Graphene based nanomaterials for neuro-engineering: Recent advances and future prospective

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Abstract

Graphene unique physicochemical properties made it prominent among other allotropic forms of carbon, in many areas of research and technological applications. Interestingly, in recent years, many studies exploited the use of graphene family nanomaterials (GNMs) for biomedical applications such as drug delivery, diagnostics, bio-imaging and tissue engineering research. GNMs have been successfully used to the design of scaffolds for controlled induction of cell differentiation and tissue regeneration. Critically, it is important to identify the more appropriate nano/bio material interface sustaining cells differentiation and tissue regeneration enhancement. Specifically, we focus this review on graphene-based scaffolds that endow physiochemical and biological properties suitable for a specific tissue, the nervous system, that links tightly morphological and electrical properties. We review different strategies to exploit GNMs for neuronal engineering and regeneration, material toxicity and biocompatibility. Specifically, we present the potentiality for neuronal stem cells differentiation and subsequent neuronal network growth as well as the impact of electrical stimulation through GNM on cells. The use of field effect transistor (FET) based on graphene for neuronal regeneration is described. This review concludes the important aspects to be controlled to make

graphene a promising candidate for further advanced application in neuronal tissue engineering and biomedical use.

1. Introduction

Graphene (GR) is a two-dimension (2D) material discovered in 2004, and constituted by coplanar sp^2 hybridized carbon atoms [1]. GR extraordinary physicochemical properties, quickly let it rise to a prominent position among the others allotropic forms of carbon, such as fullerene, carbon nanotubes (CNTs) and diamond (Figure 1) [2,3], in many research fields and technological applications [4–6]. GR was at first isolated from graphite, a three-dimensional (3D) laminar material composed of many stacked GR foils, by repeated mechanical exfoliation [7] Single-layer GR, bi-layer GR, multilayer GR, GR oxide (GO), reduced GR oxide (rGO) and chemically modified GR are the principal members of the graphene family nanomaterials (GFNs) [8]. Each member of this family possesses its properties, in terms of the number of layers, lateral dimension, defect density, oxygen content and overall chemical composition [5]. Single layer defect free GR production is quite challenging, due to its highly reactive surface and to the difficulty to suspend in water. For this reason, GO and rGO are the most preferred materials used for biological applications [5].

The properties of GFNs make them gaining more and more interest in different fields of science and technology, including physics, chemistry, material science, environmental sciences, biology, medicine and bioengineering [9,10]. This aspect is further confirmed by the exponentially increasing of published papers every year, with more than 30,000 publications in the last decade (Figure 2a). Moreover, due to their biocompatibility associated with mechanical flexibility, transparency and thermo-electrical conductivity, a large number of studies exploited the use of pristine and functionalized GR and GO for biomedical applications such as drug delivery, diagnostics, bioimaging and stem cell research (Figure 3) [11–17]. For example, at the central nervous system level, GFNs were functionalized as cell labelling and real-time live-cell monitoring [19,20]; and for the delivery of drug molecules to the brain effectively transposing the Blood Brain Barrier (BBB) [21,22]

The properties of GFNs are of particular interest for biomedical applications in neurology, for neuronal implants or bio-devices, with potential applications that range from neuro-oncology to neuro-regeneration [5,15,18] and also where conductive materials may promote electrical and chemical communication within the nervous system.. interfacing GR with neuronal cells was also proposed to be extremely advantageous for exploring their electrical behavior or facilitating neuronal regeneration [16,23,24]. Furthermore, they possess the potential to overcome the limitations of metal and silicon-based implantable devices, characterized by high stiffness, high inflammatory potential and poor long-term stability and in living physiological environment of the nervous system [25,26].

In this review, we focus on the different strategies exploiting GFNs for neuronal engineering and regeneration scaffolds and platforms. Specifically, in the framework of neuronal tissues, we overviewed material toxicity and biocompatibility, together with the potentiality of GFNs to promote stem cells differentiation, neuronal network growth, and neuronal tissue stimulation taking advantage of their high electrical conductivity.

2. Challenges in Neuronal Tissue Engineering.

The brain is characterized by a complex 3D network organization allowing communication with all other sites of the human body. The ability to understand the mechanisms regulating a 3D neuronal network model is challenging. How the brain elaborates its signals, how it propagates them along nerves and how this translates into actions are mainly unresolved questions. Such comprehension could help, in the future, to develop artificial brain models for pathological or traumatic neuronal diseases studies [30–32]. Neuronal tissue engineering is a multi-disciplinary research field that combines neuroscience and bioengineering to develop biomimetic tissue constructs for CNS/PNS regeneration, as well as for diagnostic and therapeutic research [33–36]. In this framework, biomaterials are crucial components in all tissue engineering fields as they can induce specific cellular functions, direct cell differentiation, and modulate cell-cell interactions [37–43]

Neurons, the brain basic cellular unit are composed of a a cell body, dendrites and axons,. Dendrites carry and compute the signals received from the surrounding neuronal network while the axon generates the outgoing signals, i.e. the action potentials. Assembles of axons of motor or sensory neurons are called nerves, which serve as nervous system communication paths [27]. Upon nerve injury, signal transmission between different areas of the central nervous system (CNS) or to/ from the peripheral nervous system (PNS) is compromised, limiting the functionality of these complex organs. . More than 50,000 people each year suffer from traumatic CNS or PNS injuries resulting in behavioral inabilities compromising the patient quality of life [28,29]. Unfortunately, to restore or regenerate damaged axons is a daunting task, due to the extreme complexity of the neuronal structure and the limited self-repair ability in the adult nervous system. The ability to enhance repair and regenerate neurons is thus a significant challenge in modern neuroscience and neuro-engineering. Nowadays, this challenge is being addressed through the lens of material sciences, as the regeneration is highly dependent on the extracellular environment and neuronal interactions,

However, different tissues in the body possess different mechanical and physiological properties, a single material might not mimic the physical and biological properties of all the native tissues. Therefore, an ad-hoc selection (or design) of the appropriate material (or of its components) has to be engineer in order to properly fit the requirements of every specific tissue. In this framework, the extraordinary mechanical and electronic properties of GFNs [14] have induced researchers to investigate their use in tissue engineering and regenerative medicine [44]. In particular, GR ability to be combined with a variety of other bioactive structures opens to novel and original approaches to design materials for neuro-engineering applications. Although GR-based materials have been widely utilized to fabricate films [45,46] or 3D scaffolds [47,48] able to sustain neuronal development and nerve fibers regrowth, there are ongoing studies in order to extend the versatility and functionality of GR and its chemical derivatives for neuronal regenerative medicine. The positive role of GR and its derivatives has also been confirmed in electrical stimulation of neuronal cells for the growth, differentiation, and the development of neuronal lineage cells. Additionally, the tunable surface and machining properties of GFNs and their nanocomposites are suitable to fabricate neuronal tissue-like structures able to induce neuronal cells arrangement in a controlled way, suggesting their use for neuronal tissue engineering applications. These promising results are discussed in detail in the following sections.

2.1. Graphene Nanomaterials in Neuronal Tissue Engineering.

While neurons are the main functional units of the nervous systems, they cannot regenerate and are prone to permanent damage due to injury and disease [49]. Recently, neuronal tissue engineering showed great potential to help neuronal cells recover using platforms as 3D-scaffolds able to sustain or stimulate nerve regeneration. In particular, the possibility to engineer biocompatible and flexible materials incorporating therapeutic molecules paved the way for the development of platforms supporting cellular attachment and migration [49,50]. In this process, a key role is also played by nanotechnology. Apart to improve or tune surface and bulk nanomaterial properties for neuro-engineering applications, nanotechnology demonstrates its ability to offer alternative solutions in the development of scaffolds promoting neuronal regeneration [51,52]. Allowing neurons to reconstruct synaptic networks in appropriate space coordinates, and in the presence of homeostatic abilities expressed by neuroglia in 3D, may provide crucial insights into the integration of signals in health and disease [53]. This approach promoted the emergence of a new generation of culture models aimed at mimicking tissue complexity *in vitro*, in particular 3D neuronal arborization. Ideal properties of a scaffold are biocompatibility, controlled biodegradability with non-toxic degradation products, poor inflammatory responses, three-dimensional features with appropriate mechanical properties to mimic the extracellular matrix (ECM) [54], porosity allowing ongoing vascularization and cell migration. In this framework, the unique properties of GFNs, as well as the possibility to be easily manipulated, show great potential in mimic *in vivo* cues as the strategy for the treatment of neuronal injuries and diseases. It was shown that GFNs are suitable for the design of electroactive porous scaffolds that may be able to transmit the externally applied electrical signal to promote neuroregeneration [55] and, in this way, providing a unique environment for future neuroregenerative therapies [48]. These porous scaffolds have been shown to improve the differentiation of neuronal stem cells and functional neurons [56,57]. For example, Li et al. and collaborators were cultured neuronal stem cells on 3D-GR platforms, have shown the ability of these cells to grow within the scaffold and then differentiate in functional neurons, astrocytes and oligodendrocytes [58]. Serrano *et al.* studied the differentiation of embryonic neuronal progenitor cells using 3D porous GO scaffolds (Figure 2b), showing, after 2 weeks, a good cell differentiation towards neurons and glia [59]. Similarly, Jiang *et al*. demonstrated the ability of 3D-GR scaffolds to significantly increased the number and average size of neuro-spheres favoring neuronal stem cell migration [60]. Feng and collaborators prepared GR scaffolds with excellent physicochemical stability, biocompatibility, electrical conductivity and softness (Figure 4) [61], showing an accelerated growth and development of the primary motor neurons, in a long-term culture period (Figure 5) [61]. 3D-GR scaffolds can be obtained using nickel foam template for chemical vapor deposition of GR. Growing neuronal stem cells on these substrates allows not only a more physiological condition but also a substrate that can be electrically stimulated [56] [5]. Neuronal dissociated hippocampal cultures, grown on 3D-GR scaffolds were also able to reestablish the coexistence of local and global electrical activity, in the form of correlated electrical activity varying in space and time [62]. Furthermore, 3D-GR scaffolds possess the ability to impact a 3D neuronal circuit, boosting spontaneous network activity and tuning the excitation/inhibition ratio [47]. In a different strategy, Martìn *et al.* built hybrid hydrogels combining GR with polyacrylamide (PAM). This study demonstrates that GR improves the biocompatibility of 3D scaffold [10] promoting neuronal growth. Microglia cells were also cultured in 3D-GR substrates [63], but in this case, the 3D structure negatively affected the neuroinflammatory response, probably because of the spatial constraints. *In vivo*, 3D-GR scaffolds were implanted in injured rat spinal cord, showing no local or systemic toxic response and encouraging further investigation of these materials as promising platforms for CNS repair [16,64]. Exploiting their electrical conductivity, 3D-GR scaffolds were also used as stimulating electrodes, to promote neuronal growth and differentiation [16,56]. Overall, the use of GR materials as 3D neuronal implants is still limited, but we expect that using strategies improving the biocompatibility of the implants will lead to the development of functional 3D-GR platforms for nervous system applications.

2.2. Graphene neurocompatibility

The biocompatibility of a nanomaterial is crucial to its application in tissue engineering and subsequent exposure to organs, tissues and cells [65,66]. Among a large number of nanomaterials investigated worldwide, only a limited amount is suitable for biological applications. If we further think about the nanomaterials for neuroengineering applications, the number shrink, due to the complexity of the CNS. In this challenge, GFNs have made significant contribution due to its good biocompatibility, related to its chemical properties that allow strong and non-destructive interactions at the cellular level [16,49,67,68]. The biocompatibility of GFNs have been studied *in vitro* using different human cell cultures, such as fibroblasts [69], epithelial cells [70], oligodendroglia cells, fetal osteoblasts [71], red blood cells [72] as well as neuronal cells [73] and spinal cord slices [74].

