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Graphene Based Materials in Neural Tissue Regeneration

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Abstract

Due to its extraordinary features such as large surface area, high electrical conductivity, chemical stability and mechanical properties, graphene attracts great interest in various fields of biomedical sciences including biosensors, cancer therapy, diagnosis and regenerative medicine. The use of graphene-based materials has been of great interest for the design of scaffolds that can promote neural tissue regeneration. Recent studies published over the last few years clearly show that graphene and graphene based materials promote adhesion, proliferation and differentiation of various cells including embryonic stem cells (ESC), neural stem cells (NSC), mesenchymal stem cells (MSC) and induced pluripotent stem cells (iPSC). Therefore graphene based materials are one of the promising nanoplatforms in regenerative medicine for neural tissue injury. With its unique topographic and chemical properties, graphene is used as a scaffold that could provide a bridge between regenerating nerves. More importantly, as a conductive substrate, graphene allows the continuation of electrical conduction between damaged nerve ends. The integration of supportive cells such as glial, neural precursor or stem cells in such a scaffold shows higher regeneration when compared to currently used neural autografts and nerve conduits. This review discusses the details of such studies involving graphene based materials with a special interest on neural stem cells, mesenchymal stem cells or pluripotent stem cells.

Keywords

Graphene oxide · Mesenchymal stem cells · Neural stem cells · Pluripotent stem cells

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Abbreviations

2D	Two dimentional
3D	Three dimentional
1 step-G	One-step growth
2 step-G	Two-step growth
BDNF	Brain-derived neurotrophic factor
b-FGF	Basic fibroblast growth factor
CNS	Central nervous system
Cu	Copper
ECM	Extracellular matrix
EGF	Epidermal growth factor
ELF-EMF	Extremely low frequency electromagnetic fields
ESCs	Embryonic stem cells
FGF-2	Fibroblast growth factor 2
G	Graphene
GO	Graphene oxide
hADMSCs	Human adipose-derived mesenchymal stem cells
hMSCs	Human mesenchymal stem cells
hNPCs	Human neural progenitor cells
hNSCs	Human neural stem cells
IFNγ	Interferon-γ
iPSCs	Induced pluripotent stem cells
LIF	Leukemia inhibitory factor
LPS	Lipopolysaccharide
MSCs	Mesenchymal stem cells
NGLC	Nanocrystalline glass-like carbon film
NGF	Nerve growth factor
NGO	Nanosized graphene oxide
NPCs	Neural progenitor cells
NSCs	Neural stem cells
PADM	Porcine acellular dermal matrix
PCL	Polycaprolactone
PDGF	Platelet-derived growth factor
PDMS	Polydimethylsiloxane
PEDOT	Poly (3,4-ethylenedioxythiophene)
PEG	Poly (ethylene glycol)
PN	Peripheral nerve
PNI	Peripheral nerve injury
PNS	Peripheral nervous system
PU	Polyurethane
rGO	Reduced graphene oxide
SCI	Spinal cord injury
SCs	Schwann cells
SDIA	Stromal cell-derived inducing activity
siNPs	Silica nanoparticles
TBI	Traumatic brain injury
TCPS	Tissue culture polystyrene
TiO_2	Titanium dioxide

1 Introduction

The mammalian brain is an extraordinary organic machine that has fascinated scientists and clinicians for hundreds of years. A complex network of chemical and biochemical components of more than a dozen million neurons leads to the emergence of thought, emotion, memory and life. In contrast, fine imbalances or damage to this system can cause serious complications in physical, motor, psychological, and cognitive functions. Furthermore, the loss of inevitable nerve tissue due to degenerative diseases and traumatic injuries is destructive due to the limited regenerative ability of the central nervous system (Shah et al. 2016).

Currently, there are nanotechnology-based approaches developed to direct neural differentiation and regeneration. Among these approaches, stem cell based regenerative medicine shows the greatest hope for repairing and regenerating damaged nerve tissue. However, the establishment of controlled and reliable methodologies (eg, neurons and oligodendrocytes) that direct stem cell differentiation to specialized cells has been a major problem in the field.

