Graphene and graphene-based materials in axonal repair of spinal cord injury

Abstract
Graphene and graphene-based materials have the ability to induce stem cells to differentiate into neurons, which is necessary to overcome the current problems faced in the clinical treatment of spinal cord injury. This review summarizes the advantages of graphene and graphene-based materials (in particular, composite materials) in axonal repair after spinal cord injury. These materials have good histocompatibility, and mechanical and adsorption properties that can be targeted to improve the environment of axonal regeneration. They also have good conductivity, which allows them to make full use of electrical nerve signal stimulation in spinal cord tissue to promote axonal regeneration. Furthermore, they can be used as carriers of seed cells, trophic factors, and drugs, to effectively construct scaffolding and microenvironmental conditions for spinal cord repair. These materials promote axon regeneration and construct a local microenvironment after spinal cord injury. However, to achieve clinical adoption of graphene and graphene-based materials for the repair of spinal cord injury, further research is needed to reduce their toxicity.

Key Words: axonal regeneration; graphene; graphene oxide; nerve axon regeneration; reduced graphene oxide; spinal cord contusions; spinal cord injury; spinal cord trauma

Introduction
Spinal cord injury (SCI) is a severe trauma of the central nervous system that results in a high rate of disability (Slusarczyk et al., 2019) through motor, sensory, and sphincter dysfunctions, dystonia, and pathological reflexes. Considering the increasing incidence of car accidents and fall-related injuries (Hamid et al., 2018), the incidence of SCI is also increasing annually. SCI can be divided into two stages—primary injury and secondary injury (Manchikanti et al., 2009; Ghatas et al., 2019). Primary injury is mechanical injury caused by the trauma. This leads to SCI via external factors, such as compression, dislocation, and other mechanical forces that damage intramedullary cells, destroy axons, and cause Wallerian degeneration (Singh et al., 2014; Vismara et al., 2017). Secondary injury is caused by a series of subsequent pathological reactions, which lead to neuropathy. This further harms the nervous system and leads to irreversible injury. Secondary injury is the most important obstacle affecting nerve regeneration and late functional repair (Singh et al., 2014; Vismara et al., 2017).

Surgical decompression and high-dose corticosteroid pulse therapy are mainly used in the immediate clinical treatment of SCI. However, these treatments only alleviate the primary symptoms and reduce secondary damage, without establishing connections between the axons at the injured site to restore their physiological function (Rouanet et al., 2017; Vismara et al., 2017). In addition, stem cell transplantation, neurotrophin delivery, and implantable biomaterials are being developed to promote nerve repair after SCI. The purpose of these treatments is to delay the process of injury, promote nerve axonal regeneration, remove growth inhibitory factors, repair damaged myelin sheath, restore normal transmission of nerve stimulation signals, and restore the function of the corresponding part of the human body (Vazquez et al., 2017; Kato et al., 2019; Wang et al., 2019b). Although the effectiveness of these treatments has been confirmed by clinical studies, issues remain in terms of their safety (Chung et al., 2010; Mortazavi et al., 2016; Kasi et al., 2017). For example, in stem cell transplantation, stem cells secrete a variety of cytokines and can be induced to differentiate into neurons and other cells to promote nerve regeneration (Iyer et al., 2017; Robinson et al., 2017); however, transplantation of bone marrow mesenchymal stem cells has been shown to lead to tumorigenesis (Kobayashi et al., 2012). Using cytokines secreted by cells derived from macrophages can improve the local microenvironment of SCI and promote the repair of SCI; however, this does not prevent the development of SCI (Kong et al., 2018; Zhang et al., 2021).

The treatment of SCI with implantable biomaterials is one of the most important research areas in the field of SCI repair. These biomaterials can be a single material, an implant that carries a nerve regeneration drug, or a biological tissue engineering scaffold combined with seed cells for tissue construction. The biomaterials implanted at the site of SCI can build a suitable three-dimensional space for the growth of cells and guide the extension of neuronal axons, which is beneficial to the establishment of functional connections of regenerated axons. Graphene and graphene-based materials have better mechanical properties than more mature biological scaffold materials (e.g., humanoid collagen, chitosan, various gels, polyactic acid, and polyethylene glycol). These properties result in a high swelling rate of graphene-based composite scaffolds; reduce the possibility of secondary injury; and increase the affinity between the scaffold surface and the loaded cells. Moreover, the good conductivity of these biomaterials allows them to effectively conduct nerve electrical stimulation, thereby accurately guiding the formation of axons, shortening the repair cycle, and improving the rate of axon repair. Furthermore, because graphene can induce embryonic stem cells to differentiate into neurons, can induce the formation of human neuron synapses, and has excellent drug carrier properties, it can play an important role in overcoming secondary SCI (Min et al., 2017; Saravanan et al., 2018; Agarwal et al., 2020; Dominguez-Bajo et al., 2020; Usmani et al., 2020; Yang et al., 2021).

