitro and in vivo [99]. Electrical stimulation of nerve cells on PANI substrates have been studied by many research groups [100–102]. H9c2 rat cardiac myoblast on PANI/gelatin nanofibrous scaffolds has been investigated by Li et al. [103]. Li and coworkers prepared biocompatible fibrous blend of conductive camphorsulfonic acid–doped emeraldine PANi (C–PANi) with gelatin showing to support the proliferation of H9c2 rat cardiac myoblasts [104]. Jeong et al. investigated the cell adhesion on electrospun PANI/poly (L–lactide–co–ecaprolactone) (PLCL) scaffolds and applied electrical stimulation to NIH–3T3 fibroblasts [105]. Ghasemi–Mobarakeh et al. fabricated conductive PANI/PG nanofibrous scaffolds by electrospinning. The electrical stimulation of NSCs through conductive nanofibrous scaffolds enhanced the cell proliferation and neurite outgrowth more significantly than the nonstimulated scaffolds, indicating that they are suitable substrates for nerve tissue engineering [106].

Compared with PPy and PANi, poly(3,4–ethylenedioxythiophene) (PEDOT) is the other popular conducting polymer due to its ordered and well–defined chemical structure, which exhibits outstanding conductivity, stability, fast response time, small electronic band gap (Eg ¼ 1.6eV, 775nm), low redox potentials, and facile fabrication in a doped form [107–110]. Recently, researchers have demonstrated the ability to dramatically improve the electrical properties of neural [111, 112] and cochlear electrodes by surface modification with PEDOT [113]. Cui and Hendricks have reported that the electrical properties of neural electrodes can be significantly improved by surface coating with PEDOT [111, 113]. In additions, various methods have been explored to improve biocompatibility and drug release capabilities of the PEDOT films. Despite its advantages and well-defined outlook, conventional PEDOT films still need to improve their physical and chemical properties in order to be a promising coating material for neural electrodes. Cui and Jan have indicated that the long–term stability of PEDOT coatings during chronic electrical stimulation was not satisfied [111, 114]. As PEDOT coatings may form cracks or delaminate under stimulation, which could lead to further coating detachment and debilitating the function of the electrode. SH–SY5Y neuroblastoma cells were found to grow and adhere well on the conductive and electro-active 3D–scaffolds from electrospun PEDOT-pTs coated fibers by Maria [115]. Collazos–Castro et al. reported the combination of electrochemical and molecule self–assembling methods to consistently control neural cell on PEDOT doped with polystyrene sulfonate (PEDOT:PSS), while maintaining very low interfacial impedance [116]. Neurite extension was strongly inhibited by an additional layer of PSS or heparin, which could be either removed electrically or further coated with spermine to re-activate cell growth. Binding basic fibroblast growth factor (bFGF) to the heparin layer inhibited neurons but promoted proliferation and migration of precursor cells. This method provides a potential way to control neural cell behavior on electro active polymers via improving cell/electrode communication in prosthetic devices, and to develop a platform for tissue repair strategies.