2 Graphene and Its Properties

Graphene is a two-dimensional crystal formed by sp2 hybridization of carbon atoms arranged in the form of a honeycomb and composed of single atomic layers of graphite (Bitounis et al. 2013; de Lázaro et al. 2014; Novoselov 2011). Even though the existence of single grafic plates is theoretically debated (Slonczewski and Weiss 1958), the presence of two-dimensional

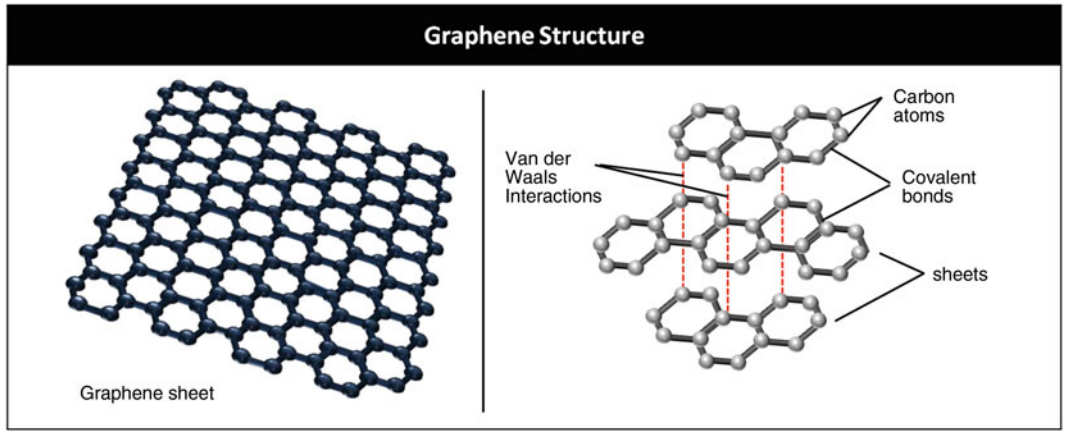


Fig. 1 Graphene structure: Graphene is an allotrope of carbon consisting of a single layer of carbon atoms arranged in a hexagonal lattice. Graphite is composed of stacked layers of graphene sheets, which are held together by the weak Van der Waals interactions

atomically fine crystalline materials was considered physically impossible (Venables et al. 1984). For the first time in 2004, Novoselov and Geim isolated and characterized a single graphene layer by 'Scotch Tape' method (Novoselov et al. 2004). Novoselov and Geim were awarded the Nobel Prize in 2010 for their discovery of this new carbon allotrope (Geim 2011; Novoselov 2011). The simple planar arrangement of the carbon atoms within the single layer of graphene and the covalent bonding between these carbon atoms give graphene unique properties (Fig. 1).

The generation of novel or improved graphene based biomaterials has had a major impact on nanotechnology. Due to its two-dimensional structure, high surface area, high electrical conductivity, chemical stability and bendable properties, graphene attracts great interest in various fields of biomedical sciences including biosensors, cancer therapy, diagnosis and regenerative medicine (Okan et al. 2016; Saner et al. 2010). All these features provide the ability to immobilize many substances such as metals, drugs, biomolecules, fluorescent probes and cells on graphene surface (Reina et al. 2014).

The application of graphene based materials in cell biology and physiology allows targeted interactions at the basic molecular level. In neuroscience, for example, it requires specific interactions with neurons, glial cells or other neuronal cells. Exemplary investigations include development of advanced molecular imaging technologies, engineering hybrid materials used in neural regeneration and developing technologies designed for targeted delivery of drugs and small molecules to blood-brain barrier and neuroprotection (Silva 2006).

2.1 Biocompatibility of Graphene Based Materials

Numerous studies have been conducted to improve the use of graphene in the biomedical field and to understand the toxicity profile in preclinical studies. By engineering surface chemistry, colloidal properties, water solubility and size, graphene-based biomaterials can become more biocompatible (Bussy et al. 2013). The toxicity profile of graphene based materials has been studied extensively in both in vitro and in vivo systems and there are promising results suggesting that these materials will be able to translated into clinical settings in the future (Seabra et al. 2014). In vitro studies have shown that when oxidised (referred to as graphene oxide-GO), or surface functionalized (with biodegradable polymers), toxicity related to graphene could

be abolished (Ali-Boucetta et al. 2013). Supporting cell culture studies, animal models have also shown that graphene-based materials which are small and single layer or conjugated to polymers such as PEG can be eliminated from living systems (Bussy et al. 2013). Therefore, considering such studies, it is possible to produce biocompatible biomaterials which will not cause toxicity.