This review summarizes the advantages of these materials in the treatment of SCI (Figure 1). We discuss their potential in overcoming the difficulties in the treatment of SCI and provide a reference for researchers to perform further investigation.

Search Strategy
We performed an online search of PubMed and Web of Science to retrieve related articles using the search terms “spinal cord injury” and “graphene” with the time range from inception to June 2021. A total of 86 related articles were retrieved according to the following inclusion and exclusion criteria in this narrative review. Inclusion criteria: (1) basic and clinical studies of graphene and graphene-based materials involved in the repair of spinal cord injury; (2) basic studies on the effects of graphene and graphene-based materials on nerve cells. Two researchers (SXW and YBL) independently read and screened the articles by reading the titles and abstracts and then combined the screening results. In the event of disagreement between the
Graphene and Graphene-Based Materials

Overview of graphene and graphene-based materials

Graphene is a two-dimensional carbon nanomaterial with a hexagonal honeycomb lattice composed of carbon atoms with $sp^2$-hybridized orbitals. Graphene has the characteristics of high strength, high flexibility and bendability, super-hydrophobicity, super-lipophilicity, and good thermal conductivity (Luo et al., 2020; Kim et al., 2021; Liu et al., 2021). Driven by increasing demand for various applications, different derivatives of this material have been fabricated (Table 1). The double-bonded structure of graphene can attach to specific functional groups or molecules through addition reactions (Zhang et al., 2017a; White et al., 2018; Bao et al., 2019; Li et al., 2020). Graphene oxide (GO) is an intermediate for the preparation of graphene. The oxygen-containing functional groups of graphene oxide make it hydrophilic and more widely used than graphene itself (Narayanan et al., 2020). Reduced graphene oxide (rGO) has a large specific surface area owing to the small number of oxygen-containing groups on its surface, giving it a sufficiently high conductivity to promote stem cell growth (Bianco et al., 2016). Finally, graphene substrates have also been shown to promote nerve differentiation (Hong et al., 2014), and graphene and graphene-based materials have been used as stents implanted into the injured spinal cord to initiate a beneficial tissue response (Domínguez-Bajo et al., 2017).

Physicochemical Properties and Advantages of Graphene and Graphene-Based Materials in Axonal Repair after Spinal Cord Injury

Biomaterials have been increasingly used in the treatment of SCI, with increasing attention paid to graphene and graphene-based materials because of their suitability for axonal repair after SCI (Table 2). There is also an increasing number of in vitro cytological studies addressing the effects of graphene and graphene-based materials on nerve cells (Table 3). Graphene and graphene-based materials are used in the treatment of SCI because of their good biocompatibility, neuronal induced orientation, adsorption, electrical conductivity, and flexibility.

Because of their excellent optical and electrical properties, graphene-based materials have attracted considerable attention for application as electrochemical sensors and biosensors, and in biological imaging. For example, exfoliated graphite nanosheets are widely used for blood glucose measurement, giving much higher detection sensitivity than the traditional method based on carbon nanotubes (Lu et al., 2008; Shi et al., 2015). Graphene quantum dots have excellent photoluminescence properties because of the boundary effect and quantum confinement effect. For example, Qu et al. (2015) synthesized nitrogen-doped graphene quantum dots capable of emitting a variety of colors (blue, green, and yellow). These quantum dots had low cytotoxicity and good biocompatibility, making them attractive as biological imaging agents. Such materials also have great potential in photochemical therapy of tumor replacement therapy (Yang et al., 2012; Chen et al., 2016).

Because of its excellent mechanical properties, graphene is often combined with different biomaterials to make scaffolds for bone tissue regeneration and stem cell differentiation. For example, Ignat et al. (2019) used GO scaffolds to induce cell differentiation and found that higher scaffold concentrations promoted more osteogenic differentiation to greater extent. These findings were consistent with those of Elkenany et al. (2015). In another study, Schwann cells were shown to have a significant proliferative effect on targeted amnionized poly-L-lactide nanofiber scaffolds coated with GO nanoparticles, highlighting the potential of this material in nerve regeneration (Zhang et al., 2016). Finally, graphene substrates have also been shown to promote nerve differentiation (Hong et al., 2014), and graphene and graphene-based materials have been used as stents implanted into the injured spinal cord to initiate a beneficial tissue response (Domínguez-Bajo et al., 2017).

