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Review Article

The applications of conductive nanomaterials in the biomedical field

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Abstract: As their name suggests, conductive nanomaterials (CNMs) are a type of functional materials, which not only have a high surface area to volume ratio, but also possess excellent conductivity. Thus far, CNMs have been widely used in biomedical applications, such as effectively transferring electrical signals, and providing a large surface area to adsorb proteins and induce cellular functions. Recent works propose further applications of CNMs in biosensors, tissue engineering, neural probes, and drug delivery. This review focuses on common types of CNMs and elaborates on their unique properties, which indicate that such CNMs have a potential to develop into a class of indispensable biomaterials for the diagnosis and therapy of human diseases. © 2015 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 104A: 322–339, 2016.

Key Words: conductive nanomaterials, biosensor, tissue engineering, neural probe, drug delivery

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INTRODUCTION

Prior to the development of nanotechnology, conductive polymers (CPs) were one of the main conducting materials used in biomedical applications.[1] Although CPs have good electrochemical and optical properties, they have some drawbacks such as relatively high resistance. With the discovery of nanomaterials, research on CPs has shifted into nanoscale; particularly, the high surface-to-volume ratio at this scale can effectively assist electron transfer. Recently, conductive nanomaterials (CNMs) have been widely used in the biomedical field such as in biosensors,[2–4] tissue engineering (include cardiac,[5–7] bone,[8,9] and neural[10]), neural probes,[11,12] and drug delivery.[13]

In general, conductive materials could be divided into the following categories: CPs, carbon-based materials, metals, and conducting composites. These conducting materials have been prepared into nano-scale materials such as nanoparticles, nanorods, nanotubes, nanofibers, and nanowires.[2,4,5,9]

CPs as electroactive organic materials were first prepared in the 1970s. CPs possess intriguing properties of common polymers, such as environmental stability and ease of synthesis, but they also exhibit good biocompatibility and electronic properties. CPs have a conjugated backbone that contains a series of alternating single and double bonds, which gives rise to an extended π network. The movement of electrons within the network makes CPs possible to

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possess semiconductive properties like metals.[14] Moreover, their electrchemical and physical properties can be altered and controlled through stimulation such as electricity and pH. Currently, nanostructured CPs such as polypyrrole (PPy), polyurethane, polyaniline (PANI) and their derivatives have been used in biosensors, neural probes, and controlled release systems, etc.[15–17]

Carbon-based nanomaterials, including graphenes, carbon nanotubes (CNTs), nanowires, and nanoribbons, have been used as biomaterials. Among them, CNTs are generally classified as single-walled, doubled-walled, or multi-walled structure nanotubes.[18] Especially, CNTs show the remarkable ability to direct stem cell differentiation and could act as extracellular matrix to offer favorable environment to induce cellular attachment and growth.[19] In the research of electroactive tissue repair and regeneration, graphene has also been extensively employed as the promising biomaterials due to their unique properties: two-dimensional planar structure, large surface area, super conductivity, good chemical and mechanical stability and biocompatibility.[20]

The common utilized metal nanoparticles include nano-Ag, nano-Cu, and nano-Au. It is known that nanoscale metals possess a better orientation as well as large and porous surface areas, and excellent conductivity. For example, gold nanoparticles (AuNPs) have been extensively applied in biomedicine owing to their large surface area, high surface free energy and good biocompatibility.[18]

In recent years, conductive nanocomposites usually combine with different types of CNMs. The combination could overcome the limitation of single CNM and effectively improve the overall performance of biomaterials via the synergistic effect. In order to improve the biodegradability and biocompatibility, the nanocomposites are further mixed with biodegradable natural or synthetic materials; herein, natural or synthetic materials usually provide good biocompatibility and biodegradability to maintain the activity of biomolecules. The conductive nanocomposites generally form valid electrical pathways and provide a large surface to promote electron transfer and regulate biomolecules. The as-prepared biocomposites could possess increased surface area, functional groups, mechanical performance, and good biocompatibility.[21] Indeed, considerable attentions have been drawn to synthesize the above-mentioned biomaterials. For example, Tung et al. prepared single-walled CNTs/poly(3,4-ethylenedioxythiophene) (PEDOT) nanocomposite by the poly(ionic liquid)-mediated coupling method. The composites were found to be thermally stable, showing less surface resistivity as compared to single PEDOT-based films.[22]

CNMs USED IN BIOSENSORS

Normally, a biosensor combines a recognition layer with a transducer (detector), which could measure quantitatively an analyte. An ideal biosensor should possess exceptional detection capabilities, such as selectivity, sensitivity and stability. As it is well known, the sensitivity is a central concern of biosensor design, which depends on the ability of singal amplification. Recently, CNMs have offered interesting prospects in biosensing, which have been used as biomolecule immobilizers, electrode surface modifiers, carriers to load labels, or directly as signal reporters. Their high surface-to-volume ratio provides a suitable microenvironment for biological elements (for example, enzymes, antibodies, nucleic acids, cells, and tissue sections). Their conductivity and electrocatalytic property enable the electron signal to transfer quickly through electrodes and biological elements.[23] The CNMs have therefore been employed to magnify and measure the electrochemical signal. Herein, we discuss mainly about the application of CNMs in protein and nucleic acid biosensors.

Protein-based biosensors

Protein (for example, enzyme or antibody) is the most common recognition element in the process of preparing sensors. The crucial issue in protein-based biosensors is stable immobilization of proteins. However, conventional immobilization methods, including covalent attachment, entrapment, cross-linking, and encapsulation, have some drawbacks in the fabrication of biosensors. For example, functional reagents are able to form stable covalent bonds between the interface of protein-protein and protein-substrate, but the active sites in the layer are decreased accordingly and even the reaction conditions maybe do harm to the protein and conductivity of substrate.[24] To minimize these limitations, CNMs have been extensively employed as stable immobilizers or labels for biomolecules like enzymes, antibodies, antigens and so forth and they have shown great application prospects because they can increase the amount of antibodies and retain their bioactivity with simple immobilization process.

Enzyme-based biosensors. Numerous enzyme-based biosensors have been investigated through the years. In particular, glucose oxidase (GOx), which help to monitor glucose level in the blood or urine samples for the treatment and control of diabetes, have been often employed in the fabrication of glucose biosensors.[25] To improve the sensitivity and selectivity of biosensing, CNMs, such as polyaniline (PANI),[26] graphene,[27] and polyurethane (PU),[28] have been employed to detect glucose. For instance, Pt nanoparticles, PANI, and multi-walled carbon nanotubes (MWCNTs) were prepared into porous nanocomposite layers by Zhong et al.[26] The Pt/MWCNT-PANI membrane exhibited a large surface-to-volume ratio, which enhanced the loading capacity of GOx. The good biocompatibility of the nanocomposite retained bioactivity of the enzyme and the synergistic electrocatalytic effect of hybrid film made the biosensor exhibit high sensitivity. Likewise, Zhai et al. fabricated a glucose sensor with higher sensitivity (96.1 $\mu A m M^{-1}$ cm^{-2}). In their work, Pt nanoparticles were combined with nanostructured PANI hydrogel. The hydrogel facilitated efficiently the catalysis reaction of GOx.[2] Recently, PtPd bimetallic nanoparticles and MWCNTs have been respectively used as GOx immobilizers and surface modifiers by Chena et al. The study demonstrated that the biosensor possessed excellent

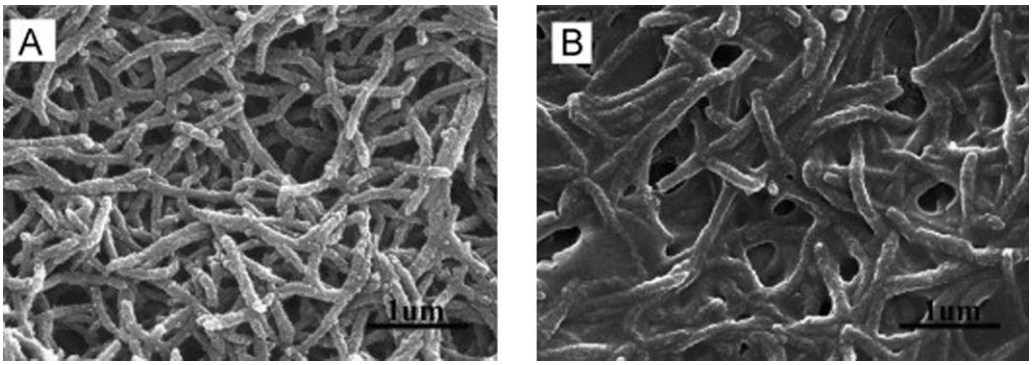

FIGURE 1. SEM images of the surface morphologies of the sensor. A: PANI nanofibers covered on the electrode. B: PANI nanofibers and GOx enzyme crosslinked by glutaraldehyde. (Adapted with permission from Ref. 28. Copyright 2014 Elsevier Ltd).

electrochemical properties, rapid response, and the detection limit for glucose was 0.031 m*M*. The biocomposite enlarged surface area and promoted electron transfer ability.[27] In fact, many types of mental nanoparticles have been applied in glucose biosensors. They have excellent electrocatalytic activity and high conductivity, which improve the efficiency of charge transport, promoting therefore the sensitivity and selectivity of biosensing and shortening obviously respond time; they possess a large surface area and good adsorption capacity, which lead to more enzyme immobilized on the sensor; they provide a biocompatible electroactive surface for enzyme immobilization, which help to retain the biological activity of GOx with high stability.[3]

The synergistic effect of conductive nanocomposites could greatly improve electron transfer ability, electroncatalytic performance and the redox reaction on the electrode surface, and facilitating more GOxs to be successfully immobilized on the electrode surface. To enhance the capabilities of glucose biosensor, Fang et al. prepared a Pt/PANI/GOx/PU glucose sensor based on biodegradable electroactive composite. In their work, the PANI nanofiber film was electrodeposited on the electrode. SEM images of the surface morphologies of the sensor was shown in Figure 1. Their result showed that the sensor maintained its initial sensitivity for 20 days in 37°C bovine serum. This indicated that the biodegradable hybrid improve effectively the stability of glucose biosensor.[28] Similarly, the immobilization of GOx was achieved in a straightforward method without any cross-linking agents or modifiers. The as-prepared biosensor exhibited a wide linear range of $0.1-27$ m*M* and a quick response.[29] The Au@Ag nanoparticles were used to modify electrode for the analysis of biosensors by Yang, the biosensor exhibited high electrocatalytic activity toward glucose oxidation and good selectivity.[30] In addition, a CuNWs-MWCNTs-based glucose sensor was synthesized by Huang et al, they found that the nanocomplex could evidently enhanced the performance of the biosensor; especially, the sensitivity of the glucose sensor attained to 1995 μ*M*.[31] Several other kinds of CNMs modified glucose biosensors were also explored these years.[32–35] The analytical performance of the different GOx electrodes is listed in Table I.

