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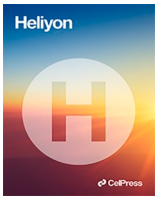

Review article

Graphene and its hybrid nanocomposite: A Metamorphoses elevation in the field of tissue engineering

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ABSTRACT

In this discourse, we delve into the manifold applications of graphene-based nanomaterials (GBNs) in the realm of biomedicine. Graphene, characterized by its two-dimensional planar structure, superconductivity, mechanical robustness, chemical inertness, extensive surface area, and propitious biocompatibility, stands as an exemplary candidate for diverse biomedical utility. Graphene include various distinctive characteristics of its two-dimensional planar structure, enormous surface area, mechanical and chemical stability, high conductivity, and exceptional biocompatibility. We investigate graphene and its diverse derivatives, which include reduced graphene oxides (rGOs), graphene oxides (GOs), and graphene composites, with a focus on elucidating the unique attributes relevant to their biomedical utility. In this review article it highlighted the unique properties of graphene, synthesis methods of graphene and functionalization methods of graphene. In the quest for novel materials to advance regenerative medicine, researchers have increasingly turned their attention to graphene-based materials, which have emerged as a prominent innovation in recent years. Notably, it highlights their applications in the regeneration of various tissues, including nerves, skeletal muscle, bones, skin, cardiac tissue, cartilage, and adipose tissue, as well as their influence on induced pluripotent stem cells, marking significant breakthroughs in the field of regenerative medicine. Additionally, this review article explores future prospects in this evolving area of study.

1. Introduction

Carbon is a ubiquitous component that has a significant blueprint in the fields of science and technology. Carbon nanostructures can be categorized into zero, one, two, and three-dimensional configurations, with fullerenes, graphene quantum dots, and carbon dots representing zero-dimensional forms, and nanofibers and nanotubes comprising one-dimensional carbon nanomaterials [1,2].

The recently discovered two-dimensional carbon nanostructures offer intriguing properties. Graphene, a widely recognized two-dimensional nanostructure, forms the basis for the creation of three-dimensional graphene superstructures and nanotube-graphene hybrids, predominantly composed of one and two-dimensional structural components, thereby yielding the strongest and thinnest material currently employed. Mostly, it is made of two-dimensional sheets that are only 10 nm thick [3]. Graphene and its derivatives, composed of carbon atoms exhibiting sp^2 hybridization and arranged in a honeycomb lattice structure, find extensive application

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across various scientific domains owing to their distinctive physical and chemical attributes, including exceptional surface area, robust mechanical properties, transparency, electrical conductivity, and biocompatibility [4]. Graphene enhances the mechanical and structural properties of materials and holds promise for a wide array of applications in disease diagnosis and treatment, bioimaging, drug delivery, cancer therapy, and genetic research, supported by its unique surface attributes that confer superior biocompatibility compared to other carbon nanostructures. The physicochemical attributes of graphene, encompassing factors such as layer count, chemical functional groups, surface charge density, among others, exert an influence on its toxicity profile, and while numerous publications exist regarding the toxicity and biocompatibility of graphene-based nanostructures, comprehensive and exhaustive examinations in this regard remain incomplete [5].