### Table 1 | Overview of graphene and graphene-based materials

| Type               | Physicochemical properties                         | Main applications                  |
|--------------------|---------------------------------------------------|------------------------------------|
| Graphene           | High electrical conductivity, high thermal conductivity, high mechanical strength and good optical properties | Electrochemical biosensor          |
| GO                 | High specific surface energy, good hydrophilicity and mechanical strength properties | Antibacterial                      |
| rGO                | Good optical properties                            | Promote the adhesion and proliferation of stem cells; |
| Nitrogen-doped graphene | Good biocompatibility and strong electrocatalysis activity | Promote osteogenic differentiation of stem cells; |
| Graphene           | Semiconductor properties                           | Promote the adhesion and proliferation of stem cells; |

**Figure 1 | Advantages of graphene and graphene-based materials in solving the key problems in the treatment of spinal cord injury.**

Two researchers on the inclusion of a particular article, XXW was consulted to arrive at a decision. Any articles unrelated to SCI or axon regeneration and duplicated studies were excluded.

The search formula used in the PubMed database was: (("spinal injury"[Title/Abstract] OR "Spinal Cord Trauma"[Title/Abstract] OR "Spinal Cord Injury"[Title/Abstract] OR "Spinal Cord Contusions"[Title/Abstract]) OR "Spinal Cord Injuries"[Mesh]) AND ((Graphene[Title/Abstract] OR rGO[Title/Abstract] OR CNTs[Title/Abstract] OR "graphene oxide"[Title/Abstract] OR "reduced graphene oxide"[Title/Abstract]) OR ("Graphite"[Mesh] OR "graphene oxide"[Supplementary Concept])).

**Figure 2 | Biomedical applications of graphene and graphene-based materials**

- Good biocompatibility
- Inducing differentiation of seed cells
- Improving electrical conductivity
- As carriers
- Supporting axonal regeneration

**Table 2 | Physicochemical Properties and Advantages of Graphene and Graphene-Based Materials in Axonal Repair after Spinal Cord Injury**

| Biomaterials | Properties | Advantages |
|--------------|------------|------------|
| Graphene     | High electrical conductivity, high thermal conductivity, high mechanical strength and good optical properties | Electrochemical biosensor, Promote the adhesion and proliferation of stem cells, Promote osteogenic differentiation of stem cells, Optical biosensor, Drug carrier, Gene vector, Photodynamic therapy, Photothermal therapy, Biological imaging, Antibacterial |
| GO           | High specific surface energy, good hydrophilicity and mechanical strength properties | Promote the adhesion and proliferation of stem cells, Promote osteogenic differentiation of stem cells, Optical biosensor, Drug carrier, Gene vector, Photodynamic therapy, Photothermal therapy, Biological imaging, Antibacterial |
| rGO          | Good optical properties                            | Promote the adhesion and proliferation of stem cells, Promote osteogenic differentiation of stem cells, Optical biosensor, Drug carrier, Gene vector, Photodynamic therapy, Photothermal therapy, Biological imaging, Antibacterial |
| Nitrogen-doped graphene | Good biocompatibility and strong electrocatalysis activity | Promote osteogenic differentiation of stem cells, Optical biosensor, Photodynamic therapy, Antibacterial |
| Graphene     | Semiconductor properties                           | Promote the adhesion and proliferation of stem cells, Promote osteogenic differentiation of stem cells, Optical biosensor, Photodynamic therapy, Biological imaging, Antibacterial |

**Table 3 | Overview of graphene and graphene-based materials**

| Type               | Physicochemical properties | Main applications                  |
|--------------------|---------------------------|------------------------------------|
| Graphene           | High electrical conductivity, high thermal conductivity, high mechanical strength and good optical properties | Electrochemical biosensor          |
| GO                 | High specific surface energy, good hydrophilicity and mechanical strength properties | Antibacterial                      |
| rGO                | Good optical properties    | Promote the adhesion and proliferation of stem cells; |
| Nitrogen-doped graphene | Good biocompatibility and strong electrocatalysis activity | Promote osteogenic differentiation of stem cells; |
| Graphene           | Semiconductor properties   | Promote the adhesion and proliferation of stem cells; |
GO could have a positive role in the recovery of neurological function after SCI (animal experiments) in vivo experiments). The exploration of graphene has progressed greatly for tissue engineering applications (review). An effective design of an injectable, anti-inflammatory, and conductive material for SCI repair (in vitro and in vivo experiments). The material as a carrier for the further application in the central nervous system injury (in vitro experiments) and this films were promising to apply for the electrical stimulation therapy of peripheral nerve repair in vivo (cell experiments). The potential of scaffolds to promote regenerative features at the injured spinal cord (in vivo experiments).