GR films were shown to possess excellent biocompatibility promoting the growth of primary murine hippocampal cultures as well as neurite sprouting and outgrowth [75]. Fabbro *et al.* demonstrated that GR can preserve the basal physiological neuronal activity [76], maintaining neuronal passive properties, spontaneous synaptic activity, synaptogenesis and short-term plasticity. More recently, GR was reported to tune the extracellular ion distribution at the interface with hippocampal neurons, a key regulator of neuronal excitability. The ability to trap ions by GR is maximized when a single layer GR is deposited on substrates electrically insulated. These biophysical changes caused a significant shift in neuronal firing phenotypes and affected network activity [7]. Several other studies demonstrated the ability of GR based substrates to promote neurites sprouting and outgrowth [77], to enhance neuron electrical signaling [78] and to reduce the inflammatory response [63].

Similar results were obtained by Li *et al.* that observed good biocompatibility of GR films toward mouse neuronal cells (Figure 6a), confirming that GR could efficiently promote neuronal cells growth [58,79]. Moreover, they showed the ability of GR to increase neurite number and average length, boosting neurite sprouting and outgrowth, without affecting cell morphology [58]. In another study, Rastogi and collaborators investigated the viability of neuronal and non-neuronal cells grown on GR substrates, showing their ability to promote cell adhesion and cell proliferation of both cells types without any adverse side effect [80], encouraging the use of GR in biomedical applications.

However, the mechanisms of interaction of GFNs with neurons and astrocytes are still poorly investigated and unclear, depicting an undefined scenario mainly dependent on GR intrinsic characteristics, as well as the oxygen content, lateral size or the number of layers. For primary neuronal cultures, no changes in neuronal and glial cell viability were detected upon GR exposure, both *in vivo* and *in vitro* [32,81–84]. However, primary neuronal cultures exposed to GO nanosheets displayed evident alterations in several physiological pathways, such as calcium and lipid homeostasis, synaptic connectivity and plasticity [26,32]. Defterali *et al.* using thermally synthetized rGO, showed good neuronal and glial biocompatibility, as well as neuronal induction in neuronal stem cells. These pieces of evidence open to its use as scaffold for neuronal induction and growth in *in-vivo* experiments [85], or as a smart material to treat neurodegenerative diseases such as Parkinson's.

Not only the properties of GR can affect its biocompatibility, but also the method of preparation (Figure 6c) [86]. Compared to chemical methods, physical methods can produce GR with lower toxicity, higher quality and purity. Among different techniques, chemical vapor deposition (CVD) method is one of the widely used for preparing GR. Lee *et al.* investigated the cytocompatibility of GR monolayer grown through CVD, using human neuroblastoma (SH-SY5Y) cell culture. GR substrates were able to induce neurite outgrowth even in the absence of neurogenic factor suggesting the use of GR as a platform for neuronal regenerative medicine [87]. Meng and collaborators compared the biocompatibility of cortical neuronal cells of CVD-GR films with GR films prepared by spray coating, showing better biocompatibility for CVD-GR films, also promoting neurite outgrowth (Figure 6b) [86]. Furthermore, the higher conductive properties of CVD-GR have been used to electrically stimulate human neuronal stem cells and hence direct their differentiation toward a neuronal phenotype [88,89]. The combination of all these measurements holds the potential to use GR as a promising tool for neuronal implants and biomedical applications [86].

Since many applications of GR are as neuronal interface or substrate, the toxicity could also be reduced after coating with biopolymers improving the development of devices. GR, GFNs and its derivatives have physicochemical properties that facilitate the easy functionalization with different functional groups, for this reason, GFNs have been conjugated with several natural biopolymers, as functionalizing agents, for drug delivery applications. Weaver and Cui, for example, utilizing carboxylic acid, functionalized GO sheets by cross-linking with Interferon-gamma (IFNg) to stimulate neuronal stem cells differentiation towards neurons or oligodendrocytes [90]. The functionalization of GR has the ability to modify the charge on the surface and, in this way, ameliorating the material properties. Tu *et al*. functionalized the methoxy terminated groups of GO with amino, poly-m-aminobenzene sulfonic acid, resulting in a different surface charge. The resulting charged GO substrates were used to investigate the neurite outgrowth and branching for primary rat hippocampal neurons (Figure 7a) [24]. The study revealed that comparing the differently charged GO, the positively charged one was more beneficial for neurite outgrowth and branching. However, further investigation is needed to explore the possibility to extend the study to clinical studies [24]. Silica nanostructures are widely studied in different areas, including biomedical and tissue engineering. The advantage of silica is easy tuning the structure, shape and surface functionalization. Solanki and colleagues, demonstrated that GO functionalized with silica nanoparticles induces enhanced neuronal differentiation and axonal alignment in human nerve stem cells (Figure 7b-c) [91], compared to silica nanoparticles alone. Moreover, laminin functionalization of GO sheets resulted in a further improved attachment and growth of cells on GO, with a higher expression of neuronal markers (e.g. β-tubulin III, microtubule-associated protein 2 and synapsin) after 2 weeks. Hence, nanocomposites of GFNs with silica can be a promising nanomaterial in neuroengineering.

Natural biopolymers are biocompatible, biodegradable and have low immunogenicity that can minimize the toxic effects of GR [92] and also improve its physicochemical properties. Zhou *et al.* used electrospinning to prepare scaffolds made of polycaprolactone (PCL) with or without GR and coated with poly-L-lysine (PLL). The scaffold implanted into the striatum and subventricular zone, from 7 to 21 days, showed a decreased microglial intensity in GR-PLL coated scaffold compared to PCL-coated scaffold [93]. In another study, Shan *et al*. functionalized GO with PLL via conjugation of epoxy groups of GO with the amines of PLL, resulting in a more biocompatible composite that can be used in to load bioactive molecules or for the release of drugs [94].

An efficient method is covalently modifying GR or its derivatives with polymers, including polyethylene glycol (PEG), poly [2-(dimethylamino)-ethyl-methacrylate] (PDMAEMA), chitosan, pluronic F127 (PF 127), poly (N-isopropylacrylamide) (PNIPAM) [66,95]. For example, PEG was used to functionalize GO by conjugation of carboxylic acid groups with PEG amino groups resulting in nano-sized PEG-GO nanocomposites with good stability in a variety of physiological solutions [96]. Following this, many studies have been performed with functionalized PEG-GO for *in vitro* and *in vivo* biomedical applications [92,97,98]. Similarly, polyethyleneimine (PEI)-GO conjugates exhibited excellent ability to condense DNA/siRNA and were used for gene delivery [99]. This approach led to a series of studies exploring GFNs in drug delivery [92]. In this framework, Wen *et al.* developed a PEGylated-GO with redox-responsive detachable PEG shell using disulphide linkages (NGO-SS-mPEG) [100], which rapidly released encapsulated payload at tumor-relevant glutathione (GSH) levels.

Similarly, Yang *et al.* developed a conjugate using folic acid (FA) modified β-cyclodextrin (β-CD) linked to GO carrier [101]. *In vivo* biodistribution study in mice showed no appreciable toxicity by PEGylated GO over 3 months [92]. Recently, Xiao and collaborators (2016) used GR conjugated to a neuro-protective peptide and once injected intravenously in a murine model of Alzheimer disease, they were able to increase learning and memory, dendritic spines formation and decrease pro-inflammatory cytokine levels (Figure 8a) [26,102]. As described, GR is strongly explored as a novel platform for the local delivery of therapeutic molecules, and the results are encouraging. Functionalization of GR and GO can tailor their properties and strongly enhance their application as carriers of therapeutic molecules.

Among the different possible strategies to increase GR/GO biocompatibility and lower its toxicity, an important note should be given to modulate its lateral dimension. It does determine the maximum dimension of the material, which is relevant for cell uptake, renal clearance, BBB transport, and many other biological phenomena that depend on particle size [8]. Yue *et al.* investigated the toxicity of differently sized GO flakes on various cells, and results reveled that larger GO sizes are more toxic than nanosize ones [103]. This effect is in some way in contrast with what was observed in nanoparticles of different materials, which showed enhanced toxicity as particles' size is reduced [103]. This might be correlated with concentration: increasing the size of GR, indirectly increases the concentration of GR resulting in higher toxicity. Rauti *et al.* also attempted to investigate the effect of increased lateral size of GO nanosheets (with a lateral dimension in the few micrometer range) on cultured hippocampal cells. However, after 6-8 days of incubation, they measured a significant reduction in both neuron and glial cell densities indicating cell toxicity that prevented any further functional measurements [32]. In another study, Das and collaborators used GO and rGO with different size and investigated the cytotoxicity on neurovascular endothelial cells for 48 h. Results showed that rGO is less toxic than GO that, instead, gave rise to a large number of dead cells floating and less adhered cell. Importantly, smaller sheets (0.4 µm) demonstrated to be more toxic than larger ones (0.8 µm) [104]. Different researchers thoroughly studied size dependent toxicity. For example, Agarwal *et al,* demonstrated the cytotoxicity of rGO micron sheets on different cells such as neuroendocrine PC12, oligodendroglia cells and osteoblasts [71].

The *in vivo* biocompatibility and toxicity of GR nanomaterials after local/systemic administration also needs attention. Future emphasis on investigating the mechanisms of clearance and toxicity as well as tissue distribution is required to realize their true potential since knowledge of the *in vivo* behavior of different GR-based materials will eventually expand their biomedical applications. Zha *et al.* utilized GR and GO substrates to study *in vivo* toxicity, by implanting them for several months, into the subcutaneous tissue of rats. Blood biochemistry, hematological analysis, histological examination and behavioral test were used for analysis and interestingly no *in vivo* toxicity was observed [105]. In another study, Li and collaborators studied the *in vivo* toxicity of GO, using ^{125}I labelled GO with 10-800 nm size and 5 µg/kg concentration aqueous suspension intravenously injected to male mice at the tail vein. Toxicity was analyzed based on quantification of the radioactivity of ^{125}I indicating that 55.9, 10.0, 2.2% and <2.0% of radioactivity in liver, lung, spleen and other organs was observed after 10 min (Figure 8b-c) [106]. The high reduction in radioactivity was observed for PEG-GO as compared to GO pure after 10 min and 360 min. They concluded GO more toxic to liver, lung and spleen when administered intravenously [106]. However, many studies reported that *in vivo* toxicity of GFNs in liver, lung and spleen depends on the type of material, concentration and can be reduced by functionalizing it [5,107,108]. For example, it was shown that PEGylation of GO reduces the toxic effects in mice, and similarly, no toxicity was measured *in vivo* upon administration of GO as injectable hydrogels for tissue engineering [109]. Recently, PEGylated GFNs showed no uptake via oral administration, indicating limited intestinal absorption of the nanomaterial, with almost complete excretion.

Overall the studies reported up to now suggested that GR and its derivatives are characterized not only by outstanding biocompatibility but also by the ability to enhance cellular functionality, including cell growth, proliferation and differentiation [89,110,111]. However, the interactions of graphene with biological systems depend on many parameters, including their size, shape, surface functional group, and preparation method [5], thereby, research on the biomedical applications of GR nanomaterials is still needed.

