Review Article
The application of graphene-based biomaterials in biomedicine

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Abstract: Graphene-based nanocomposites have attracted more and more attention recently in the field of biology and biomedicine. Graphene and its derivatives have been integrated with drugs, nucleic acids, antibodies, and other molecules. And these materials could be use as nanocomposite carriers or scaffold materials taking advantages of their enormous specific surface area, good elasticity and ductility, excellent biocompatibility, and outstanding mechanical strength. In addition, these composites have strong near-infrared absorbance and can act as photothermal agents to kill target cells through physical or chemical mechanisms. Along with significant advances in cell and organ transplantation, many of these materials have been explored in recent years for use in tissue engineering and regenerative medicine. Tissue engineering includes bone, nerve, heart, and muscle tissue engineering based on two-dimensional and three-dimensional graphene-based matrices or scaffolds possessing certain mechanical strengths and electrical conductivities, and the aim is to produce bioactive tissues to replace or repair natural tissue by promoting osteogenic, neuronal, and myogenic differentiation and myocardial cell growth. In this review, the basic properties of graphene-based complexes are systematically described and the biomedical applications of graphene-based materials in vivo and in vitro are summarized. This review first discusses the safety of graphene-based materials in terms of their biocompatibility and toxicity, and then it discusses these materials' applications in biosensing, photothermal therapy, stem cell culture, and tissue engineering. This review therefore provides a comprehensive understanding of graphene and its derivatives and their present and future applications.

Keywords: Graphene, biocompatibility, toxicity, biomedical, tissue engineering

Introduction

Graphene is a 2D carbon nanomaterial composed of carbon atoms in an sp2 hybrid orbital hexagonal honeycomb crystal lattice [1]. The physicists A. Gem and K. Novoselov of the University of Manchester in the UK successfully separated graphene from graphite by micromechanical stripping, and this discovery led to their jointly winning the 2010 Nobel Prize in Physics. Traditional preparation methods of graphene and its derivatives can be generally classified as mechanical stripping [2], redox methods [3], orientation epiphysis [4], chemical vapor deposition [5], graphitization [6], solvo-

thermal methods [7], organic synthesis [8], liquid exfoliation of graphite [9], thermal exfoliation and liquid intercalation [10], electrochemical exfoliation [11], chemical reduction of graphene oxide (GO) [12], thermal reduction of GO [13], and photothermal reduction of GO [14]. These graphene-based nanomaterials have been used in many applications such as surface-enhanced Raman scattering [15-22], electrochemistry [23-29], catalysis [30-34], fuel cells [35-38], and biomedicine [39-45].

Among the nanomaterials used for biomedical applications, graphene-based biomaterials have attracted much scientific and technological
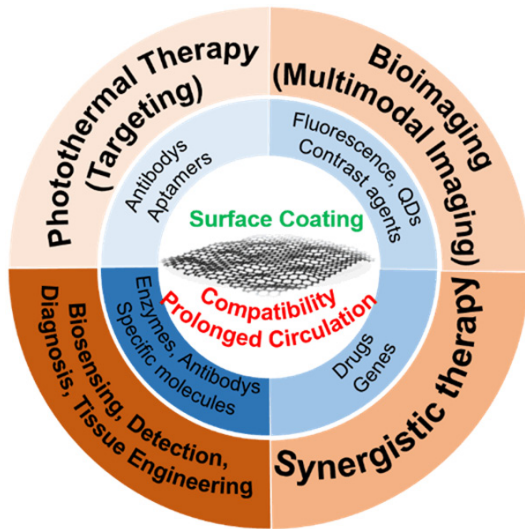
Figure 1. The primary applications of graphene-based nanomaterials in biomedicine.

interest in recent years [46-49] and show great promise as antibacterial agents [50], biosensors [51, 52], photothermal therapies (PTT) [53], bioimaging tools [54-56], and as stem cell and tissue engineering components [57, 58]. Surface coatings with graphene and its derivatives involve in covalent bonds, Van der Waals forces, π-π reactions, or electrostatic interactions to improve the biocompatibility and to prolong the circulation time of these graphene-based biomaterials. Aptamers and specific antibodies can be ligated to the surface of graphene to achieve targeting of lesions, which can be modified with fluorescent molecules, quantum dots, and contrast agents to achieve multimodal imaging, combined with drugs and genes to achieve synergistic therapies, and also linked to enzymes, specific molecules, antibodies, *etc*., for applications in molecular detection, biomarker detection, disease diagnosis, PTT, tissue engineering, and cell or exosome capture and detection (**Figure 1**).

