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Review Article

Graphene: One Material, Many Possibilities—Application Difficulties in Biological Systems

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Energetic technologies, nanoelectronics, biomedicine including gene therapy, cell imaging or tissue engineering are only few from all possible applications for graphene, the thinnest known carbon configuration and a basic element for other more complicated, better discovered and widely used nanostructures such as graphite, fullerenes and carbon nanotubes. The number of researches concerning graphene applications is rising every day which proves the great interest in its unique structure and properties. Ideal pristine graphene sheet presents a flat membrane of unlimited size with no imperfections while in practice we get different flakes with irregular edges and structural defects which influence the reactivity. Nanomaterials from graphene family differ in size, shape, layer number, lateral dimension, surface chemistry and defect density causing the existence of graphene samples with various influence on biological systems. Whether graphene induces cellular stress and activates apoptosis, or on the contrary facilitates growth and differentiation of the cells depends on its structure, chemical modifications and the growth process. A certain number of in vitro studies has indicated cytotoxic effects of graphene while the other show that it is safe. The diversity of the samples and methods of the production make it impossible to establish clearly the biological impact of graphene.

1. Introduction

The age of stone, copper, and iron is now prehistory. Nowadays, steel, carbon, and almost perfect silicone are the materials of choice [1]. Nevertheless, carbon is the one which still gets a lot of attention. Known as a nonrenewable source of energy, carbon is a ubiquitous molecule capable of forming many allotropes with many potential applications. The major ones are graphite and diamond [2]. Recently, carbon has become again an object of intense scientific research which resulted in many discoveries. In 1996 Smalley and his colleagues received the Nobel Prize in Chemistry "for their discovery of fullerenes," zero-dimensional nanospheres formed by 60 carbon atoms which resemble the soccer ball and exist only in the molecular form of carbon in contrast to the crystalline forms of the graphite and diamond [3]. Iijima in 1991 described one-dimensional helical microtubules of graphitic carbon called nanotubes [4]. Again the Nobel Prize in Physics in 2010 was awarded jointly to Andre Geim and Konstatntin Novoselov "for groundbreaking experiments regarding the two-dimensional material graphene" [5]. The scientists obtained 2D graphene only one atom thick, strikingly reminiscent to a honeycomb structure by the simple use of ordinary sticky tape. Not only did they make graphene but they also studied and described its unique properties and possible applications [5–8]. Graphene is a basic element for the other graphitic materials and it is possible to transform one structure to another under appropriate conditions (Figure 1). The last 20 years of research have shown many possibilities of chemical functionalization of different synthetic carbon varieties such as fullerenes or carbon nanotubes which led to many significant achievements in improving the solubility, processing capabilities, fixing with other compounds, and exploring the unique properties of graphene [9]. Different distribution of the benzene rings in the two-dimensional structure of graphene determines the shape, size, edges, and number of layers and additional covalent or noncovalent bonds with other atoms which results in modifications of the electrical or chemical properties of graphene. Like in semiconductors

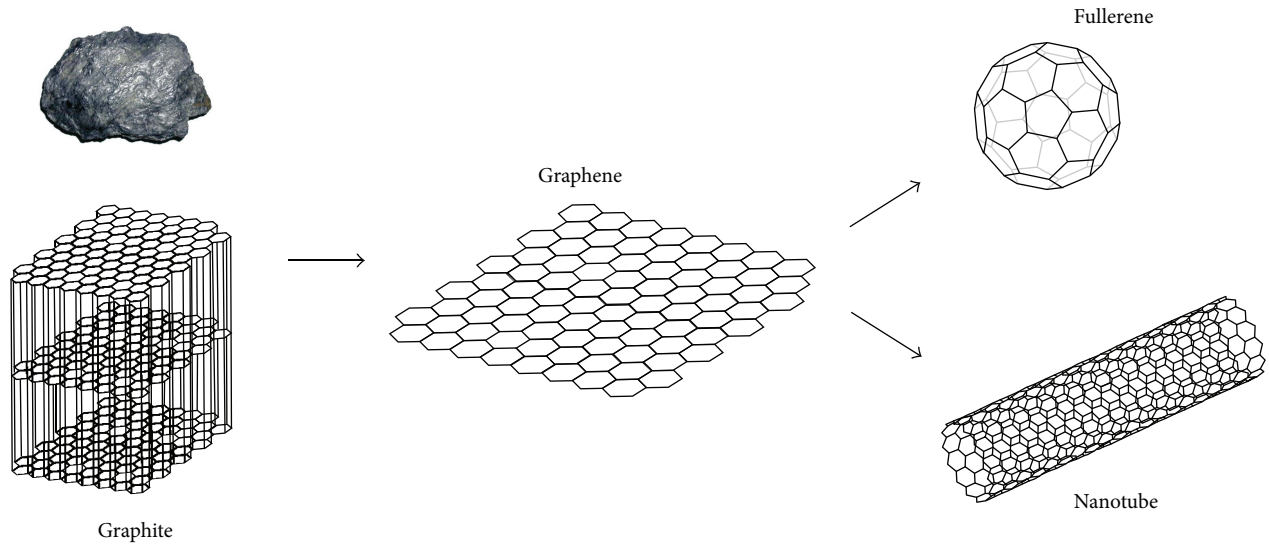

Figure 1: Graphite is a common and naturally occurring mineral in nature. Graphene is a basic element for the other graphitic materials and it is possible to transform one structure to another in appropriate conditions. *Reprinted from The Nobel Prize in Physics 2010. The Royal Academy of Sciences.*

and in graphene the electrical current is transferred either by negatively charged electrons or by the positively charged holes that are left behind. However, on the contrary to conventional semiconductors the two-dimensional graphene layer exhibits a strong ambipolar electric field effect in the room temperature with a minimum band gap between the valence and the conduction bands. It enables ballistic electron transfer over long distances and with high speed that is only 300 times slower than that of the light speed and from 10 to 100 times greater than that in the currently used silicon chips. Graphene is a flexible, transparent, and well-conductive material with remarkable mechanical properties and is the thinnest known structure being 200 times stronger than steel and harder than diamond [10–12]. The ubiquitous and widely available carbon is the basal unit of graphene and it is not surprising that it has become a rising star in the present world of nanomaterials, while being competitive for silicone, an essential material of modern electronics. To enable the use of graphene and its unique properties and to assure cost-effective production and application in the industry it would be necessary to bypass some major obstacles. Those are high cost and difficulties in the production of graphene on a massive scale and the quantitative and qualitative control of the resulting product [13]. Moreover, each of the graphene production methods results in various types of the same material which has different properties, quality, and number and type of defects. Gathering evidence from laboratories concerning unusual properties of graphene has been carried out on small, high quality, and single layer samples without impurities or structural defects, which may not necessarily be reflected in graphene obtained on a large scale. Creating ideal crystals composed of a single layer at low cost and precise manipulation on the microlevel is still a big challenge despite the numerous known methods of graphene production and synthesis [14–16]. Researchers from different fields of science are interested in graphene and are still learning how to work with it and take advantage of its unlimited potential which can be confirmed by a growing number of publications regarding its recent applications [15–22].