2.3. Graphene substrates for neuronal interfaces

Neurological applications of GFNs represent a field in continuous exponential expansion. Traditional treatments of CNS disorders present different challenges, thus developing a tool able to improve neuronal regeneration is one of the main goals of modern neuroscience. As already mentioned, researchers have started exploring the use of graphene for neuronal cell cultures, to deliver molecules to the brain, or recreate 3D-architecture. Also, GR substrates can be explored as neuronal interfaces for facilitating neuronal regeneration [5,107,112], opening new avenues in neuro-therapeutics, including neuro-oncology, neuro-surgery and neuro-regeneration.

When the nerve gets damaged, it is important to push their regeneration, attempting to restore their full functionality. There is a number of nanomaterials already under investigation for nerve regeneration applications, anyhow the outstanding physicochemical and electrical properties of GFNs make it the best candidate among them. In recent years GFNs impact was boosted by the discovery of its ability to improve neuronal cell differentiation and growth. Yang *et al.* investigated the effect of GR and GO on differentiation of mouse embryonic stem cells, revealing that GO can enhance dopaminergic neuron differentiation enormously and further improve gene expression (Figure 9a-b), underlying the possibility to use GO as a promising material for cell transplantation therapy [113]. Sahni *et al.* demonstrated the ability of GR surfaces to improve mouse hippocampal cell culture as well as branching and regrowth of neuronal circuit [114]. Similarly, Akhavan and collaborators demonstrated that on GR nanogrids, neuronal stem cells attachment and proliferation was better than other materials (e.g., quartz), with elongated morphology and neurite outgrowth [115,116]. As already mentioned, Li *et al.* investigated the effect of GR on mouse hippocampal culture model and observed not only excellent biocompatibility of GR with increase cell viability but also a substantial enhancement of neurite sprouting, during the early phase.

Furthermore, improved expression of growth associate protein-43 (GAP-43) by GR, results in an increased neurite outgrowth, a sign of nervous system development [58]. In the same way, Tang *et al.* investigated the formation of neuronal network and its performance once grown on GR substrates, using stem cell cultures. Results revealed that GR improved neuronal growth, performance and electrical signaling through calcium imaging and electrophysiological recordings. Heo and collaborators investigated neuronal cell-to-cell interactions using GR/polyethylene terephthalate (PET) films. Cell viability and proliferation enhancement were observed for GR/PET film substrates compared to conventional culture dish [89]. These results suggest that GR can be an excellent material as a neuronal interface, improving neuronal stem cells adhesion and differentiation for long-time along with neuronal prosthetics which helps neuronal regenerative medicine [89] improving nerve regeneration or repair [78].

Wang *et al*. studied the differentiation of cells into neurons using GR substrates and the effect on retinoic acid, a crucial inductive agent in neuroengineering. Results revealed that cells interfaced to GR showed higher cell differentiation and increased presence of retinoic acid [117]. This investigation opened a new window to identify other chemical molecules that show good inductive effect due to the presence of GR.

The next advancement in GR-based neuronal interfaces was provided by using GR as a coating material. Results revealed that using GR and GO as coated substrates support cell adherence and proliferation and no difference in viability was observed when comparing GR/GO to other substrates such as Poly-dimethyl siloxane (PDMS), PET and glass slide [118]. Ryoo and co-authors reported that GFNs, including GO and rGO, can be immobilized onto glass substrates treated with 3-aminopropyltriethoxysilane (APTES) via electrostatic interactions [119], showing that the presence of GO and rGO not only supports cell adhesion, spreading and proliferation but also improves the gene transfection efficiency of cells when compared to uncoated glass substrates [111]. Furthermore, they found an improved differentiation of neuronal stem cells towards neurons than glial cells on GR-coated substrates compared to glass substrates (Figure 9c-e) [89]. In another study, Park *et al.*

found that the GR coated substrates increased the differentiation of human neuronal stem cells into neurons [111], enforcing the idea that GR coated substrates are highly cell-friendliness and that can be readily employed as surface coating materials in biomedical applications, such as implants, cell culture platforms and cell-interfacing systems [111]. In addition, Tang *et al.* cultured neuronal stem cells on GR-coated substrates and investigated the neuronal network activity, monitoring the intracellular spontaneous and synchronous calcium oscillations, showing that the neuronal cells were able to form functionally active neuronal networks [78].

In another scenario, the GR-patterned arrays have been spotlighted as a novel strategy for guiding and stimulating cellular behaviors, because GR can provide desirable topographical and biochemical guidance cues [111,116,120] Moreover, Zhang *et al*. found out that the width of GO-patterned arrays can directly affect cell migration, alignment, morphology and cell adhesion [121]. They showed in fact that the cytoskeleton contractility, intracellular traction and actin filament elongation were significantly enhanced when the width of the GO-patterned arrays was similar to the cell dimension. Kim *et al*. also revealed that the shape of GO-patterned arrays could determine cell morphology, migration distance, speed and directionality [122]. Therefore, GFNs patterned arrays fabricated with sophisticated control of structures and properties can provide unique opportunities for biomedical applications.

Recently, the possibility to precisely control the direction of neuronal growth gained more and more interest due to the key advantages in neuroengineering it could bring. Different nanomaterials, such as aligned magnetic nanoparticles patterned substrates, aligned fibers, hydrogels and other scaffolds were investigated to promote controlled neuronal growth. Lorenzoni *et al.* interfaced patterned substrates of CVD single layer GR (SLG) with primary embryonic hippocampal neurons showing highly aligned neuron adhesion and growth (Figure 10a-b) [123]. Wang and collaborators created micro-channels of fluorinated CVD-GR containing parallel lines of PDMS, demonstrating the ability of GR to improve cell adhesion and aligned growth, compared to PDMS alone (Figure 11) [117]. To note, they also found an improved expression of specific neuronal markers Tuj1 and MAP2 in cell cultured on fluorinated CVD-GR even in the absence of chemical inducers [117]. Yang *et al.* developed GO-patterned substrate composed of micro grooves/nanoridge to thoroughly understand the growth of human neuronal stem cells and other properties such as differentiation, elongation, extension and adhesion and comparing with substrates without GO (Figure 12a-d) [124]. Results showed that compared to other substrates, GO patterned substrates induced increased growth with elongated, aligned neurite extension and focal adhesion, highlighting the possibility to use them as an exciting platform in neuro-engineering or stem-cell therapy for neuronal diseases treatment [124].

2.4. Graphene-based interfaces for neuronal stimulation and field effect transistors.

As every class of neuron has its specific electrical behavior, hence it is essential to retain its electrical functionality after regeneration or repair. Electrically conductive scaffolds can be fabricated by the combination of conductive polymers and carbon-based materials such as carbon nanotubes (CNTs), graphite, and GR [6,17]. It has been demonstrated that GR-based substrates are not only biocompatible but also can improve neuronal cell growth. The investigation of the effect of GR on the electrical activity of neuronal networks has provided further outstanding surprises since GR films could be used as neuronal-stimulation electrodes [17,125].

Indeed, graphene possesses all the desirable properties for use in stimulation/recording electrodes: (1) GR-based electrodes have been successfully developed and do not seem to inflict tissue damage; (2) its high conductivity has the ability in lowering electrode impedance and increasing charge transfer; (3) it possesses exceptional flexibility and high electrochemical surface area, important parameters in neuronal stimulation [5]; and (4) GR electrodes produce slightly higher values of charge injection compared to common noble metal electrodes, such as Pt or Au [16,68].

Tang *et al.* investigated the neuronal response to electrical stimulation utilizing CVD-GR as substrate (Figure 13a-b) [78]. Similarly, Heo and collaborators, through non-contact electrical field stimulation, were able to control neuronal cell-to-cell interaction, growing SHSY-5Y human neuroblastoma cells on film composed of CVD GR/PET (Figure 13c-d) [126]. Results revealed that a week electrical field stimulation (4.5 mV/mm, 10 s pulse duration for 32 min) was highly effective in shaping cell-to-cell interactions of SHSY-5Y human neuroblastoma cells (Figure 14a-c) [126]. In another study, it has been shown as neuronal regeneration might be improved using highly conductive GR-nanofilm as neuronal substrates. Indeed, neuronal-like PC12 cells, grown on these devices showed enhanced neurite elongation, after being exposed to constant electrical stimulation frequency [60]. Yang *et al.* fabricated a conductive silk fibroin scaffold integrated with variable percentages of GR to improve the mechanical and electrical properties of the scaffold. The scaffold was used in *in vitro* analysis of rat bone mesenchymal stem cells (rBMSCs) and results highlighted the enhanced cells growth and expansion (Figure 14d) [127], as well as the potential use of this substrate to induce local electrical fields in cell cultures, biological interfaces and *in vivo* studies [127].

Hess *et al.* has achieved further progress in the detection of the electrical activity of electrogenic cells, using arrays of GR-based solution-gated field-effect transistors (G-SG-FETs) Figure 15a) [128]. They resolved and tracked the action potential of cardiomyocyte-like HL-1 cells across these transistor arrays (Figure 15c) [128]. The signal-to-noise ratio of G-SG-FETs was better than most of the known devices, and their large transconductive sensitivity make them promising devices for biomedical applications [128].

Electrogenic cells were interfaced with GR field-effect transistor (GR-FETs) and GR and nanowire field-effect transistors (NW-FETs). Interestingly, in GR-FET and (Silicon nanowires (SiNW)) SiNW-FETs, peak to peak signal-recording width increased with the area of the devices. It indicates that the signal is the average of different points of beating cell's outer membrane. Both devices showed different distinct and complementary capabilities. Thus, GR and NW-FETs represent important devices to explore further opportunities in the field of future bioelectronics for neuronal recording or stimulation [129].

Recently, Li *et al.* investigated the use of 3D-GR foams as scaffolds for cell electrical stimulation, further showing the ability of GR to significantly enhance electrical stimulation performance (Figure 15b) [56]. Similarly, Serrano *et al.*, through biocompatible freeze casting technique, fabricated 3D free-standing porous GO scaffold for stable growth of embryonic neuronal progenitor cells. The conductive scaffold acts as a platform for electrical stimulation to induce neuronal stem cells differentiation (Figure 16a) [59]. A series of 1-100 ms monophasic cathodic pulses at intervals of 10 s was used, pointing out a stimulation threshold current of 20-30 mA, and improved cellular growth. As already mentioned, GR exhibits electrochemical capabilities for neuronal recordings similar to Pt or Au, which have been for long the standard electrode materials for neuronal recording. Indeed, recent studies revealed the successful recording of local field potentials from the rat cortex using GR-based electrodes [18,125]. Recently, Kostarelos and collaborators investigated GR performances by recording *in vivo* brain activity using porous GFNs with a good signal-to-noise ratio [88]. In a more advanced setting, Liu *et al.* developed an implantable GR-based neuronal electrode to detect electrophysiological and neurochemical signaling *in vivo* [130]. They constructed an rGO/Au_2O_3 nanocomposite-coated electrode to detect the concentration of H_2O_2 in an *in vivo* hyperacute stroke model (Figure 16b-c) [130]. This rGO-modified electrode provided high H_2O_2 sensitivity, low detection limits, and stronger electron transfer between tissue and electrode interfaces than traditional gold electrodes (Figure 17) [130,131].

All the studies reported highlighted the great potential of GR and its derivatives as promising tools for biomedical applications in the CNS. It is expected that the high attention given nowadays to GR devices, including brain interfaces, will further improve its use in medical applications.