The nanofibres might be an effective carrier for insulin-like growth factor delivery (in vitro experiments). The nanofibre is an effective carrier for insulin-like growth factor 1 and brain-derived neurotrophic factor delivery (in vitro and in vivo experiments). The marriage of nervous system and nanotechnology disciplines may provide a solution to many central nervous system disorders (review).

Primary data suggest hints of rGO sheets dissociation and eventual degradation at the injured spinal cord for the first time (in vivo experiments). The scaffold represents a new class of nanomaterial which can work in nerve regeneration (in vitro and in vivo experiments). The material can support wound healing and tissue repair after implantation (in vitro experiments). Promising materials to serve as nerve conduits (in vitro experiments) and the work focused on materials used in biomedicine (review).

Favorable biological responses of neural cells and tissue interacting (in vitro and in vivo experiments).

Evaluate the biocompatibility of the scaffold in a SCI model (in vivo experiments). Investigate for the first time chronic tissue responses to 3D scaffolds composed of partially rGO when implanted in a rat model of SCI (in vivo experiments). This study reported for the first time implantation of 3D porous and flexible rGO scaffolds at the injured rat spinal cord (in vivo experiments). This study is the first to report on the utility of these coatings in stimulation applications (in vitro experiments) and the composites can enhance neurite sprouting and outgrowth (in vitro experiments). The material resulted in the formation of highly aligned axons from the differentiating neural stem cells (in vitro experiments). Further use of graphene as a bioscaffold require surface modification (in vitro experiments).

Aminated carbon nanotubes significantly recover function following stroke in rats—something specific to the materials (review). The scaffolds can promote neuronal differentiation of human embryonic stem cells (in vitro experiments). Use of carbon nanotubes as a growth promoting substratum (Meeting presentation).rGO materials in the shape of microfibers.

PLGA/GO nanofibres with immobilized insulin-like growth factor 1 and brain-derived neurotrophic factor delivery (in vitro and in vivo experiments). The marriage of nervous system and nanotechnology disciplines may provide a solution to many central nervous system disorders (review).

Primary data suggest hints of rGO sheets dissociation and eventual degradation at the injured spinal cord for the first time (in vivo experiments). The scaffold represents a new class of nanomaterial which can work in nerve regeneration (in vitro and in vivo experiments). The material can support wound healing and tissue repair after implantation (in vitro experiments). Promising materials to serve as nerve conduits (in vitro experiments) and the work focused on materials used in biomedicine (review).

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**Figure 2** | Advantages of graphene and graphene-based materials in solving the key difficulties in the treatment of spinal cord injury (SCI, 2009–2021). PLGA: Poly(lactic-co-glycolic acid).
A summary of animal model experiments (in vivo) on graphene and graphene-based materials in the repair of spinal cord injury