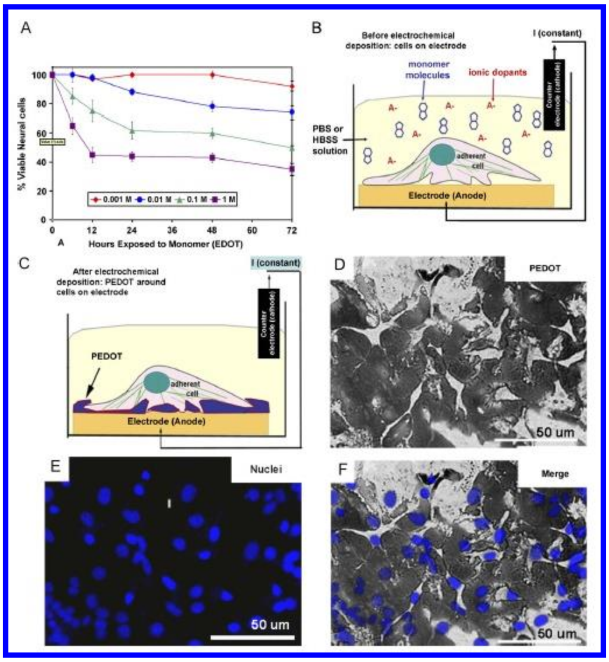

Figure 4. (A) MTT cytotoxicity assay for exposure of SY5Y neural cells to increasing concentrations of EDOT in monomer solution (all with 0.02 M PSS) for 0–72 h. (B) Diagram representing the electrochemical deposition cell and the neural cell monolayer cultured on the surface of the metal electrode prior to polymerization. (C) Diagram representing PEDOT polymerized around living cells. (D) PEDOT (dark substance) polymerized in the presence of a monolayer of SY5Y neural cells cultured on an Au/Pd electrode. (E) Nuclei of SY5Y cells stained with Hoechst 33342 (blue florescence). (F) Merged image showing nuclei of cells around which PEDOT is polymerized.

Besides above three main CPs (PPy, PANi, and PEDOT), there are also other kinds of conducting polymers, such as Poly(L–lactic acid) (PLLA), which possesses good mechanical integrity, biodegradability and biocompatibility. It has also been utilized for fabrication of scaffolds for nerve regeneration with longer degradation behaviors [117]. PLLA microfilaments used as structural support for long lesion nerve gap regeneration have also been reported [118]. Yang et al. fabricated nano–structured PLLA scaffolds, which can facilitate NSCs differentiation and neurite outgrowth in great degree [119]. Molamma et al. reported the synthesis PLLA/PANi nanofibers using electrospinning can enhance the neurite outgrowth under electrical stimulations, providing possibility for application of electrical stimulation as a potential clue for nerve tissue regeneration [120].

3. Carbon nanomaterials

Carbon nanotubes (CNTs), an important type of carbon nanomaterials, are cylindrical structures having high aspect ratios with long axial lengths (up to millimeters) and a few Ångströms in diameter. They are tough and robust materials with ultimate electrical and thermal conductivities and mechanical strength. These carbon hollow nanomaterials can be viewed as ‘‘rolled–up’’ structures of one or more layers of graphene sheets for single–walled (SWNT) or multiple–walled (MWNT) carbon nanotubes, respectively. The unique mechanical, chemical and electrical properties of CNTs provide a wide range of opportunities and potential applications in biology, medicine industry, which allow them to be one of the most promising materials for application in neural Prosthesis [121].

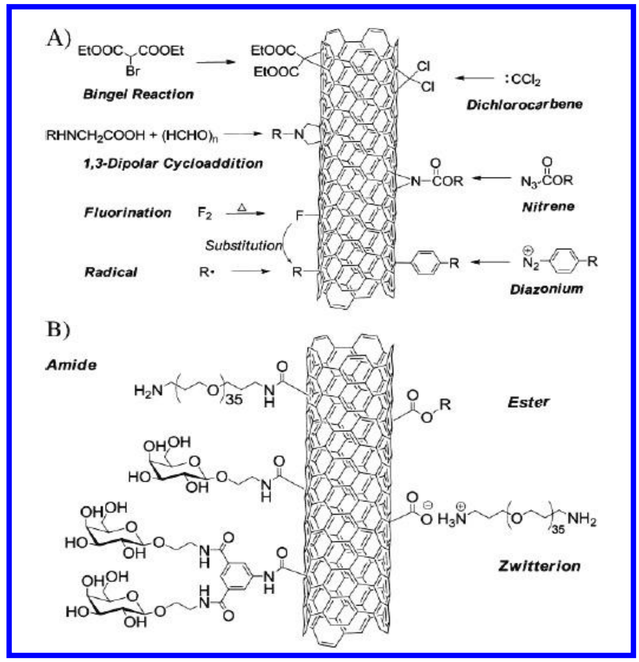

Figure 5. A) Covalent addition reactions on the sidewall of carbon nanotubes. B) Reactions targeting carboxylic acids (derived from nanotube surface defects). Adapted from Ref. [130].