Conclusions and Future prospective

The ultimate goal of neuronal tissue engineering research is to understand how the brain functions and signals propagate, translating this knowledge into actions: the unravel principles will be used to rebuild tissue and to interface with the nervous system. One approach is to design artificial nervous tissues. Indeed nano/bio materials are promising interfaces in supporting and modulating the organization of 3D neuronal networks, but it is critical to identify the appropriate materials able to induce cell differentiation and tissue regeneration enhancement. In the nervous system, that is composed of excitable cells, the conductivity of the materials is also a major parameter. Many studies exploited the use of GFNs for neuronal tissue engineering due to their extraordinary mechanical and electrical properties. In particular, graphene ability to be combined with a variety of other bioactive structures opens to novel and original possibilities. Here we reviewed graphene-based scaffolds that endow physicochemical and biological properties fit for the nervous system that links tightly morphological and electrical properties. In this review, we have discussed various aspects of why GFNs have gained more importance in recent years in the context of neuronal tissue engineering applications. We reviewed the three most essential aspects of GFNs.

Firstly, we thoroughly discussed the cytotoxicity of GFNs and concluded that it depends on various parameters such as size, surface charge, surface functional group, number of layers, time-dependent toxicity, concentration-dependent toxicity, and preparation technique. According to the recent findings, graphene and its derivatives have been revealed to have not only outstanding biocompatibility but also the ability to enhance cellular functionalities, including cell growth, proliferation and differentiation. We concluded that most GFNs and composites are non-toxic at the concentration required for their use in neuronal tissue engineering.

Secondly, we present the potentiality for neuronal stem cells differentiation and subsequent neuronal network growth. Various GFNs and GFNs composites with polymers, other nanomaterials, electrospun nanofibers, films, and 3D graphene foam are shown and considered promising candidates for generation of neuronal networks. 3D GFNs and 3D scaffolds composed of GFNs have shown superior results in the development of 3D neuronal network with good biocompatibility.

Finally, we discussed the impact of GFNs on cells through electrical stimulation. We also discussed the possibility to use FET based on GFNs for electrical sensing and stimulation of cells for neuronal regeneration. Graphene presents promising results leading to the conclusion that GFNs based scaffolds are suitable for regeneration or repair of neurons with retention of electrical behavior. Moreover, electrically conductive scaffolds can be fabricated by a combination of conductive polymers and graphene. It has been demonstrated that graphene-based substrates are not only biocompatible but also can improve neuronal cell growth. When investigating the effects of graphene on the electrical activity of neuronal networks, studies show that graphene is providing characteristics that can be used for neuronal-stimulation electrodes. Additionally, the tunable surface and machining properties of graphene-based materials are suitable to fabricate the neuronal tissue-like structures in order to align the arrangement of neurons suggesting that these nanomaterials have enormous potentials for neuronal tissue engineering applications.

Further research is needed for focusing on how to engineer the GFNs for advanced applications in the fields of neuroengineering. The biocompatibility, biodegradability, biostability and mechanism of interaction need to be further studied. This review covers the most crucial aspects that need to be controlled to make graphene a promising candidate for further advanced bioactive applications in neuronal tissue engineering and biomedical use.

Acknowledgements

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"Author 1 and Author 2 contributed equally to this work."

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Figures and captions

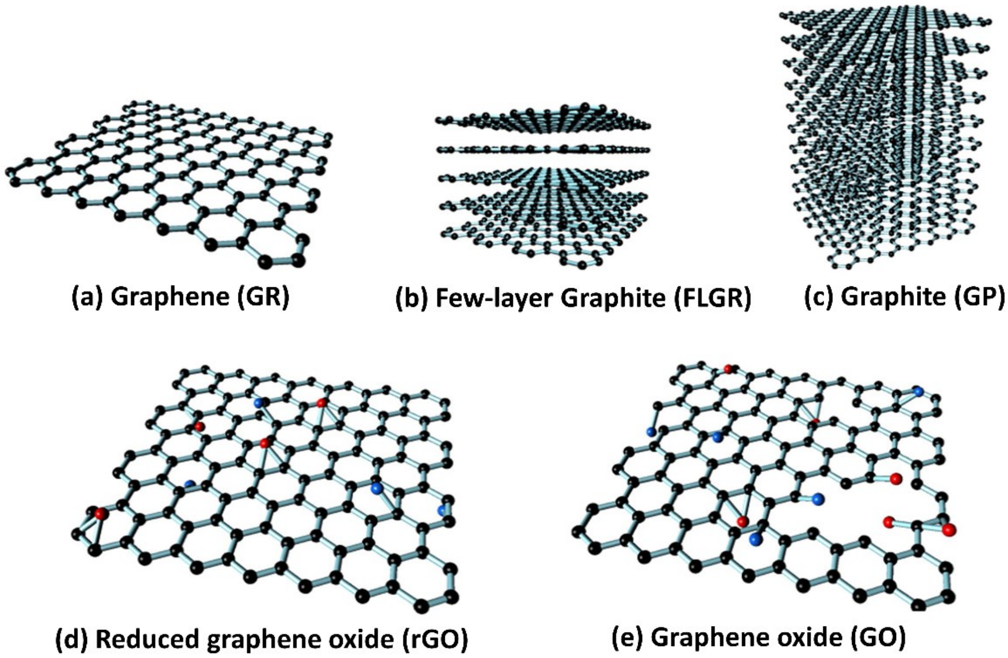

Figure 1. Classification of carbon allotropes based on dimensionality. Reproduced with permission from ref. [3]. Copyrights 2016, Elsevier.

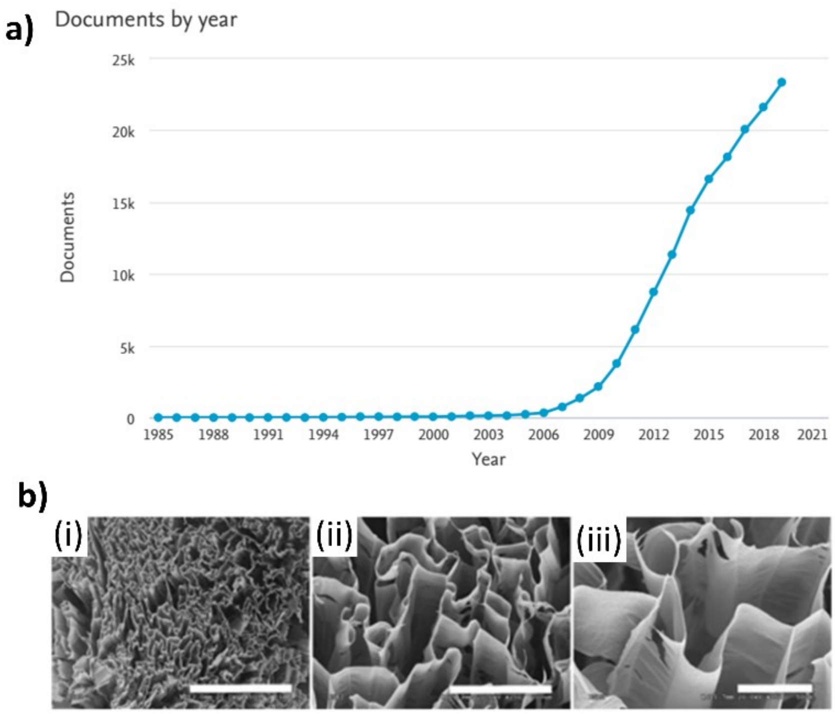

Figure 2. (a) progress of number of documents year by year on graphene family nanomaterials for neuronal tissue engineering. **(b)** Representative SEM images of 3D GOx scaffolds obtained by ISISA; Scaffolds after thermal treatment are shown. Scale bare represents 1 mm (i), 200 µm (ii), and 50 µm (iii). Reproduced with permission from ref. [59]**.** Copyrights 2014 The Royal Chemical Society.

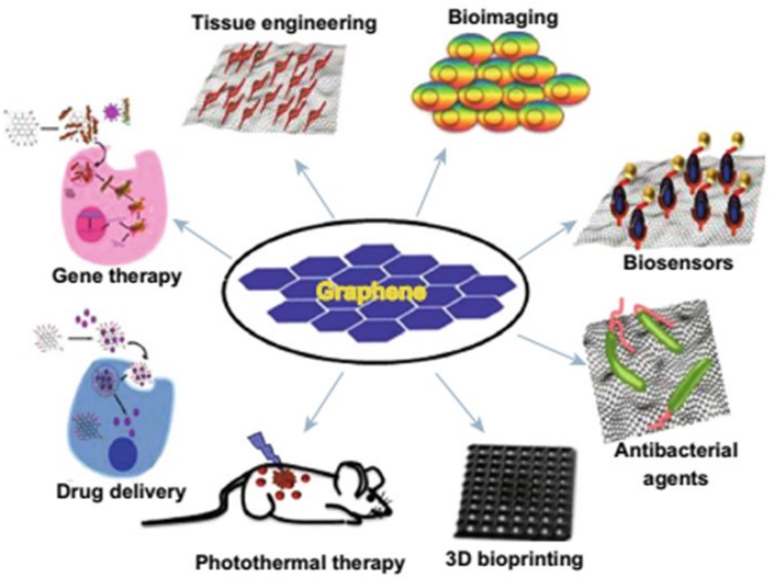

Figure 3. Diverse biomedical applications graphene. Reproduced with permission from ref. [11]. Copyrights 2019 open access Springer Nature.

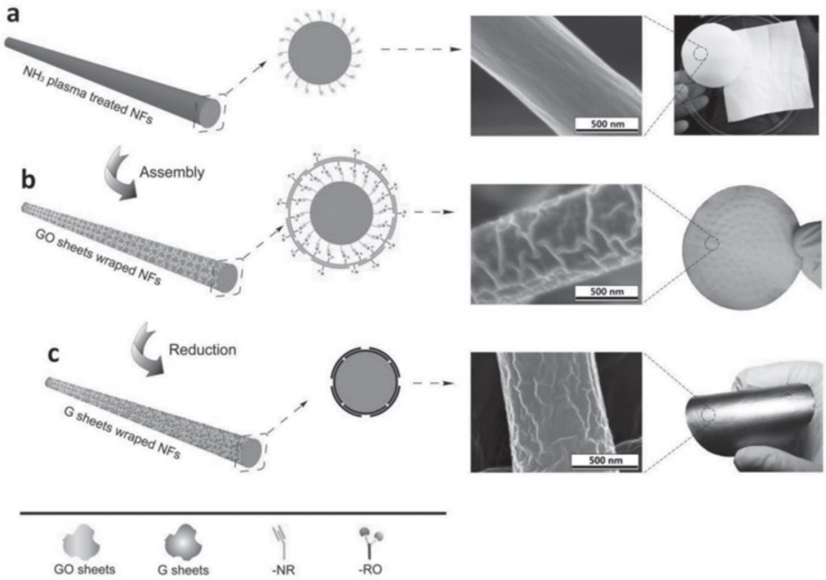

Figure 4. Schematic representation of the fabrication of G-NFs. **(a)** Modification of electrospun poly(vinyl chloride) nanofibers by NH_3 plasma treatment to render positively charged surface, **(b)** assembly of negatively charged GO sheets onto the surface of the modified nanofibers, **(c)** chemical reduction to obtain G-NFs. SEM and optical images of nanofibers obtained in every step. Reproduced with permission from ref. [61]. Copyrights 2015 Wiley-VCH.