3 The Nervous System

The nervous system is a network of signals that allows the brain and other parts of the body to function simultaneously. Neurons are electrically excitable cells which are using a concentration gradient of various ions (sodium, potassium, calcium, chloride etc.) and provide electrical conduction through the release neurotransmitters such as acetylcholine. Since neurons do not have division capabilities (suspended in G0 phase), it is of great importance to establish new treatment options for neural diseases by using the ability of stem cells to differentiate into neuronal cells (Fraczek-Szczypta 2014; Shin et al. 2016).

Glial cells are the most abundant cell type in the nervous system. Virchow first explained that there were cells present in neural tissue other than neurons. The first characterization of glial cells is a result of microscopic studies and metallic impregnation techniques developed by Ramon y Cajal and Rio Hortega in particular. Using gold impregnation, astrocytes; a few years later, oligodendrocytes and microglia, using silver carbonate impregnation were named (del Río-Hortega 1921, 1928).

Figure 2 summarizes the different types of cells present in CNS and PNS (Amoh et al. 2005; Frostick et al. 1998; Gardin et al. 2016; Liu et al. 2000; Nedergaard 1994; Ramírez-Jarquín et al. 2014; Scholz and Woolf 2007; Sedaghati et al. 2011; Spassky et al. 2005; Zhou et al. 2010)

The most common injuries affecting the nervous system are peripheral nerve injuries (CNS), spinal cord injury (SCI), and traumatic brain injury (TBI) (Gardin et al. 2016). The incidence of peripheral nerve injury (PNI) is estimated to be between 13 and 23 per 100,000 people per year in developed countries and causes partial or complete loss of motor, sensory and autonomic function in the relevant segments of the body. On the other hand, spinal cord injuries with an incidence of 750 out of every 1,000,000 people in the world are seriously threatening life (Wyndaele and Wyndaele 2006).

Most of the peripheral nerve injuries are repaired using nerve autografts, but they are limited to the source of donor nerves and may cause morbidity of the donor site (Zhou et al. 2010). Typically, axons in the micro perimeter of the peripheral nerve (PN) may be self-regulating at a relatively short distance (not greater than 5 mm). Regeneration of PN begins with the separation of Schwann cells (SCs) from axons, occurs as a result of incision of myelin sheaths and is phagocytozed by glial cells. SCs that break from the axons can multiply and enhance axonal guidance (Terenghi 1999). On the other hand, the cell loss during spianl cord injury can not be replaced by the body itself, and thus the spinal cord function is permanently lost. Due to the limited internal regenerative abilities, experts from different fields are encouraged to seek new ways to regenerate damaged or diseased nervous system, such as many pharmacological approaches, stem cell treatments, delivery of neurotrophic factors and biomaterial use (Lee-Kubli and Lu 2015).

4 Graphene in Regenerative Medicine

Ding and colleagues stated that a Web of Science search for “graphene” and “tissue engineering” showed that the majority of studies with graphene-based materials were based on bone and neural tissue regeneration (Ding et al. 2015). This trend clearly demonstrates the global importance of the graphene and the increase in interest of scientists in this field. In short, novel biomaterial platforms that can be used in regenerative medicine.

As confirmed by the increase in the number of publications, it is not surprising that the graphene has shown great interest in nanomedicine and

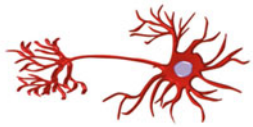
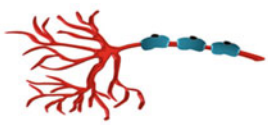
Types of cells in nervous system	Functions
Astrocytes 	• have functional neurotransmitter receptors, modulating the conduction properties of neighbouring neurons (Nedergaard 1994) • feature inhibition mechanisms to the regenerating neurites (Zhou et al. 2010) • have important roles in the homeostasis of the CNS (Gardin et al. 2016)
Ependymal cells 	• Provide the transport of cerebrospinal fluid and in brain homeostasis (Spassky et al. 2005)
Microglial cells 	• are macrophages of the CNS and permanent cells in the brain's immune system (Gardin et al. 2016)
Neurons 	• are the most common cell type in the CNS and PNS (Ramírez-Jarquín et al. 2014) • manipulate information to communicate neural networks (Gardin et al. 2016)
Oligodendrocytes 	• produce myelin and myelinated host axons in the central nervous system (CNS) (Liu et al. 2000) • provide axonal regeneration in CNS.
Satellite cells 	• encompass the cell bodies of dorsal root ganglia neurons (Scholz and Woolf 2007) • enhance the expression of glial fibrillary acidic protein (Scholz and Woolf 2007)
Schwann cells 	• support neuron regrowth (Amoh et al. 2005) • form myelin sheaths surrounding axons (Amoh et al. 2005) • provide regeneration of peripheral nerve (Frostick et al. 1998) • are important in axonal regeneration because they secrete neurotrophic factors (Sedaghati et al. 2011)