2. Synthesis

The discovery of graphene in 2004 was not the first contact with this revolutionary material. Already in 1986 Boehm and coworkers identified a monolayer graphene and officially named it using a combination of the word graphite and the suffix "en" relating to polycyclic aromatic hydrocarbons [23]. It was the development of the new techniques which allowed to identify and characterize the single-atom structure of graphene and its unusual properties, though. Graphene is probably produced every time while using a pencil. However, it is difficult to observe it among other stacked layers of graphite. In fact, the observation of graphene is not possible using traditional visualization techniques (with the exception of electron microscopy or atomic force) due to the lack of the clear visible differences in the atomic structure of graphene monolayer and many layers of graphite [15]. To obtain the desired image intensity and contrast and to identify the single-atom structure of graphene in the optical microscope it is required to select the appropriate substrate of certain thickness and wavelength. Except of the optical microscopy there are techniques useful in observing and determining the structure of graphene such as Atomic Force Microscopy (AFM), Transmission Electron Microscopy (TEM), Angular Distribution Photoemission Spectroscopy (APRES) and the Spectroscopy of Rayleigh and Raman [16]. Raman spectroscopy is one of the best methods for the insight into the structure of graphene, fast and accurate characterization, and direct measurements of electron interactions which allow determining the number of layers and identify defects and

impurities. The Raman spectrum presents three major bands: the G band ~1580 cm^{-1}, band D ~1350 cm^{-1} activated by defects, and band 2D ~2700 cm^{-1} which informs about the value of the cumulative load and number of layers [24]. Increase in the ratio of the D peak intensity to that of the G peak and broadening of both of them are caused by increased number of defects on the surface of graphene. The Raman spectroscopy particularly gives information about the consistency of graphene carbon skeleton. In turn, the intensity of D peak increases gradually together with the number of holes in the structure or new centers with sp^3 hybridization created by covalent bonds (Figure 2). However, it is hard to distinguish between them. In the presence of less than 1% of the structural defects the analysis of the D band can be used to determine the quality of graphene obtained by reducing the graphene oxide or to state the degree of the functionalization [13].

Andre Geim and Konstantin Novoselov used simple and effective method to obtain graphene by the repeated use of the adhesive tape to rip off thin flakes from graphite and then attached them to a plate of oxidized silicon SiO_2 to reveal and determine the number of graphene layers. Mainly due to the use of high quality crystals of graphite (HOPG: highly ordered pyrolytic graphite) as a starting material the mechanical exfoliation still remains one of the best methods to obtain structurally and electrically homogenous and single-layer graphene [25]. Despite being limited by its low production and the possibility of practical use, the mechanical exfoliation method led to numerous discoveries of graphene electronic and mechanical properties and resulted in the development of new methods of the production [14–20].

There are two approaches to obtain graphene (Figure 3, Table 1). One involves the graphite and weakening of the van der Waals forces to separate the layers from each other and the second is based on the alternative carbon sources. The effective dissection without damaging the structure and protection against reaggregation are the key elements to obtain graphene from graphite. The disadvantages of these methods are low yield, many steps of the production, and the fact that natural graphite is not easily available and it is listed in the European List of Critical Raw Materials [26].

In turn, graphite synthetic form obtained at high temperatures is not suitable for production of high quality graphene because of its irregular structure. Noncovalent interactions between each layer in natural graphite form extremely stable and thermodynamically favorable arrangement which is the reason of the reaggregation of graphene flakes, for example, during graphite exfoliation in solution using chemical methods [27].

In assumption, the methods of production of graphene de novo are much easier and allow getting larger area surfaces on chosen substrates but require high temperatures and generate lots of defects and structure damage. These methods include epitaxial growth [28–30] on solid substrate and chemical vapor deposition [31, 32] which requires specialized equipment, strict control, and laborious preparation techniques. The epitaxial growth method originally involves placing silicon carbide in a vacuum at a temperature of 1300°C which results in sublimation of Si atoms and reorganization of the remaining carbon atoms. The exact time and temperature control of the process should lead to the formation of thin layers of graphene on the entire surface of SiC wafers which occasionally forms monolayer. However, the detailed studies of these structures show very weak bonding and rotation of the individual layers, surface roughness, and presence of numerous holes and cavities [16]. Over time and in order to obtain more stable and homogenous graphene monolayers the new improvements have been introduced such as the change of the temperature or atmosphere of the sublimation process.

The methods based on the distribution of hydrocarbons and deposition of the graphene on the chosen substrate have gained a lot of interest because of its previous application in the carbon nanotubes production [33]. The chemical vapor deposition on metallic surfaces which catalyzes the supported growth of graphene requires specific conditions. In early method the process was carried out in the ethylene atmosphere at 800 K temperature with Pt as a surface substrate. In order to improve the process, the temperature, type of the gas (e.g., benzene), and metal surface (e.g., Ru, Ir, Ni, or Cu) were changed. The combination of the terms of the chemisorption of the graphene on metal surfaces allowed obtaining regular and homogenous graphene layer which could be transferred later on any type of material [32]. The growth of the monolayer graphene was also achieved by the thermal decomposition of polymer films or small molecules and even from unconventional carbon sources such as biscuits, chocolate, grass, or cockroach legs [34, 35].

The methods of supported growth of graphene require expensive templates which would be useful in more demanding and high performance applications. Much more attractive from an economic point of view are the chemical methods for the production of graphene in the solution [27, 36–41]. They give the possibility of the mass production with the satisfactory electrical and optical properties of graphene. However, during the production of graphene from graphene oxide [42] the strict control of the process conditions is required in order to prevent excessive oxidation, release of CO_2, and formation of impossible-to-remove lattice defects. Graphene oxide is a highly oxidized form of chemically modified graphene produced by oxidation of crystalline graphite in solution preceded by sonication or other methods of dispersion. The oxidation of graphene to GO allows effective separation of layers from each other but in order to ensure that they can remain in the free state they should by fixed to the surface, masked with the functional groups, or separated with surfactant molecules. The size of graphene oxide flakes in the solution ranges from 10–100 nm to 100 μm and for biological applications scientists use those with a minimum dimension, even 20 nm, in order to facilitate the entry into the cells [43]. To restore the electrical conductivity and other properties of pristine graphene it is necessary to receive reduced graphene oxide (rGO) which is the product of the reduction of graphene oxide (GO) by the use of reducing agents such as high temperature or chemicals. Most of the researches concern GO and its reduced form rGO due to the ease of the preparation, good solubility, and stability

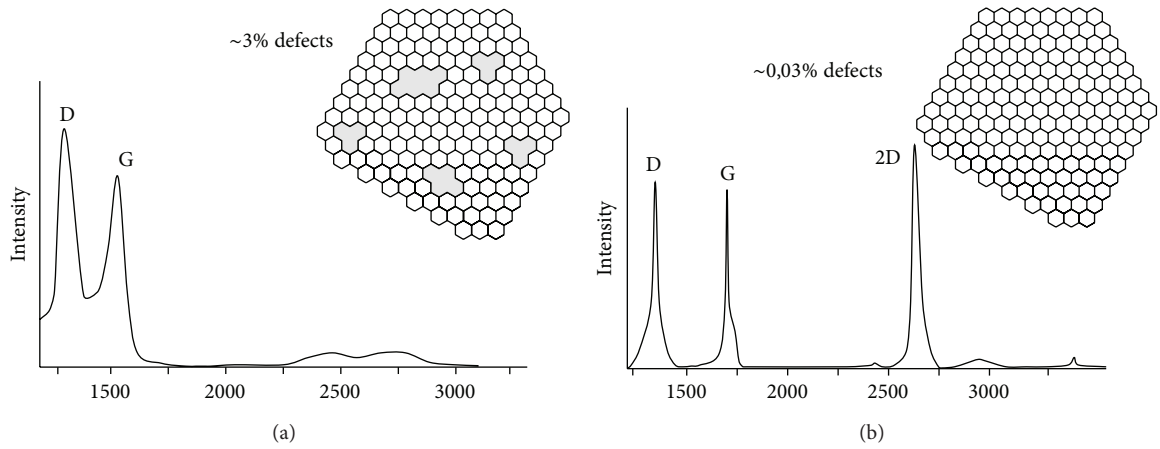

Figure 2: Raman spectra of graphene with simplified structural models. (a) GO with defect density of 1–3%. (b) GO with almost intact carbon framework with 0,03% defects. *Reprinted from [13].*