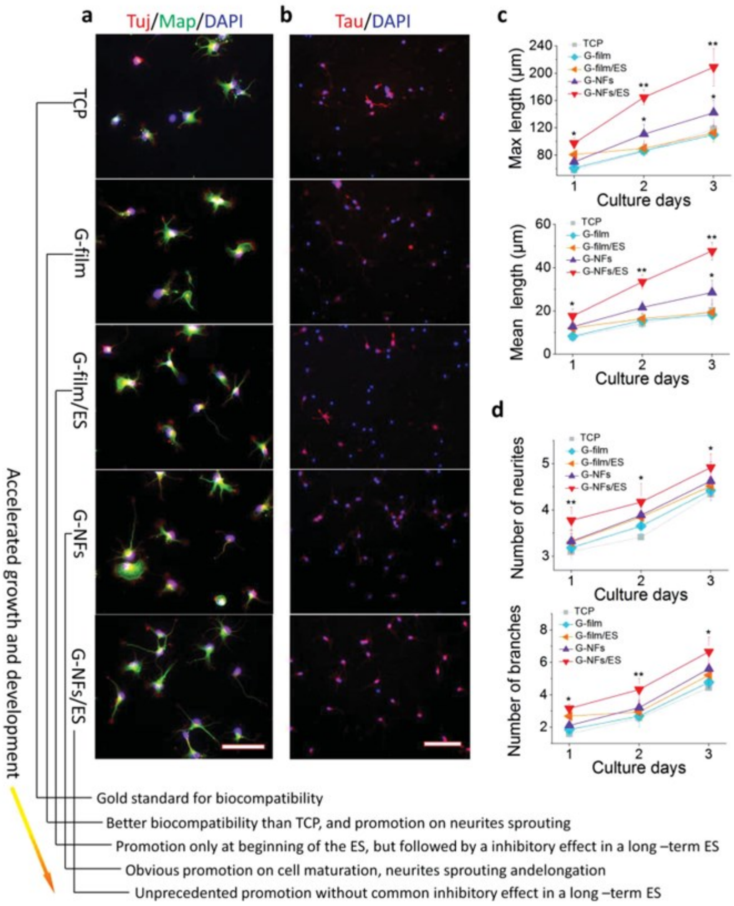

Figure 5. The growth and development of motor neurons on different substrates. Fluorescent images of motor neurons after 3 d in culture for **(a)** neuritogenesis (red: the neuronal marker protein of III β-tubulin (Tuj) for filopodia, green: dendrite marker protein of the microtubule-associated protein-2 (Map) for neurites); and **(b)** cell maturation (red: neuraxon marker protein of tau expression), **(c)** Neurites elongation: max and mean lengths of neurites, (d) Neurites sprouting: the mean number of neurite and the branches of neurite. Blue: nuclear. Scale bar a): 80 μm; b) 200 μm. Reproduced with permission from ref. [61]. Copyrights 2015 Wiley-VCH.

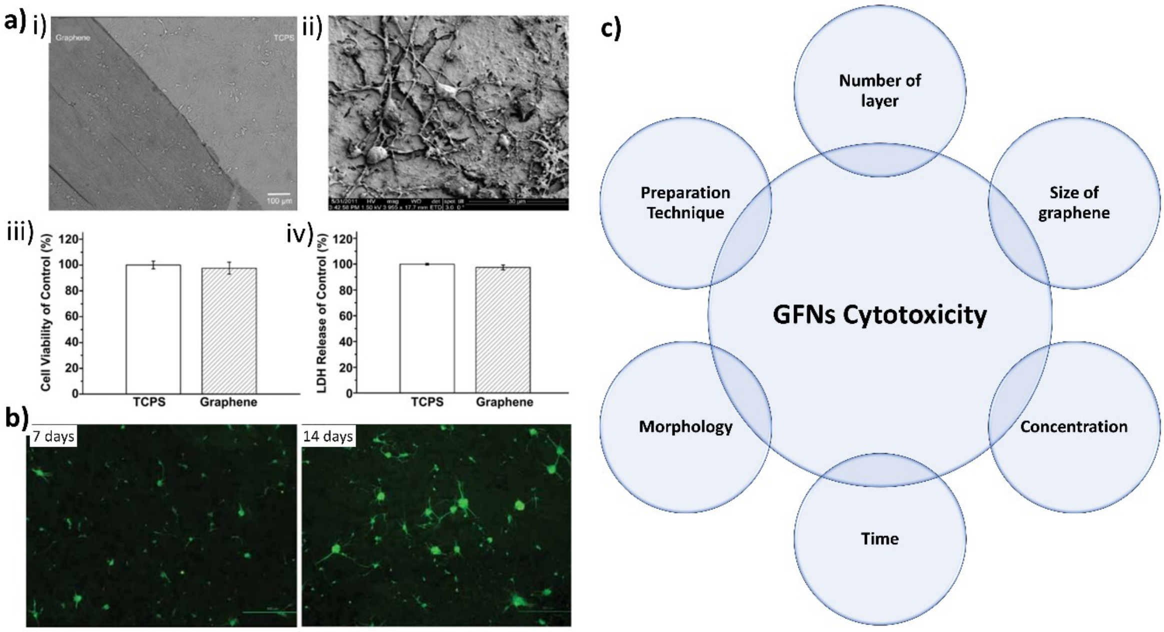

Figure 6. (a) Neurons cultured on different substrates. **(i)** An optical image of neurons cultured on the border of graphene (left) and TCPS (right), **(ii)** scanning electron microscopy image of neurons on graphene, **(iii)** MTT-measured viability of neurons cultured on TCPS and graphene after 7 days, **(iv)** LDH activity of neurons after 7 days incubation on TCPS and graphene. Reproduced with permission from ref. [58]. Copyrights 2011 Elsevier. **(b)** a comprehensive view of neurite number and length of primary neuron cells with a relatively low magnification. (scale = 500 µm) Cells were cultured onto nonfunctionalized graphene nanosheet film (NGNF) for 1 week and 2 weeks. Alexa Fluor® 488 ant-Rabbit green staining showed that both the neurite length and number were increased after an additional 7 days of growth. Reproduced with permission from ref. [86]. Copyrights 2016 open access wichtig. **(c)** Various factors effecting toxicity of GFNs.

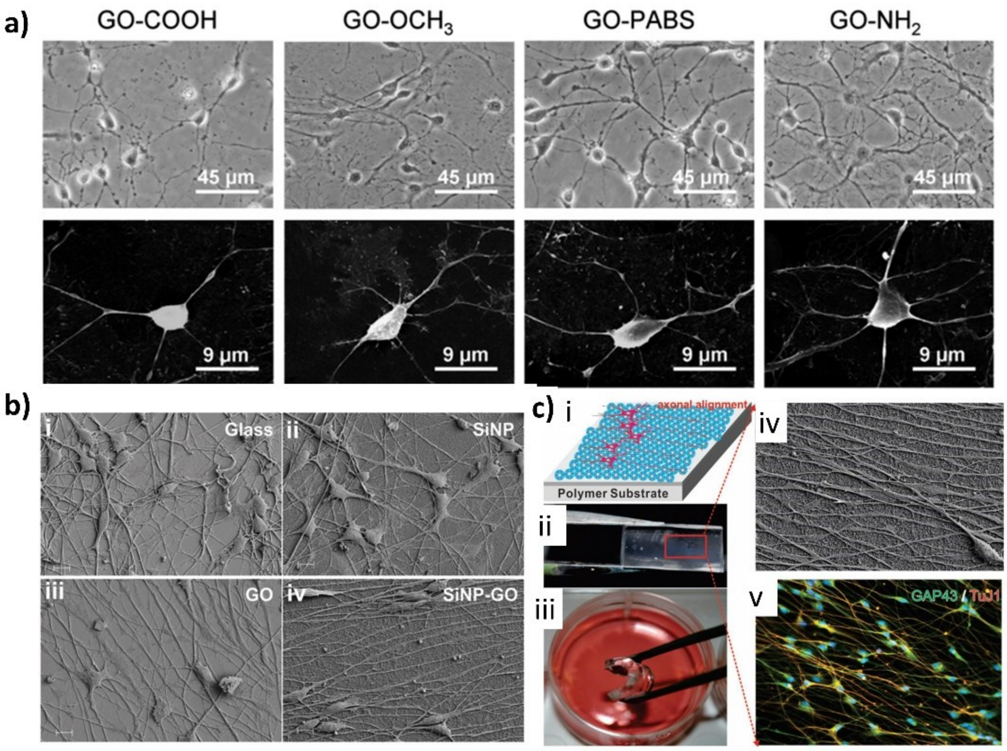

Figure 7. (a) Observation of the neuron morphology, first row-optical images and second row- SEM (single cell) images of hippocampal neurons after 7 days of culture on GO–COOH, GO–OCH3, GO–PABS, and GO–NH2 (from left to right). Reproduced with permission from ref. [24]**.** Copyrights 2014 The Royal Chemical Society. **(b)** SEM showing the behavior of hNSCs on GO and SiNP-GO. SEM images confirm that the axons do not align on (i) control and (ii) SiNP substrates and they align on (iii) GO and (iv)SiNP-GO substrates; **(c)** Axonal alignment of differentiated hNSCs on SiNP-GO on flexible and biocompatible substrates made from polydimethylsiloane (PDMS), (i) schematic diagram of axonal alignment of differentiated hNSCs on SiNP-GO on polymer substrates, (ii) SiNP-GO monolayer on PDMS, (iii) Flexible PDMS substrate with SiNP-GO in media for culturing hNSCs, (iv) SEM image of SiNP-GO on PDMS substrate showing highly aligned axons from hNSCs on Day 14, (v) Immunocytochemistry results showing the expression of neuronal marker TuJ1 and axonal marker GAP43 in hNSCs. Reproduced with permission from ref. [91]**.** Copyrights 2013 Wiley-VCH.

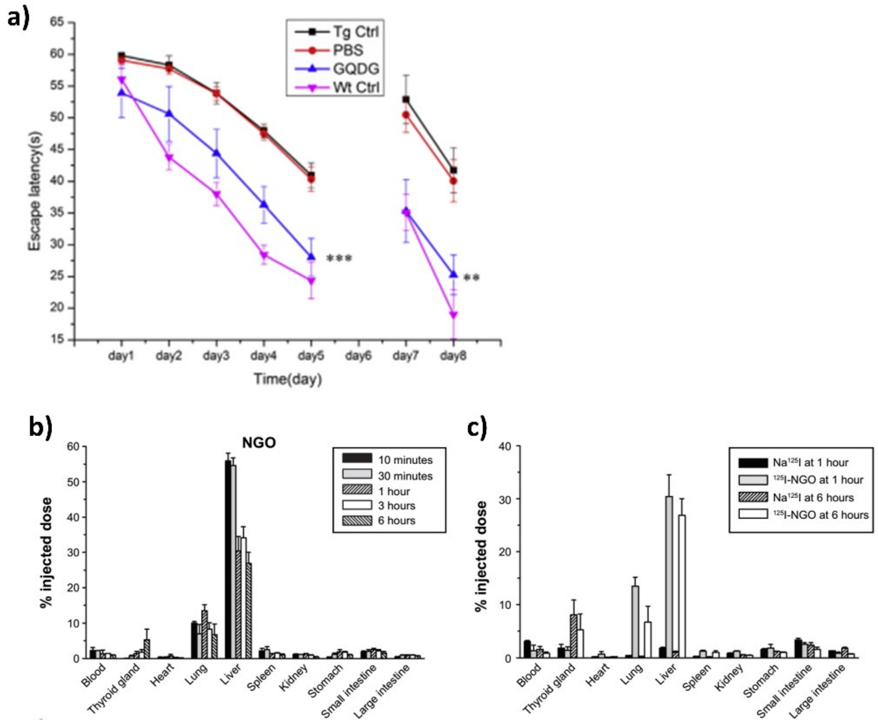

Figure 8. (a) GQDG improves the spatial learning abilities of APP/PS1 mice in MWM, comparison of latency time of each group in learning trails. Reproduced with permission from ref. [102]. Copyrights 2016 Elsevier. **(b)** Distribution of ^{125}I-NGO in the blood and main organs of mice at different time points; **(c)** comparative distribution of Na^{125}I and ^{125}I-NgO in mice at one and 6 hours; Reproduced with permission from ref. [106]. Copyright 2014 Open Access, Dove Medical Press.