Recently, great efforts have been developed in biological applications of CNTs at molecular and cellular levels, such as nanoscale biosensors [122, 123], electroanalytical nanotube devices [124], electromechanical actuators for artificial muscles [125, 126], and laser heating cancer therapy [127]. Futhermore, unique properties of CNTs such as diameter and aspect ratios similar to neural processes such as dendrites, good mechanical strength with flexibility, make them be able to maintain scaffolds’ structural integrity during cell growth. Good conductivity of the CNTs based scaffold can provide extra advantages for electrical stimulation. Additionally CNTs can also be used in vivo devices that could directly interact with neurons. All these unique propertiess make CNTs well suited in the design of novel neural biomaterials [128].

For well applications in neural regeneration, a good scaffold should not only conduct electrical current but also support neuron growth. The functionalization of CNTs or CNF–based scaffolds can provide further advantages such as improvement in bioactivity, and conjugation with various functional groups such as bioactive agents, nucleic acids and therapeutic agents [129]. Figure 4 exhibited various methods for functionalization of CNTs with different functional groups such as bioactive agents, nucleic acids and therapeutic agents after being functionalization [130] .

Mattson et al. studied for the first time in application of carbon nanotube technology to neuroscience research. They found that neurons extend only one or two neuritis on unmodified nanotubes, which exhibit very few branches. After incorporation of CNTs with the bioactive molecule 4–hydroxynonenal (4–HNE) used as scaffold, neuritis exhibit extensive branching [131]. These results provide possibility for using nanotubes as substrates for nerve cell growth and as probes of neuronal function at the nanometer scale. Hu et al. reported that the control of neurite outgrowth by manipulating the charge carried by functionalized CNTs [132]. Gaby et al. realized neuronal cell patterning using nano–topography constructed with islands of high–density fabrics made of CNTs [133]. These results suggest that CNTs are biocompatible as neuronal substrates and have potential applications in neural prostheses. Anava et al. and Sorkin et al. have developed a unique carbon–nanotube (CNT) based MEA in which the CNT electrodes are used to position and stabilize the cells and the network between the neurons and the CNTs, respectively [134, 135]. The highly–conductive CNTs can be used as recording and stimulation sites, forming an optimized interface with the neurons to achieve long–term electrical recordings. Moreover, Greenbaum et al. reported a new result about using specially designed CNT substrates to pattern predefined small size networks of locust frontal ganglion neurons and record their electrical activity [136].

CNTs were also considered as a good candidate for implants due to the good stability and non–biodegradation, making the effect studies of CNTs on neurons to be very necessary. So far, many efforts have been made on it. Lovat et al's recent report demonstrated that purified CNTs are ideal sustrates for the growth of neurons and helpful for the enhancement in the efficacy of neural signal transmission [137]. Authors attributed the increase in the efficacy of neural signal transmission to the specific properties of CNTs, which provided a pathway allowing direct electrotonic current transfer, and causing a redistribution of charge along the surface of the membrane. This result can be attibuted to the reinforcement of a direct electrical coupling between neurons. Meanwhile, Cui et al. found that SWCNTs inhibited the proliferation of HEK293 cells (human embryo kidney cells) by decreasing their cell adhesiveness in a dose– and time dependent manner [138]. Cellot and co–workers further investigated the efficiency of signal transmission of neurons grown on a conductive nanotube meshwork. Their results provide a new mechanistic insight into how nanotubes target the integrative properties of neurons. Authors proposed a mathematical model to explain phenomena and consequences for the enhanced signal transmission of neurons cultured on nanotube substrate, linking the electrical phenomena in nanomaterials to neuronal excitability for the first time [139]. Mazzatenta et al. developed an integrated SWNT–neuron system to test whether electrical stimulation delivered via SWNT can induce neuronal signaling. Hippocampal cells were grown on pure SWNT substrates and patch clamped [140]. Results indicate that SWNTs can directly stimulate brain circuit activity and facilitate to understand the electrical coupling between neurons and SWNT. Fabbro et al. reported that direct nanotube–substrate interactions with the membranes of neurons would affect single neuron activity and promote network connectivity and synaptic plasticity in mammalian cortical circuits in culture [141, 142]. Moreover, they used organotypic cultures of the embryonic mouse spinal cord interfaced with CNT scafolds to investigate whether and how the interactions at the monolayer level are translated to multilayered nerve tissues. The results indicated that the effects rely on direct and indirect MWCNT interactions [143]. Matsumoto et al. reported that low concentrations of functionalized CNTs modified by amino groups could promote outgrowth of neuronal neurites in dorsal root ganglion (DRG) neurons and rat pheochromocytoma cell line PC12h cells in culture media. In addition, they investigated the signal transduction pathways (extracellular signal–regulated kinase (ERK) signaling pathway and Akt signaling pathway) stimulated by CNTs [144].