Herein, we review the preclinical biocompatibility and cell toxicity of graphene-based nanomaterials and advanced applications in biosensing, PTT, and tissue engineering. Furthermore, we discuss the challenges and future prospects of graphene-based biomaterials.

Biocompatibility and toxicity

The biocompatibility and toxicity of a nanomaterial is the primary consideration for its biomedical applications. The properties of gra-

phene and graphene-based nanomaterials, such as morphology, size, surface modifications, etc., can have diverse implications for cells, tissues, and even the body as a whole, so the potential toxicity must be well explored. Many recent studies have been conducted to investigate the potential toxicity of graphene-based materials in their interaction with cells and tissues in vitro and in vivo, and this is critical for identifying suitable candidates for clinical applications.

Zhou et al. [59] used large-scale all-atom molecular dynamics simulations to investigate the potential toxicity of graphene on cells at the molecular level and found that hydrophobic protein-protein interactions can be interrupted by graphene. Disruption of the cell's metabolism or even the cell's survival was proposed to be due to the separation of 2-deoxyribose-5-phosphate aldolase and phosphoglucose isomerase. Wang et al. [60] used A549 cells as the model to investigate the influence of GO on cell morphology, viability, mortality, and membrane integrity, and they found that the GO caused dose-dependent oxidative stress in A549 cells, leading to slightly decreased cell viability. Recently, Chu et al. [61] performed a systematic evaluation of graphene quantum dot toxicity in male mice and showed the low toxicity of graphene quantum dots in terms of sexual behaviors, reproduction, and offspring viability. Furthermore, graphene materials have been tested for their antibacterial activities, and this activity has been shown to be related to the material's lattice size [62], number of layers [63], shape [64], surface modifications [65-67], agglomeration, and dispersion [68, 69]. In addition, the interactions of the graphene materials with lipids, proteins, and DNA/RNA have been shown to lead to lipid extraction [70], protein disruption [59], and reactive oxygen species production [71], resulting in the death or inactivation of microorganisms [72-74]. These results above suggest that graphene could efficiently bring up the toxicity to bacteria and the in vitro toxicity to cells.

Although the toxicity of graphene can be applied in the field of antibacterial, this defect just hinders the development of graphene in the field of biomedicine. Appropriate biocompatibility modifications are necessary for graphene materials to be used in a wider range of applications.

The application of graphene-based materials

As emerging nanomaterials with simple, inexpensive, and good physical and chemical properties, graphene-based nanomaterials are widely used in many biomedical fields. The following mainly introduces the application of graphene-based nanomaterials in biosensing, PTT, and tissue engineering.

Biosensors

The high surface-to-volume ratio of graphene provides sufficient loading of the required ligand, and its excellent conductivity and small bandgap makes graphene useful for sensitive electrical and electrochemical sensing. In addition, its tunable optical properties make it suitable for using as a fluorescence and plasmonic sensor. In general terms, graphene-based sensors consist of a receptor, such as an antibody [75], single-stranded DNA [76], or enzyme [77] can combined with the target ions, molecules, nucleic acids, or even whole cells or microorganisms. The graphene-based nanomaterials also serve as the transducer to convert chemical information regarding the interactions between the receptor and the target molecules into a measurable signal.

Rapid and sensitive detection of glucose has always been a popular research, and many different methods for its detection have been reported over the past few decades. Graphene-based glucose biosensors generally comprise immobilized glucose oxidase and a graphene surface, such as the glucose oxidase-functionalized tilted fiber grating developed by Zhang et al. [77] to detect low concentrations of glucose. In that work, the amino groups of glucose oxidase were covalently connected to the graphene oxide via the assistance of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysulfosuccinimide, and the glucose was oxidized and the changes in the refractive index of the local microenvironment were converted into measurable information with a sensitivity of ~ 0.25 nanometers per millimolar. A non-enzymatic glucose sensor was also developed by Lai et al. [78] through the self-assembly of phenoxyl-dextran on electrochemically reduced GO (rGO) to compete with glucose for binding to the biosensor.