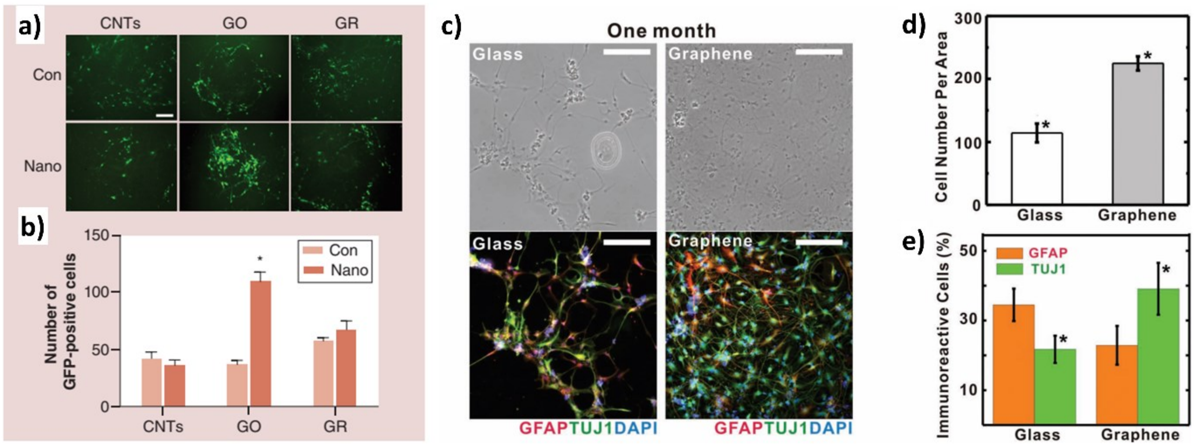

Figure 9. (a-b) Different effects of carbon nanotubes, graphene oxide and graphene on dopamine neuronal differentiation of GFP-reported endothelial stem cells. Reproduced with permission from ref. [113]. Copyrights

2014 Future Medicine, 2011 Wiley-VCH. **(c)** Enhanced neuronal-differentiation of hNSCs on graphene films. (i) Bright-field (top row) and fluorescence (bottom row) images of hNSCs differentiated on glass (left) and graphene (right) after one-month differentiation. The differentiated hNSCs were immunostained with GFAP (red) for astroglial cells, TUJ1 (green) for neuronal cells, and DAPI (blue) for nuclei. **(d)** Cell counting per area (0.64 mm^2) on graphene and glass regions after one-month differentiation. **(e)** Percentage of immunoreactive cells for GFAP (red) and TUJ1 (green) on glass and graphene. Reproduced with permission from ref. [89]. Copyrights 2014 Future Medicine, 2011 Wiley-VCH.

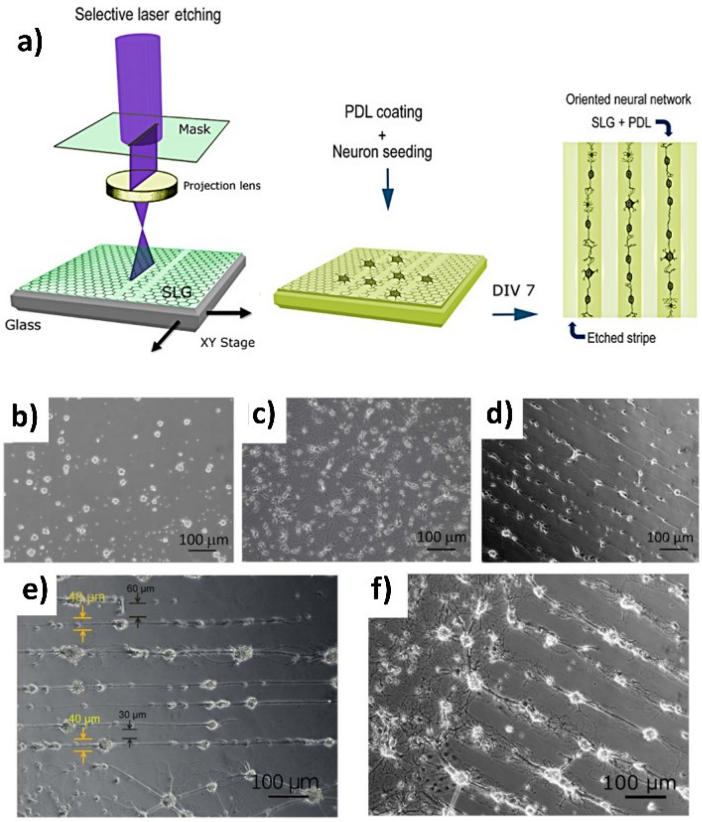

Figure 10. **(a)** Schematics of the steps proposed to create an ordered neuronal network on SLG substrate, development of the neuronal network. Wide field transmission images of neurons at DIV 7, **(b)** No neuronal network development was found on bare glass/graphene substrates: the adhered cells appear dead. In **(c)** a widespread neuronal network on PDL coated glass/graphene substrates is shown. In **(d-f)** neuronal networks oriented along line patterns. Reproduced with permission from ref. [123]. Copyrights, 2013 open access, Nature Scientific Reports.

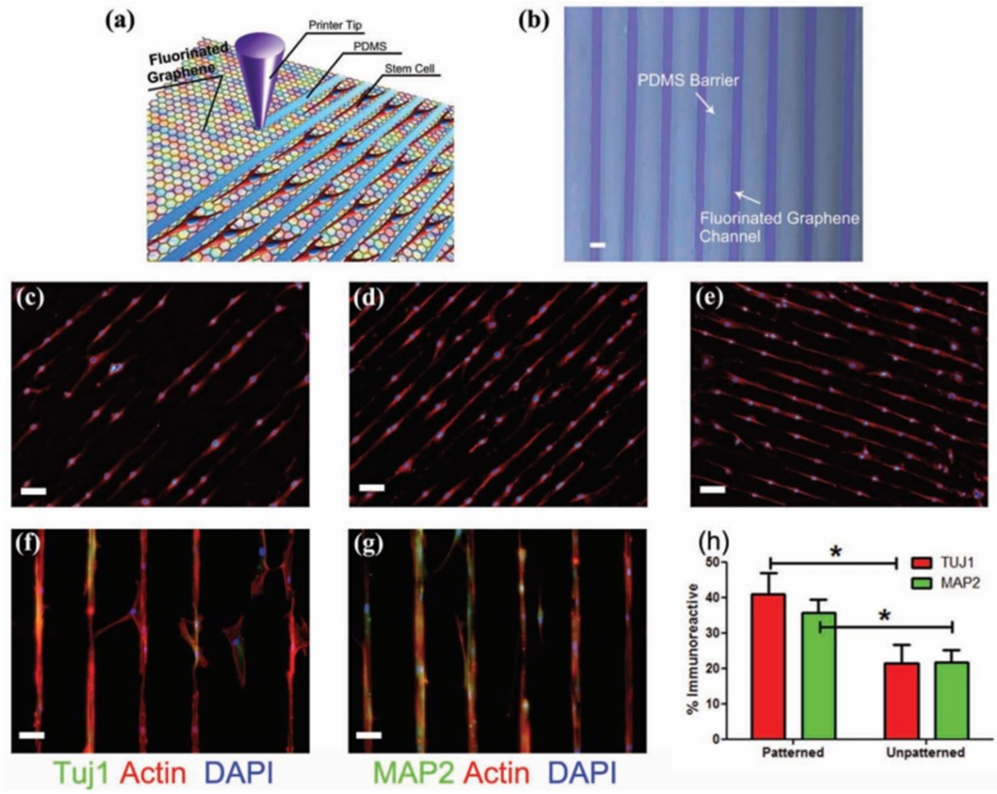

Figure 11. (a) schematic drawing of patterning MSCs by printing PDMS barriers on graphene films directly. **(b)** optical microscope image of printed PDMS on fluorinated graphene film. **(c-g)** The aligned growth of stem cell on graphene; **(h)** Percentage of immunoreactive cells for Tuj1 and MAP2 on un-patterned and patterned FG strips. Reproduced with permission from ref. [117]. Copyrights 2012 Wiley-VCH.

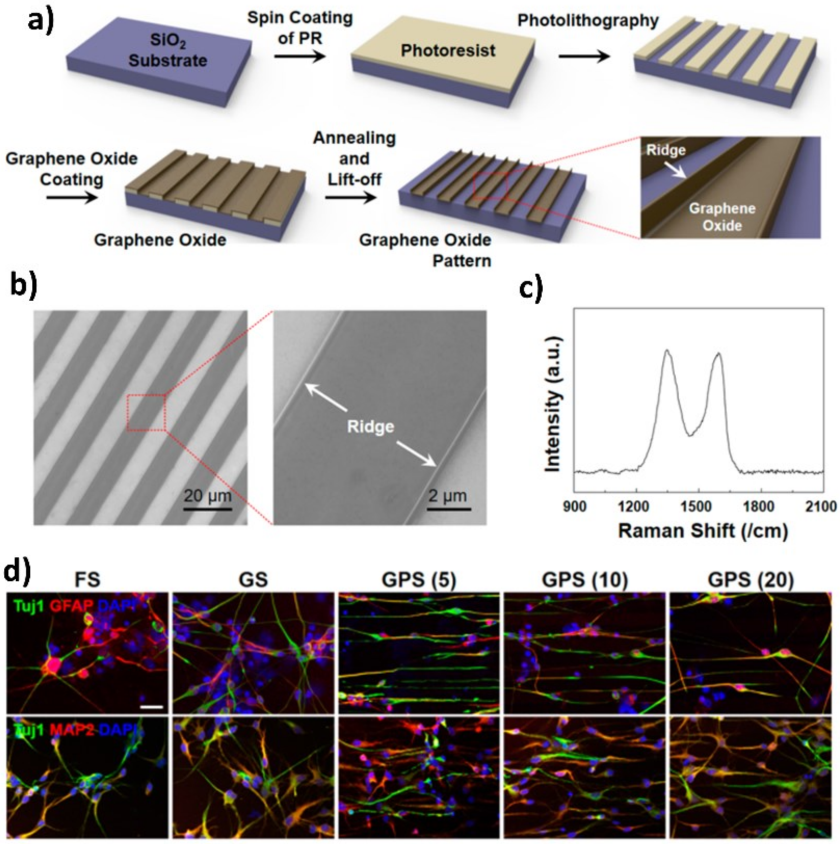

Figure 12. Preparation of GO-based patterned substrates, **(a)** Schematic illustration of GPS fabrication, **(b)** High-magnification SEM images and, **(c)** Raman spectroscopy analysis of the GPS; **(d)** Enhancement of neuronal differentiation of hNSCs on the GPS after 5 days in culture, Immunofluorescent staining to check for the expression of Tuj1 and MAP2 (neuronal markers) and GFAP (astrocyte marker) in hNSCs cultured on each substrate; Reproduced with permission from ref. [124]**.** Copyright 2016 American Chemical Society.