Fig. 2 **Cells in the nervous system**: Neurons and various types glial cells are found in the nervous system

biomedical applications. Recent studies published over the last few years clearly show that graphene and graphene based materials promote adhesion, proliferation and differentiation of various cells such as embryonic stem cells (ESC), neural stem cells (NSC), mesenchymal stem cells (MSC) and induced pluripotent stem cells (iPSC) and thus graphene based materials are known to be one of the promising nanoplatforms in regenerative medicine (Bressan et al. 2014).

Currently, there are various preclinical applications of graphene-based biomaterials showing bone tissues regeneration (Lee et al. 2011; Nayak et al. 2011), partial repair of muscle mass and loss of function (Kenry et al. 2018), usage in cardiac therapies (Park et al. 2014), regeneration of adipose tissue (Gomillion and Burg 2006). Due to the complexity of the anatomy and physiology of the nervous system compared to the other tissues, the repair and regeneration of injured and malfunctioning neural tissue via graphene based materials still need to be investigated further (Fraczek-Szczypta 2014).

5 Graphene, Neurons and Glia Cells

Recently, the use of graphene-based materials has been of great interest for the design of scaffolds that can promote neuron regeneration. With its unique topographic and chemical properties, graphene is a promising scaffold that could provide a bridge between regenerating nerves. More importantly, as a conductive substrate, graphene allow the continuation of electrical conduction between damaged nerve ends. The integration of supportive cells such as glial and neural precursor cells in such a scaffold can enhanec regeneration when compared to currently used neural autografts and nerve conduits.

Differentiation of stem cells to neurons or neuronal cells for neural regeneration is a critical step while developing stem cell based therapies. Several types of graphene scaffolds have been studied which supported significant stem cell differentiation.

Park et al. used a graphene scaffold as an inducer to differentiate human neural stem cells (NSCs) into neurons (Park et al. 2011). Wang et al. showed that human MCS underwent more neuronal differentiation on fluorine-functional graphene sheets compared to pristine graphene material (Wang et al. 2012). Selective differentiation of stem cells to neurons or oligodendrocytes for regeneration of central nervous system is a highly preferred situation and Shah et al. showed that even in the absence of growth factors, NSCs can differentiate into oligodendrocytes by using only GO-coated cell culture surfaces (Shah et al. 2014).

One of the greatest advantages of using graphene in nerve tissue damage, which also makes it more preferred than other conventional biomaterials, is the ability to create functional neural network connectivity. For, example, when embryonic neural progenitor cells were cultured on three dimentional GO-layers, it was observed that there were not only neurons and glial cells differentiated on these layers, but also a neural network rich in dendrites, axons and synaptic connections (Serrano et al. 2014).

6 Graphene and Neural Tissue Injuries

Currently, there is no cure for spinal cord injury (SCI), however, various scientific studies have recently begun to investigate the potential use of stem cells for SCI. In these cases, stem cell therapies focus on the introduction of neurons and oligodendroglia cells at the injury site in order to create a good microenvironment for the regenerating cells (Salewski et al. 2010). In this regard, mesenchymal stem cells, Schwan cells, glia cells and neurons from olfactory mucosa, neural stem cell and progenitor cells and embryonic stem cells have been used (Schroeder et al. 2016).

In spinal cord injury, there are two potential approaches in which stem cells are used: transplantation of stem cells into the injury site or use of nerural precursor cells at the damaged spinal cord. Transplantation of stem cells is a risky task and

requires a precise surgical procedure, and there is a possiblity that the immune system may reject new cells (in cases of embryonic and neural stem cell use). The enhancement of the treatment potential with the help of biomaterials such as graphene has been suggested in various studies. According to these, when graphene-based materials are applied together with stem cells or neural precursor cell, they induce the differentiation towards neurons, oligodendroglia and astrocytes (Barnabé-Heider and Frisén 2008; Nayak et al. 2011; Park et al. 2011; Wang et al. 2012).

