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Forum

Graphene in Regenerative Medicine: Focus on Stem Cells and Neuronal Differentiation

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Emerging graphene-based materials offer numerous opportunities to design novel scaffolds for neural tissue engineering. Graphene is a promising candidate due to its superior topographical, chemical, and electrical cues compared with conventional biomaterials. Here we examine the state of the art in graphene-based materials science for the neurodifferentiation of stem cells.

Graphene as a Cell Culture Substrate

Graphene is a novel material that is a rapidly rising star on the horizon of biomaterials science with emerging potential in regenerative medicine. Graphene denotes a flat monolayer of carbon atoms tightly packed into a 2D honeycomb lattice and is a basic building block for graphitic materials of all other dimensionalities. Due to its unique structure and geometry, graphene possesses remarkable physical–chemical properties, including a high fracture strength, excellent electrical and thermal conductivity, fast mobility of charge carriers, large specific surface area, and good biocompatibility. These properties offer an excellent capability to immobilise a large number of substances, including metals, drugs, biomolecules, fluorescent probes, and cells [1]. Therefore, it is unsurprising that graphene has generated great interest in nanomedicine and biomedical applications, as confirmed by the increasing number of publications. Exciting literature reports published over the past few years have clearly indicated that graphene and its related substrates are excellent nanoplatforms for promoting the adhesion, proliferation, and differentiation of various cells such as embryonic stem cells (ESCs), neural stem cells (NSCs), mesenchymal stem cells (MSCs), and induced pluripotent stem cells (iPSCs) [2]. So, the graphene 'gold rush' has begun. Nevertheless, although most of these literature reports describe *in vitro* studies of specific cells, future *in vivo* investigation will ultimately lead to graphene's utilisation as an implantable tissue engineering material.

Due to their limited intrinsic regenerative capabilities, there is considerable interest in the use of stem cells for the treatment of the injured or diseased nervous system (Box 1). Although it is possible to obtain stem cells from nerve biopsies, their access is limited and these cells have a poor capacity to expand *in vitro*. An alternative source of cells is stem cells recovered from non-neural adult tissues, which can be differentiated into a neurological phenotype. A major challenge in utilising stem cells for regenerative therapies is the poor control over the survival, differentiation, and functional integration of the transplanted cells. In this view, a promising opportunity in therapies for neural regeneration may result from the use of graphene-based substrates, which seem able to drive not only the differentiation of stem cells into neural cells but also the formation of functional neural networks.

Stem Cell Differentiation and Neural Networks on Graphene-Based Scaffolds

Promoting stem cell differentiation into neurons is a critical challenge in therapies for neural regeneration. Park *et al.* [3] tested a graphene scaffold as an inducer of human NSC differentiation into neurons. The most remarkable data confirmed that a greater degree of differentiation rate into neurons occurred on a graphene substrate, whereas more

Box 1. Organisation and Disorders of the Nervous System

The nervous system is anatomically and physiologically organised in a complex architecture that comprises the CNS and the PNS. The CNS receives information from and sends information to the PNS. The main components of the CNS are the brain and spinal cord, but it also comprises the optic, olfactory, and auditory systems; the PNS is a collection of nerves, sensory receptors, and ganglia outside the CNS [12].

Neurons and neuroglia are the two main cell categories in the nervous system. Neurons are the central components of the brain and spinal cord of the CNS and of the ganglia of the PNS. Morphologically, these cells comprise soma, axons, and dendrites. Neurons are the information-processing cells of the nervous system and can connect to each other to form neural networks. Neuroglia are the supporting cells; they are more abundant than neurons and able to divide. Neuroglia refer to astrocytes, oligodendrocytes, and microglia in the CNS and Schwann cells in the PNS. Oligodendrocytes and Schwann cells synthesise the myelin sheath, which surrounds axons facilitating rapid impulse conduction. Unlike oligodendrocytes, Schwann cells produce neurotrophins and extracellular matrix (ECM) molecules, which promote nerve regeneration. Astrocytes play important roles in CNS homeostasis and are a major source of ECM proteins and adhesion molecules in the CNS. Microglial cells are considered the macrophages of the CNS and function as the resident representatives of the immune system in the brain. Besides neurons and neuroglia, the nervous system contains NSCs able to self-renew and differentiate into neurons, astrocytes, and oligodendrocytes.