Protein biomarkers are the basis for the prediction, detection, diagnosis, etc., of many diseases and cancers. Although the detection of some biomarkers has been used clinically, there are still likely to be many unknown biomarkers for certain diseases that are only present at very low concentrations, and this poses a challenge for current biomarker detection. In addition, the need for non-invasive, fast, real-time, and accurate detection of biomarkers has also limited the development of the field. Owing to the good biocompatibility, low-cost, and high sensitivity, graphene-based nanomaterials have shown excellent performance in the selectivity of biomarker detection. A representative study was that of Nezhad et al. [79] developed a method to detect glial fibrillary acidic protein (GFAP), which is an intermediate filament protein expressed by certain cells in the central nervous system. In their study, a polyethylenimine-coated graphene electrode, covalently bound with GFAP antibody, was used for electrochemical biosensing of GFAP with a dynamic linear response in the range of 1 pg/ml to 100 ng/ml. Detection of living cells is another effective method for disease detection, and graphene-based nanomaterials are becoming more and more widely used in this field. Xing et al. [80] reported an ultra-sensitive and ultra-fast label-free method for detection of whole living cancer cells using a graphene-based optical biosensor. The corresponding sensitivity was increased, and the detection time was as short as 260 ns, and their device showed great promise for use in cell-based research. In addition, Sun et al. [81] fabricated a "turn-on" magnetic fluorescent biosensor based on graphene quantum dots, Fe_3O_4, and molybdenum disulfide nanosheets for rapid, efficient, and sensitive separation and detection of circulating tumor cells with a linear range of 2 to 64 nM and a limit of detection of 1.19 nM for Epithelial cell adhesion molecule detection.

Photothermal therapy

In recent years, graphene-based nanomaterials have been used in PTT due to their special physical and chemical properties. Graphene, carbon nanotubes, and their derivatives have strong near-infrared absorbance for acting as photothermal agents and have large surface areas that can be functionalized with anti-cancer drugs, biocompatibility-enhancing molecules, and specific cell-targeting biomolecules for use as anti-cancer nanodrugs. Liu et al. first reported the use of GO combined with PTT to

suppress tumors [82]. When PEGylated GO absorbs near-infrared light, it releases energy, thus causing excessive heat in the cells and inducing apoptosis. Meanwhile, covalent binding of Cy7 can trace the metabolism of GO.

To further improve the efficacy of cancer treatment, PTT is being combined with other strategies. For example, a chemo-photothermal method has been reported based on PEGylated GO loaded with doxycycline (DOX) [83-85]. Wang et al. combined Fe_2O_3@Au with reduced-GO (rGO) to increase PTT, and the material was loaded with DOX as chemotherapy [84]. In that work, DOX was transported under the guide of a magnetic field, and synergistic results were seen with the combination of chemotherapy and PTT. The mixing of anticancer drugs loaded onto GO has also been reported [85], wherein sulfonic acid groups were loaded to enhance the stability, folic acid molecules were bound to increase the specificity, and camptothecin and DOX were used as anticancer drugs. Their work was the first to identify the ability of GO to act as a carrier for controlled loading and release of multiple drugs.

Photosensitizers have also been loaded onto GO to combine photodynamic therapy and PTT methods [86-89]. Folic acid-conjugated GO loaded with Ce6 was reported by Cui and colleagues [86]. In their study, the Ce6 was specifically delivered to targeted cells, and the controlled photohyperthermia and singlet oxygen generation worked synergistically. In addition, methylene blue [88], hypocrellin B [89], and other photosensitizers have also been used to kill cancer cells.

Due to limited laser irradiation depth, it is difficult to perform PTT and photodynamic therapy (PDT) in solid tumors in deep tissues. To overcome this obstacle, ultrasound-activated PDT was developed. By using ultrasound instead of a laser, the depth of the treatment has been greatly increased and the invasiveness of the method has been reduced [90]. Chen et al. [91] proposed a mesoporous silica (MSN) grown on reduced graphene oxide nanosheet (nrGO)-PEG-conjugated iron-oxide nanocarrier (ION) for sonodynamic therapy and ultrasound hyperthermia. This nanocarrier is an rGO, mesoporous silicon, and superparamagnetic composite structure with high photosensitivity, high biocompatibility, and magnetic targeting. PE-Gylated nrGO/MSN-ION loaded with Rose Bengal (sonosensitizer, maximum concentration of 0.5 µM) can generate singlet oxygen under burst-mode ultrasound irradiation. In addition to the uses mentioned above, graphene-based nanomaterials have also been combined with gene therapy [92, 93], specific target molecules [94], and modifications intended to prolong circulation in the blood [95, 96].

Tissue engineering

Graphene-based materials have also led to new approaches to regenerative medicine and tissue engineering [97, 98]. Loss of tissue or organs caused by injury or disease is a serious health problem that negatively affects the patient's life. Traditional surgical treatments use the patient's own tissues to repair or replace damaged tissues or organs or use allografts of certain organs. However, these traditional methods face limitations, in particular the lack of suitable organs and tissue donors, and thus tissue engineering has been seen as a promising way forward [99]. Tissue engineering utilizes different scaffold materials to control cell proliferation, differentiation, etc., and to stimulate the extracellular matrix of the target tissue to provide a suitable microenvironment for cell adhesion, migration, proliferation, and differentiation so as to produce biologically functional tissues [100]. Tissue engineering technology has great prospects for clinical applications because it avoids immune rejection and reduces the need for suitable autologous and allogeneic materials. Tissue engineering can be applied to the skin, bone, muscle, cardiovascular system, digestive tract, liver, kidney, reproductive system, nervous system, etc., but here we mainly discuss the development of tissue engineering techniques for bone, nervous, muscle, and cardiac tissues.