In addition to the two dimentional stem cell culture system, there are studies involving three dimentional graphene based materials which better mimic the in vivo microenvironment. Li et al. has shown that they can achieve proliferation and differentiation of neural stem cells using a 3D graphene scaffold (Li et al. 2013; Nayak et al. 2011). In another study, 3D graphene oxide scaffolds were tested in spinal cord injury-induced rats, and an increase in tissue repair was observed (López-Dolado et al. 2015). However, in this study even though no local or systemic toxicity was observed, the use of diisocyanate-containing cross-linkers which was involved during the long-term production processes graphene, increases the toxicity risks of this application. In addition, the researchers have only investigated one type of graphene and the effects of different surface chemistry and derivatives with topography have not been investigated and the mechanism of tissue repair has not been explained.

7 Graphene and Stem Cells

With the purpose of neural tissue regeneration, graphene based materials have been tested with various cell sources and mostly with neural stem/progenitor cells and other stem cells including mesenchymal, embryonic or induced pluripotent stem cells. Various graphene based materials have been combined not only with polymers or differentiation factors but also under electrical or magnetic field. This review will discuss the details of studies performed with neural stem cells, mesenchymal stem cells or pluripotent stem cells.

7.1 Neural Stem Cells

Neural stem cells are multipotent in origin and they are present in the adult central nervous system. They can renew themselves and give rise to new neurons and supporting neuronal cells. Activation of NSCs or their transplantation into areas of central nervous system injury can lead to regeneration in animal models (Barkho and Zhao 2011). Various graphene based materials and hybrid systems have been studied with neural stem cells (Table 1). In 2011, Park et al. generated laminin coated graphene substrates and following NSC seeding, authors observed enhanced neural differentiation (Park et al. 2011). Laminin coatings have been combined with graphene based materials in various studies. It has been suggested that it improves cell seeding and survival on the biomaterial substrates. In addition to extracellular proteins such as laminin, researchers have included differentiation factors such as b-FGF, EGF, PDGF or NGF to improve the differentiation protocol (Park et al. 2011; Solanki et al. 2013).

In hybrid systems, graphene has been combined with nanofibers or other nanoparticles. For examples, graphene and Silica based nanoparticle hybrid structures successfully aligned axons, the differentiation and growth of adult hNSCs (Solanki et al. 2013). In another study, titanium oxide combined reduced GO (rGO) substrates were produced and their flash photo stimulation resulted in a $\sim$ 23-fold increase in the neural to glial cell ratio (Akhavan and Ghaderi 2013).

Another study used GO sheets coated with PCL nanofibers which were generated by electrospinning. When NSCs were seeded on these hybrid srufaces oligodendrocyte differentiation was improved (Shah et al. 2014). Weaver et al. showed that GO-poly (3,4-ethylenedioxythiophene) nanocomposite films can also induce oligondendrocyte differentiation (Weaver and Cui 2015). In another microfiber study, nanostructured rGO poly-D-lysine microfibers were demonstrated to be more successful in adhesion and proliferation of NSCs than 2D graphene film and tissue culture plate (Guo et al. 2017).

Table 1 Graphene based hybrid systems have been studied with neural stem cells

Types of graphene	Additional materials or factors	Main findings	Reference
Graphene substrates	+ Laminin + b-FGF + EGF	Long term culturing of hNSCs on laminin coated graphene films enhanced neural differentiation	Park et al. (2011)
GO nanosheets	+ ECM protein patterning + 300 nm SiNPs + Laminin + b-FGF + EGF	The graphene-nanoparticle hybrid structures successfully aligned axons, the differentiation and growth of adult hNSCs	Solanki et al. (2013)
3D graphene foams	+ EGF + FGF-2	Foams improved proliferation of NSCs and enhanced the NSCs differentiation towards astrocytes and especially neurons	Li et al. (2013)
rGO	+ rGO/TiO2 heterojunction film + Flash photo stimulator	Flash photo stimulation of human neural stem cells on graphene/TiO2 heterojunction resulted in a ~ 23-fold increase in the neural to glial cell ratio	Akhavan and Ghaderi (2013)
2D- and 3D-graphene materials	+ LPS + TCPS + EGF + FGF-2	3D graphene causes less neuroinflammation in microglia than 2D graphene For neural repair and neurogenesis, the studies on the 3D graphene topic should be increased	Song et al. (2014)
GO	+ PCL nanofibers	NSCs were seeded on GO sheets coated with electrospunned PCL nanofibers GO-nanofiber hybrid scaffolds showed enhanced differentiation into oligodendrocyte lineage cells	Shah et al. (2014)
GO/PEDOT nanocomposite films	+ PEDOT + IFNγ + PDGF	PDGF and IFNγ supported neuronal and oligodendrocyte lineage differentiation GO/PEDOT can be customized to develop therapeutic potential	Weaver and Cui (2015)
Nanostructured rGO microfibers	+ poly-D-lysine + EGF + b-FGF	Nanostructured rGO microfibers were demonstrated to be more successful in adhesion and proliferation of NSCs than 2D graphene film and tissue culture plate	Guo et al. (2017)
3D printted graphene scaffolds	+ PU + 3D printing	Graphene–polyurethane composite hydrogel enhanced the oxygen metabolism and neural differentiation of NSCs	Huang et al. (2017)
3D Graphene scaffolds	+ monophasic current stimulation + Neurobasal media	iPSC-derived hNPCs were used Electrical stimulation to enhance NPCs neurogenesis was promising and suggested further investigation of the therapy	Nguyen et al. (2018)