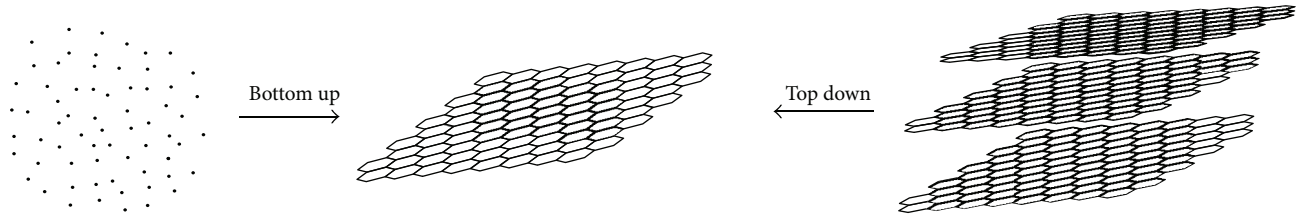

Figure 3: A schematic of bottom-up and top-down approaches for graphene synthesis. *Reprinted from [19].*

in aqueous solutions in comparison to the other forms of graphene. Pristine graphene is insoluble in organic solvents and the monolayers will be prone to reaggregate in aqueous solutions [44]. The stabilization of the structure of monolayer graphene involves weak interactions of noncovalent bonding with the surfactant molecules. Covalent modifications are accompanied by the creation of new bonds together with the change of sp^2 to sp^3 hybridization. Depending on the type of modification the electrical conductivity of the functionalized graphene is changed or reduced [45]. Numerous attempts to functionalize graphene using chemical methods with graphite as a starting material have allowed so far to obtain mainly multilayer graphene ($G < 10$) and only in a few cases a single-layer form. Although there are lots of methods to functionalize graphene, many problems still remain unsolved including the size of the flakes, quality control, the number of defects, and the distinction between single- or few-layer graphene. The method for the mass production of graphene has also not been identified.

3. Structure Defects

Nowadays, graphene is one of the most promising materials in nanotechnology. However, little is known about the influence of structural defects of graphene nanostructures caused by the synthesis on a large scale and there is a discrepancy between the ideal graphene and its excellent properties predicted by theory and in practice. Most of the laboratory studies showing its unique properties were carried out on the small, uncontaminated model samples. Since defect-free materials do not exist it is important to understand the mechanisms of their formation and the influence on the material properties mainly because when induced on purpose and in a controlled manner they can even become desirable and suitable for some new solutions [17]. Various types of defects can be formed spontaneously, can be generated during the production process depending on the temperature and chemicals used, and can be artificially introduced to change the properties of the material. The reduced dimensionality of graphene in these cases may be a disadvantage because it increases the number of possible types of defects. The cause and the probability of their formation are not known. Carbon atoms in the 2D honeycomb structure of graphene have the ability to relocate and reorganize the hexagonal lattice to form nonhexagonal carbon rings [46]. The defects in graphene form the net of carbon=carbon double bonds whose number depends greatly on the method used for the graphene production [47]. Symmetry-breaking point defects mostly occur in graphene plane and include vacancies and substitutional or interstitial impurities. In any case the changes in the graphene structure are due to the absence of one or more sp^2 carbon atoms or presence of one or more different atoms with sp^3 hybridization. The defects can also migrate which has an influence on the properties of a defective crystal. The defects change electronic structure and the susceptibility to chemical reactions which modifies the chemical reactivity of graphene [48]. The simplest example of in-plane reconstruction is the 5-7-7-5 Stone-Wales defect which lies in rotation of the two

Table 1: Two main approaches with different methods of graphene synthesis [19].

Method	Description	Advantages/disadvantages
	Top-down methods	
Micromechanical cleavage	First method used to isolate graphene from graphite using adhesive tape. Involves repeated cleavage and yields mono-, di-, and few-layer graphene.	High quality graphene sheets. Slow method, used mainly for study of the graphene properties.
Electrochemical exfoliation	Exfoliation of graphite as a sacrificial electrode and collecting graphene from the electrolyte solution (e.g., surfactant, H_2SO_4-KOH).	Produces a mixture of different thicknesses of graphite flakes with the possibility to isolate few-layer graphene by centrifugation. Surfactant molecules are difficult to remove and influence the electrical and electrochemical properties of graphene.
Solvent-based exfoliation	Solvent-assisted or thermal exfoliation methods for the production of graphene from GICs. Exfoliation of unmodified graphite via sonication in solvents. Exfoliation of graphite oxide. (Mostly used method of solvent exfoliation and reduction to obtain graphene including Hummers [81] method to synthesize graphite oxide.)	Expensive and hazardous solvents or surfactant molecules are difficult to remove and affect properties of graphene. Increased concentration of graphene is accompanied by the decrease in flake size and increase in defect contamination. Exfoliation of graphite oxide results in rGO with different properties than pristine graphene due to high levels of defects induced by the harsh conditions of the production process.
Unzipping carbon nanotubes	Few-layer graphene synthesis through unzipping single or multiwall carbon nanotubes using wet chemistry methods or physical methods.	The unzipping results in graphene nanoribbons with different widths considered as quasi-one-dimensional material with different properties than pristine graphene.
	Bottom-up methods	
Epitaxial growth	The formation of graphene on silicon carbide (SiC) at high temperatures (>1000°C) and generally in ultrahigh vacuum (UHV) conditions or in different atmospheres by the preferential sublimation of silicon from SiC surface and graphitization of the carbon atoms left behind.	Relatively high quality but rarely ≤2 layers of graphene. Possible transfer of graphene from SiC substrates. SiC substrates are commercially available but are too expensive for commercial applications.
Chemical vapor deposition (CVD)	Formation of graphene films by the high temperature carbon pyrolysis in gaseous atmosphere on metal substrates. The optimum conditions of the process depend on the chosen metal substrate. Possible synthesis of graphene nanosheets without preparing the substrate.	Production of monolayer graphene, but the number of layers strongly depends on various process factors.

carbon atoms by 90° which results in the conversion of four six-carbon rings for two pentagons and two heptagons (Figure 4(a)). Second simple defect concerning any material is the missing lattice atom. To lower the total energy of the system caused by nonlinear atomic rearrangements (Jahn-Teller distortion) two of the three bonds saturate towards the missing atom which leads to the formation of two new rings, one with the five and another with nine carbon atoms (5-9) (Figure 4(b)). Another common rearrangement of the graphene symmetry is double vacancies resulting in two pentagons and one octagon (5-8-5) instead of four hexagons which also do not affect the atomic network (Figure 4(c)). Except for 5-8-5 defect there are more possible configurations caused by two missing atoms; for example, the rotation of one of the bonds in the octagon forms three pentagons and three heptagons with even lower total formation energy (Figure 4(d)) or the rotation of the another bond resulting in a 5555-6-7777 defect (Figure 4(e)). With more than two atoms missing more possible defect configurations can occur [49–51].