Two (2D) and three (3D) dimensional architectures with interconnected cavities composed of CNTs also could be used in envisioning cell growth and tissue modeling, as shown in Figure 5 using 3D Aligned CNTs/SIBS platform. Many studies have investigated the cellular response to carbon nanofibers/nanotubes including dose–dependent effect [145, 146]. Correa–Duarte created a 3D network based on an array of interconnected MWCNTs [147]. They found that the common mouse fibroblast cell line L929 can extensive grow, spread, and adhere on the MWCNT substrate, indicating that the 3D MWCNT network was a good candidate for scaffolds/matrices in tissue engineering.

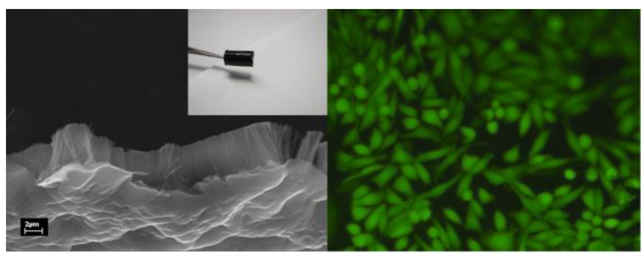

Figure 6. Left - SEM image of ACNTs/SIBS; and Right – L-929 cell culture on 3D ACNTs/SIBS platform.

As a fiberious carbon nanomaterial, carbon nanofibers (CNFs) have drawn much attention in creating interfaces between electrodes and neural tissues in electrical stimulation due to their unique properties, such as chemical stability, ultramicro size, low electrical impedance, 3D structures with high surface–to–volume ratio, and long–term biocompatibility. CNFs can provide a large active surface area for neural recording and stimulation while individual electrode sites on the substrate are scaled down. Li and co–workers reported a series of advancements in developing 3D brush–like vertically aligned carbon nanofiber (VACNFs) [148–150]. They fabricated VACNFs on a silicon wafer by plasma enhanced chemical vapor deposition using Ni as catalyst, and tested them with PC12 cells. The results indicated that the soft 3D VACNFs architecture provided a new platform to fine–tune the topographical, mechanical, chemical and electrical cues at subcellular nanoscale. Yu et al. developed a CNF–based neural chip and demonstrated its capability of both stimulating and recording electrophysiological signals from brain tissues in vitro [151, 152]. In this study, long–term potentiation (LTP) was induced and detected through CNFs arrays. Park and co–workers developed thin–film transistor (TFT)–VACNFs MEA platform [153], in which they fabricated the VACNFs on an active matrix TFT. By using this new platform, stimulating and recording could also be realized simultaneously. VACNF integrated on the TFT array enhanced the electrical selectivity to the cell, and furthermore, they provided the potential for intracellular sensing within individual cells. McKnight et al. prepared two types of VACNF electrode arrays with high aspect ratios and tested neuronal cell (specifically, rat phenochromocytoma, PC12 cells) differentiation on the VACNF substrates [154]. According to electro–analysis results at discrete electrodes after long term cell cultures, they founded that these CNF arrays were responsive for the detection of oxidized species generated by the cultured cells. They also recorded spontaneous and induced neuroelectrical activity in organotypic hippocampal slice cultures with ultra microelectrode VACNF arrays [155], suggesiting that the carbon–based electrodes may be potentially superior to conventional metal electrodes.