| Reference | Animals | Material intervention mode | Modeling method | Material intervention time | Detection method | Main results | Significance |
|-----------|---------|---------------------------|-----------------|---------------------------|-----------------|--------------|--------------|
| Kolarčik et al., 2015 | Adult male Sprague-Dawley rats | Conductive polymer poly(3,4-ethylenedioxythiophene) (PEDOT) and multi-wall CNTs were coated on the electrode surface and doped with the anti-inflammatory drug dexamethasone | A unilateral laminectomy was performed to expose the left side of the dorsal root ganglion between L5 and L6 | 14 d | (1) Confocal fluorescent microscopy; (2) Immunofluorescence | Significantly less neuronal death/damage was observed with coated electrodes and the inflammatory was also reduced. | This study was the first to report the utility of these coatings in stimulation applications. |
| López-Dolado et al., 2015 | Aged adult male Wistar rats | 3D flexible and porous scaffolds composed of partially rGO | A right lateral hemisection of approximately 8 mm² (2 mm × 2 mm × 2 mm) at the C6 segment, rostral to the bulk of triceps brachii motoneurons | 10 d | (1) Histological examination; (2) Immunofluorescence | These structures facilitated regaining tissue integrity after SCI as early as 10 d and prevent the extension of the lesion. It had no local and systemic toxic responses. | This study was the first to implant 3D porous and flexible rGO scaffolds at the injured rat spinal cord. |
| López-Dolado et al., 2016 | Adult male Wistar rats | 3D scaffolds composed of partially rGO | A right lateral hemisection of approximately 8 mm² (2 mm × 2 mm × 2 mm) at C6, rostral to the bulk of triceps brachii motoneurons | 30 d | (1) Histological examination; (2) Immunofluorescence; (3) Transmission electron microscopy | The scaffolds in injury stabilization and sealing, moreover, rGO scaffolds supported angiogenesis. | This study investigated for the first time chronic tissue responses to 3D scaffolds composed of partially rGO when implanted in the injured rat spinal cord. |
| Palejwala et al., 2016 | Wistar rats (13 males and 1 female) | Graphene nanoscaffolds were prepared by the mild chemical reduction of GO | Hemispinal cord transaction at approximately the T2 level | 3 mon | (1) Electron microscopic; (2) Histological examination; (3) Immunofluorescence | The graphene nanoscaffolds adhered well to the spinal cord tissue. | Graphene is a nanomaterial that is bioincompatible with neurons and may have significant biomedical application. |
| González-Mayorga et al., 2017 | Adult male Wistar rats | rGO microfibers as substrates for promoting nerve growth | A right lateral hemisection of approximately 8 mm² (incomplete lesion) at C6, rostral to the bulk of triceps brachii motoneurons. | 10 d | (1) Scanning electron microscopy; (2) Transmission electron microscopy; (3) Immunofluorescence | In vivo studies reveal the feasible implantation of these rGO microfibers as a guidance platform in the injured rat spinal cord, without evident signs of subacute local toxicity. | These positive findings boost further investigation for enhancing repair in the damaged central neural tissue including the injured spinal cord. |
| Domínguez-Bajo et al., 2019 | Adult male rats | 3D randomly porous foams have been prepared in mechanical compliance with neural cells and tissues (Young’s modulus of 1.3 ± 1.0 kPa) as demonstrated by atomic force microscopy techniques applied ex vivo. | A cervical unilateral hemisection at the right C6, rostral to the bulk of triceps brachii motoneurons. | 4 mon | (1) Transmission electron microscopy; (2) Magnetic resonance imaging; (3) Atomic force microscopy; (4) Immunofluorescence; (5) Histological examination; (6) Behavioral tests (1) Immunofluorescence; (2) Motor function detection; (3) Histology observations; (4) The BBB locomotor rating scale; (5) Motor evoked potential detection | The scaffolds significantly reduced perilesional damage and caused no compressive damage in the contralateral hemisected and rostral/caudal regions. It also does not either alter the rat spontaneous behavior or induce toxicity in major organs. | This study suggests hints of rGO sheets dissociation and eventual degradation at the injured spinal cord for the first time. |
| Pan et al., 2019 | Female Sprague-Dawley rats | T9 spinal cord hemisection rat model | 4 wk | (1) Immunofluorescence; (2) Motor function detection; (3) Histology observations; (4) The BBB locomotor rating scale; (5) Motor evoked potential detection | (1) Behavioral tests; (2) Immunofluorescence | These findings outline the potential of rGO-MF-based scaffolds to promote regenerative features at the injured spinal cord such as axonal and vascular growth. | This study indicated that PLGA/GO is an effective carrier for IFG-1 and BDNF delivery. |
| Domínguez-Bajo et al., 2020 | Adult male rats | rGO materials in the shape of microfibers | A right hemisection at C6 cervical level, rostral to the bulk of triceps brachii motoneurons | 10 d | (1) The BBB locomotor rating scale; (2) Electrophysiologic recording; (3) Histological analysis; (4) Immunofluorescence | GO could have a positive role in the recovery of neurological function after SCI by promoting the degradation of the scaffold, adhesion, and migration of nerve cells to the scaffold. | In this work, the regenerative potential of rGO-MFs when chronically interfaced with a cervical spinal cord injury was investigated for the first time. |
| Yang et al., 2021 | Female Sprague-Dawley rats | A conductive GO composited chitosan scaffold was fabricated by genipin crosslinking and lyophilization. | The lamina of the thoracic vertebrae T8–T10 were exposed. The spinal cord was exposed and approximately 2 mm of the spinal cord tissue at the T9 level was completely removed under an operating microscope | 10 wk | (1) The BBB locomotor rating scale; (2) Electrophysiologic recording; (3) Histological analysis; (4) Immunofluorescence | The scaffold can promote the repair of damaged nerve tissue. | In this work, the regenerative potential of rGO-MFs when chronically interfaced with a cervical spinal cord injury was investigated for the first time. |
Advantages of graphene and graphene-based materials in inducing differentiation of seed cells