Apart from detecting glucose, the biosensors also can detect other classes of enzymes. For instance, Hu et al. employed AuNPs and MWCNTs to develop an ultrasensitive e biosensing to detect mucin 1 protein (a kind of tumor markers). In their work, MWCNTs were assembled on the glassy carbon electrode (GCE) for accelerating electron transfer, and they showed good recognition ability to analyte. AuNPs acted as electronactive labels to amplify signal.[36] Additionally, CeO_2-PANI core-shell nanoparticles were

TABLE I. Comparisons of the Analytical Performance of the Different GOx Electrodes

Electrode	Applied Potential (V)	LR[a](m*M*)	DL[b] (μ*M*)	RT[c](s)	K_m[d] (m*M*)	Sensitivity (μA/m*M* cm$^{2)}$	References
GOx/CS-GA/Nafion/PtPd-MWCNTs/GCE	0.6	0.062 − 14.07	31	<5	3.3	112	27
GOx/RGO/GCE	−0.44	0.1 − 27	–	<5	–	1.85	29
GOx/Pt/PANI/PU/GCE	0.65	0 − 14	–	10	51	–	28
GOx/Au-Ni coaxial nanorod	0.4	0.0275 − 27.75	5.5	13	–	778.2	33
GOx/MWCNT/GO/GCE	−0.402	0.1 − 19.82	28	5	–	–	34
CuNWs-MWCNTs/GCE	0.55	0 − 3	0.26	<1	–	1995	31
GOD/graphene/nano-Au/GCE	0.39	0.02 − 2	17	–	–	56.93	32
GOx/Poly(BEDOA-6)/Au NPs/MPA	−0.7	0.025 − 1.25	25	–	0.81	14.97	35
GOD/Au@Ag NRs/GCE	0.54	0.02 − 7.02	0.67	<2	–	33.67	30

[a]LR, linear range.
[b]DL, detection limit.
[c]RT, response time.
[d]K_m, Michaelis-Menten constant.

prepared for the fabrication of biosensor by Gumpu et al. The biosensor could detect histamine through nanosurface controlled reactions adding diamine oxidase (DAO). Their result suggested that the biosensor exhibited a wide linear range from 0.45 to 1.05 m*M* and a low limit of detection was 48.7 μ*M*. Moreover, the response time of the biosensing was <1 s.[37]

Immunosensors. Due to the unique recognition nature of antibodies to antigens, immunosensors usually have high selective and sensitive properties, which have been used to detect cancer, biowarfare agents, and blood components for medical diagnostics, and so forth.[38] However, in spite of their large scale applications, some limitations of immunosensors still existed. For instance, no production that produced in the antigen-antiody interaction can be easily recognized.[39] CNMs modified sensors can overcome the obstacle, which has been frequently investigated in recent years.

Owing to excellent conductivity and high surface energy, AuNPs are the most widely used materials in the antigen detection. Liu and his groups used AuNPs as a sensing platform to directly detect atrazine. In this study, AuNPs with high surface free energy were loaded on the surface of gold electrode, which enlarged surface area of the electrode and offered more adsorption sites for antibodies.[40] Jin et al. developed an immunosensor for the detection of cancer biomarker. They firstly synthesized a graphene film through chemical vapor deposition. Capture antibodies coated with magnetic beads were immobilized on the platform, and then enzyme-labeled detection antibody was conjugated with AuNPs.[41] In the study, label-free graphene sheets were used as modified materials, which offered active sites for antibody; AuNPs acted as carriers for target antibody. Their result suggested that the use of these CNMs improved effectively the rate of electron transfer and amplified the sensing signal, enhancing thus the sensitivity and specificity of the biosensor.

Liu's group reported an effective sensing strategy based on AuNPs. In their work, aryl films were modified on AuNPs through the spontaneous adsorption of aryl diazonium precursors. The functionalized AuNPs were covalently anchored to glass carbon electrode by Au-C bonding formed in aryldiazonium salt reaction. AuNPs were modified on the surface of the electrode only by one step. This work provides a simple approach for the immobilization of AuNPs. In the end, they further confirmed that the AuNPs facilitated electron transfer between biomolecules and electrode, and greatly enhanced the coupling efficiency of biomolecules.[42] A sandwich structure of immunosensor can simultaneous detect two cancer markers was reported by Wu et al. The sensitivity of the biosensor was enhanced via the use of graphene and mesoporous carbon materials.[43] Yang's group developed an ultrasensitive biosensing for the detection of α-fetoprotein by using functionalized single-walled carbon nanohorns as label and two enzymes (horseradish peroxidase and glucose oxidase) were employed to induce biocatalytic precipitation. The enzymatic reactions formed many nonconductive insoluble products that can be employed for signal amplification. In the study, cyclic voltammetric confirmed that the nanohorns further amplified the sensing singal, enhanced the conjugation of biomolecules and provided more sites for insoluble products.[43]

Genosensors

Genosensor was used in diagnostic tests for gene mutation, early cancer detection, and analysis of gene sequences as an analytical device.[45,46] Electrochemical methods have widely employed in sensing nucleic acid biomolecules for its rapidity and portability. The genobiosensings usually present increased specificity and simplicity through the addition of CNMs, which also help to reduce respond time and increase the amount of nucleic acid. For the above reasons, CNMs have been used to conduct different types of nucleic acid biosensors.

DNA biosenors. The detection of specific DNA sequences has been attracted increasing attention over the past few years. Typically, single strand DNA probes are first immobilized on the surface of a substrate in the construction of DNA biosensor. The immobilization of DNA directly affects the overall performances of the biosensor, such as the accuracy of sensor and connection of probe DNA and target DNA.[45,47] CNMs can provide a large active site network that helps to the stable immobilization of DNA strand. The dimensions and morphology of CNMs, such as nanotube, nanoparticle, and nanorob, have an effect on the sensitivity of the biosensor. Metal nanoparticles (for example, AuNPs) and graphenes are widely used to fabricate DNA biosensors in recent years. The insertion of these CNMs could enhance effectively the rate of charge transfer and amplify detect signal. Moreover, AuNPs present negative electricity, which can easily immobilize DNA in the form of Au-NH or Au-S chemical bond.[48]

In 2011, Alagar et al. developed a dual label biosensor based on AuNPs. The AuNPs modified electrochemical sensor could be used to detect mycobacterium sp. genomic DNA.[49] Similarly, Dong and his coworkers reported a sensing platform by assembling AuNPs and electrochemically reduced graphene oxide on electrode. They successfully immobilized single strand DNA on the surface of AuNPs. In this research, they found that the AuNPs greatly increased the DNA anchor points and accelerated electron transfer. The DNA biosensor possess high sensitivity and selectivity.[50] Norouzi et al. incorporated AuNPs into nanocomposite carbon paste electrode to detect methyl parathion DNA. The DNA biosensing presented enhanced comductivity and good stability, which attributed to the electrocatalytic ability and effective surface area of the nanoparticles.[37]

Graphene have also drawn much attention in the biosensing, due to their unique performances like excellent conductivity and large surface area. However, the dispersion property of graphene is poor in aqueous solution, and DNAs are not easily immobilized on it. Combined with other biocompatible materials may produce unexpected synergistic action that improves the immobilization of biomolecules.

Recently, Huang et al. conducted graphene/Au nanorod/polythionine nanocomposite to synthesize electrochemical DNA biosensing without labels and enzymes.[51] Graphene and Au nanorods were firstly electrodeposited onto the GCE. The nanocomposite electrode displayed the electro-transfer resistance was 330 Ω, which was much less than the bare one (725 Ω). The authors claimed that the novel biosensor presented high sensitivity and selectivity to detect the DNA. The nanostructured film provided a suitable surface for the DNA immobilization. Indeed, the result ascribed to the tunable and strong absorption ability and large cross-sections of Au nanorods.