Bone tissue engineering: Bone tissue engineering, which aims at regenerating bone, is a promising solution for faster healing and for reconstruction of large bone defects [101], and graphene and its derivatives have a wide range of applications in bone tissue engineering. Madhu Dhar et al. [102]. evaluated the effect of graphene on in vitro growth and differentiation of goat adult mesenchymal stem cells compared with growth on polystyrene-coated tissue culture plates. In that study, they found that the materials were cytocompatible and facilitated
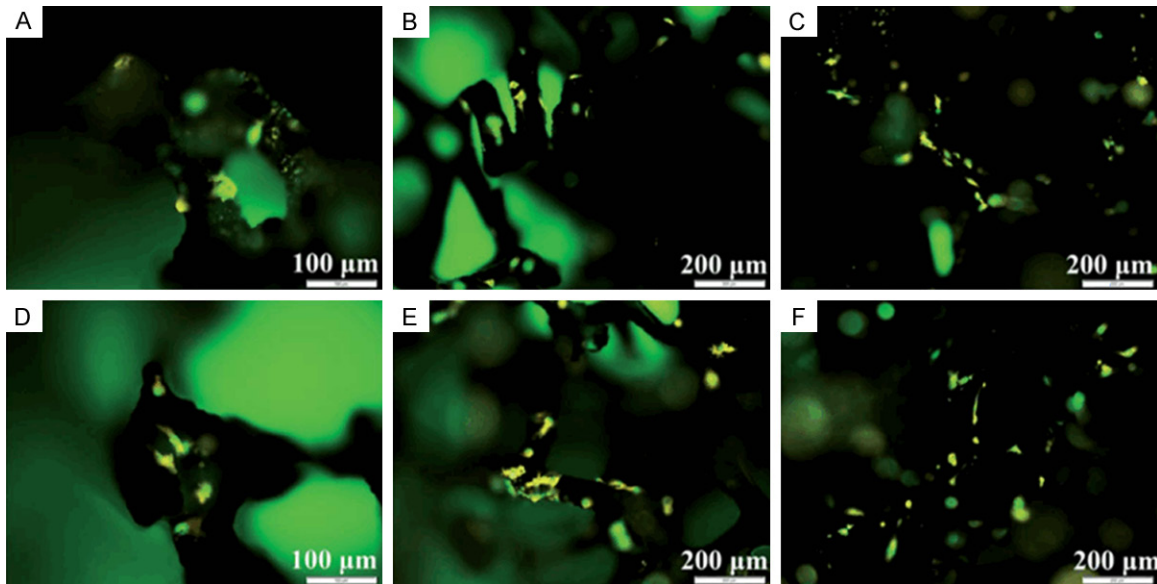
Figure 2. Fluorescence images of MC3T3-E1 cells on 3D rGO/PPY (A, D), 3D rGO/PPY/CPP10 (B, E), and 3D rGO/PPY/CPP20 (C, F) at Day 2 (A-C), and Day 4 (D-F) of culture, respectively.

cell adhesion and proliferation and enabled osteogenic differentiation in fetal bovine serum-containing medium without the addition of any glucocorticoids or specific growth factors. In the work of Zhai et al., capsaicin was modified on rGO with the purpose of increasing its stability and avoiding aggregation effects [103]. This graphene-based material promoted the differentiation and proliferation of osteoblasts, indicating that it has important applications in bone tissue engineering and for treating diseases such as osteoporosis. Fu et al. prepared a nanofiber matrix of poly (L-lactic-co-glycolic acid)/hydroxyapatite/graphene oxide by electrospinning and used this to promote MC3T3-E1 cell adhesion and proliferation. The role of the poly (L-lactic-co-glycolic acid) component was to provide a uniform and smooth nanofibrous substrate with a three-dimensional porous structure possessing excellent biocompatibility and biodegradation properties. GO was also used to enhance the tensile strength of the constructed poly (L-lactic-co-glycolic acid)/hydroxyapatite/graphene oxide nanofibrous matrices, and hydroxyapatite was employed to improve the biocompatibility and conductivity of the substrate. It is worth noting that the synergistic effects of the two components were demonstrated by the material's ability to promote protein adsorption and to induce osteogenic function [104]. The material was also used to accelerate and induce proliferation and differentiation of human mesenchymal stem cells [105]. Jie et al. reported that the three-dimensional biomaterial of rGO decorated with casein phosphopeptide/polypyrrole matrix synthesized by electrospinning had remarkable hydrophilicity and water absorption capacity [106]. The biofunctionalized casein phosphopeptide that self-assembled on the surface of the scaffold could promote the formation of hydroxyapatite in a cost-effective manner, and the performance of the composite scaffold was superior to that of the conventional rGO/polypyrrole matrix (**Figure 2**).