2D two dimensional, *3D* three dimensional, *b-FGF* basic fibroblast-growth factor, *ECM* extracellular matrix, *EGF* epidermal growth factor, *FGF-2* fibroblast growth factor 2, *GO* graphene oxide, *hNPCs* human neural progenitor cells, *hNSCs* human neural stem cells, *IFNγ* interferon-γ, *iPSCs* induced pluripotent stem cell, *LPS* lipopolysaccharide, *NSCs* neural stem cells, *NPCs* neural progenitor cells, *PCL* polycaprolactone, *PDGF* platelet-derived growth factor, *PEDOT* poly (3,4-ethylenedioxythiophene), *PU* polyurethane, *rGO* reduced graphene oxide, *siNPs* silica nanoparticles, *TCPS* tissue culture polystyrene, TiO_2 titanium dioxide

In addition to the above reports with 2-dimentional (2D) graphene based materials, there are also studies performed in 3-dimention (3D). One of the first studies showed that, in the presence of FGF-2 and EGF, 3D graphene foams improved the proliferation of NSC and enhanced their differentiation towards astrocytes and especially neurons (Li et al. 2013). In another comparison study between 2D and 3D graphene based materials, Song et al. suggested that 3D graphene causes less neuroinflammation in microglia than 2D graphene (Song et al. 2014). Furthermore, monophasic electrical stimulation was also shown to enhance NPCs neurogenesis on 3D graphene scaffolds (Nguyen et al. 2018). Recently, 3D printing technologies have been also combined with graphene based substrates and Huang et al. reported that 3D printed graphene–polyurethane composite hydrogel enhanced the oxygen metabolism and neural differentiation of NSC (Huang et al. 2017).

7.2 Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) which are having multipotent differentiation potential, can be obtained from bone marrow or fat tissue. MSCs are currently being investigated preclinically for the treatment of various diseases and are being tested in clinical trials (Ullah et al. 2015). Similar to NSCs, MSCs have been also investigated with different graphene based materials in order to test their efficacies for neural tissue regeneration (Table 2). When GO was combined with porcine acellular dermal matrix, human adipose derived mesenchymal stem cells (hADMSCs) showed differentiation to ectodermal cells, especially neurons in the presence of differentiation factors (Kim et al. 2015b). On the other hand, there were studies which reported enhanced differentiation even in the presence of such factors. For examples, cell alignment using printed PDMS channel arrays on fluorinated graphene enhanced the neuro-induction of hMSCs in the absence of growth or differentiation factors (Wang et al. 2012).

Similar to NSC studies, 3D printing was also combined with MSCs. In 2015, when MSCs were incubated on 3D printed graphene polylactide-co-glycolide scaffolds, in vitro studies showed neurogenic differentiation with significant upregulation of glial and neuronal genes in the absence of differentiation factors. In vivo studies suggested that 3D printed scaffolds showed promising biocompatibility over the course of at least 30 days (Jakus et al. 2015). In another recent study, 1 step- and 2- step growth graphene were shown to be effective at neuronal differentiation of bone marrow-derived human mesenchymal stem cells (Lee et al. 2018)

Electrical stimulation also improves the MSCs differenation on graphene based substrates. For examples, Lee et al. reported that extremely low frequency electromagnetic fields exposure synergistically increased biological efficacy of neuronal differentiation of hMSCs grown on graphene-coated substrates (Lee et al. 2015). Later, in 2016, electric pulses generated by the triboelectric nanogenerator were shown to induce neural differentiation of MSCs when cells were grown on rGO- Poly (3,4-ethylenedioxythiophene) hybrid microfibers (Guo et al. 2016b).