Shi and his co-workers reported the application of Au nanomaterials (AuNPs and Au nanorods) and graphene in electrochemical detection of a specific-sequence target DNA.[52] They demonstrated that these CNMs enhanced the sensing performance of the biosensor. In their work, Au nanorobs and reduce graphene oxide were used to modify the electrode. Moreover, AuNPs were functioned as carrier to load reporter DNA, which obviously amplified the detection of target DNA. Likewise, Qi et al. used chitosan/Co_3O_4 nanorod/graphene nanocomposite film to immobilize single strand DNA. They confirmed that the film provided good conductivity to analyze target DNA, which decreased the interface resistance and enlarged effectively the signal of biosensing. Due to special porous structure and appropriate surface area of the conductive film, the prepared detector was capable to measure lower concentrations of single strand DNA sequence (the DNA sequence only was 3.7%).[53]

Actually, other types of CNMs have also qualified in acting as electrode modified materials to construct a suitable sensing platform for the immobilization and analysis of target DNA.[48] In some cases, these CNMs can be used as carriers to load signal elements such as enzymes and dyes, or as nanotracers to increase electrochemical responses or direct detection. For example, Sui et al. fabricated a novel DNA biosensing with V_2O_5 nanobelts/MWCNTs/chitosan modified carbon ionic liquid electrode. The synergistic effect of conductive nanocomposites amplified sensing signal and increased the immobilization amount of single strand DNA.[54] Qiu et al. investigated a lateral flow biosensor to detect amine-modified DNA based on MWCNTs. In their work, MWCNTs were used as carriers to enhance the DNA loading quantity and realize more effective hybridization, which also led to the visual detection of target DNA as a marker.[55]

RNA biosensors. MicroRNAs (miRNAs) are a family of 18- to 25-nucleotide-long, endogenous RNA molecules,[56,57] which are related to diverse biological processes like cell differentiation and proliferation, and the onset of cancer. They have been employed in developing electrochemical RNA biosensors over the past decades, but the detection of miRNAs still presents many challenges, owing to the small size and low content in RNA samples.[58] So far, many researchers have reported that CNMs could be used for signal amplification and advancement of hybridization efficiency, further improving the overall properties of miRNA biosensor, especially its sensitivity.[56,57,59] Compared with conventional methods like PCR, label-free electrochemical methods, CNMs have great advantages, such as reducing time and cutting down the cost of detection.

Yin and his group reported the use of graphene nanosheets and AuNPs for the detection of microRNA-21.[59] The sensing signal of label-free biosensor was amplified three times. The method they took was that dendritic gold nanostructure and graphene nanosheet modified glass carbon electrode, the biotin-functionalized DNA were loaded onto the surface of AuNPs and the AuNPs were hybridized with the target microRNA, the streptavidin functionlized horseradish peroxidase reacted with terminal biotin molecules of barcode DNA. Based on the above steps, the microRNA biosensor had high sensitivity, and the detection limit was as low as 0.06 pM. Liu et al. also proposed multiple signal amplication based on AuNPs and enzymes to detect the target microRNA sequence.[56] In their work, the label-free biosensing with a lower detection limit was 3 fM.

Additionally, CNTs have excellent electrochemical property for guanine oxidation in the nucleic acid detection. Li et al. used firstly MWCNTs modified GCEs to develop a microRNA-24 biosensing without label. In this study, the capture probes were assembled on the surface of the electrodes by covalent binding with the carboxyl group of the MWCNTs. The nanotubes provided a favorable platform and increased the guanine oxidation, which led to the prepared biosensor presented high selectivity and good reproducibility.[44]

CNMs USED IN TISSUE ENGINEERING

Tissue engineering aims to repair or regenerate tissues that have been damaged or lost, which can act as a potential alternative to organ transplantation. The strategy of tissue engineering is to replace a defective organ with an artificial scaffold composed of cells, growth factors, and biomaterials that function as an extracellular matrix (ECM).[60] The success of tissue engineering mainly depends on two essential elements: cells and biomaterials. It is generally acknowledged that a close imitation to the natural ECM could provide a proper environment to support cell functions. Electrical signals are critical physiological stimuli factors that control the adhesion and differentiation of certain cell types (for example, nerve, cardiac, and bone). To retain cellular bioactivity and enhance overall properties of scaffold, CNMs have lately emerged as promising candidates in producing scaffolds to better mimic the natural ECM and to efficiently replace defective tissues.[61] For instance, Hsiao et al. prepared aligned PANI/poly(lactic acid-*co*-glycolic acid) (PLGA) conductive nanofibrous mesh via electrospinning technique. Their result demonstrated that the nanocomposite promoted cardiomyocyte adhesion due to the positive charges they transformed, and the fluorescence micrographs were shown in Figure 2.[62] Particularly, CNMs with nanoscaled structure and excellent conductivity were proven to can modulate early stage of stem cell lineage commitment without the aid of induction factors.[60]

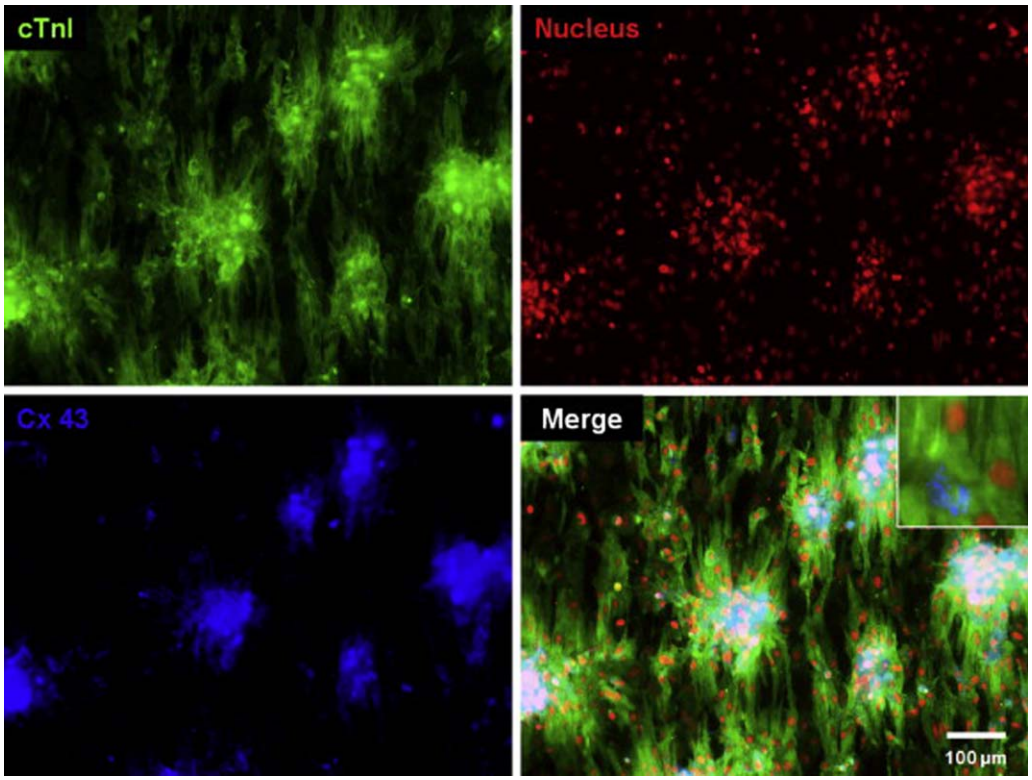

FIGURE 2. Fluorescence micrographs of the neonatal rat cardiomyocytes cultivated on test meshes using the immunofluorescence staining for cardiac troponin I (cTnI, green), connexin 43 (Cx 43, blue), and nucleus (red). The inset in the panel shows a higher magnification image (Adapted with permission from Ref. 62. Copyright 2013 Elsevier Ltd).

Moreover, electrospinning has emerged as a useful tool to prepare spatially organized materials for tissue engineering. Electrospun nanofibrous scaffolds are able to mimic the architecture and biological functions of the ECM. The as-prepared CNMs could provide electrical impulses to cells and regulate cellular functions, including attachment, proliferation, migration, and differentiation.[63]

Cardiac tissue engineering

The cardiac muscle is a significant electroactive tissue, which retains heart beat and transfers electrical signals.[20] Previously, natural and synthetic polymers, such as polyurethane, polycaprolactone (PCL), gelatin and their blends, were often used to fabricate scaffolds. However, these scaffolds exhibit high electrically resistant at biologically relevant frequencies (heart muscles DC conductivity ~0.1 S/m), resulting in noncommunication among cells.[5,6] By now, a considerable amount of efforts have been devoted for the development of conductive scaffolds. In recent years, many researchers have employed CNMs as scaffold materials, whereby electrophysiological coupling between seed cells and tissues could be promoted effectively. So far, the composites or blends composed of CNMs and polymers have been widely studied in cardiac tissue engineering. For instance, CNTs/gelatin methacrylate hydrogels could act as scaffolds for cardiac regeneration. Cardiac tissues cultured on the hydrogels could resist damage by a model cardiac inhibitor and a cytotoxic compound. In this hydrogel system, CNTs enhanced the mechanical integrity and electrophysiological function of the hydrogels and promoted cell attachment and proliferation.[7]

Similarly, cardiomyocytes cultured on nanofibers of GelMA-coated CNTs and gelatin (PG) could retain their viability, contractile activities, and exhibit stronger spontaneous and synchronous beating behavior than those cultured on pure PG scaffolds. This result can be attributed to the higher electrical conductivity, toughness and fiber alignment offered by the nanofibrous scaffolds. Because CNTs possess nanoscale geometries that can structurally mimic collagen fibers, which can greatly influence cell adhesion, proliferation, and differentiation.[64] In addition, PLGA and conductive PANI were also prepared into a nanofiber mesh by electrospinning. This research suggested that the aligned PANI/PLGA nanofibers obviously promoted cell adhesion and helped to isolated cell cluster to form the adhered cardiomyocytes along the long axis of the fibers. The adsorption amount of negatively charged adhesive proteins increased due to the positive electrical properties of the nanocomposite.[62] Stout et al. evaluated cardiomyocyte and neuron functions based on PLGA (50 : 50 wt %)-carbon nanofiber (CNF) composites. The research indicated that the composites further promoted the adhesion and proliferation of cardiomyocytes and neurons, and increased the conductivity compared to pure PLGA. As the amount of CNFs increased,