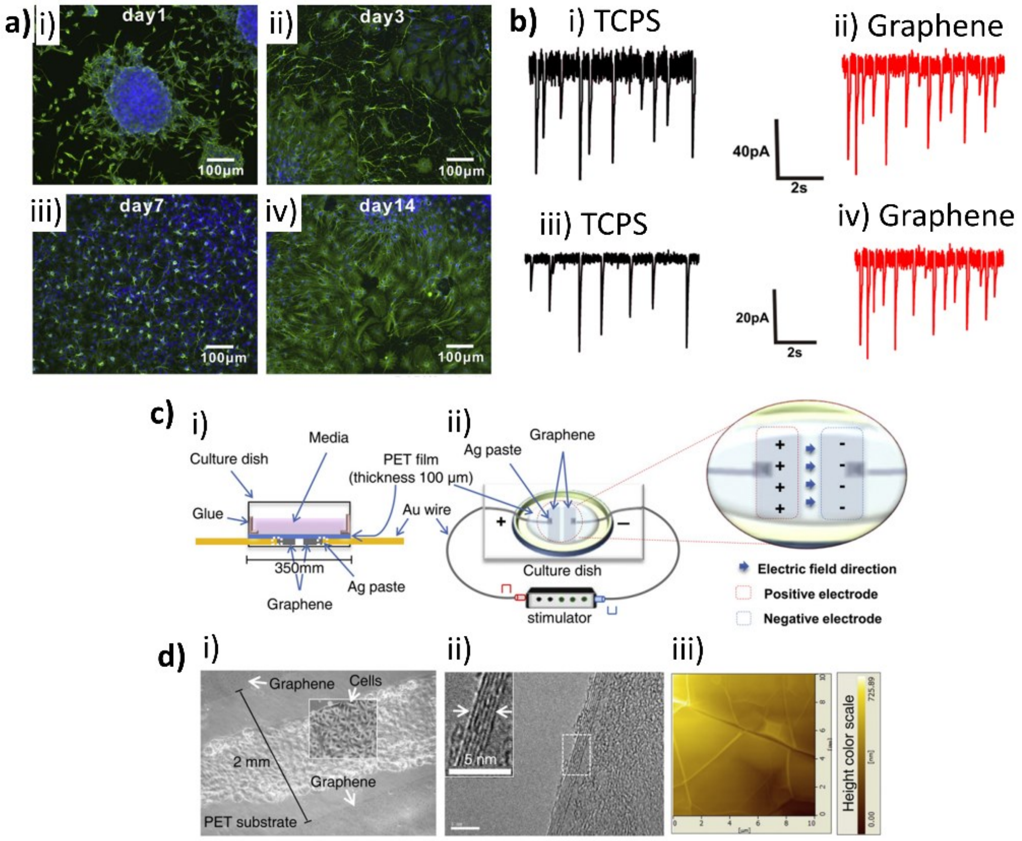

Figure 13. (a) The development of neuronal networks by NSC differentiation on graphene substrates, (i-iv) Representative images immunostained by antibody against b-tubulin at different culturing times (day 1 to day 14); **(b)** Graphene substrate increases spontaneous synaptic activity and firing and miniature synaptic activity. Representative spontaneous synaptic currents (sPSCs) (i ii) and miniature synaptic current (mPSCs) (iii, iv) are shown in both TCPS and graphene groups. Reproduced with permission from ref. [78]**.** Copyrights 2013 Elsevier, 2010 Elsevier. **(c)** Schematic illustrating the morphological features of the graphene electric field stimulator and electric field stimulation protocol. (i) Side view and (ii) top view of the PET/graphene film stimulator. The electrical field forms between two graphene electrodes. Neuronal cells located between two electrodes were observed by live optical microscopic imaging. **(d)(i)** Optical microscopic images showing that the two graphene electrode edges were separated by a 2 mm gap. Neuronal cells were placed between graphene electrodes. (ii) TEM images depicting cross-section view of 6 layers of the graphene stimulator. Total thickness of six layers is 2.3 nm. (iii) AFM images of the graphene surface. Reproduced with permission from ref. [126]**.** Copyrights 2013 Elsevier, 2010 Elsevier.

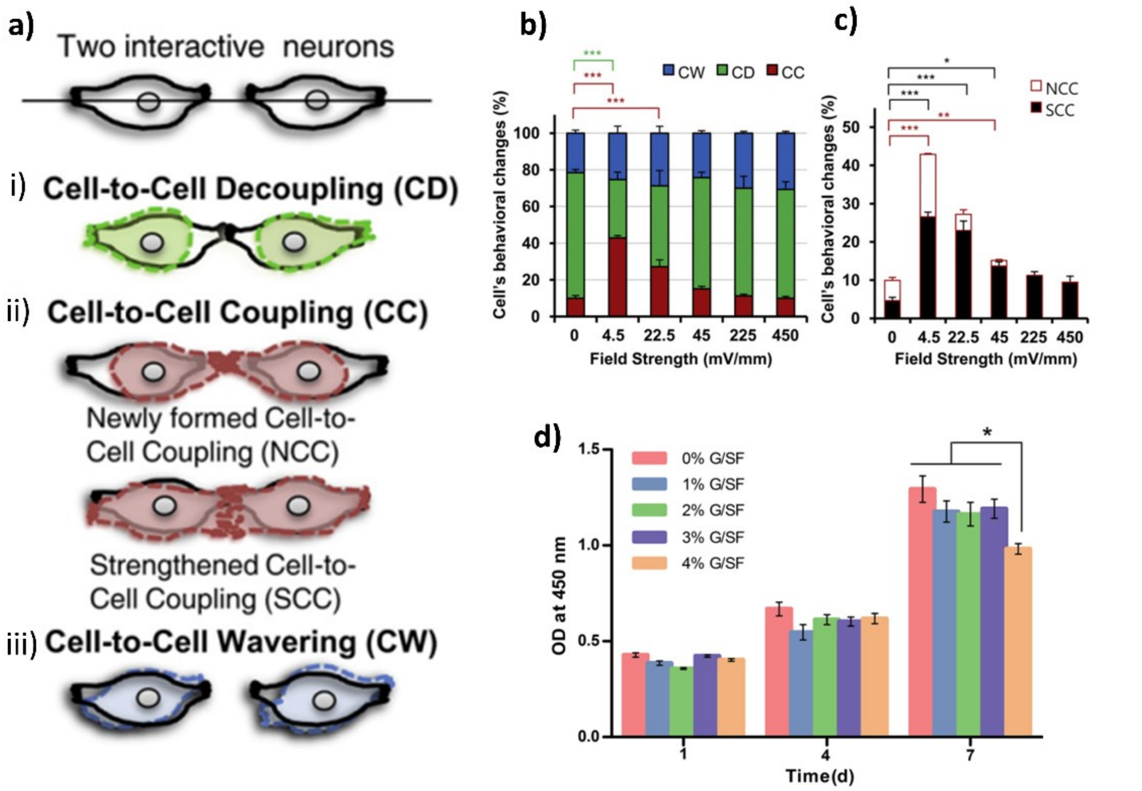

Figure 14. Cell–to-cell interactive reactions to electric field stimulation. **(a).** Schematic illustrations of cell-to-cell interactive reactions between two separated cells under electric field stimulation. i) Cell-to-cell decoupling (CD). Cells belonging to the CD group separated from each other after stimulation. ii) Cell-to-cell coupling (CC). The CC group was further classified into two groups: The newly formed cell-to-cell coupling (NCC) group and the strengthened cell-to-cell coupling (SCC) group. The NCC represents a group of cells that respond to electric field stimulation by forming new contacts between cells. The SCC represents a group of cells strenthening existing contacts between cells after electric field stimulation. iii) Cell-to-cell wavering (CW). Cells belonging to the CW group exhibit a wavering behavior following electric field stimulation. Reproduced with permission from ref. [126]. Copyrights 2010 Elsevier, 2017 The Royal Chemical Society. **(b)** A bar graph categorizing behavioral reactions to electric field strengths. **(c)** The categorization of CC cells. When we further categorized CC into two groups, there was a clear effect of electric field on NCC; **(d)** CCK-8 assays of rBMSCs cultured on G/SF fibrous scaffolds. Reproduced with permission from ref. [127]**.** Copyrights 2010 Elsevier, 2017 The Royal Chemical Society.

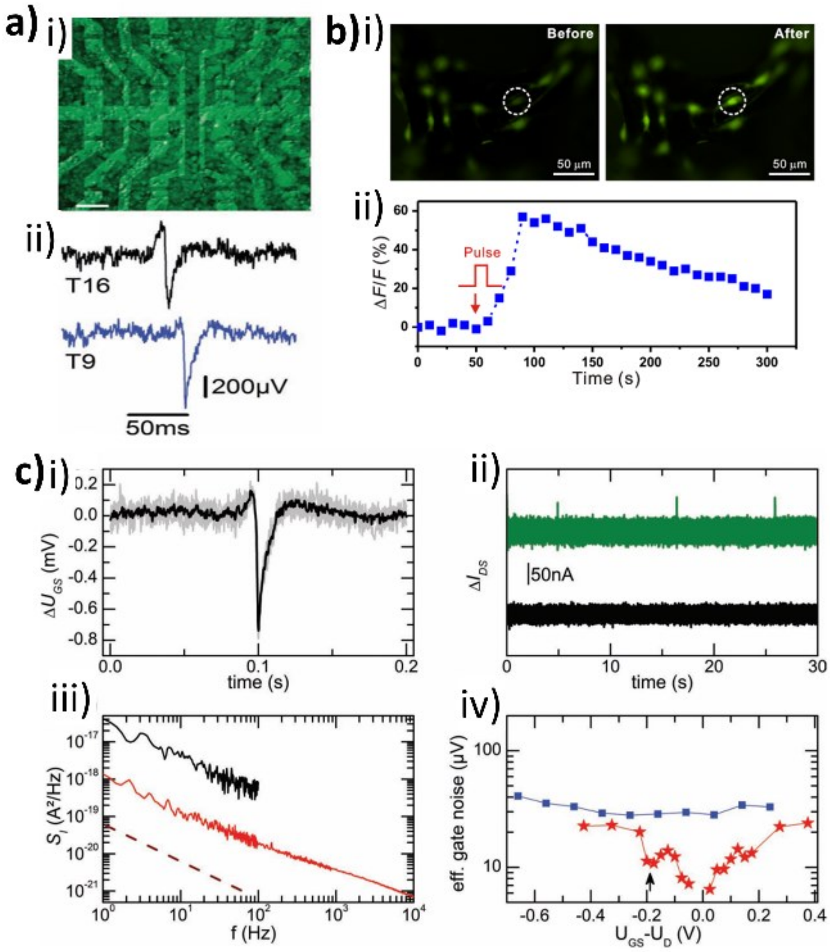

Figure 15. **(a)** (i) Combination of an optical microscopy image of a transistor array and a fluorescence image of the calcein-stained cell layer on the same array. (ii) Exemplary single spikes. The current response has been converted to an extracellular voltage signal. The upper spike resembles a capacitive coupling followed by the opening of voltage-gated sodium channels whereas in the bottom one the ion channels dominate over the capacitive coupling; **(c)** (i) Five consecutive spikes recorded with one transistor (grey) and their average (black). (ii) Recording of the current of a G-SGFET (top) and a 3.6 kΩ resistor. The resistance of the transistor was 3 kΩ. The transistor current is shifted up for clarity. (iii) Exemplary current noise power spectral density of a G-SGFET in a low-noise setup (red) and a 1 kΩ resistor measured in the cell setup (black). The dashed line serves as a guide to the eye showing a 1/*f* dependence. (iv) Effective gate noise of a graphene (red stars) and a silicon SGFET (blue squares). *U*D refers to the *U*GS at which the minimum of the current is observed. The arrow marks the point of maximum transconductance. Reproduced with permission from ref. [128]**.** Copyright 2011 Wiley-VCH, 2013 Open Access Nature Scientific Report. **(b)** Electrical stimulation of the cells differentiated from NSCs on 3D-GFs. (i) Fluorescence imaging of the cells pre-incubated with Fluo-4 AM dye on 3D-GFs before (left) and after (right) electrical stimulation. Panel (ii) plots the relative fluorescence intensity change DF/F of the circled cell in panel (a) versus the stimulation time period. Reproduced with permission from ref. [56]**.** Copyright 2011 Wiley-VCH, 2013 Open Access Nature Scientific Report.