Other types of defects change the charge of the graphene to a positive or negative or alter the total atomic weight of the whole crystal. In fact, these defects degrade charge mobility but when introduced intentionally facilitate the manipulation of the charge transport. These impurities can be introduced into the carbon lattice by substitution or functionalization of some original carbon atoms [51]. The doping of graphene can be done by intrinsic defects modification or by adding foreign atoms to the graphene structure [52]. Additional atoms replace one or two carbon atoms and depending on the size or charge much larger ones will be displaced outward from the graphene plane. Graphene planarity is determined by the arrangement of defects mainly because of placing an atom in in-plane position which engages the third dimension and changes in hybridization. However, when two carbon atoms migrating over the graphene surface meet each other and form a dimer, they can be incorporated into the sp^2 carbon lattice but with the expense of local curvature. The properties of graphene depend on the bonding between foreign atoms and graphene. If the van der Waals interactions

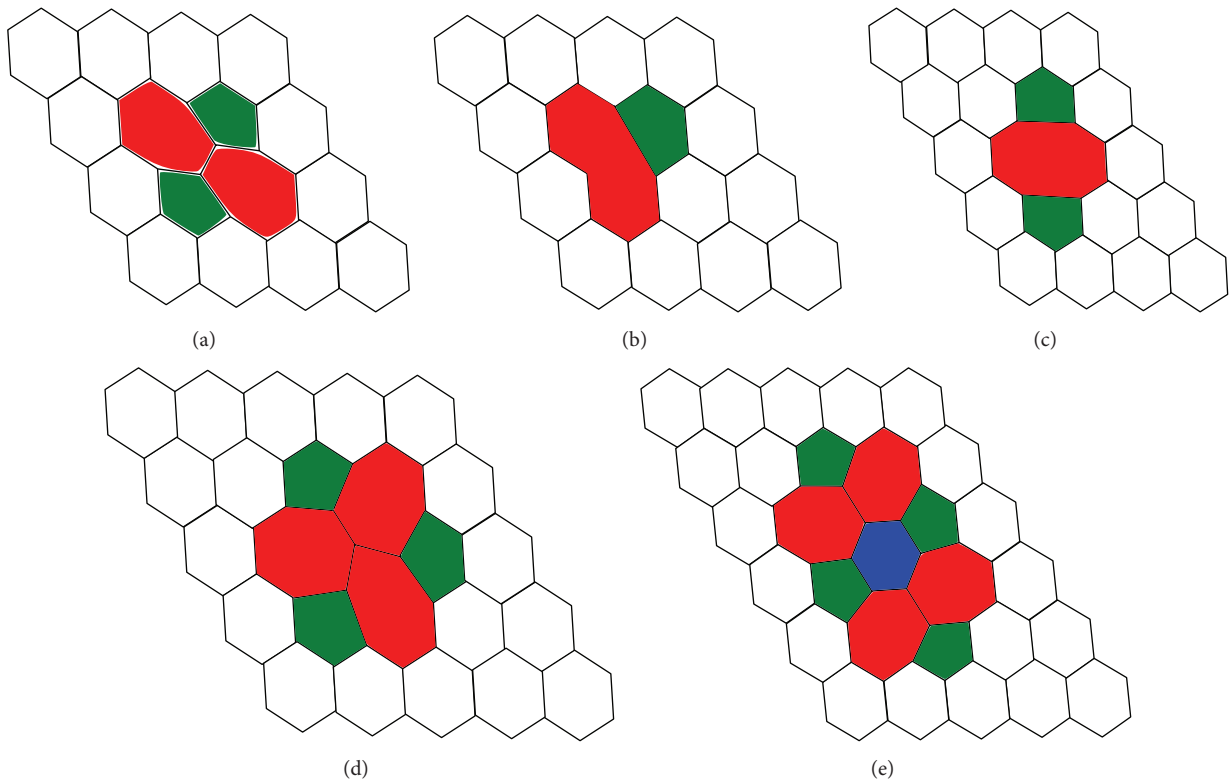

FIGURE 4: Point defects of graphene structure ((a)–(d)). (a) Stone-Wales defect SW(5-7-7-5). (b) Single vacancy defect V_1(5-9). (c) Double vacancy defect V_2(5-8-5). (d) Double vacancy defect V_2(555-777). (e) Double vacancy defect V_2(5555-6-7777) formed from (c). *Reprinted from [46].*

occur the bonding is weak. When foreign atom covalently bonds with the carbon the interaction will be much stronger. Different strength involves various bonding configurations which changes the symmetry and occurs mainly on top of a carbon atom, on top of the center of a hexagon, or on top of the bridge position [46].

Together with in-plane variations the grain-boundary defects can also be observed especially in graphene prepared by chemical vapor deposition or mechanical exfoliation. Zigzag and armchair configurations are two main present graphene boundary terminations and show different electrical and magnetic properties. There are other possible terminations but these two are the mostly preferable. Synthesis of graphene structure with defined edges is not an easy task and for the majority of graphene materials consists of a mixture of the two motifs. Local changes in the reconstruction type or sustained removal of carbon atoms from the edge provides the defects. Armchair edges can be transformed into the zigzag ones and all the intermediates can be considered defective together with chemical groups that can saturate dangling bonds at the edge [46, 49–51]. Obviously, sharp edges and dangling bonds can directly break the membranes of cells and living organisms regardless of graphene properties.

Many outstanding properties of graphene are due to the low concentration of defects but the commercial production of graphene on a large scale introduces changes in curvature, sharpens edges, and makes holes, cavities, or protrusions. All structural defects change physical and chemical properties even at low concentrations. Defectless pristine graphene is relatively inert and exhibits low chemical reactivity with high anisotropic charge transport. It may not be the ideal for some electronic applications but it can be used as a safe and biocompatible biomaterial in various biological systems.

4. Graphene as a Biomaterial

The biomaterial is any substance other than drug or a combination of natural or synthetic substances intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ, or function of the body [European Society for Biomaterials]. Tissue engineering is a combination of the knowledge about the cells, cellular environment, and materials used to improve or regenerate tissues and their function. No matter what biomaterial has been used, a suitable scaffold for tissue engineering should meet certain requirements. First of all any biomaterial must be biocompatible. After implantation the scaffold cannot induce immune response which could reduce the healing process and allow the cells to adhere, proliferate, migrate, and function normally. As implants are not intended to be permanent, the biomaterial should be biodegradable and allow the cells to produce their own extracellular matrix (ECM). All byproducts left after the degradation should not be toxic and should be able to leave the body without any harm. Ideal biomaterial should have specified mechanical properties together with porous architecture to allow cell infiltration and adequate diffusion of

nutrients. And finally the manufacturing technology should be cost effective and possible for scale-up processing and should be made available to the clinicians [53]. Choosing the right synthetic or natural biomaterial plays a critical role in tissue engineering. The material properties such as surface chemistry (e.g., hydrophobicity, functional groups, and types of bonding) and topography, roughness, or porosity can modulate cell response and it is well known that biomaterial surface plays an important role influencing cell phenotype and other growth essential factors. The cell viability, proliferation, and fate depend strongly on the extracellular matrix. A scaffold that mimics ECM and regulates cell behavior and tissue progression would be essential in clinical applications. Owing to its ideal properties, graphene has found potential in a wide range of areas, including tissue engineering. Graphene can provide a perfect surface for cell culture because of its good biocompatibility, chemical inertness, high elasticity, flexibility, and electrical conductivity. Graphene-based materials are a promising tool for tissue engineering, mainly because of its compact, regular structure susceptible to functionalization and modification, and ability to bind a variety of molecules such as proteins, DNA, or drugs [54, 55]. Together with the fact that carbon is the basic element of all biological structures graphene-based nanomaterials can serve as a bioactive scaffold and as a structural reinforcement for other biomaterials currently being used in tissue engineering [56].