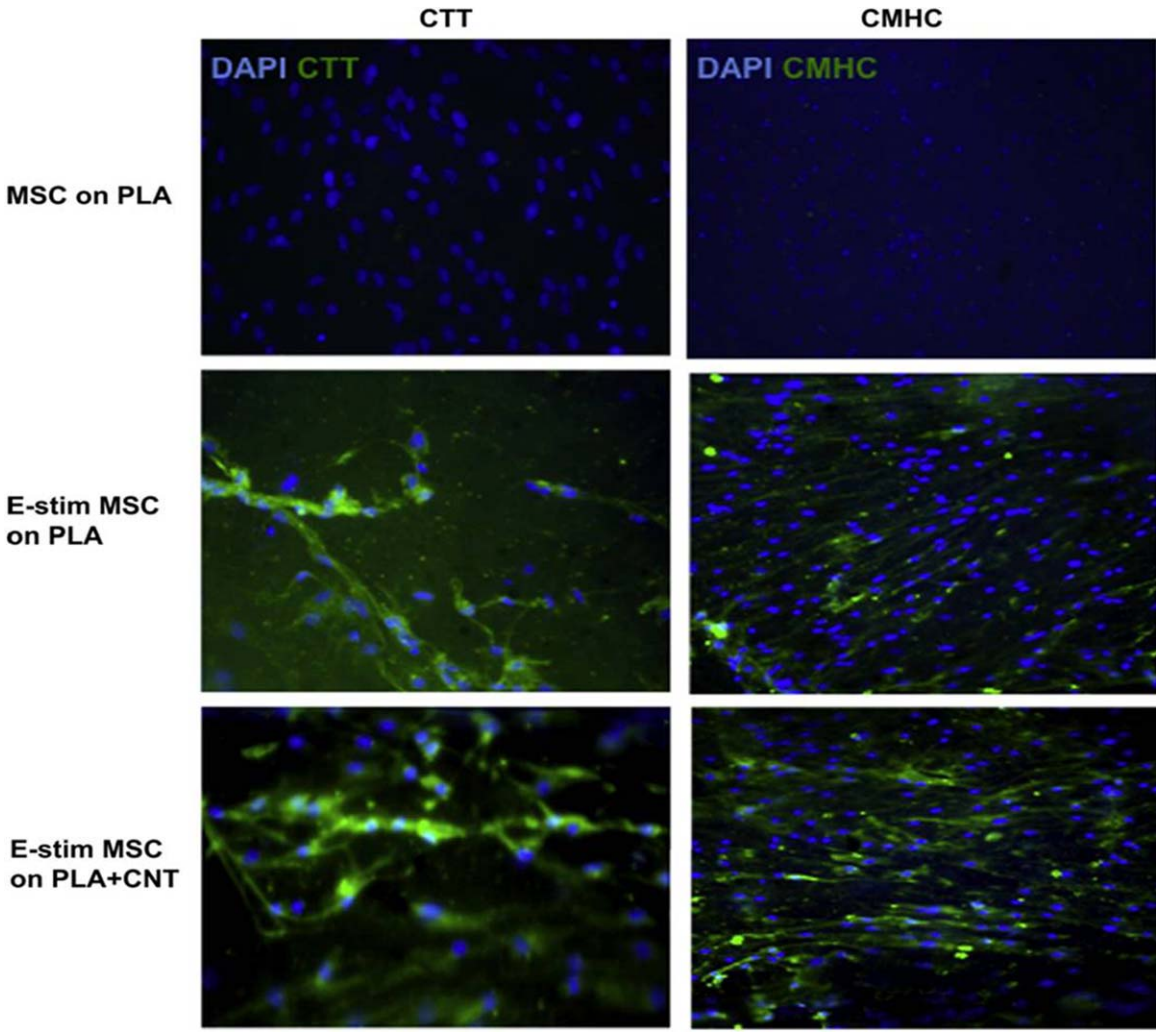

FIGURE 3. A: Examination of protein expression for a range of cardiac markers using (i) Western blot analysis and quantification using (ii) densitometric analysis. Cardiac marker expression was normalized to CuZn SOD levels with MSC alone as the control cultures (Results are representative of two independent donors). B: Immunofluorescence staining for detection of cardiac troponin T, cardiac myosin heavy chain, and connexin43 for (i) MSC exposed to medium containing CNT and (ii) MSC seeded on CNT scaffolds. Scale bar 130 μm. In both cases there was an upregulation in protein expression, more noticeably with CNT present after electrical stimulation. (Adapted with permission from Ref. 20. Copyright 2012 Elsevier Ltd).

the cardiomyocyte density became greater for up to 5 days. Owing to the biodegradability and biocompatibility of PLGA, the as-prepared composites might be suitable for clinical applications in cardiac tissue engineering.[65]

A poly(*N*-isopropylacrylamide) (PNIPAAM)/MWCNTs hydrogel was fabricated by Chen et al. The hydrogel scaffold possessed higher elastic moduli and hydrophobicities than pure PNIPAAM scaffolds. In general, pure PNIPAAM hydrogels can not be directly employed to culture anchorage-dependent cells, but the hydrogel can serve as cell culture substrates and promote cell proliferation. This result indicated that MWCNTs can regulate cell functions and maintain cellular bioactivity.[66] In another study, PNIPAAM hydrogel was combined with SWCNTs. Therapeutic efficacies were evidently enhanced after myocardial infarction while the hydrogel was applied as a carrier. The PNIPAAM/SWCNTs hydrogel showed higher cell adhesion and proliferation to adipose-derived stem cells (BASCs) *in vitro* compared with the base PNIPAAM hydrogel. In this research, SWCNTs enhanced the bioactivities of PNIPAAM hydrogel. Their nanostructure nature provides a large and rough surface to cell attachment.[67]

Given that cardiomyocytes possess poor regeneration capability, many scholars have devoted to study the functional cells and develop suitable scaffold materials to repair injured myocardium. In the recent years, the potential ability of CNTs to conduct electrical impulses has been verified *in vivo* cardiomyogenesis. Mesenchymal stem cells (MSCs) were directly exposed and seeded on CNT/polylactic acid (PLA) scaffolds by Mooney and his colleagues (Fig. 3). Their result showed that the majority of cells presented elongated morphology and reoriented perpendicularly to the direction of the electrical current at an angle between 0 and 10° via electrical stimulation. Additionally, the expression amount of

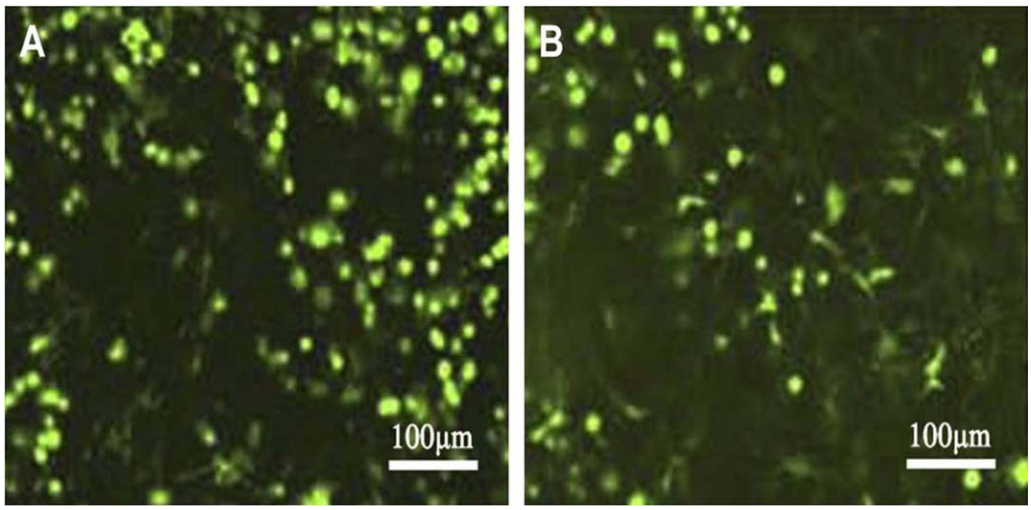

FIGURE 4. Confocal microscopy images of the HASCs conventionally cultured for 7 days on: MWNTs (A) and GP (B) (Adapted with permission from Ref. 71. Copyright 2012 Elsevier Ltd).

the relevant proteins increased for cell-seeded scaffolds under the electrical stimulation; thereby, the synergy between ES and CNTs regulated the pre-differentiation of MSCs to create cardioprogenitor cells.[20] Very similar results were also found on conducting polymers.[68] Conductive PANI nanoneedles networks have demonstrated to promote charge transfer within the PANI/PCL composites, and PANI facilitated the cardiogenic differentiation of human mesenchymal stem cells (hMSCs). Moreover, the hMSCs showed a relatively high survival rate cultured on PANI/PCL composites in the early stage.[69]

Bone tissue engineering

It has been known that bone tissue is associated with the electrical signals. Osteogenic maturation of the osteoblast is crucial for bone formation. Typically, electric stimulation is vital to the attachment and proliferation of osteoblasts, and CNMs could link with the cells though transfer electrical signals, which have been confirmed to can promote the proliferation and differentiation of osteoblast cells.[70] For example, Zhang et al. fabricated nanocomposites of octadecylamine-functionalized nanodiamonds (ND-ODA) and poly(l-lactic acid) (PLLA) via solution casting. The nanocomposite based scaffold exhibited excellent mechanical properties relative to the pure PLLA scaffold, especially when adding 10%wt. ND-ODA in composites. Moveover, the mineralization capability of scaffolds was increased on ND-ODA/PLLA surface.[8]