As a layered carbon nanomaterial, graphene, a fascinating 2–dimensional monolayer of carbon atoms, has recently emerged with many intriguing properties including electrical, thermal, optical, sensing, high surface area and biocompatibility. As the single or fewer layered structure of graphene provides richness for diversified surface chemistry on both sides of the sheet including edges, significant progresses have been made for the utilization of grapheme in nanocomposites [156] and biological systems as well, such as detection of DNA and metal ion [157, 158], protein and pathogen [159–161], design of cell/bacterial nanodevices [162–164] and drug delivery carriers [165, 166]. Meanwhile, much attention has also been paid in designing novel neural biomaterials based on graphene for neural regeneration since neural cells are electro–active and functions of nerve systems are related to electrical activities. As neuronal stimulation and monitor are needed for a variety of clinical diagnostics and treatments, unique electrical properties of graphene offer great advantages for the therapeutic or other purposes. Another reason for develping graphene based materials for neural regeneration is that the electronic properties of the nanostructured graphene can be tailored to match the charge transport required for electrical cellular interfacing. In addition, chemically stable properties of graphene facilitate the integration with neural tissues. For example, they can be used as neural chips, implanted electrodes and drug/gene vectors [167–169]. Li and co–workers demonstrated that graphene films grown from CVD have excellent biocompatibility for primary culture of mouse hippocampal neurons and are even capable of promoting neurite sprouting and outgrowth, especially during the early developmental phase [170]. In order to use human neural stem cells (hNSCs) for brain repair and neural regeneration, it is critical to induce hNSC differentiation which is directed more towards neurons than glial cells [171–174]. However, most previous studies reported that hNSCs, without biochemical motifs or co–culturing, differentiated more towards glial cells than neurons [175–177].

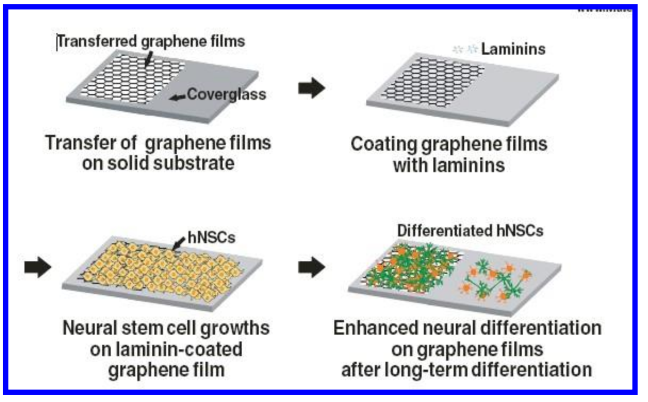

Figure 7. Schematic diagram depicting the growth and differentiation of hNSCs on graphene. Adapted from Ref. [178].

Park et al. discovered that the neuronal differentiation of hNSCs on graphene was greatly enhanced under electrical stimulation [178]. In a typical research, as schematically shown in Figure 6, graphene worked as an excellent cell–adhesion layer and induced differentiation of hNSCs more toward neurons rather than glial cells, which would open up tremendous opportunities in stem cell research, neuroscience, and regenerative medicine. Authors also found that grapheme had a good electrical coupling with the differentiated neurons. Their results suggested that graphene ccould be used as excellent nanostructured scaffolds for promoting NSC adhesion and differentiation for long–term periods as well as possible neural prosthetics.

Heo et al. prepared a non–cytotoxic graphene/ polyethylene terephthalate (PET) film [179]. The transient non–contact electric field was produced by charge–balanced biphasic stimuli through the graphene/PET electrodes, which significantly increased the number of cells forming new cell–to–cell couplings and the number of cells strengthening existing cell–to–cell couplings. These findings may facilitate the development of a new therapeutic stimulator for neurological diseases and cell transplantation therapy in CNS. Feng and coworkers developed a reusable graphene–based electrochemical aptasensor for label–free cancer cell detection [180]. Typically, 3,4,9,10–perylene tetracarboxylic acid (PTCA), a water–soluble perylene derivative was strongly adsorbed on graphene through pep stacking and hydrophobic interactions was used to avoid graphene aggregation and introduce more negatively–charged –COOH groups on graphene surface, without further destroying the conjugated p–system of graphene.