7.3 Pluripotent Stem Cells

Pluripotent stem cells are classified in two categories, embryonic and induced pluripotent. Embryonic stem cells (ESCs) are derived from the inner cell mass of a blastocyst whereas induced pluripotent stem cells (iPSCs) are generated from somatic cells following forced expression of reprogramming factors (Chin et al. 2009; Takahashi and Yamanaka 2006; Thomson et al. 1998). In one of the first studies involving graphene and pluripotent stem cells (Table 3), mouse iPSCs were cultured on both graphene or GO surfaces spontaneously differentiated into ectodermal and mesodermal lineages, and authors reported that these materials could be a promising strategy to use in differentiation protocols in pluripotent stem cell cultures (Chen et al. 2012).

Table 2 Graphene based hybrid systems have been studied with mesenchymal stem cells

Types of graphene	Additional materials or factors	Main findings	Reference
GO	+ PDMS arrays	Cell alignment using printed PDMS channel arrays on fluorinated graphene enhanced the neuro-induction of hMSCs even in the absence of growth or differentiation factors	Wang et al. (2012)
NGO (100 nm)	+ 3D PADM + b-FGF + BDNF + NGF	hADMSCs were differentiated to ectodermal cells (neuron) on NGO grid patterns	Kim et al. (2015b)
3D printable graphene	+ polylactide-co-glycolide	In vitro studies showed neurogenic differentiation with significant upregulation of glial and neuronal genes in the absence of differentiation factors In vivo studies suggested that 3D printed scaffolds showed promising biocompatibility over the course of at least 30 days	Jakus et al. (2015)
Graphene substrate	+ ELF-EMF + Hydrocortisone + Forskolin + Valproic acid + Insulin	ELF-EMF exposure synergistically increased biological efficacy of neuronal differentiation of hMSCs grown on graphene-coated substrate	Lee et al. (2015)
Graphene monolayers	+ 3D spheroid cultures of hMSCs	Graphene monolayers regulated the interactions at cell-substrate or cell–cell interfaces, consequently promoting the neurogenesis of hMSCs as well as the outgrowth of neurites	Kim et al. (2015a)
rGO microfibers rGO-PEDOT hybrid microfibers	+ PEDOT + Electrical stimulation + b-FGF	By inducing electric pulses generated by the triboelectric. Nanogenerator, neural differentiation of MSCs was dramatically improved	Guo et al. (2016b)
rGO nanosheets	+ PADM scaffold	rGO-assembled PADM scaffold enhanced the differentiation of MSCs into neuronal cells 7 days after seeding	Guo et al. (2016a)
1 step-G and 2 step-G Graphene	+ 35-mm-thick Cu foils	1 step- and 2- step growth graphene were effective at neuronal differentiation of bone marrow-derived human mesenchymal stem cells	Lee et al. (2018)

1 step-G one-step growth, *2 step-G* two-step growth, *3D* three dimensional, *ADSCs* adipose derived stem cells, *BDNF* brain-derived neurotrophic factor, *b-FGF* basic fibroblast growth factor, *Cu* copper, *ELF-EMF* extremely low frequency electromagnetic fields, *GO* graphene oxide, *hADMSCs* human adipose-derived mesenchymal stem cells, *hMSCs* human mesenchymal stem cells, *MSCs* mesenchymal stem cells, *NGF* nerve growth factor, *NGO* nanosized graphene oxide, *PADM* porcine acellular dermal matrix, *PDMS* polydimethylsiloxane, *PEDOT* poly (3,4-ethylenedioxythiophene), *rGO* reduced graphene oxide

Later in 2012, when mouse embryonic stem cells were seeded on graphene or graphene oxide surfaces under stromal cell-derived inducing activity, only GO effectively promoted mouse embryonic stem cell differentiation towards dopamine neurons compared to only graphene treated cells or negative control groups (Yang et al. 2014). In a recent study, transgenic mouse embryos were used to derive substantia nigra dopaminergic cells which were then cultured on graphene flakes containing nanocrystalline glass-like carbon films. The results demonstrated neuronal capability and there was a direct relationship between the thickness of the films and cell maturation (Rodriguez-Losada et al. 2017).