Li et al. evaluated the effects of MWCNT and graphite compacts with the same size on human adipose-derived stem cells cultured *in vitro* and the formation of ectopic bone *in vivo*. The result indicated that MWCNTs promoted the expression of the osteogenic genes and the adsorption of protein relative to graphite, which might attribute to the special surface structure (Fig. 4). A large number of absorbed proteins were benefit to the attachment and proliferation of cells. In Figure 5, ALP stands for the formation of osteogenic cells. This indicated that MWCNTs might induce human adipose-derived stem cells to differetiate into

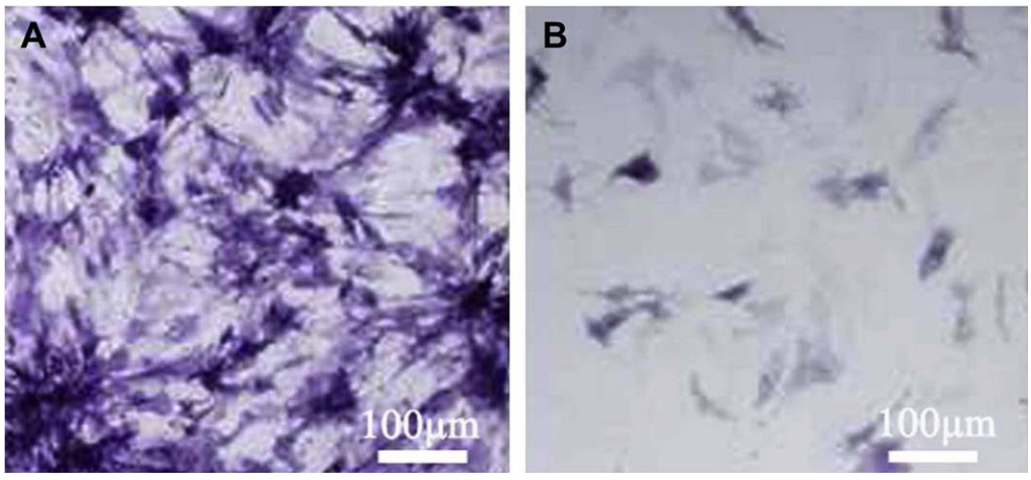

FIGURE 5. ALP staining on MWNTs (A) and GP (B) at cell culture time of 7 days with adsorption of rhBMP-2 in advance (Adapted with permission from Ref. 71. Copyright 2012 Elsevier Ltd).

osteogenic cells. Moreover, MWCNT induced ectopic bone formation while graphite did not. Therefore, CNTs have potential in modulating downstream stem cellular response without adding exogenous growth factors.[71]

The mechanical properties of CNMs have a significant influence on regulating stem cell differentiation. PANI nanofibers was functionalized by carboxylic acid, which exhibited higher solubility and biodegradability than uncoated PANI. The elastic modulus of the nanofibers were significantly enhanced, and the composite nanofibres supported the stem cells attachment and proliferation, as compared with pure PANI.[72] For another instance, rat bone marrow derived MSCs and adipose tissue derived MSCs in passages 2–4 were cultured on different stiffness of substrates. It was found that the stiff substrate facilitated the osteogenic differentiation of the two kinds of stem cells, the cells attached easily on soft substrates relative to the stiff counterparts. However, the proliferation of the cells showed no obvious change.[73]

In order to study the effect of graphene on osteogenic differentiation of human mesenchymal stem cells (hMSCs), Nayak et al. coated graphene onto different substrates, including polydimethylsi-loxane (PDMS), polyethylene terephthalate (PET), glass slides, and silicon wafers. They concluded that graphene coating accelerated the differentiation of hMSCs, which was the driving force of bone cell formation.[74] Furthermore, Lee et al. studied the effect of graphene film on the proliferation and differentiation of bone marrow derived mesenchymal stem cells (MSCs). In this work, a higher density of MSCs was found on the graphene film compared to the polydimethylsiloxane film. Moreover, the graphene film had the ability to preconcentrate osteogenic inducers (dexamethasone and β-glycerolphosphate), thus graphene could enhance MSC osteogenic differentiation. Herein, the high adsorption capacity for dexamethasone can be attributed to π-π stacking between the aromatic rings in the biomolecules and the graphene basal plane.[75]

Similarly, it was found that rat MSCs cultured in normal stem cell medium (without growth factors) on the CNT composite scaffold can differentiate into the osteogenic lineage and express high levels of bone marker ALP under the electrical stimulation.[76] Additionally, Shao et al. fabricated random oriented and aligned conductive PLA/MWCNTs nanofiber meshes by electrospinning in 2011.[9] Their result showed that osteoblasts grew along the axis of aligned nanofibers, and cell proliferation was better on the aligned PLA/MWCNTs nanofiber meshes than on random oriented nanofibers via electrical stimulation. The conductive nanofibers possess great potential in coupling bone regeneration and healing fracture with the use of an appropriate electrical stimulation. The electrospun nanofibers could simultaneously provide topographical and electrical cues to cells. Clearly, the combination of electrical stimulation and topographical cues of the conductive scaffolds may be an effective technique to promote osteoblast functions.

Native skeletal muscle responds to electrical stimuli via contracting neuromuscular junctions, and therefore generated forces. The contraction of skeletal muscle mainly depends on electrical stimulation unlike the heart that is voluntarily controlled.[6] It is necessary to enhance the formation of myotubes, because the regeneration capacity of muscular is too low. For example, Chen et al. prepared conductive biodegradable composite nanofibers composed of PANI and PCL to study the function of mouse C2C12 myoblasts.[77] The electroconductibility of PANI/PCL nanofibers containing 3 wt % PANI enhanced significantly to 63.6 ± 6.6 mS cm^{-1} relative to pure PCL fibers. The aligned nanofibrous scaffolds enhanced myoblast differentiation and myotube formation, and induced muscle cell alignment relative to randomly oriented nanofibers (R-PCL). Moreover, the amount, length, fusion index, and maturation index of myotubes on the aligned-PCL/PANI nanofibers were further enhanced, especially myotube numbers increased ~80% compared with R-PCL scaffolds. These results indicated that PANI could provide two different types of guidance cues, including topographical and electrical cues, to promote effectively myoblast formation, and well-aligned fibers can provide better topographical cues for cell growth compared with random fibers.[78] The poly(L-lactide-*co*-epsilon-caprolactone) (PLCL)/conductive PANI nanofibers exhibited similar functions, the electrically conductive properties and formation of myotubes on the PLCL/PANI nanofibers were distinctly enhanced (Fig. 6), but the mechanical properties decreased compared to the pure PLCL fibers, due to the brittleness of PANI.[79]

Neural tissue engineering

A significant amount of researches has been conducted to optimize the topographic, chemical, and electrical cues of scaffolds for enhancing neurocyte functions. CNMs, which are used to mimic the hierarchical structure of the ECM, can promote selectively proteins adsorption and retain their bioactivity. Moreover, they could provide a large surface area and high conductivity in favor of nerve stem cells (NSCs) attachment and establish the intercellular communication.[80] Particularly, electrical stimulation also can alter the local electric field of ECM proteins and regulate the protein adsorption to the surface of scaffold, and subsequently affect the neurite outgrowth.[10]

During the *in vitro* electrical stimulation of NSCs cultured on polypyrrole/PCL nanofibers scaffolds, the scaffolds improved the NSCs differentiation and neurite outgrowth.[10] Likewise, CNTs have also been applied in neural tissue engineering for promoting neuron growth and increasing the transmission signal among neurons. To improve the properties of CNT, carboxylated MWCNTs films deposited onto a collagen-coated polystyrene dish by Chen et al. The carboxylated MWCNTs up-regulated the neural growth and transcription factors, which make for the long-term differentiation of human bone marrow mesenchymal stem cells toward neurons, providing protein adsorption ability for neural growth and differentiation.[81] Carboxylated SWCNTs also had the similar effects. The human mesenchymal stem cells cultured on the SWCNT films showed homogeneously focal adhesion distribution throughout the cell body, because the nanostructure of the SWCNTs has