The most common injuries affecting the nervous system are spinal cord injury (SCI) and traumatic brain injury (TBI) in the CNS and PNI in the PNS. Other frequent neurological disorders include Parkinson's and Alzheimer's diseases and epilepsy. The common feature of all of these disorders is the loss of neurons or additionally the loss of supporting neural cells. Although the CNS and PNS have a very limited regenerative capability, responses to injury or disease vary greatly between the two systems due to their different intrinsic physiological properties. The absence of Schwann cells together with the formation of an impermeable glial scar in the CNS leads to a more difficult repair process compared with the PNS, where the natural regeneration of nerve transection injuries can succeed well if the gap is of small dimensions.

glial cells than neurons were found on a glass surface. In an attempt to significantly influence stem cell differentiation, several configurations of graphene scaffolds were studied. For instance, Wang *et al.* [4] tested human MSCs on fluorinated sheets of graphene, observing an enhancement of neuronal differentiation compared with cells seeded on graphene. The authors hypothesised a neuroinductive effect of the fluorinated graphene scaffold through spontaneous cell polarisation. Yang *et al.* [5] compared the effects of carbon nanotubes, graphene oxide (GO), and graphene nanoparticles on the dopamine neural differentiation of mouse ESCs. They demonstrated that only GO nanoparticles could promote the differentiation of ESCs into dopamine neurons, whereas carbon nanotubes and graphene nanoparticles did not show any important promotion of dopamine neural differentiation.

For central nervous system (CNS) regeneration, the selective differentiation of stem cells into either neurons or oligodendrocytes is highly desirable. Shah *et al.* [6] developed a graphene-based nanomaterial for the design of hybrid nanofibrous scaffolds to guide rat NSC differentiation into oligodendrocytes. The authors demonstrated that GO is an effective coating material in combination with electrospun nanofibers for the selective differentiation of NSCs into oligodendrocytes, even in the absence of inductive factors in the culture medium. They found that the GO coating promoted the overexpression of several integrin-related signalling molecules that are known to induce oligodendrocyte survival, differentiation, and myelination. The behaviour of rat NSCs on a composite scaffolding material based on GO nanosheets has recently been investigated by Weaver and Cui [7]. The authors demonstrated that the functionalisation of the free carboxylic acid groups of GO with different biomolecules was able to preferentially promote either neuronal or oligodendrocyte lineage differentiation.

Another intriguing characteristic of graphene is its capability to form a functional neural network. In a study by Tang *et al.* [8], neurospheres derived from NSCs were seeded on graphene substrates and after 14 days of culture the process of network formation was visualised by beta-tubulin immunostaining. Serrano *et al.* [9] used 3D GO-based scaffolds for seeding embryonic neural progenitor cells and observed the presence of both neurons and glial cells on these scaffolds and the formation of interconnected neural networks rich in dendrites, axons, and synaptic connections. Also, Li *et al.* [10] discovered the great potential of using graphene for neural interfacing, as it could promote neurite sprouting and outgrowth in primary culture of hippocampal neurons, enhance neural performance in networks differentiated by NSCs, and be used as an electric field stimulator for effective cerebral blood volume enhancement. Novel applications of graphene as a neural interface material seem to be encouraged by a recent study of Song *et al.* [11], who established anti-inflammatory effects of 3D graphene foams cultured with murine microglial cells – the macrophages of the brain and spinal cord that are first activated by damage caused by nanomaterials. The pioneering work described here demonstrates the potential of graphene for applications in tissue engineering of the CNS.

Regarding the peripheral nervous system (PNS), peripheral nerve injury (PNI) represents a clinically relevant problem. Patients with milder-severity nerve injuries improve spontaneously as peripheral neurons spontaneously sprout new axons after injury. When large nerve defects occur, implantation of a nerve graft is necessary to bridge the gap. Nerve autografts still represent the gold standard, but this approach is associated with several clinical complications. Allografts and xenografts are valid alternatives; nevertheless, systemic immune rejection remains a major concern. Tissue-engineering researchers have been attempting to contribute to the scientific and medical communities by developing synthetic grafts for the treatment of PNI. However, the optimal nerve graft remains to be found. Studies of animal trials showed that the combination of nerve conduits with stem cell technologies could be the best choice to obtain the maximum functional recovery. In this view, the excellent electrical properties of graphene could represent a promising advantage over other materials. At the time of writing, there is no published trial on the use of graphene for directing nerve electrical stimulation after PNI.

Future Outlook: Graphene-Based Biomedical Devices

The development of biomaterials that can improve neuronal growth after nerve lesions in both the CNS and the PNS is expected to have a big impact on future neuroregenerative therapies. The global nerve repair and regeneration market was worth US$4.1 billion in 2014 and is expected to increase at a compounded annual growth rate of 11.5% in the next 5 years. Several factors, such as the rapidly aging population and the growing number of nerve injuries and associated diseases, drive the growth of this market.

Recently there has been great interest in the use of graphene-based materials for the design of scaffolds that could promote neuroregeneration. Thanks to the aforementioned unique properties, graphene may represent a promising scaffold to bridge nerve defects favouring nerve regeneration. The integration of glial and nerve precursor cells within such a scaffold could improve regeneration compared with the nerve autografts and artificial nerve tubes currently in use. Most importantly, as a conductive substrate graphene may provide cues to the developing cells that reinforce their electrical connections, thus opening a completely new scenario for neural tissue engineering and regeneration applications.

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