A recently developed method of preparing a free-standing rGO matrix takes advantage of chemical reduction, and the GO solution is filtered through nanofiber membranes under vacuum. The proteins decorating the synthesized rGO film produce a rough surface, and this roughness can be controlled by changing the parameters of the electrospinning. In addition, the synergistic effects of surface-functionalized protein and rGO have been shown to improve the biocompatibility, roughness, and specific surface area of the basement membrane with the aim of promoting cell adsorption and the proliferation of human bone marrow mesenchymal stem cells [107].

Nervous tissue engineering: Central nervous system damage, including traumatic brain inju-

ry and spinal cord injury and neurological diseases such as Alzheimer's disease, Parkinson's disease, and Huntington's disease, is difficult to repair due to the limited regenerative capacity of nerve tissue. Neural stem cells (NSCs) have the ability to differentiate into neurons, and they have high neuronal protein expression, extended axon projection and arrangement after differentiation, and secretion of various neurotrophic factors, indicating that NSCs therapy is a promising approach to repairing nervous system damage. Graphene has also been widely used in nervous tissue engineering due to its superior performance (**Table 1**).

At present, the most important difficulty in the regeneration of nervous system damage is how to effectively differentiate NSCs into uniaxially arranged neurons. Recently, a novel composite scaffold structure including aligned electrospun silk nanofibers and conductive reduced graphene paper was used to enhance directional SH-SY5Y cell growth and neuron differentiation [116]. This neatly arranged directional matrix of nanofibers supported the cultivation of cells and stimulated the growth of neurites. The rGO paper contributed of good biocompatibility and excellent conductivity in the complex, and it increased neurological activity under electrical stimulation. The formation of extended axons and the expression of β3-tubulin and Nsetin as the neuronal markers were observed by immunolabeling and confocal imaging, indicating that the conductivity and aligned silk nanofiber-reduced graphene oxide paper (AS-rGOP) could effectively induce SH-SY5Y cells to differentiate into neurons and then guide the arrangement of the resulting neurons to form a functional neural network (**Figure 3**). Another graphene-based hybrid nanofibrous scaffold has been proposed to guide NSCs differentiation into oligodendrocytes [117]. In that work, GO was coated on nanofibers, and by varying the amount of GO coating changes in the expression and concentration of key neuronal markers were observed, where a high concentration of GO coating promoted the differentiation of NSCs into mature oligodendrocytes. Furthermore, Gao et al. investigated composites of aligned and aminated poly-L-lactide coated with GO without destroying the alignment [121]. Scanning electronic microscopy was used to characterize the topological structure, and X-ray photoelectron spectroscopy and water contact angle showed the modification and GO coating. The material promoted the differentiation and proliferation of rat pheochromocytoma 12 (PC12) cells.

Muscle tissue engineering: Muscles tissue engineering has great clinical value for the regeneration and reconstruction of muscle tissue in the treatment of muscle damage and muscle atrophy and for the repair of smooth muscles in the heart, intestines, and urinary system, and graphene-based nanomaterials have been widely used in this field. For example, Park et al. investigated myotube formation on graphene-based nanomaterials [122]. In their work, GO was covalently linked to an aminated glass surface via a carboxyl group to act as the substrate for the culture of mouse C2C12 myoblasts. They studied the adhesion, proliferation, and differentiation on bare and laminin-coated graphene foam (GF) by measuring the expression of myogenic proteins and differentiation-specific genes and the formation of multinucleated myotubes and demonstrated GO's ability to stimulate myogenic differentiation. Estrada et al. used laminin-coated GF as a biocompatible platform to study the cellular activity of C2C12 myoblasts [123]. The expression of myosin heavy chain protein was used as a marker for demonstrating that the laminin-coated GF could induce C2C12 myoblasts cells to differentiate into myotubes (**Figure 4**).

Cardiac tissue engineering: Cardiac tissue damage is associated with high mortality because damage to heart tissue is irreversible due to the inability of the organ to carry out tissue regeneration and to repair itself [124]. Clinical treatment is limited to heart transplants, but this has the drawbacks of a lack of donors, immune rejection, etc. The development of cardiac tissue engineering technology brings the promise of expanded therapeutic options.