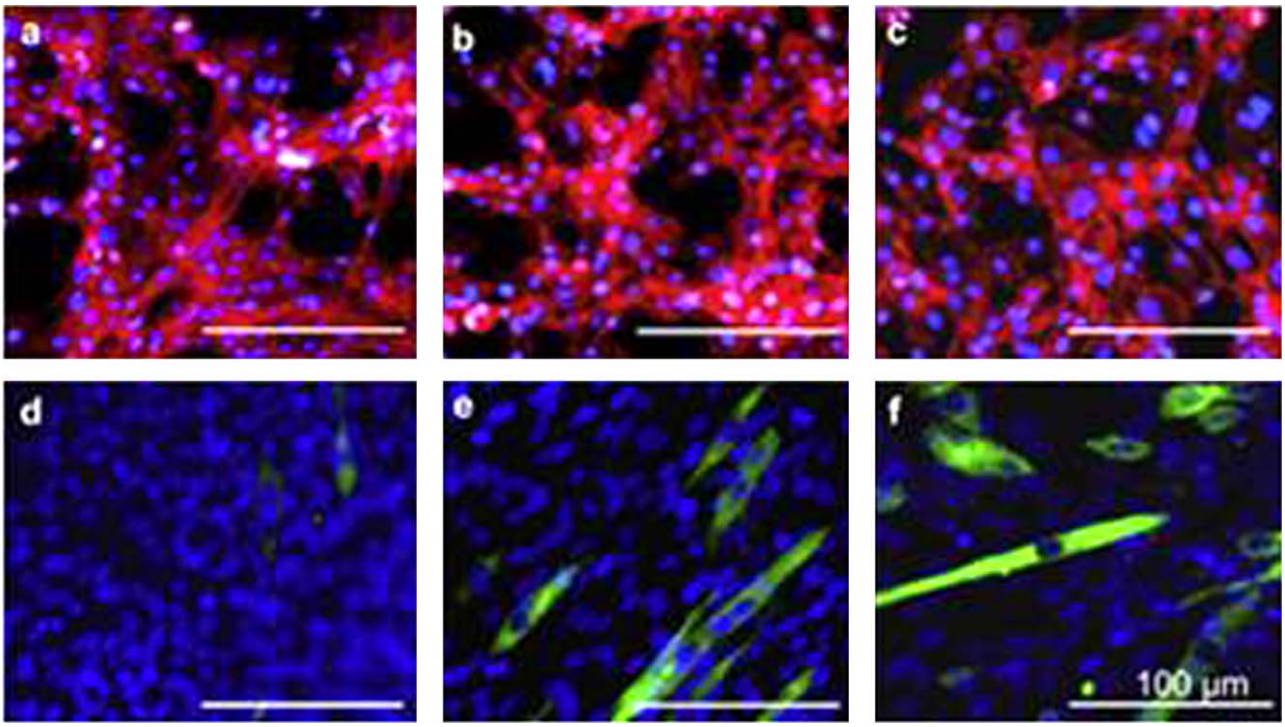

FIGURE 6. Fluorescence images of C2C12 myoblasts cultured on PLCL/PANi-0 (a,d), PLCL/PANi-15 (b,e), and PLCL/PANi-30 (c,f) for 1 and 4 days, respectively (Adapted with permission from Ref. 79. Copyright 2009 Elsevier Ltd).

significant effects on the cell morphology. Furthermore, the SWCNT films exhibited good biocompatibility and upregulated neurogenic gene markers during the initial phase of cell culture.[82] In 2014, Serrano et al. fabricated three-dimensional (3D) porous scaffolds cultured with embryonic neural progenitor cells based on chondroitin sulphate (CS)/MWCNT composites, and compared with 2D scaffolds with the same components. The SEM images of CS/MWCNTs are shown in Figure 7. In this research, a great number of neuron cells attached, for up to 20 days, on the viable networks that were supplied by the 3D porous scaffolds. The result suggested that the neural-enriched networks showed calcium dynamics and active mitochondria.[83] When these scaffolds are implanted in the acute phase after injury, the 3D structure of the scaffolds have more advantages in the enhancement of neural network formation compared with the 2D scaffolds, and CNTs are a type of suitable candidates in neural tissue devices.

In addition, graphene could enhance effectively the gene transfection in NIH-3T3 fibroblasts, graphene films have found to can promote significantly the sprouting and growth of neurons in the initial phase, especially in day 2, the number and length of neurons on the graphene films were higher relative to tissue culture polystyrene. The graphene films maintain cell viability and morphology, facilitating the expression of the neuronal differentiation marker growth associate protein.[84] Likewise, a 3D graphene foam was used to culture NSCs. The research demonstrated that the conductive 3D scaffold kept the cells at an active proliferation state, and enhanced the NSCs differentiation toward astrocytes and neurons, especially into neurons.[85] Indeed, biodegradable electroactive composites based on conducting polymer are also suitable for the neural tissue engineering. A research indicated that cellular adhesion and proliferation on the surface of polypyrrole (PPy)-coated PCL nanowire was significantly higher than on the uncoated PCL nanowire surface. The electrical resistivity of the PPy-coated nanowires reduced to some extent under the electrical stimulation, as is shown in Figure 8.[86] Furthermore, the functionality of PC12 cells and expression of key neuronal markers were enhanced on the nanowire surface significantly compared to smooth surface.[87]

In conclusion, CNMs are widely used to construct scaffolds to assist the reconstruction or regeneration of electroactive tissues (that is, bone, nerve and cardiac muscle). The nanocomposite composed of CNMs and natural or synthetic polymer are often used in tissue engineering. They could transfer effectively electrons and promote the proliferation and differentiation of cells, and retain the bioactivity of cells cultured on the scaffold.

CNMs USED IN NEURAL PROBES

Neural probes are composed of an array of microelectrodes to record brain activity. Recently, they have exhibited great potential in human-brain computer interface applications.[88] However, numerous problems need to be solved in this field such as high electrode impedance, mechanical mismatch, poor reliability, and longevity.[88,89] In general, the size of

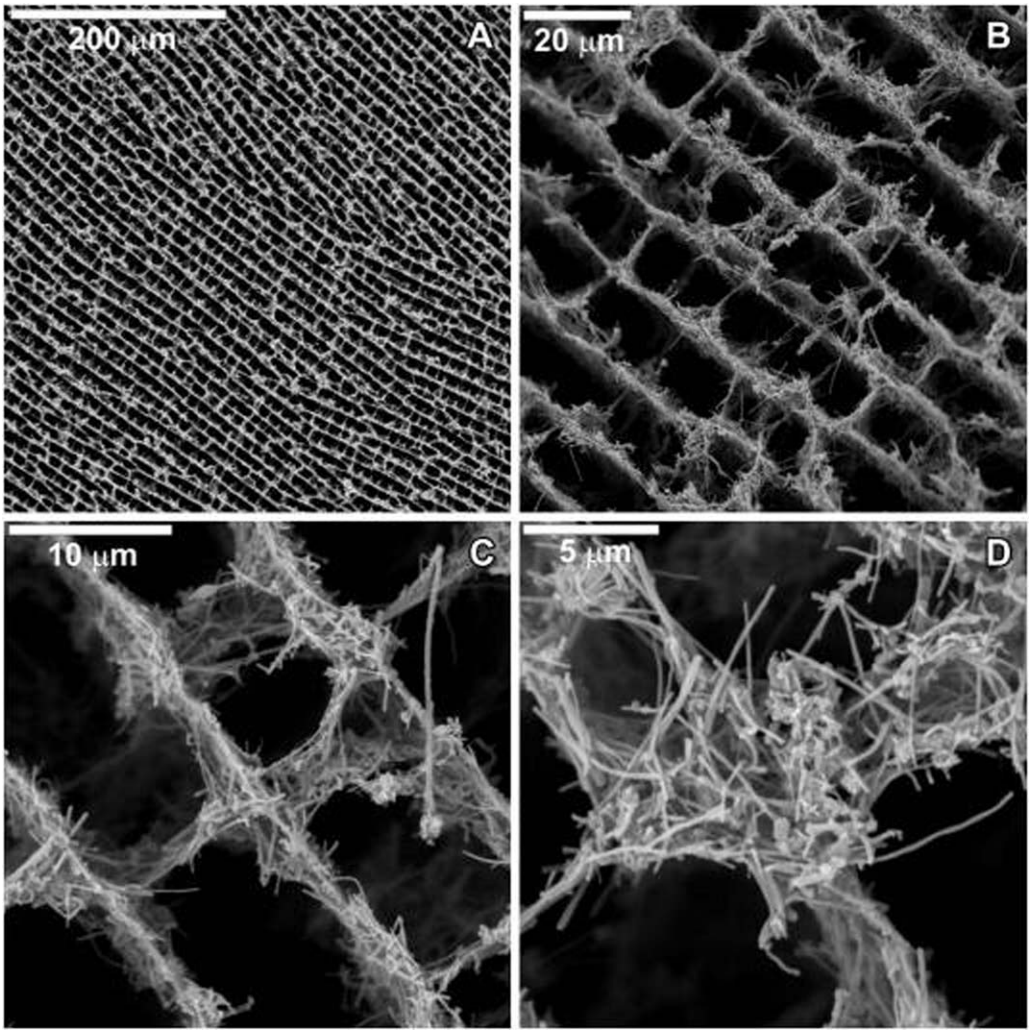

FIGURE 7. SEM images illustrating the architecture and surface topography of CS/MWCNT scaffolds. (Adapted with permission from Ref. 83. Copyright 2014 Elsevier Ltd).

neural probes must be small to precisely monitor and control neural circuit activity and increase the density of electrodes. Unfortunately, the reduction in size sharply increases electrode impedance and thermal noise, limiting the recording sensitivity, and the maximum stimulating current deliverable through an electrode.[90] Furthermore, when neural probes are implanted into human brains, the impedance of the electrode-neuron interface inevitably increases due to the glial encapsulation and the phagocytosis of immune system.[91] High electrode impedance means high potential at the interface, which decreases the charge transfer capability of microelectrodes and causes irreversible chemical reactions or heat accumulation that can lead to degradation of the probe material.[92,93] Thereby, reducing the interfacial impedance of microelectrodes is of great importance in neural probes. To extend longevity of neural probes, the microelectrodes must be biologically compatible, stable, and possess high a charge injection density. At present, CNMs are frequently used as probe materials to address these issues due to their unique properties. For example, Su et al. found that CNT-modified electrodes of neural probes can improve the charge delivery ability and reduce the impedance of the electrode.[12]