A promising strategy to promote axonal regeneration after SCI is to induce seed cell differentiation (Lee-Kubli et al., 2015). Tissue engineering scaffolds made from graphene or its derivatives construct a suitable space for nerve repair in the later stage of SCI and axon regeneration. When combined with seed cells, they also facilitate proliferation and differentiation (Silver et al., 2014; He et al., 2016). Using a novel amino-functionalized graphene-crosslinked collagen-based nerve conduit, Agarwal et al. (2020) confirmed that graphene-based scaffolds, under certain conditions, can make bone marrow mesenchymal stem cells differentiate into neurons, promote neuronal cell proliferation and migration, and inhibit neuro-inflammation. GO can also induce embryonic stem cells to differentiate into neurons. For example, GO can effectively enhance the dopamine neuronal differentiation of embryonic stem cells and enhance the expression of dopamine neuron-related genes (Yang et al., 2014). Another report found that graphene can promote neural stem cell differentiation into neurons rather than glial cells because of its unique surface properties (Guo et al., 2021). In this work, the regenerative potential of rGO-MFs when chronically interfaced with a cervical spinal cord injury was investigated for the first time. These positive findings encourage further investigation of these microfibers as attractive biomaterials to interact with neural cells.

Table 3 | Summary of in vitro cytological studies on the effects of graphene and graphene-based materials on nerve cells

| Year of publication | Cell type | Nerve cell sources | Culture method | Detection method | Main results | Significance |
|---------------------|-----------|--------------------|----------------|-----------------|--------------|--------------|
| Chen et al., 2012   | Human embryonic stem cells | Wicell (Madison, WI, USA; passages 32 to 55) | A neuron induction medium consisting of F12/DMEM, N2 supplement, and FGF2 (20 ng/mL) | Immunochemistry and fluorescence measurement; scanning electron microscopy | The results demonstrated that cells on silk-CNT scaffolds have a higher β-III tubulin and nestin expression, suggesting augmented neuronal differentiation. | The silk-CNT composite scaffolds developed here can serve as efficient supporting matrices for stem cell-derived neuronal transplants. |
| Solanki et al., 2013 | Neural stem cells | Unclear | Proliferated in culture media containing basic fibroblast growth factor (BFGF, 20 ng/mL) and epidermal growth factor (EGF, 20 ng/mL) | Quantitative RT-PCR; scanning electron microscopy; immunostaining | This work has demonstrated that the graphene nanoparticle-coated hybrid structures lead to enhanced neuronal differentiation of HNSCs along with significant axonal alignment. | The hybrid nanostuctures have tremendous implications for the potential use of GO as an ECM component especially in the field of neurobiology. |
| Tu et al., 2013      | Primary rat hippocampal neurons | Prepared from postnatal Sprague-Dawley rat pups (aged 1 to 3 d) | The DMEM medium supplemented with 10% fetal calf serum, 0.03 mM L-glutamine, 0.03 mM L-glutamine, 0.03 mM L-glutamine; 0.03 mM L-glutamine, 0.03 mM L-glutamine, 0.03 mM L-glutamine, 0.03 mM L-glutamine; 0.03 mM L-glutamine, 0.03 mM L-glutamine, 0.03 mM L-glutamine | Scanning electron microscopy; immunocytochemistry; western blotting | These biomimetic choline-like GO composites can significantly boost neurite outgrowth and outgrowth. | All results demonstrate the potential of DDAEMA- and MPC-modified GO composites as biomimetic materials for neural interfacing. |
| González-Mayorga et al., 2017 | Embryonic neural progenitor cells | Obtained from cerebral cortices of E18 Wistar rat embryos | 500 μL of complete neurobasal media containing B-27 supplement (2%), streptomycin (100 μg/mL), penicillin (100 μg/mL), and L-glutamine (1 mM) | Scanning electron microscopy; confocal laser scanning microscopy; transmission electron microscopy | These microfibers behave as supportive substrates of highly interconnected cultures composed of neurons and glial cells for up to 23 days, and the colonization by meningeal fibroblasts is dramatically hindered by N-cadherin coating. | These positive findings indicated that DDAEMA- and MPC-modified GO composites can be applied for the ES therapy of peripheral nerve repair in vivo. |
| Min et al., 2017     | Human neuroblastoma cells | American type culture collection (Manassas, VA, USA) | The DMEM medium supplemented with 10% heat-activated fetal bovine serum and 1% antibiotics (penicillin and streptomycin) | Fluorescence imaging; confocal imaging; Raman spectroscopy | The study developed a novel MF-driven GO hybrid pattern that was highly effective for controlling synaptogenesis. | This work provides treatment and modeling of brain diseases and spinal cord injuries. |
| Serrano et al., 2018 | Embryonic neural progenitor cells | Obtained from cerebral cortices of E18 Wistar rat embryos | 500 μL of complete neurobasal media containing B-27 supplement (2%), streptomycin (100 μg/mL), penicillin (100 μg/mL), and L-glutamine (1 mM) | Confocal laser scanning microscopy; inflammatory cytokine detection; flow cytometry | The capacity of rGO microfibers to inhibit the proliferation of RAW264.7 macrophages, without affecting their viability and cell cycle profiles. | These findings encourage further investigation of these microfibers as attractive biomaterials to interact with neural cells. |
| Pan et al., 2019     | Neural stem cells | Isolated from the cerebral cortex of embryonic mice (E11.5) | The growth media contained neurobasal media, B27 neural supplement, 100 μg/mL penicillin-streptomycin, 20 ng/mL epidermal growth factor and 20 ng/mL basic fibroblast growth factor. | MTX assay; immunofluorescence; quantitative real-time PCR analysis | PLGA/GO nanofibers loaded with IGF-1 and BDNF not only protected NSCs from oxidative stress induced by H2O2 but also enhanced NSC proliferation and neuronal differentiation in vitro. | The study indicated that immobilization of IGF-1 and BDNF onto PLGA/GO nanofibers has a great potential as a nerve implant for spinal cord injury applications. |
| Dominguez-Bajo et al., 2020 | Embryonic neural progenitor cells | Isolated from cerebral cortices of gravid Wistar rats | Samples were covered with 500 μL of complete neurobasal media containing B-27 supplement (2%), streptomycin (100 μg/mL), penicillin (100 μg/mL), and L-glutamine (1 mM). | Immunocytochemical studies by confocal laser scanning microscopy | This work confirmed the capacity of rGO-MFs to support the growth of ENPCs in vitro. | In this work, the regenerative potential of rGO-MFs when chronically interfaced with a cervical spinal cord injury was investigated for the first time. |
| Li et al., 2020      | Primary rat Schwann cells | The spinal nerves of Sprague-Dawley rats (newborn to postnatal d 4–5) | The cells were cultured with laminin-coated culture plate or bottle in DMEM/F12 containing 15% FBS and 1% penicillin-streptomycin. | Scanning electron microscopy; western blotting; MIT assay; immunofluorescence | The results of cell experiments indicated the better adhesion and higher expression of neural proteins in rat Schwann cells (RSCs) on PDA/GO/PPy-PILLA films. | These results indicated that PDA/GO/PPy-PILLA films were promising to be applied for the ES therapy of peripheral nerve repair in vivo. |