The development of traditional biomaterials is limited by low conductivity, but new graphene-based nanomaterials meet the need for both biocompatibility and conductivity. Sonkusale et al. proposed a three-dimensional GF deposited with a 10 nm layer of titanium and a 50 nm layer of gold to create a scaffold for recording the electrical activity of cardiac cells [125]. The results demonstrated that the scaffold had good biocompatibility and excellent conductivi-

Table 1. Summary of the main results reported based on graphene and its derivatives for nervous tissue engineering

Material	Coating	Cells	Results	Ref.
Graphene film	Poly-L-lysine	Mouse hippocampal neurons	Promoted neurite sprouting and outgrowth and the expression of growth-associated protein-43	[111]
Graphene rod	Gelatin	MSCs	Profound effect on the differentiation of MSCs to Schwann cell-like phenotypes and on their paracrine activity	[112]
3D hybrid graphene	None	NSCs	Facilitated the differentiated NSCs to bridge the spacing between skeletons and promoted the formation of neural networks	[113]
Graphene oxide	Polyvinylidene fluoride	PC12 cells	Promoted cell proliferation and could easily be converted into a nerve guidance conduit	[114]
Graphene oxide	None	NSCs	Selective differentiation of NSCs into mature oligodendrocytes	[117]
Reduced graphene oxide	None	Schwann cells, PC12 cells	Enhanced Schwann cell migration, proliferation, and myelination and induced the differentiation of PC12 cells	[115]
Reduced graphene oxide	None	SH-SY5Y cells	Oriented the growth of SH-SY5Y cells and enhanced SH-SY5Y cell neuronal differentiation and the formation of neural networks	[116]
Reduced graphene oxide	Collagen	MSCs	Supported MSC attachment, maintained cells in a more active proliferation and neural differentiation state, and promoted neurite sprouting and outgrowth	[118]
Reduced graphene oxide	Polycaprolac-tone	PC12 cells	Supported neural differentiation of PC12 cells	[119]
Graphene foam	None	NSCs	Supported NSC growth, maintained cells in an active proliferation state, and enhanced the NSC differentiation towards astrocytes and especially neurons	[120]

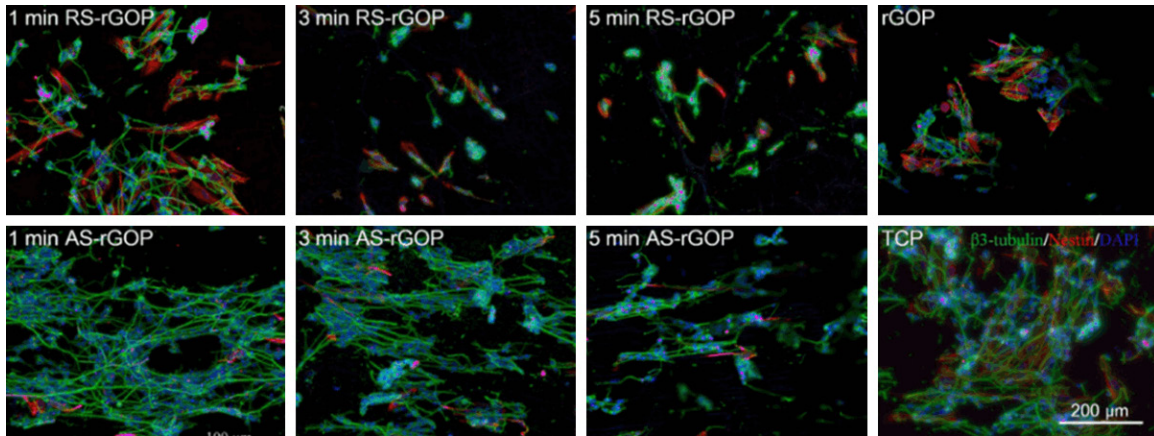MSC = mesenchymal stem cell, NSC = neural stem cell.