In general, the surface of CNT contains hydrophobic group that restricts its application. However, the treatment of the surface can promote the solubility of CNT, and therefore maintain effectively cellular bioactivity. O_2 plasma to treat (OT) the surface of CNTs enabled them to become more hydrophilic, and the OT-CNT probe reduced the impendence by half and increased the interfacial capacitance more than two orders of magnitude compared to the as-grown-CNT probes. Moreover, the 3D cone-shape probe possessed a larger surface area and higher spatial resolution of neural recording at the same footprint area compared to the planar-type CNT electrodes.[12] Similarly, Yen et al. used amine groups grafted MWCNT surfaces to promote neuronal cell growth on electrodes. The amino-functionalization (AF-MWCNTs) electrode reduced the impedance from 0.37 to 0.19 $k\Omega\ mm^{-2}$ and increased the interfacial capacitance from 2.1 to 22 $F\ mm^{-2}$. Additionally, the impedance fluctuation of the probe reduced, resulting in the further enhancement of long-term recording capabilities.[94] It revealed that

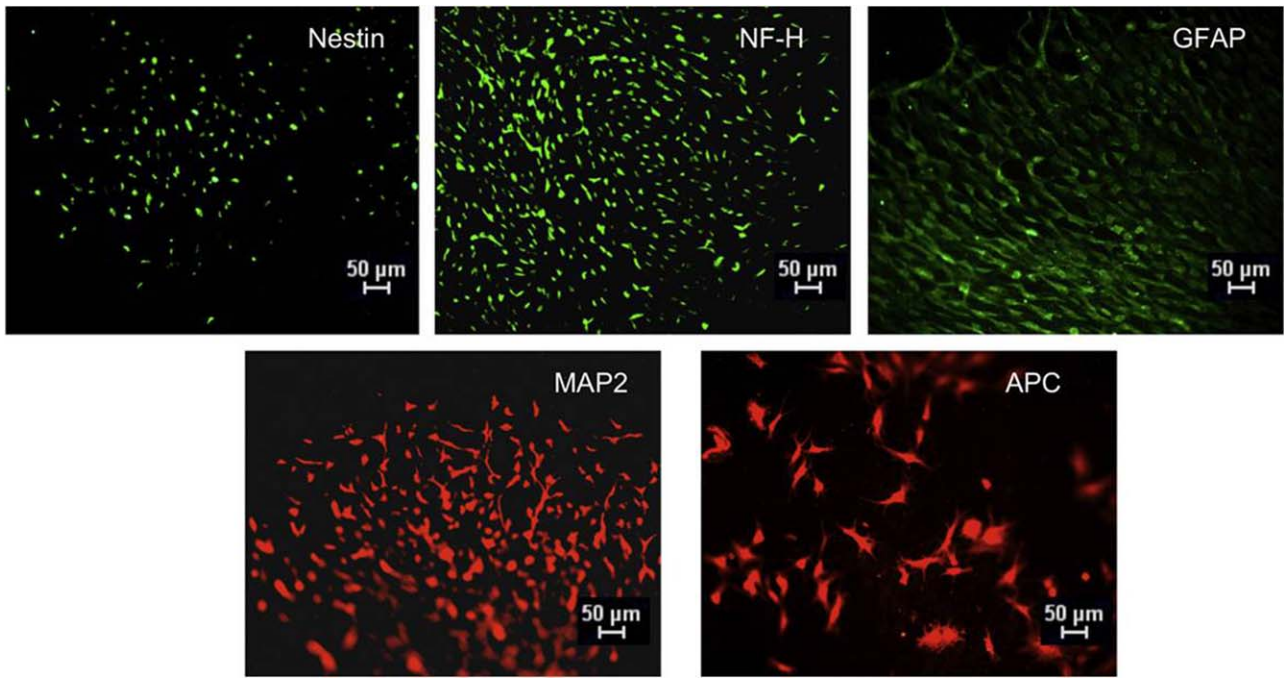

FIGURE 8. Representative immunofluorescence images showing expression of Nestin, NF-H, GFAP, MAP2, and APC on PPy-NW surfaces, confirming that the NSCs have differentiated into all neural lineages. (Adapted with permission from Ref. 86. Copyright 2011 Elsevier Ltd).

the surface of MWCNTs was significantly improved via combining with the amine groups. The AF-MWCNTs exhibited higher sensitivity to neural signal measurements as compared with the traditional suction glass pipette.

To further enhance the charge storage capacity, conductive nanocomposites have also used in the fabrication of neural probes for their synergistic effect. For instance, MWCNTs and Au nanoparticles were coated onto silicon electrodes to prepare neural probes by Zhang et al. Their work showed that the cathodic charge storage capacity of the neural probe was up to 35 mC cm^{-2}, and the impedance was reduced to 21 ± 3 kΩ. These datas indicated that the nanocomposites could sharply decrease electrochemical impedance and conductive properties of neural probes.[95] Similarly, Tsang et al. reported CNT/Au nanocomposites based probe that served as bi-directional insect-machines. The nanocomposite modified flexible neural probes were able to deliver electrical signal and capture neuronal signal to the ventral nerve cord of the moths. Moreover, the stimulation experiments suggested that the composite improved the local placement of the electrodes near axons.[11] In conclusion, due to their high conductivity, CNTs can activate resident immune cells in neural tissues. The high surface area of CNTs can increase charge injection capacity and decrease the interfacial impedance with neuronal cells.

Numerous investigations showed that mechanical mismatch between the probe and tissue may cause the chronic tissue response in the central nervous system. Flexible neural probes (FNPs) can reduce the mechanical strain at the probe-tissue interface.[89,96] This concept is based on the hypothesis that the mechanical mismatch far away (for example, a few hundred micrometers) from the electrode will not affect the local tissue response.[96] However, there are some drawbacks with FNPs; particularly, they have difficulty in penetrating through the brain due to their low elastic modulus. Many studies have been conducted to enhance the implantation of FNPs such as taking advantage of CNT,[97] PANI,[98] CNT-Au[11] nanocomposite modified microelectrodes to synthesize FNPs. The sufficient stiffness is necessary to FNPs. For example, maltose-coated polyimide flexible neural probe was prepared, the coated maltose can increase the stiffness of polyimide FNPs and it is dissolved by body fluids a few seconds after implantation.[97] Therefore, the stiff coating on the flexible polyimide probes allows for efficient implantation, after which the coating is dissolved, leaving the flexible probes to create a low mechanical mismatch with the tissue. Moreover, the ultra-thin flexible polyimide neural probe can reduce the glial sheath growth on the probe body, and its flexibility can minimize the micromotion of the probe-brain tissue. In addition to maltose, nanoporous alumina coatings can also increase the stiffness of FNPs and improve the charge delivery ability and reduce the impedance of titanium neural probes.[93]

It is well known that the electrochemical properties of electrode mainly depends on the interface of electrode materials. To reduce the impedance of electrode, a common approach is that suitable materials are coated on the surface of electrode.[97] For example, Di et al. fabricated PANI-coated platinum electrodes by *in situ* polymerization. The morphology of the nanoparticle was maintained for 6 months under

the electrical stimulation.[98] The probe cannot only promote neurite outgrowth, but also resist corrosion of the probe. Wang et al. synthesized nanostructured films by coating PANI nanoparticles on the surface of Pt electrode. The films had anti-eroding properties. In this work, PANI nanoparticles increased protein adsorption and improved the biocompatibility of the tissue-implant interface.[15] Moreover, in contrast to uncoated platinum electrodes, the increased surface roughness and surface energy by PANI coatings promoted initial protein adsorption. The increased amount of protein adsorption was mainly ascribed to the nanostructure films, which have a large surface area and high surface energy. These performances were able to facilitate specific protein interactions beneficial for cellular functions. Under the action of electrical stimulation, bovine serum albumin aggregates can form the nanostructure PANI films that can inhibit such aggregation, thus PANI nanoparticles can be used as a good coating material.[15] Furthermore, it was found that the electrochemical modification of the electrode active area with poly(3,4-ethylenedioxythiophene) (PEDOT) greatly decreased the electrical impedance. However, there are many parameters such as temperature, monomer concentration, applied voltages, etc, that influence the ultimate morphology of the PEDOT electrodeposition. Desai et al. prepared PEDOT/MWCNT nanocomposite film modified on microelectrode arrays to improve the detection capability. They found that the nanocomposite modified microelectrode exhibited higher charge storage capacity and charge injection limit, compared to bare gold and pure PEDOT coated electrodes. Moreover, neural signals in response to acoustic stimuli were recorded from the inferior colliculus of rats and high SNR signals >15 dB were recorded using the modified microelectrode arrays. The extraordinary characterization of the nanostructured film could as an excellent optional model to improve neural interfaces in bioengineering.[92]

CNMs USED IN DRUG DELIVERY

Drug delivery is a vital field of medicine and healthcare. Currently, a promising approach in the field is targeted drug delivery, which is designed to directly deliver drug toward the tissues.[17] Controlled drug delivery system can release quantitative drugs at specific time and position, especially stimuli-response drug delivery. The stimuli, such as pH, light, temperature, enzymes, and electric/magnetic fields, has been explored to prepare drug delivery device.[99] However, drug delivery still has difficulty in developing for clinical applications as it often causes undesirable systemic nonspecificity toxicity, narrow therapeutic window, and multi drug resistance.[100] Hence, the development of effective methods to deliver drugs has attracted wide attention in tumor therapeutic and tissue engineering. In recent years, CNMs have emerged as promising candidates to effectively deliver drugs, biomolecules and genes due to their unique structure and properties. For example, SWCNTs have often grafted with biodegradable polymer to enhance the biocompatibility and realize drug delivery.[13,100] The targeted SWCNT-lipid-paclitaxel significantly suppressed tumor growth in a human breast tumor xenograft mouse model. When the drugs were loaded on the SWCNT sidewalls, the CNT-based drug conjugated freely with other kinds of molecules. The membrane of artificial cells could prevent drug degradation from the harsh gastrointestinal environment via oral delivery.[101] Likewise, a research focused on the MWCNT/polyethylenimine (PEI) composites was conducted by Wu et al. In their work, the composites were conjugated with fluorescein isothiocyanate (FITC) and prostate stem cell antigen monoclonal antibody by covalent interaction method. The as-prepared CNT-PEI (FITC)-mAb possessed good biocompatibility and inhibited their growth on PC-3 tumor-bearing mice, which could targeted delivery drug to the tumor.[102]