Table 3 Graphene based hybrid systems have been studied with pluripotent stem cells

Types of graphene	Additional materials or factors	Main findings	Reference
Graphene and graphene oxide substrates	+LIF	Mouse iPSCs cultured on both G and GO surfaces spontaneously differentiated into ectodermal and mesodermal lineages	Chen et al. (2012)
Graphene and graphene oxide	+SDIA	Only GO effectively promoted mouse embryonic stem cell differentiation towards dopamine neurons compared to graphene treated cells or control group	Yang et al. (2014)
Graphene flakes	+ NGLC composed of curved graphene flakes joined by an amorphous carbon matrix	Substantia nigra dopaminergic cells were derived from transgenic mouse embryos Culturing on NGLC demonstrated neuronal capability to a certain extent There was a direct relationship between the thickness of the films and cell maturation	Rodriguez-Losada et al. (2017)

ESCs embryonic stem cells, *G* graphene, *GO* graphene oxide, *iPSCs* induced pluripotent stem cells, *LIF* leukemia inhibitory factor, *NGLC* nanocrystalline glass-like carbon film, *SDIA* stromal cell-derived inducing activity

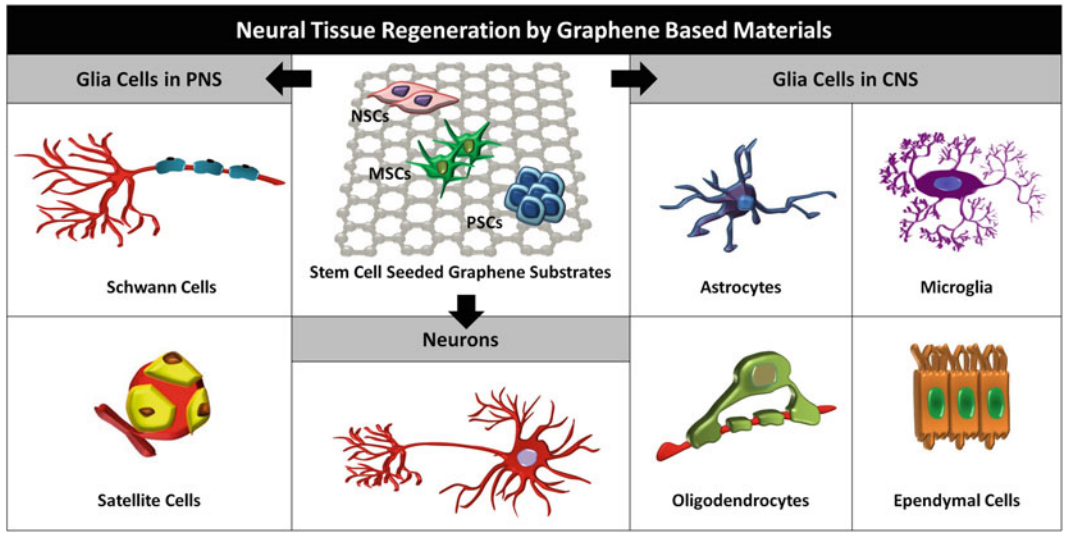


Fig. 3 Neural tissue regeneration by graphene based materials: Seeding neural stem cells, mesenchymal stem cells or pluripotent stem cells on graphene based scaffolds results in the differentiation towards neurons and glial cells

8 Conclusion an Future Perspectives

Graphene has a tremendous interface and a very conductive path for the conduction of electricity (Chen et al. 2011). Neurons in this count are electro-active and electrical stimulation can affect the behavior of stem cells (Chang et al. 2011; Ghasemi-Mobarakeh et al. 2011). For this reason, graphene has been used in various neural tissue regeneration studies which are summarized in Fig. 3. With the development of graphene derivatives, more specific needs for injury repair can be met. Most of the studies have examined the differentiation potential of Schwann cells, oligondendrocytes or neurons, however more detailed experiments are needed to understand the effect of graphene based materials on differentiation of other glial cells such as astrocytes, microglia or ependymal cells, since a healthy microenvironment would be needed for efficient neural tissue repair.

In addition to the development of novel graphene derivatives, 3D printing technologies are getting improved. As seen from the above Tables, there are already reports in literature which involves printing of graphene with additional polymers and growth factors. Most of the time, following the production of these 3D printed scaffolds, cells are seeded on their surface to evaluate differentiation potential. With the help of advanced bioprinting technologies, researchers will be able to print cells and materials together which can anatomically and histologically mimic the healthy neural tissue.

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