CGO: Carboxylic graphene oxide; CNs: carbon nanotubes; DDAEMA: dimethylaminomethylethylmethacrylate; DMEM/F12: Dulbecco’s modified Eagle media: nutrient mixture F-12; ECM: extracellular matrix; EGF: epidermal growth factor; ENPCs: embryonic neural progenitor cells; FBS: fetal bovine serum; FGF: fibroblast growth factor; MF: magnetic force; MPC: 2-methacryloyloxyethyl phosphorylcholine; NSCs: neural stem cells; PDA: polypodamine; PPA: p-lactic acid; PPy: polypyrrole; TH: L-theanine.
Review

Many studies (Windle, 1956; Kimura-Kuroda et al., 2010; Dolma and Kumar, 2021; Hart and Karimi-Abdolrezaee, 2021) have confirmed that the inflammatory response of secondary injury activates a large number of astrocytes around the injured foci. They also have confirmed that the glial scar not only produces a direct physical barrier to the regeneration of nerve injured axons, but also releases a series of factors to inhibit axonal regeneration and growth. However, Andersen et al. (2016) concluded that the formation of astrocyte glial scar does not just the secondary injury to axonal regeneration after nervous system injury, it also contributes to central nervous system axonal regeneration under appropriate stimulation. The dual role of glial scars needs to be further explored.