The phenomenon of proteins binding to nanoparticles (including CNTs), known as the "corona effect", has already been demonstrated.[103] Inevitable protein adsorption on CNTs following *in vivo* administration may alter the release of Doxorubicin (DOX) by competitively displacing DOX from the surface of CNTs. In fact, the desorption of DOX from oxidized MWCNTs was accelerated by two times in a release medium of neutral pH comprising of bovine serum albumin (BSA) or immunoglobulin G versus blank PBS.[104] A few studies, however, demonstrated negligible differences between the release profiles of DOX from MWCNTs covalently functionalized with hyaluronic acid (HA) and noncovalently functionalized SWCNTs in buffer and serum. This suggested possibly that the effects of protein binding on DOX release may be influenced by the different functional groups/coating present on the surface of CNTs.[105] Furthermore, a PPy-based drug delivery system has been developed. In this system, PPy acted as the drug carrier and the drug was directly released without an external power source, and the massive drug release only occurred at around human body temperature. Because the system had no cytotoxicity and was constructed by direct deposition of the drug-contained PPy film on the surface of titanium foil, it can reasonably find applications in the field of titanium-related orthopedics to facilitate the wound healing process.[106]

The graphene oxide (GO) and graphene are also able to act as nanotherapeutic drug delivery platforms to carry different therapeutic molecules include small drugs, antibodies, DNA, proteins, and genes.[102] GO is more widely used than graphene for biomedical applications due to the presence of carboxylic, epoxy, and hydroxide groups, which allows for a wide range of reactions and functionalization opportunities. The large π-conjugated structure, abundant structural defects on R-GO and functional groups on GO are also advantageous when they have assembled with nanoparticles, including Au, Ag, Pd, Pt, Ni, Cu. All these NPs have been successfully incorporated with graphene and graphene derivatives for a variety of drug delivery applications.[107–109] For example, graphene with conjugation of iron oxide nanoparticles possesses super paramagnetic and can be effectively used in drug delivery. Similarly, a pH-dependent drug release system was reported by Fan et al. The 5-FU-loaded

nanohybrid system was composed of graphene nanosheets (GN), CNT, and Fe_3O_4, which exhibited significant cytotoxicity toward $HepG_2$ cells.[110] Moreover, the blank GN-CNT-Fe_3O_4 complex was efficiently internalized by $HepG_2$ cells without any toxic effects *in vitro* even at high concentrations of 80 μg/mL. Biodegradable conducting polymer composites show special advantage in drug delivery, because they can degrade *in vivo* rather than aggregation, which reduces inflammatory reaction and the damage to the normal cell. Based on their unique property, Hardy et al. reported a biodegradable electroactive copolymer composed of oligoaniline-based electroactive blocks, polyethylene glycol or polycaprolactone blocks. The copolymer exhibited a high drug loading of 16 or 31 wt % for their good solubility. Their result demonstrated that the polymer film promoted cell behavior *in vitro* and released dexamethasone phosphate (anti-inflammatory drug) under the electrochemical stimuli. Therefore, the biodegradable conducting polymer have a promising prospect in clinical application of drug delivery.[99]

THE BIOCOMPATIBILITY AND BIODEGRADABILITY OF CNMs

For the successful biomedical applications of CNMs, it is essential to understand their biocompatibility and biodegradability. The evaluation on the biocompatibility of materials is involved with the potential toxicity and biological function. The function of CNMs has been discussed in above biological field, herein, we will focus on their toxicity. Actually, CNMs have been shown to be biocompatible with many biological molecules *in vitro*, including neural, cardiac, and bone cells. However, studies on the potential toxicity of CNMs have generated conflicting opinions. No a systematic method could measure their potential toxicity. Nanoscaled size, shape, degree of aggregation, and chemical functionalization could influence the biosafety. For instance, unmodified CNT is insoluble in aqueous media, which results in nanotube aggragetion; moreover, the longer length of CNT could be adverse to phagocytosis. MWCNT produced higher cytotoxicity over SWCNT *in vivo* for longer lengths and more aggragetion.[111] Similarly, gold nanoclusters of 18 nm have been shown to cause lower toxicity relative to minor counterparts (1.4 nm).[112] Due to the difference of shape, spherical gold nanomaterials were less cytotoxic relative to gold nanorods to human HeCaT keratinocytes.[113]

Actually, exposure to most of pristine CNMs have been shown to cause low cytoxicity at higher concentrations (both *in vivo* and *in vitro*). The biocompatibility of CNMs could be easily improved by surface functionalization or binding with biocompatible molecules or materials. PEDOT nanotube-coated neural microelectrodes implanted into the barrel cortex of rats caused a better tissue response relative to their uncoated counterparts.[114] Likewise, PPy-PLGA composite exhibited better biosafety to embryonic hippocampal neurons relative to pure PPy.[115]

Additionally, the degradability of CNMs is an important factor that limits their applications in the biological field. For instance, scaffolds are used to support the new bone formation in bone tissue engineering. Scaffold materials should totally degrade once the process of bone repair comes to end.[116] Moreover, in terms of the long-term toxicity, nondegradable materials could accumulate in body and produce adverse effects to the biomolecules. On the contrary, the toxicity can be caused when the degradation products of materials is toxic.[111] Typically, untreated CNMs are nondegradable biomaterials. However, their biodegradability can be improved by changing the synthesis route, combining biodegradable materials or functionalizing surface. To overcome the non-degradability, blends and composites that contain CPs and biodegradable polymers have been prepared. For example, PANI/gelatin nanofiber was synthesized by electrospinning. The nanofiber was found to promote the attachment and prolification of H9c2 rat cardiac myoblast cells.[116] Guo et al. prepared an electroactive degradable porous tubular scaffolds based on polycaprolactone and a hyperbranched conducting polymer.[117] The hyperbranched polymer was composed of niline pentamer and polycaprolactone, which was degradable and fabricated by them.

Further research on biocompatibility has to be conducted to systematic analyze the toxicity of CNMs and to confirm the cause. The long-term toxicity of these CNMs *in vivo* should be further studied. Moreover, CNMs with controlled biodegradability and non-toxic degradation products also should be developed in the future.

CONCLUSIONS AND PERSPECTIVES

This review compiles the numerous advantages of using CNMs in biomedical applications. Many benefits of CNMs such as high conductivity, large surface area and preferred electrocatalysis properties have made them preferable in a wide biomedical applications. In electrochemical-based biosensor, CNMs can accelerate electrical signal transfer between electrode and biomolecules, and provide a compatible environment for the biomolecules via electrostatic interaction. In tissue engineering, CNMs could regulate cellular behaviors and induce specific cellular response, and therefore promote the regeneration of defective tissues, especially in electroactive tissue. In neutral probe, CNMs can increase charge injection capacity and decrease the interfacial impedance with neuronal cells, which further improve the detection sensitivity of probe. In drug delivery, CNMs can act as nanocarriers, which help to targeted delivery drug. Especially, CNMs can regulate cell behavior and release drug at the fixed time and location in the electric field.

Recent studies have mainly combined CNMs with natural or synthetic polymers to fabricate composites or blends at the nanoscale, which possess promising properties, resulting in meeting more biomedical requirements. The aligned nanocomposites with 3D porous structure are benefit to the cellular growth. Particularly, the biocompatibility and biodegradability of CNMs can be improved by mixing them with specific materials. When CNMs are used in tissue engineering or neural probes, electrical stimulation helps enhance the cellular function and control the growth of cells. Furthermore, CNMs can be used in biosensors and targeted drug delivery.

Ever though CNMs have been used in many biomedical fields, more studies on controlling the amount of CNMs and improving their properties still need to be carried out. Previous studies have proved the function of stem cell can be influence by the stiffness of biomaterials. For example, stiff substrates significantly promoted the osteogenic differentiation of the rBMCs and rAMCs, the stiff substrates also help MSCs differentiate into osteoblast.[73] However, the soft substrates contribute to the attachment of the stem cell. Therefore, choosing the suitable hardness of CNMs as substrates will be an interesting research direction in bone tissue engineering. Besides, the employment of CNMs in neural probe has been drawing increasing attention. It is believed that more and more CNMs will be investigated in the future for this area. For example, CNTs and several other kinds of CNMs or polymers could be used to modify microeletrodes. Given that CNTs are not biodegradable, they can be implanted for long-term extracellular recording. Combined with the conductivity, nanostructure mechanical character, and biocompatibility of component materials, the as-prepared neural probes should possess excellent properties and could be applied in clinical medicine. Furthermore, CNMs with good biocompatibility and biodegradability will be a promising research direction, and the fabrication methods of nanocomposites, like electronspun nanofibres or hydrogels, have a crucial influence on the composite properties. We hold the opinion that the exploration of CNMs with low cytotoxicity and good biodegradability will be useful in developing biosensors, scaffolds for future transplantation studies and other applications. In summary, CNMs are a promising class of biomaterials, which will be an interesting item in future studies.

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