So far graphene and its derivatives have been used as a substrate for the stem cells in regenerative medicine [57]. To use stem cells in transplantation and tissue engineering it is necessary to stimulate them properly and provide suitable synthetic or natural growth conditions. An additional benefit would be a scaffold material which has controllable and interactive interface with the living cells. There are mainly three types of stem cells used in regenerative medicine: pluripotent embryonic stem cells isolated from inner cell mass of a blastocyst (ESC), adult stem cells (ASC) with similar regenerative potential but reduced ability to differentiate, and induced pluripotent stem cells (iPS) generated in vitro from somatic cells [58]. Mesenchymal stem cells can be isolated from many adult tissues and can differentiate into adipocytes, osteocytes, or chondrocytes depending on the growth conditions. The first research of Kalbacova and coworkers showed that graphene produced through chemical vapor deposition (CVD) on copper foils is not toxic for human osteoblasts or for mesenchymal stem cells (hMSC) but is even suitable for their growth, proliferation, and later differentiation into osteoblasts [59]. Studies of Nayak et al. also showed that graphene does not affect the morphology, viability, or growth of hMSCs. Moreover, they proved that mesenchymal stem cells differentiate into bone cells in a comparable manner to samples with addition of appropriate growth factors used for osteogenic differentiation [60]. A little bit later Lee et al. proposed the theory due to which hMSCs are so likely to differentiate into osteoblasts on graphene and its derivatives by connecting the strong noncovalent graphene binding abilities towards different growth agents and the degree of π–π stacking [61].

The graphene biocompatibility to mouse iPSCs with good adherence and proliferation was also proved by Chen and his colleagues together with the implication to the great potential of graphene coated materials as platforms for various medical applications and not only for hard tissue engineering [62]. For neuronal regeneration and brain repair it is critical to induce the proper hNSC differentiation towards neurons. The research revealed that graphene substrates possess excellent electrical and electrochemical properties and support the growth of functional neurons and improve their performance and electrical signaling [63]. The cells growth can be stimulated by the change of the surface charge through the contact of graphene with the voltage-gated ion channels. Park and his team observed the promoted cell adhesion, attachment, and enhanced neural differentiation on graphene films in contrast to conventional substrates such as glass [64]. Numerous reports suggest that graphene is an excellent material for the studies with adherent cells especially in the form of film where it can exhibit good biocompatibility with no viability inhibition. The research on adherent human fibroblasts cells NIH-3T3 grown on graphene films and human cancer epithelial cells A549 results in good attachment and favorable growth without inducing deleterious effects while enhancing cellular functions [65, 66]. The studies also demonstrate that graphene oxide paper enhances the attachment and proliferation of mammalian cells without inducing cytotoxic effects [67].

The safety of nanomaterials is a key element for their biomedical applications [68]. However, there are some elements that influence the biocompatibility/toxicity of graphene-related materials and determine how graphene will be incorporated onto the scaffold such as the number of layers, lateral size, surface chemistry, defect density, quality of individual graphene sheets, purity, and first of all the method of production [69, 70]. Graphene is a part of a bigger family including graphene oxide (GO), reduced graphene oxide (rGO), few-layer graphene, and graphene nanosheets, flakes, dots, and ribbons. All these members have different chemical and physical characteristics than pristine graphene and also different toxicological profile. Toxicity of graphene and its derivatives has been established in many studies both in vitro and in vivo, however, the conclusions are often different and sometimes even contradictory [71]. Some of the studies clearly showed toxic effects of graphene but the majority of them have focused on the nonadherent cells cultured in the suspensions with graphene derivatives [72, 73]. The interaction between dispersed graphene materials using cell cultures demonstrates that graphene could be cytotoxic in size-, dose-, and time-dependent manner and can enter the cells and cause ROS generation, hinder nutrient uptake, and activate MAPKs and TGF-related signaling pathways in macrophages [74, 75]. Chen et al. demonstrated inflammatory response regulated by the TLR pathway and autophagy in GO stimulated macrophages [76]. Sasidharan et al. studies showed that additional graphene carboxyl functionalization attenuates the cytotoxic effect on cells [77, 78]. However, there are some chemical modifications on nanosurfaces which increase their solubility in water or serum, prevent aggregation, enhance their biocompatibility, and allow creating new functions for

other applications. Even different oxidation state of graphene which regulates only the dispersibility gives similar toxic response to HepG2 cells but through a different mechanism. GO induced toxicological effect through TGFβ1 signaling pathway whereas rGO elicited innate immune response mediated by TLR4-NFkB pathway [79]. To reduce the toxic effect of graphene there have been attempts to conjugate with known biocompatible polymers such as PEG, PEI, or chitosan or other functional groups [77–79].

Before the systemic administration it is also important to evaluate the hemocompatibility as the graphene enters the blood stream and encounters the blood components. Research indicates that graphene shows little hemolysis of red blood cells and does not influence the coagulation pathways; however, it is difficult to compare all the results because of the different graphene origin and functionalization [71]. Undoubtedly, the surface charge has a strong influence on the stability of erythrocytes, which can be explained by the interaction of negatively charged oxygen groups of GO and positively charged phosphatidylcholine on the surface of red blood cells or the hydrophobic interaction of pristine graphene with the cell membranes. Sasidharan et al. using human primary blood components showed that graphene samples did not interfere with intrinsic and extrinsic coagulation pathways [78]. Liao and his coworkers demonstrated that the particle size, state, and oxygen content of graphene have a strong impact on the red blood cells [80]. Graphene oxide prepared by Hummers Jr. and Offeman [81] method showed strong hemolytic activity at the smallest size, whereas aggregated graphene sheets exhibited the lowest hemolytic activity, suggesting that the more extensive and homogenous the layer of graphene the better the hemo- and biocompatibility.

Controversial reports on the cytotoxicity of graphene depend on the different preparation method, size of the sheets, and the functionalization which greatly restricts its potential applications. Analyzing the surface functionality and reactivity of graphene nanomaterials is necessary to determine the influence and the physiochemical properties which further modulate the biological effects on living cells and allow predicting the modes of action of graphene-based materials.