So far, considerable progresses have already been made in the related fields, while solutions for many critical issues in neural biology/medicine are still underway due to the availability of specialized nanomaterials.

4. Carbon nanomaterials and conducting polymers composites

The function and longevity of implantable microelectrodes for chronic neural stimulation depends greatly on the electrode materials or coatings with high charge injection capability and high stability. Though conducting polymers have been coated on neural microelectrodes and shown promising properties for chronic stimulation, their practical applications have been limited due to their drawbacks, e.g. the fragile characteristics, weak adhesion to the electrode substrate, and the poor electrochemical stability [181]. CNT–modified electrodes have exhibited good cytocompatibility and stability, suggesting their possible applications as in vivo devices to interact directly with neurons. Their Q_{inj}, however, are found to be in the range much lower than IrOx electrodes, limiting their further applications.

Recent reports have shown that CNTs can be incorporated into conducting polymers to prepare composite materials with enhanced properties, such as lower electrode impedance, higher capacitance and faster charge transfer rate as well as better mechanical stability [182]. Keefer and co–workers synthesized PPy/SWCNT deposited microelectrodes to record neural signals in vivo [183]. Peng et al. prepared composite films from CNTs and conducting polymers e.g. polyaniline (PANI), polypyrrole (PPy) or poly[3,4–ethylenedioxythiophene] (PEDOT). The composite films were prepared via electrochemical co–deposition from solutions containing acid treated CNTs and the corresponding monomers of conducting polymers [184]. The CNTs served as the charge carriers during electro–deposition, the backbone of a three–dimensional micro– and nano–porous structure and the effective charge–balancing dopant within the polymer. All composites showed improved mechanical integrity, higher electronic and ionic conductivity, and larger electrode specific capacitance than the pristine polymers. In the indentified conditions, the capacitance was enhanced significantly after incoporation of conducting polymers with CNTs. Bhandari et al. fabricated composite films of of PEDOT-enwrapped functionalized multiwalled carbon nanotubes (MWCNTs) over multiple length scales by electropolymerization of the monomer without the use of any other supporting electrolyte [185]. In this work, as schematically shown in Figure 7, the functionalized MWCNTs were incorporated into the positively charged polymer deposit as counterions during oxidative electropolymerization.

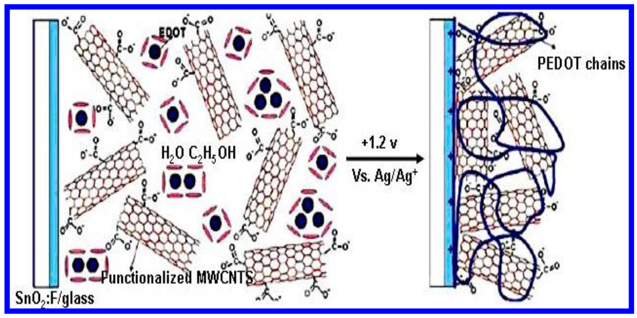

Figure 8. Schematic showing the formation of the PEDOT-MWCNT film from the solution containing EDOT, functionalized MWCNTs in a mixture of polyethylene glycol, water, and ethanol under a constant potential of +1.2 V. Adapted from Ref. [185].