Figure 3. Immunostaining images of SH-SY5Y cells growing on the arranged directional matrix of nanofibers with staining for DAPI (blue), β3-tubulin (green), and Nestin (red). The upper row of images shows the differentiated cells that were supported on randomly oriented silk nanofiber-reduced graphene oxide paper (RS-rGOP) after electrospinning for 1, 3, and 5 min, with rGOP as the control. The lower row of images shows the differentiated cells growing on AS-rGOP after different electrospinning for 1, 3, and 5 min, with tissue culture plate as the control. The neuron-specific marker β3-tubulin was expressed to the greatest extent on AS-rGOP with an electrospinning time of 1 min.
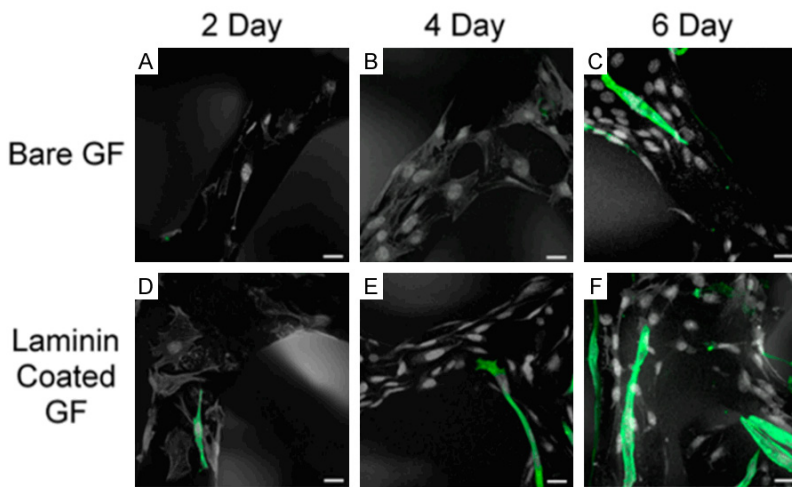

Figure 4. Immunofluorescence of MHCs indicative of C2C12 differentiation. Differentiation of C2C12 grown on bare GF (A-C) for 2, 4, and 6 days and on laminin-coated GF (D-F) for 2, 4, and 6 days. The scale bars are all 20 μm.

ty. Another electronically conductive hybrid hydrogel was engineered based on gelatin methacryloyl (GelMA) hybrid hydrogels composed of homogeneously dispersed rGO nanoparticles [126]. The efficiency of UV-induced crosslinking of GelMA was decreased with the GO incorporation, thus the GO was reduced with ascorbic acid to improve the electrical conductivity and biocompatibility, and the GelMA acted as the biocompatible substrate to provide active chemical groups. This composition provided a natural cell microenvironment for cardiomyocyte culture and maintained proper cell attachment. Overall, these results suggest that GO is a promising material in cardiac tissue engineering applications (**Figure 5**).

Conclusion and future perspectives

Graphene-based nanomaterials have been widely used in the biomedical field because of their various unique properties. The large specific surface area of graphene and its derivatives supports the absorption and binding of antibodies, adapters, drugs, genes, enzymes, and other molecules. Among these processes, aptamers and specific antibodies have been employed to target binding to the lesion site, and modifications involving fluorescent molecules, quantum dots, and contrast agents have been used for achieving multimodal imaging. In addition, the combinations of drugs and genes that are possible with graphene nanomaterials have been shown to have synergistic therapeutic effects. High priority should be given to linkages of enzymes, specific molecules, and antibodies for applications
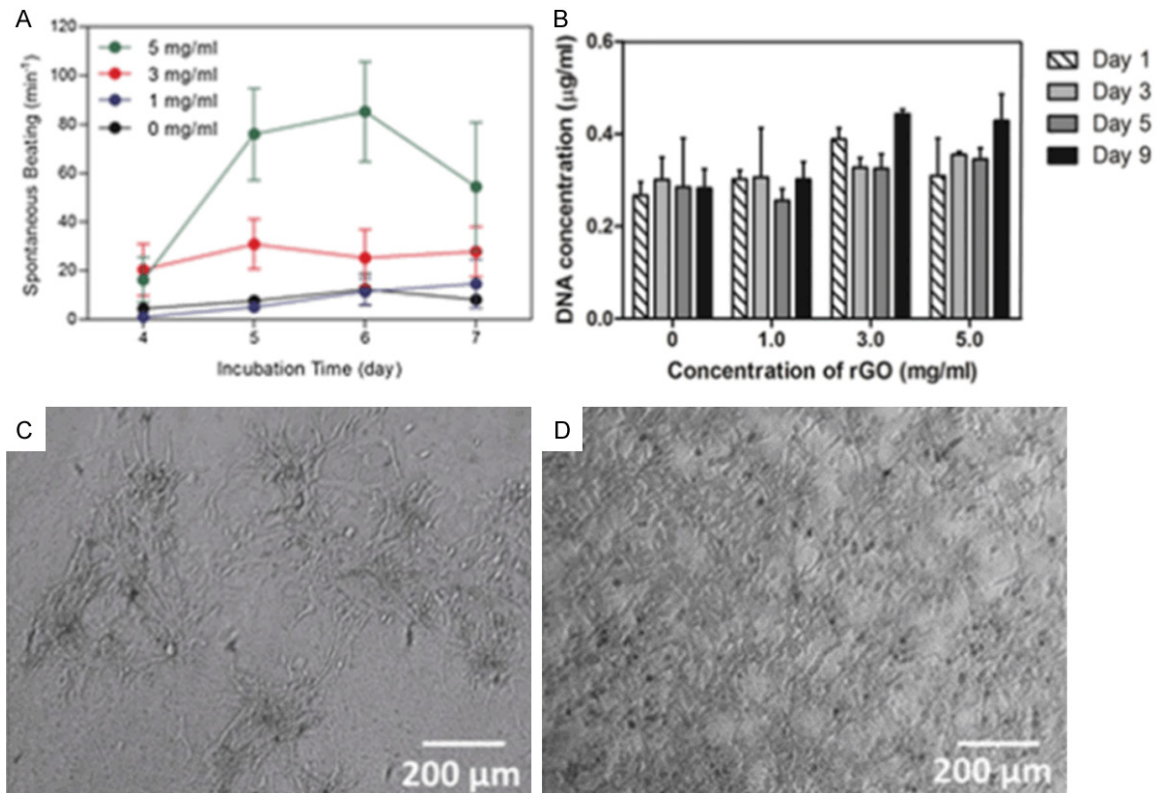
Figure 5. Characterization of the electrophysiological properties and compatibility of the graphene-based material. (A) Spontaneous beating rates of cardiomyocytes seeded on GelMA incorporated with different amounts of rGO (0, 1, 3, 5 mg/ml) plotted against incubation time. (B) Stability of cells (in terms of DNA content) cultured on GelMA alone and on rGO-GelMA hydrogels. Phase contrast images of cells cultured on (C) GelMA and (D) rGO-GelMA hydrogels at 6 days after seeding.