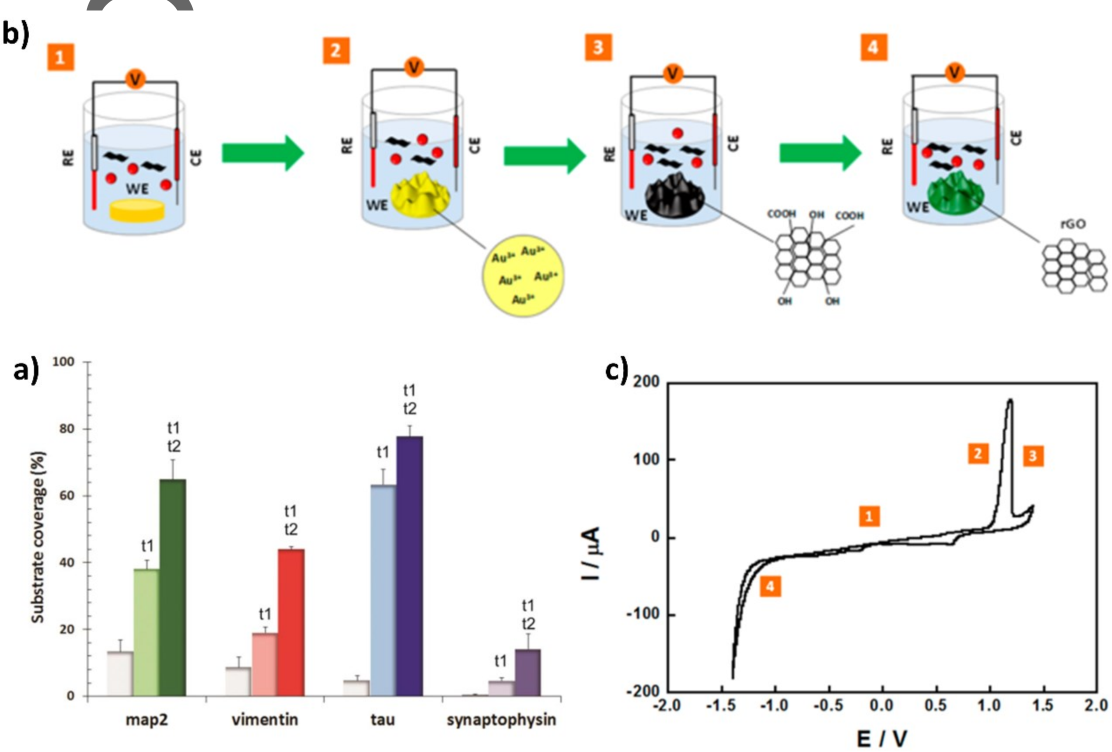

Figure 16. (a) Quantification of ENPC differentiation on 3D scaffolds over time. Histograms show the percentage of substrate area positively stained for map-2, vimentin, tau, and synaptophysin. Time points: 1 day (light grey), 7 days (light color) and 14 days (dark color) Reproduced with permission from ref. [59]. Copyright 2013 The Royal Society of Chemistry; **(b)** Schematic illustration of rGO/Au_2O_3-modified gold electrode using a one-step electrochemical process and **(c)** real-time CV plot for the formation of the rGO/Au_2O_3 nanocomposite was indicated with the numbers corresponding to the schematic. Reproduced with permission from ref. [130]**.** Copyright 2015 American Chemical Society.

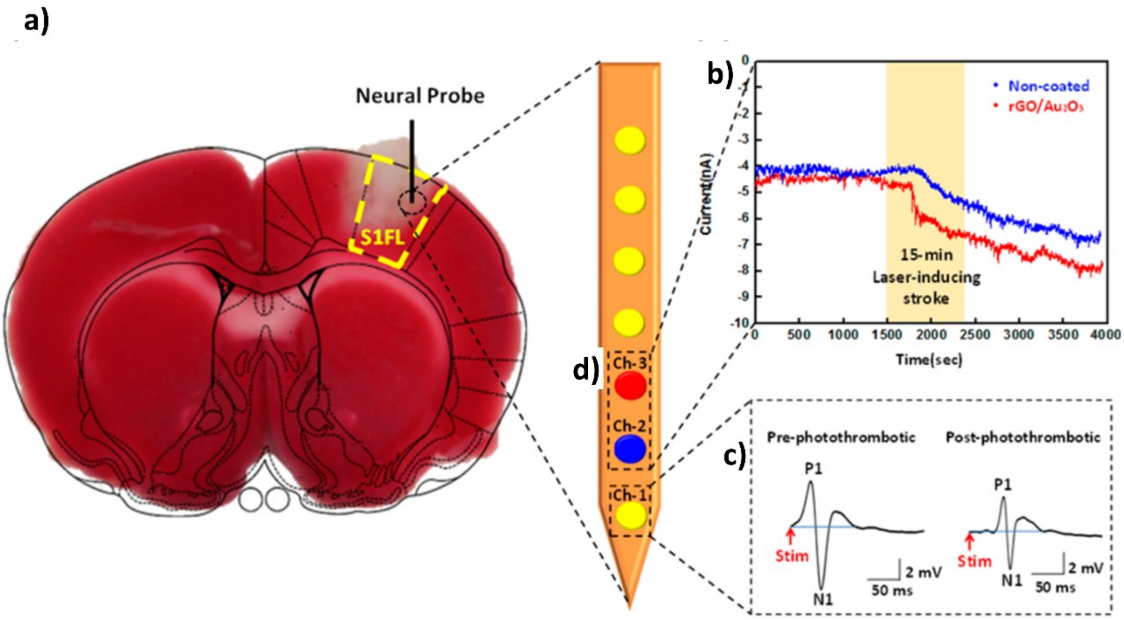

Figure 17. The photomicrograph of a TTC- stained coronal section at 2 mm anterior to bregma, **(a)** The neuronal probe was implanted into the S1FL for real-time monitoring of the relative changes in electrochemical detection and real-time SSEPs recording (marked as black line), **(b)** The amperometric responses to H_2O_2 were recorded by noncoated gold electrode (Channel-2) and rGO/Au_2O_3 electrode (Channel-3) at -0.5 V. At the time of 1500 s, the S1FL brain was illuminated by laser light for 15 min to induce photothrombotic ischemic stroke (marked as square pale yellow area), **(c)** The first bare gold electrode (Channel-1) of the developed neuronal probe was used to record the changes in neuronal activities (SSEPs) before and after ischemic stroke. Reproduced with permission from ref. [130]**.** Copyright 2015 American Chemical Society.

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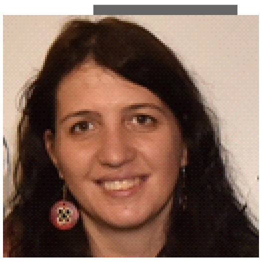
Rossana Rauti



Rossana Rauti is a Postdoc in the Department of Biomedical Engineering at Tel-Aviv University, Israel. She graduated in Chemistry and Pharmaceutical Technologies at the University of Calabria, Italy, in 2010, and obtained a Ph.D. in Neuroscience and Cognitive Sciences by the University of Trieste, Italy, in 2014. Then, she moved as a Post-Doc at the Department of Neuroscience of the International School for Advanced Studies, SISSA, Trieste, Italy where she was investigating the interactions between living neurons and carbon-based nanomaterials, such as carbon nanotubes and graphene. Currently, her main research focuses on developing new tools, such as Organs-on-Chip, to deeply investigate the neurovascular crosstalk, in physiological and pathological conditions.

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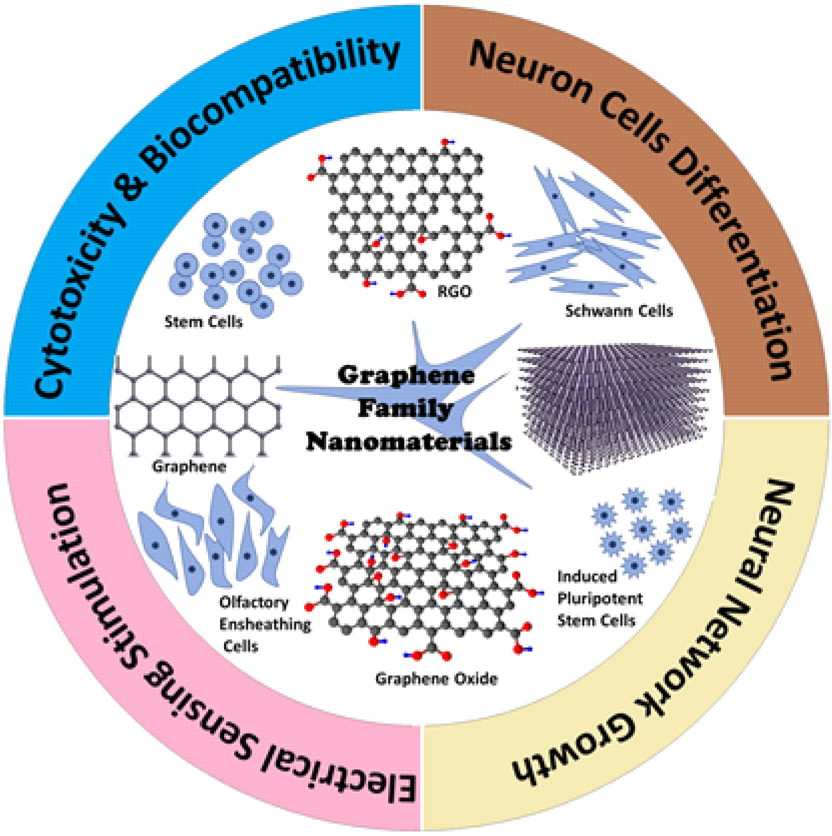

Synopsis: Here, we have discussed on different strategies to exploit graphene family nanomaterials for neuronal engineering and regeneration. Specifically, we present the potentiality for neuronal stem cells differentiation and subsequent neuronal network growth as well the impact of electrical stimulation through GNM on cells. It concludes the important aspects need to be controlled to make graphene a promising candidate for neuronal tissue engineering.