5. Summary and Conclusions

A lot of researches are focused on the use of graphene in electronics because of the most explored aspects of graphene physics and electronic properties. However, graphene and its derivatives are now rapidly developing in the field of biotechnology and biomedicine. There are many methods for the production of graphene and graphene-based materials such as graphene oxide (GO), reduced graphene oxide (rGO), graphene powder, flakes, or solution, and graphene on the surface of a metal or on any other material (e.g., Cu, SiC). Each of these methods gives the same product but different enough to make it useful in so many applications. Together theoretical and experimental studies show that the properties of graphene strongly depend on its morphology, lateral size, and edge structure [82, 83]. With the rapid development of the new production methods and functionalization approaches graphene has a large potential in genetic engineering, drug delivery approaches, cancer therapy, bioimaging, or biosensing. A controlled method used to tune the lateral size of GO in the nanometer range and tailor its edge structure by oxidation with periodic acid was firstly used to produce fluorescent GO nanosheets for metal ion detection showing the modification of graphene properties together with the change of oxidation state and size in nanometer scale [83]. Graphene nanomaterials have also shown a promise in the area of regenerative medicine as a scaffold in tissue engineering, substrates for stem cell differentiation, and components of implant devices [21, 22]. Different forms and functionalities of graphene determine the hydrophobicity/hydrophilicity, solubility, surface charge, topography, and stability in water solutions. Most of synthesis techniques produce the mixture of graphene samples which differ in size, shape, or number of layers. The various mechanical or chemical methods generate structural defects and contaminations with hydrocarbons or organic molecules which change completely the surface energy and alter the interactions with cells, tissues, or organs. The major obstacle is the uncontrollable and large scale graphene synthesis process which cannot produce good quality material reproducibly. A number of different routes to synthesize graphene have been demonstrated and some of them are better for certain applications than others. The high quality and defect free graphene is not soluble, so, instead, the majority of graphene samples have been produced using graphene oxide or reduced graphene oxide which allows better dispersion and better interactions with the cells but disrupts the original sp2 hybridization and changes all the pristine grapheme unique physical properties.

Ideally flat graphene membrane with no defects and superlative properties actually rarely exists. There is a large gap between the theoretically predicted graphene properties and the actual state of view. In reality, 2D graphene membranes have a tendency to crumble, form ripples and bubbles, rearrange, and create all kinds of defects [84]. Together Meyer et al. revealed that suspended graphene sheets exhibit roughening such that the membrane surface varies out of the plane and that graphene structures cannot exist in the free state and are always a part of larger three-dimensional arrangements [85]. Pure graphene tends to form irreversible agglomerates or restack to graphite, so the key challenge in synthesis and processing a large amount of graphene sheets is to control the aggregation. However, the presence of some defects or structure modifications can alter electronic or chemical properties of graphene in a useful way but researchers must first learn how to introduce them in controllable ways. The physiochemical properties together with the biological effect of graphene family materials depend on the particle state, size, and shape, surface functionalities, and modifications or presence of contaminants. Different fabrication methods generate different amounts of defects or even different oxidative treatments result in the production of graphene oxide with dissimilar properties [82, 83, 86].

There are many potential applications for graphene which developed rapidly in the past few years. However, making and manipulating graphene still remain a challenge like the

controlled, large scale synthesis of high quality graphene. The method of graphene synthesis depends on its application and the toxicity of graphene is related to the exposure environment (aggregation) and mode of interaction with cells (adherent cells versus suspension). However, because of the inconsistency between studies and researches it is difficult to compare and establish whether graphene is in fact safe or toxic. There is still a limited amount of publications concerning the impact of graphene on the immune system and there is a need to standardize the terminology and toxicology testing methods and fabrication techniques for better understanding of graphene characteristics and interactions with living cells in biological systems.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] M. Segal, "Material history: learning from silicon," *Nature*, vol. 483, no. 7389, pp. S43–S44, 2012.

[2] E. H. L. Falcao and F. Wudl, "Carbon allotropes: beyond graphite and diamond," *Journal of Chemical Technology and Biotechnology*, vol. 82, no. 6, pp. 524–531, 2007.

[3] R. E. Smalley, "Discovering the fullerenes (Nobel Lecture)," *Angewandte Chemie International Edition in English*, vol. 36, no. 15, pp. 1594–1601, 1997.

[4] S. Iijima, "Helical microtubules of graphitic carbon," *Nature*, vol. 354, no. 6348, pp. 56–58, 1991.

[5] K. S. Novoselov, A. K. Geim, S. V. Morozov et al., "Electric field in atomically thin carbon films," *Science*, vol. 306, no. 5696, pp. 666–669, 2004.

[6] A. K. Geim, "Graphene: status and prospects," *Science*, vol. 324, no. 5934, pp. 1530–1534, 2009.

[7] A. K. Geim, "Nobel Lecture: random walk to graphene," *Reviews of Modern Physics*, vol. 83, no. 3, pp. 851–862, 2011.

[8] K. S. Novoselov, "Graphene: materials in the flatland," *Reviews of Modern Physics*, vol. 83, no. 3, pp. 837–849, 2011.

[9] L. Yan, F. Zhao, S. Li, Z. Hu, and Y. Zhao, "Low-toxic and safe nanomaterials by surface-chemical design, carbon nanotubes, fullerenes, metallofullerenes, and graphenes," *Nanoscale*, vol. 3, no. 2, pp. 362–382, 2011.

[10] M. J. Allen, V. C. Tung, and R. B. Kaner, "Honeycomb carbon: a review of graphene," *Chemical Reviews*, vol. 110, no. 1, pp. 132–145, 2010.

[11] M. S. Fuhrer, C. N. Lau, and A. H. MacDonald, "Graphene: materially better carbon," *MRS Bulletin*, vol. 35, no. 4, pp. 289–295, 2010.

[12] V. Singh, D. Joung, L. Zhai, S. Das, S. I. Khondaker, and S. Seal, "Graphene based materials: past, present and future," *Progress in Materials Science*, vol. 56, no. 8, pp. 1178–1271, 2011.

[13] S. Eigler and A. Hirsch, "Chemistry with graphene and graphene oxide—challenges for synthetic chemists," *Angewandte Chemie*, vol. 53, pp. 2–21, 2014.

[14] R. van Noorden, "Production: beyond sticky tape," *Nature*, vol. 483, no. 7389, pp. 32–33, 2012.

[15] B. Y. Zhu, S. Murali, W. Cai et al., "Graphene and graphene oxide: synthesis, properties, and applications," *Advanced Materials*, vol. 22, no. 35, pp. 3906–3924, 2010.

[16] C. Soldano, A. Mahmood, and E. Dujardin, "Production, properties and potential of graphene," *Carbon*, vol. 48, no. 8, pp. 2127–2150, 2010.

[17] D. Wei and Y. Liu, "Controllable synthesis of graphene and its applications," *Advanced Materials*, vol. 22, no. 30, pp. 3225–3241, 2010.

[18] M. Hakimi and P. Alimard, "Graphene: synthesis and applications in biotechnology—a review," *World Applied Programming*, vol. 2, no. 6, pp. 377–388, 2012.

[19] R. S. Edwards and K. S. Coleman, "Graphene synthesis: relationship to applications," *Nanoscale*, vol. 5, no. 1, pp. 38–51, 2013.

[20] H. Y. Mao, S. Laurent, W. Chen et al., "Graphene: promises, facts, opportunities, and challenges in nanomedicine," *Chemical Reviews*, vol. 113, no. 5, pp. 3407–3424, 2013.

[21] D. Bitounis, H. Ali-Boucetta, B. H. Hong, D.-H. Min, and K. Kostarelos, "Prospects and challenges of graphene in biomedical applications," *Advanced Materials*, vol. 25, no. 16, pp. 2258–2268, 2013.