involving biomarker detection, disease diagnosis, PTT, tissue engineering, and cell or exosome capture and detection. At the same time, a large number of active groups on the surface of graphene-based materials can be functionalized by physical or chemical reactions, and this can improve the specific properties of the material to promote the material's biocompatibility, thus reducing the biological toxicity and achieving targeted functions. The graphene substrates can be modified with functional groups or molecules through covalent bonds, Van der Waals forces, π-π reactions, or electrostatic interactions with the purpose of improving the material's biocompatibility and prolonging its time in circulation. In addition, graphene has unique optical properties and can be used in PTT. Given this premise, these composites with strong near-infrared absorbance capabilities can act as photothermal agents, and these materials can rapidly release their cargo at specific targets when exposed to near-infrared irradiation, and this enables them to kill target cells by increasing cell permeability.

What needs to be noted in particular is that the excellent mechanical strength, biocompatibility, and electrical conductivity of graphene and its derivatives need to be taken into consideration when constructing matrices or scaffold materials with different morphologies and surface roughness. The interaction between cells and two-dimensional and three-dimensional graphene-based matrixes or scaffolds can stimulate stem cells to proliferate and differentiate into specific lineages, indicating their applications in biosensing, PTT, and tissue engineering. With regard to these applications, developments in tissue engineering have attracted more and more attention and have benefited from recent advances in materials science and bioengineering. In this review we have systematically elucidated the progress in bone, nerve, muscle, and cardiac tissue engineering with

the goal of producing bioactive tissues to replace or repair damaged or degraded tissue function by promoting osteogenic differentiation, neuronal differentiation, myogenic differentiation, and myocardial cell growth.

Although most of the reported studies are still in the primary stage, great progress has been made. Many efforts have been made to develop a better understanding of the basic mechanisms and signaling pathways involved in the interaction between graphene-based materials and cells, and the material's biotoxicity needs to be further studied in pre-clinical applications. These efforts to overcome problems and the advances that have been made have pushed the development of these graphene-based materials for clinical application.

With an in-depth understanding of graphene and its derivatives, more and more studies have been devoted to reducing the biological toxicity of these materials. Unfortunately, the findings of material toxicology research have not yet provided an authoritative conclusion because the different production processes that have been used have led to significant differences in the size, active groups, thickness, stability, and other parameters of the prepared graphene-based materials, and this has affected the interaction between the materials and the cultured cells. Moreover, different experimental methods and performance evaluations of substrates and scaffolds have aroused heated discussions in academic circles. It is exciting to note that current research in the field is focused on improving the properties of graphene-based materials through the formation of composite materials or functional modifications in order to overcome current limitations of the materials and to open the way for further developments in clinical research. All in all, continued efforts in these areas are in keeping with the goal of solving all of the current problems with graphene-based materials, and thus it is not difficult for us to predict that graphene and its derivatives will pave the way to significant breakthroughs in future clinical and biomedical research.

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Disclosure of conflict of interest

None.

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