Lu et al. investigated co–deposited PPy/SWCNT films on Pt for improving the electrode–neural tissue interface which are suitable for the application of neural stimulating electrodes [186]. The PPy/SWCNT microelectrode exhibited a particularly high capacitance and lower impedance when compared to the Pt microelectrode. Introduction of SWCNT into conducting polymers enhanced mechanical and electrochemical stabilities than the pristine conducting polymer films. Furthermore, the PPy/SWCNT film also showed excellent biocompatibility both in vitro and in vivo, suggesting possibilities for developing chronic implantable neural probes based on conducting polymers and CNTs for the purpose of electrical neural microstimulation and recording. Luo et al. reported the synthesis of PEDOT/CNT composite electrochemically deposited on the Pt microelectrode arrays [187]. The resulting electrode exhibited much lower impedance, higher charge storage capacity, and a high Q_{inj} (2.5 mC/cm^2). The resulting film also exhibited good stability under both long–term biphasic pulse stimulation and aggressive cyclic voltammetric stimulation, and great biocompatibility in vitro. Supronowicz et al. reported the application of nanocomposites consisting of polylactic acid and CNT blends on cell electrical stimulation [188]. Chao et al. prepared a 2D thin film scaffold composed of biocompatible polymer [poly(acrylic acid)] grafted carbon nanotubes (CNTs), which can selectively differentiate human embryonic stem cells into neuron cells while maintaining excellent cell viability [189]. Neuron differentiation efficiency of poly(acrylic acid) grafted CNT thin films was significantly greater than that on poly(acrylic acid) thin films. The surface analysis and cell adhesion study have suggested that CNT–based surfaces can enhance protein adsorption and cell attachment. This finding indicates that CNT–based materials are excellent candidates for hESCs' neuron differentiation.

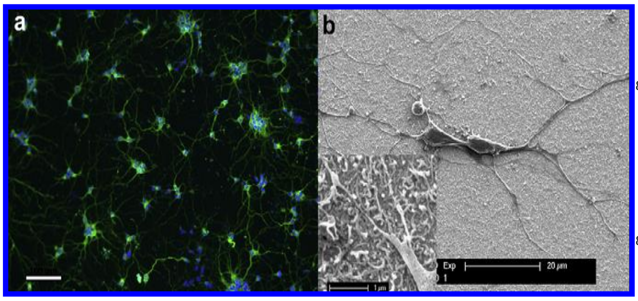

Figure 9. Fluorescent (a) and SEM (b) images of neurons cultured on PEDOT/CNT surfaces. For the fluorescent image, the scale bar represents 100 mm. The inset in (b) shows the SEM of neurites grown on the PEDOT/CNT surface with high magnification.

Recently, Nguyen–Vu and colleagues fabricated a vertically aligned carbon nanofiber (VACNF) electrode coated with a thin film of conductive PPy for neural implants [190, 191]. The nanoelectrode array had more open and strong 3D structures, and better electrical conductivity. The study showed that the vertical CNF arrays helped to form an intimate neural–electrical interface between cells and nanofibers for neural prosthesis. McKenzie et al. investigated astrocyte (one of the glial scar tissue forming cells) function on CNFs/polycarbonate urethane (PCU) composites [192]. They demonstrated for the first time that astrocyte adhesion could be effectively inhibited when incorporating and increasing the surface energy of CNFs in the polymer composites. Furthermore, CNFs could also support neuron growth and neurite extension. Webster et al. described the cellular response of neuron and osteoblast cells to composites made up of CNFs as "fillers in polycarbonate urethane substrates". The cell response to the composite may result in successful integration of neural and bone tissue implants [193]. Similarly, VACNFs coated with PPy by electrochemical deposition was also be used as for electrical stimulation [150]. CNFs, however,are easy to be bundled up, resulting in bigger, micron–sized fibers. For sucessful preparation of CNFs nanoarray, a conformal film of conducting polymers (such as PPy) was deposited onto CNFs. The PPy coated CNFs were then coated by a thin layer of type IV collagen to improve biocompatibility of the CNFs. The cell growth rate on CNF arrays with the PPy and collagen coatings was dramatically increased compared to the "bare" CNF arrays or CNF arrays coated with PPy only. This improved biocompatibility of the functionalized VACNFs, along with their 3D nanostructure and superior electrical and mechanical property, make them suitable for neural applications such as functional electrical stimulation, deep brain stimulation and neural prosthetics [148].

These studies suggeted that conducting polymer/carbon nanomaterials composite might provide extra advantages for the development of novel neural electrode based on conducting polymers or carbon nanomaterials, which are able to offer a friendly interface bridging inorganic materials to a living body. Therefore, a comprehensive study on electrochemical characteristics and biocompatibility of these composites with concern to chronic implantable neural electrodes is required.