[22] H. Zhang, G. Grüner, and Y. Zhao, "Recent advancements of graphene in biomedicine," *Journal of Materials Chemistry B*, vol. 1, no. 20, pp. 2542–2567, 2013.

[23] H. P. Boehm, R. Setton, and E. Stumpp, "Nomenclature and terminology of graphite intercalation compounds," *Pure and Applied Chemistry*, vol. 66, no. 9, pp. 1893–1901, 1994.

[24] A. C. Ferrari, J. C. Meyer, V. Scardaci et al., "Raman spectrum of graphene and graphene layers," *Physical Review Letters*, vol. 97, no. 18, Article ID 187401, 2006.

[25] J. S. Y. Chia, M. T. T. Tan, P. SimKhiew et al., "Facile synthesis of few-layer graphene by mild solvent thermal exfoliation of highly oriented pyrolytic graphite," *Chemical Engineering Journal*, vol. 231, pp. 1–11, 2013.

[26] http://ec.europa.eu/enterprise/policies/raw-materials/critical/index_en.htm.

[27] S. Park and R. S. Ruoff, "Chemical methods for the production of graphenes," *Nature Nanotechnology*, vol. 4, no. 4, pp. 217–224, 2009.

[28] X. Li, W. Cai, J. An et al., "Large-area synthesis of high-quality and uniform graphene films on copper foils," *Science*, vol. 324, no. 5932, pp. 1312–1314, 2009.

[29] W. Yang, G. Chen, Z. Shi et al., "Epitaxial growth of single-domain graphene on hexagonal boron nitride," *Nature Materials*, vol. 12, no. 9, pp. 792–797, 2013.

[30] E. Meca, J. Lowengrub, H. Kim, C. Mattevi, and V. B. Shenoy, "Epitaxial graphene growth and shape dynamics on copper: phase-field modeling and experiments," *Nano Letters*, vol. 13, no. 11, pp. 5692–5697, 2013.

[31] A. N. Obraztsov, E. A. Obraztsova, A. V. Tyurnina, and A. A. Zolotukhin, "Chemical vapor deposition of thin graphite films of nanometer thickness," *Carbon*, vol. 45, no. 10, pp. 2017–2021, 2007.

[32] A. Reina, X. Jia, J. Ho et al., "Large area, few-layer graphene films on arbitrary substrates by chemical vapor deposition," *Nano Letters*, vol. 9, no. 1, pp. 30–35, 2009.

[33] D. Wei, Y. Liu, L. Cao et al., "A magnetism-assisted chemical vapor deposition method to produce branched or iron-encapsulated carbon nanotubes," *Journal of the American Chemical Society*, vol. 129, no. 23, pp. 7364–7368, 2007.

[34] Z. Sun, Z. Yan, J. Yao, E. Beitler, Y. Zhu, and J. M. Tour, "Growth of graphene from solid carbon sources," *Nature*, vol. 468, no. 7323, pp. 549–552, 2010.

[35] G. Ruan, Z. Sun, Z. Peng, and J. M. Tour, "Growth of graphene from food, insects, and waste," *ACS Nano*, vol. 5, no. 9, pp. 7601–7607, 2011.

[36] J. S. Y. Chia, M. T. T. Tan, P. SimKhiew et al., "Facile synthesis of few-layer graphene by mild solvent thermal exfoliation of highly oriented pyrolytic graphite," *Chemical Engineering Journal*, vol. 231, pp. 1–11, 2013.

[37] C.-Y. Su, A.-Y. Lu, Y. Xu, F.-R. Chen, A. N. Khlobystov, and L.-J. Li, "High-quality thin graphene films from fast electrochemical exfoliation," *ACS Nano*, vol. 5, no. 3, pp. 2332–2339, 2011.

[38] A. V. Alaferdov, A. Gholamipour-Shirazi, M. A. Canesqui, Y. A. Danilov, and S. A. Moshkalev, "Size-controlled synthesis of graphite nanoflakes and multi-layer graphene by liquid phase exfoliation of natural graphite," *Carbon*, vol. 69, pp. 525–535, 2014.

[39] Y. Hernandez, V. Nicolosi, M. Lotya et al., "High-yield production of graphene by liquid-phase exfoliation of graphite," *Nature Nanotechnology*, vol. 3, no. 9, pp. 563–568, 2008.

[40] K. B. Ricardo, A. Sendecki, and H. Liu, "Surfactant-free exfoliation of graphite in aqueous solutions," *Chemical Communications*, vol. 50, no. 21, pp. 2751–2754, 2014.

[41] J. Xu, D. K. Dang, V. T. Tran et al., "Liquid-phase exfoliation of graphene in organic solvents with addition of naphthalene," *Journal of Colloid and Interface Science*, vol. 418, pp. 37–42, 2014.

[42] S. Stankovich, D. A. Dikin, R. D. Piner et al., "Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide," *Carbon*, vol. 45, no. 7, pp. 1558–1565, 2007.

[43] V. C. Sanchez, A. Jachak, R. H. Hurt, and A. B. Kane, "Biological interactions of graphene-family nanomaterials: an interdisciplinary review," *Chemical Research in Toxicology*, vol. 25, no. 1, pp. 15–34, 2012.

[44] K. ul Hasan, M. O. Sandberg, O. Nur, and M. Willander, "Polycation stabilization of graphene suspensions," *Nanoscale Research Letters*, vol. 6, article 493, 2011.

[45] S. Park, J. An, I. Jung et al., "Colloidal suspensions of highly reduced graphene oxide in a wide variety of organic solvents," *Nano Letters*, vol. 9, no. 4, pp. 1593–1597, 2009.

[46] F. Banhart, J. Kotakowski, and A. V. Krasheinnikov, "Structural defects in graphene," *ACS Nano*, vol. 5, no. 1, pp. 26–41, 2011.

[47] L. Rodríguez-Pérez, M. Á. Herranz, and N. Martín, "The chemistry of pristine graphene," *Chemical Communications*, vol. 49, no. 36, pp. 3721–3735, 2013.

[48] X. Gao, Y. Wang, X. Liu et al., "Regioselectivity control of graphene functionalization by ripples," *Physical Chemistry Chemical Physics*, vol. 13, no. 43, pp. 19449–19453, 2011.

[49] J. Lu, Y. Bao, C. L. Su, and K. P. Loh, "Properties of strained structures and topological defects in graphene," *ACS Nano*, vol. 7, no. 10, pp. 8350–8357, 2013.

[50] I. Zsoldos, "Effect of topological defects on graphene geometry and stability," *Nanotechnology, Science and Applications*, vol. 3, no. 1, pp. 101–106, 2010.

[51] P. T. Araujo, M. Terrones, and M. S. Dresselhaus, "Defects and impurities in graphene-like materials," *Materials Today*, vol. 15, no. 3, pp. 98–109, 2012.

[52] T. Kuila, S. Bose, A. K. Mishra, P. Khanra, N. H. Kim, and J. H. Lee, "Chemical functionalization of graphene and its applications," *Progress in Materials Science*, vol. 57, no. 7, pp. 1061–1105, 2012.

[53] F. J. O'Brein, "Biomaterials & scaffolds for tissue engineering," *Materials Today*, vol. 14, no. 3, pp. 88–95, 2011.

[54] A. M. Pinto, I. C. Gonçalves, and F. D. Magalhães, "Graphene-based materials biocompatibility: a review," *Colloids and Surfaces B: Biointerfaces*, vol. 111, pp. 188–202, 2013.