Advantages of graphene and graphene-based materials as drug carriers
Graphene and graphene-based materials have excellent drug carrier properties and are able to carry drugs directly to the action target and maintain effective concentrations. For example, Wang et al. (2019b) and Yang et al. (2018) reported ultrahigh drug loading capacity of doxorubicin on GO of approximately 400% and 238%, respectively. This is mainly because of the ultrahigh specific surface area of GO, which can adsorb drugs through electrostatic interactions and r-n stacking (Zhang et al., 2010b). Moreover, the rich functional groups of GO can directly (through electrostatic interactions) immobilize enzymes without the need for cross-linking agents or additional modification (Zhang et al., 2010a). Using a polylactide-glycolic acid nanofiber scaffold coated by GO and methylene blue, Wang et al. (2019c) loaded and released methylene blue from the matrix to protect and regulate the function of neural progenitor cells. Although this approach was developed for the treatment of Alzheimer’s disease, it could also be used in the recovery of damaged neurons after SCI. GO has also been used to deliver growth factors to the damaged site, which is necessary for regeneration. For example, Pan et al. (2019) immobilized insulin-like growth factor-1 and brain-derived neurotrophic factor on biodegradable GO-PLGA electrospun nanofibers. This material is not only toxic to inhibit thrombosis in vitro and promoted neural stem cell proliferation and neuronal differentiation.

Biosafety of Graphene and Graphene-Based Materials
Despite the otherwise ideal properties of graphene and graphene-derived materials for use in biomedicine, their toxicity needs to be further investigated. The biotoxicity of these materials is controversial (Seabra et al., 2014). For example, some research has found that GO was more likely to cause membrane damage and oxidative stress than GO (Zhang et al., 2018), whereas other studies have reported the opposite trend (Palejwala et al., 2016; Tabish et al., 2017). The toxicity of graphene and graphene-based materials is directly related to the material size, surface functional groups and degree of oxidation (Jastrzębska et al., 2012; López-Delgado et al., 2015). For example, especially in long-term culture, larger GO particles show higher toxicity than smaller particles (Mendes et al., 2015). Moreover, excessive GO has been shown to produce cytotoxicity (Jing et al., 2015; Zhang et al., 2015). At high doses, GO has also been observed to obstruct the respiratory tract, block pulmonary vessels, and lead to dyspnea (Li et al., 2014). Although some studies suggest that there is a safe range of conditions (Sasidharan et al., 2012; Yue et al., 2012), further experiments are needed before these materials are introduced into the clinical treatment of human SCI.

Summary and Future Prospects
In summary, graphene and graphene-based materials have considerable potential as scaffold materials for nerve tissue engineering that promote axon repair and regeneration, and can provide a number of overcoming the difficulties currently faced in the treatment of SCI in its late stages.

Limitations of graphene and graphene-based materials
Some studies have shown that graphene and graphene-based materials are cytotoxic, increase oxidative stress, and block pulmonary vessels. However, the biosafety of graphene and graphene-based materials is not yet clear. The future concerns precipitate the potential of graphene and graphene-based materials to provide a number of overcoming the difficulties currently faced in the treatment of SCI in its late stages. This could come from the surface modification of graphene and comparative analysis of different materials. This will pave the way to the implementation in the treatment of SCI.
Conclusions

Effective treatment strategies for SCI are a great challenge in modern medicine. Graphene-based medical engineering may provide a solution to this problem. We summarize the advantages of these materials as follows: (1) They have good tissue compatibility, and do not cause serious toxicity or immune rejection after implantation in animal models; (2) They induce seed cells to differentiate into nerve cells, replacing the lost nerve cells in SCI, thus providing a cellular basis for the bridging of nerve axons; (3) The good electrical conductivity of graphene and graphene-based materials allows them to make full use of neuroelectrical signals in the spinal cord tissue; (4) These materials stimulate axonal regeneration; and (5) Composites based on graphene and graphene-based materials can be used as a matrix for loading seed cells, nutritional factors, and drugs to reconstruct the local microenvironment after SCI.

However, some studies have shown that graphene or graphene-based materials can cause cytotoxicity, aggravate oxidative stress, and block pulmonary blood vessels, and the biosafety research data of graphene and graphene-based materials have not been conclusive. These safety issues seem to hinder the application of graphene and graphene-based materials in the treatment of SCI. However, it is well known that it is against the spirit of science to discuss toxicity without considering the dose and molecular characteristics. Therefore, we believe that the safe dosage form and dosage range of graphene and graphene-based composites for the treatment of SCI should be explored by researchers in the future. Only after accurate experimental conclusions are obtained through a dose gradient study and a comparative analysis of different composite materials can the graphene and graphene-based composite materials be used in the treatment of SCI.

Author contributions: SXW and YBL drafted the manuscript. XXW revised the manuscript. YJW and YJS contributed to the drafting of the manuscript. XXW and MN helped in the literature retrieval and provided technical assistance. All authors approved the final version of this paper.

Conflicts of interest: The authors declare that there are no conflicts of interest associated with this manuscript.

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