5. Concluding Remarks and Future Perspective

In the future, it is necessary to integrate neural–electrical interfaces and neural–chemical interfaces together for the development of intelligent, closed–loop therapeutic devices for diagnosis and treatment of neurological diseases, realizing automatic modulation of neural activity by neurostimulation or local drug delivery responding to real–time detection of electrical and chemical information from the nervous system. The demand for developing therapies to neural disorders with strategies involving drug delivery, tissue repair, and electrical implants is urgent.

Nanoscale topological features have been shown to increase cell adhesion and viability which can be exploited to make neuron–device coupling more reliable. Novel substrate coatings offered by nanomaterials can be used to immobilize cells and increase the number of cells growing neuritis. Manufacturing of this or similar devices, although technically possible, is limited by the physical properties of the available materials. Recent work has focused on the feasibility of using high–capacitance, low resistance electrodes, with the goal of large scale integration with CNS interfaces. Many efforts have been made on developments of suitable materials includin conducting polymers, carbon nanomaterials, composites and other potential materials. In this respect, studies on the chronic long–term toxicity of these materials over the period of implantation spanning several years are also necessary. In addition, nanomaterial–based scaffolds provide possiblity to investigate the ability of multilayered nervous tissue in translating adhesive interactions into network activity in regions relatively far from the interface itself. These can, provide relevant information for the scientific community dealing with neuronal interfaces and electrodes even their unique physicochemical properties pose potential risks to the health of humans.

The biosafety issues of carbon nanomaterials in practical applications are not clear yet. An increasing amount of evidence indicates that toxicity/pharmacodynamics of carbon nanomaterials is critically influenced by the route of exposure/administration. Future developments of scaffolds/devices based on the carbon nanomaterials will therefore necessarily take into account these issues. With the development of methodologies for the chemical modification and functionalization of carbon nanomaterials, it opens up an even wider range of bioapplication opportunities, such as drug delivery, bioconjugation and specific recognition. The future design of carbon nanomaterial–based technologies will have to guarantee their stability, full biocompatibility and safety. The unique properties of CNTs and the application of nanotechnology to the nervous system may have a tremendous impact on the future developments of micro systems for neural prosthetics as well as immediate benefits for basic research. The utilization of CNFs in the nervous system, which have great potentials as multiplexing neural interfaces and intracellular neural interfaces, can provide high spatial resolution, high sensitivity, and minimal damage to neural tissue. Much attention still need to be paid for clinical applications of CNTs, such as the biocompatibility of the materials introduced in the fabrication of CNFs, the enhancement of homogeneity and yield of CNFs. Considering the well interactions between graphene and neurons, graphene can be used as implanted materials or neural chips for the tissue engineering, especially in the nervous system. Despite of the challenges, for better understanding and better use of its biological effects, the graphene biocompatibility and interactions with an organism (tissue/cell) should be well clarified.

Incorporation of anti–inflammatory drugs in the coatings and neuronal guidance toward the electrode by self–assembled scaffolds represent the directions with greatest immediate and practical significance. The combination of neural guidance and drug elution capabilities in one coating should be strongly considered. Future development of nanostructured coatings will also target significant increase of charge injection capacity and reduction of interface impedance. Nanoscale technology and/or coating with high aspect ratio features on the surface are known to improve charge injection in neurons.

The incorporation of light–sensitive materials in neural electrodes is another promising direction for the future development. The presence of photoreactions at the interface gives additional restrictions on the materials to be used such as their biocompatibility, and long–term stability. More stable inorganic semiconductor materials that are active in the visible spectrum tend to contain heavy metals, which are likely to cause problems with long–term biocompatibility. Hence developments of alternate nanostructured materials and potentially differently doped inorganic nanocolloids should be charted as one of the future tasks in this area. For an instance, fullerene coatings can serve as an intermediate solution for this offering both strong photovoltaic activity in the visible range and radical savaging. CNTs may also give adequate performance and can possibly surpass other materials as a potential candidate for an artificial retina.

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