[55] Y. Zhang, T. R. Nayak, H. Hong, and W. Cai, "Graphene: a versatile nanoplatform for biomedical applications," *Nanoscale*, vol. 4, no. 13, pp. 3833–3842, 2012.

[56] N. Li, Y. Cheng, Q. Song, Z. Jiang, M. Tang, and G. Cheng, "Graphene meets biology," *Chinese Science Bulletin*, vol. 59, no. 13, pp. 1341–1354, 2014.

[57] S. Martino, F. D'Angelo, I. Armentano, J. M. Kenny, and A. Orlacchio, "Stem cell-biomaterial interactions for regenerative medicine," *Biotechnology Advances*, vol. 30, no. 1, pp. 338–351, 2012.

[58] L. Feng, L. Wu, and X. Qu, "New horizons for diagnostics and therapeutic applications of graphene and graphene oxide," *Advanced Materials*, vol. 25, no. 2, pp. 168–186, 2013.

[59] M. Kalbacova, A. Broz, J. Kong, and M. Kalbac, "Graphene substrates promote adherence of human osteoblasts and mesenchymal stromal cells," *Carbon*, vol. 48, no. 15, pp. 4323–4329, 2010.

[60] T. R. Nayak, H. Andersen, V. S. Makam et al., "Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells," *ACS Nano*, vol. 5, no. 6, pp. 4670–4678, 2011.

[61] W. C. Lee, C. H. Y. X. Lim, H. Shi et al., "Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide," *ACS Nano*, vol. 5, no. 9, pp. 7334–7341, 2011.

[62] G.-Y. Chen, D. W.-P. Pang, S.-M. Hwang, H.-Y. Tuan, and Y.-C. Hu, "A graphene-based platform for induced pluripotent stem cells culture and differentiation," *Biomaterials*, vol. 33, no. 2, pp. 418–427, 2012.

[63] N. Li, Q. Zhang, S. Gao et al., "Three-dimensional graphene foam as a biocompatible and conductive scaffold for neural stem cells," *Scientific Reports*, vol. 3, article 1604, 2013.

[64] S. Y. Park, J. Park, S. H. Sim et al., "Enhanced differentiation of human neural stem cells into neurons on graphene," *Advanced Materials*, vol. 23, no. 36, pp. H263–H267, 2011.

[65] S. R. Ryoo, Y. K. Kim, M. H. Kim, and D. H. Min, "Behaviors of NIH-3T3 fibroblasts on graphene/carbon nanotubes: proliferation, focal adhesion, and gene transfection studies," *ACS Nano*, vol. 4, no. 11, pp. 6587–6598, 2010.

[66] Y. Chang, S.-T. Yang, J.-H. Liu et al., "In vitro toxicity evaluation of graphene oxide on A549 cells," *Toxicology Letters*, vol. 200, no. 3, pp. 201–210, 2011.

[67] O. N. Ruiz, K. A. S. Fernando, B. Wang et al., "Graphene oxide: a nonspecific enhancer of cellular growth," *ACS Nano*, vol. 5, no. 10, pp. 8100–8107, 2011.

[68] H. Shen, L. Zhang, M. Liu, and Z. Zhang, "Biomedical applications of graphene," *Theranostics*, vol. 2, no. 3, pp. 283–294, 2012.

[69] M. Yang, J. Yao, and Y. Duan, "Graphene and its derivatives for cell biotechnology," *The Analyst*, vol. 138, no. 1, pp. 72–86, 2013.

[70] Y. Wang, Z. Li, J. Wang, J. Li, and Y. Lin, "Graphene and graphene oxide: biofunctionalization and applications in biotechnology," *Trends in Biotechnology*, vol. 29, no. 5, pp. 205–212, 2011.

[71] A. Bianco, "Graphene: Safe or toxic? the two faces of the medal," *Angewandte Chemie—International Edition*, vol. 52, no. 19, pp. 4986–4997, 2013.

[72] X. Hu and Q. Zhou, "Health and ecosystem risks of graphene," *Chemical Reviews*, vol. 113, no. 5, pp. 3815–3835, 2013.

[73] A. M. Jastrzębska, P. Kurtycz, and A. R. Olszyna, "Recent advances in graphene family materials toxicity investigations," *Journal of Nanoparticle Research*, vol. 14, article 1320, 2012.

[74] Y. Li, Y. Liu, Y. Fu et al., "The triggering of apoptosis in macrophages by pristine graphene through the MAPK and TGF-beta signaling pathways," *Biomaterials*, vol. 33, no. 2, pp. 402–411, 2012.

[75] H. Zhou, K. Zhao, W. Li et al., "The interactions between pristine graphene and macrophages and the production of cytokines/chemokines via TLR- and NF-κB-related signaling pathways," *Biomaterials*, vol. 33, no. 29, pp. 6933–6942, 2012.

[76] G.-Y. Chen, H.-J. Yang, C.-H. Lu et al., "Simultaneous induction of autophagy and toll-like receptor signaling pathways by graphene oxide," *Biomaterials*, vol. 33, no. 27, pp. 6559–6569, 2012.

[77] A. Sasidharan, L. S. Panchakarla, P. Chandran et al., "Differential nano-bio interactions and toxicity effects of pristine versus functionalized graphene," *Nanoscale*, vol. 3, no. 6, pp. 2461–2464, 2011.

[78] A. Sasidharan, L. S. Panchakarla, A. R. Sadanandan et al., "Hemocompatibility and macrophage response of pristine and functionalized graphene," *Small*, vol. 8, no. 8, pp. 1251–1263, 2012.

[79] N. Chatterjee, H.-J. Eom, and J. Choi, "A systems toxicology approach to the surface functionality control of graphene-cell interactions," *Biomaterials*, vol. 35, no. 4, pp. 1109–1127, 2014.

[80] K.-H. Liao, Y.-S. Lin, C. W. MacOsko, and C. L. Haynes, "Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts," *ACS Applied Materials and Interfaces*, vol. 3, no. 7, pp. 2607–2615, 2011.

[81] W. S. Hummers Jr. and R. E. Offeman, "Preparation of graphitic oxide," *Journal of the American Chemical Society*, vol. 80, no. 6, p. 1339, 1958.

[82] H. Yue, W. Wei, Z. Yue et al., "The role of the lateral dimension of graphene oxide in the regulation of cellular responses," *Biomaterials*, vol. 33, no. 16, pp. 4013–4021, 2012.

[83] D. Wang, L. Wang, X. Dong, Z. Shi, and J. Jin, "Chemically tailoring graphene oxides into fluorescent nanosheets for Fe^{3+} ion detection," *Carbon*, vol. 50, no. 6, pp. 2147–2154, 2012.

[84] A. Fasolino, J. H. Los, and M. I. Katsnelson, "Intrinsic ripples in graphene," *Nature Materials*, vol. 6, no. 11, pp. 858–861, 2007.

[85] J. C. Meyer, A. K. Geim, M. I. Katsnelson, K. S. Novoselov, T. J. Booth, and S. Roth, "The structure of suspended graphene sheets," *Nature*, vol. 446, no. 7131, pp. 60–63, 2007.

[86] E. L. K. Chng and M. Pumera, "The toxicity of graphene oxides: dependence on the oxidative methods used," *Chemistry - A European Journal*, vol. 19, no. 25, pp. 8227–8235, 2013.