Atoms containing carbon in graphene are organized in a regular hexagonal lattice to form one-atom thick carbon layer. Graphene's distinctive combination of exceptional chemical, optical, mechanical, and electrical properties influence a broad spectrum of technologies, spanning from macroscale membrane and mechanical functions to nanoscale applications in electronics, optoelectronics, and biology. To produce graphene monolayers from graphite, groups have continuously investigated various methods [6,7]. Mechanical exfoliation and chemical vapor deposition (CVD) techniques continue to be widely used to create high-quality graphene single layers that display good sheet characteristics. Mechanical exfoliation involves the precise peeling of atomically thin layers of graphene from graphite utilizing adhesive materials like scotch tape, whereas in CVD, graphene is typically grown on specific substrates such as copper, utilizing a carbon source as a precursor within a high-temperature reaction chamber [8–10]. These processes produce graphene that is suitable for use in high-end electrical and optoelectronic devices. For instance, graphene produced by CVD can achieve electrical mobility values of 105 $cm^2V^{-1}s^{-1}$. Contrarily, the electronic mobility of silicon, a widely used semiconductor, is approximately 1000 $cm^2V^{-1}s^{-1}$. At the nanoscale, CVD-graphene has an intrinsic tensile strength of 118 GPa, higher than the often-used structural steel [11,12]. Carbon, an abundant element of paramount importance in scientific and technological domains, can yield diverse allotropes through alterations in sp, sp^2, and sp^3 hybridization arrangements, resulting in a multitude of synthesized carbon structures and compact formations documented in the scientific literature. There are a variety of potential architectures, morphologies, and characteristics for carbon nanostructures, but typically they are formed mostly of sp^2 containing carbon atoms organized in a hexagonal crystal lattice [13–15].

Graphene oxide, or GO, offers a fantastic chance to advance the field of Regenerative Medicine (RM) significantly. The capacities to cultivate a biological niche for stem cells on the surface of nanoparticles are one benefit of employing GO in Tissue Engineering (TE) and RM. As a result of their ability to self-renew, proliferate, and differentiate into specific cell types under the right circumstances, stem cells also play a major role in RM. By creating and engineering particular niches with particular components, topographical characteristics, and participation of growth factors, TE, on the other hand, seeks to stimulate specialized differentiation into stem cells [16,17]. This makes GO an intriguing framework for the control of stem cell behaviour since it may be applied to the non-invasive tracing of stem cells in vivo, the release of biologically active substances (such as pro-survival and anti-inflammatory ones) from delivery systems containing stem cells, and the intracellular delivery of substances (such as growth factors, DNA, and synthetic proteins) to regulate the differentiation and proliferation of stem cells, among other uses. Moreover, growth factors (GFs), which are essential for migration, maturation, proliferation, and the differentiation of immature precursors into functional tissues, can be transported by GO due to characteristics like surface chemistry and size. Alternatively, therapeutic elements might be used directly to enhance tissue healing and ingrowth. However, the latter strategy's usefulness is limited by its vulnerability to degeneration and its inadequate and non-specific cellular absorption. Due to this limitation, greater doses are required, which is costly and increases the possibility of negative side effects [18–20].

Nonetheless, by enabling high factor concentration and averting degeneration, nanoparticle-based delivery systems can enhance the pharmacokinetics of these medicinal factors. Because of its high potential for chemical functionalization, GO is a good choice for tissue targeting and drug delivery. The use of GFs and other bioactive substances in TE methods has drawn increasing attention. GFs can also be made more bioavailable locally by upregulating their expression by transfection and gene delivery. Since gene transfection ensures that GFs will always be accessible, it might be preferable to direct administration. Moreover, by upregulating pertinent genes, gene therapy enables the promotion of cell proliferation. The latter method's primary drawback is its inability to preserve the stability and integrity of foreign DNA. Several nanoparticle-based vectors have been developed to enable effective gene delivery, and GO has shown to be one of the most effective of them. This study focuses on the latest developments in graphene-based material science, with a specific focus on their potential uses in regenerative medicine [21–23].

1.1. The evolution of graphene and its derivatives

Over a century ago, Landau and Peierls posited that thermodynamic instability posed a hindrance to the existence of two-dimensional (2D) nanocrystals, sparking a longstanding discourse on the stability or instability of materials in two dimensions. Subsequently, this conjecture received empirical support through experimental findings by Mermin, who observed a notable reduction in the melting point of small crystals as their dimensions decreased, thus raising questions regarding the viability of 2D nanocrystals. As a result, the observed two-dimensional atomic materials formed epitaxially on the surfaces of monocrystals with complementary crystal lattices were regarded as an essential component of a 3D system. Therefore, 2D materials were assumed to be non-existent without considering such a 3D substrate. A perspective that persisted since the experimental discovery of graphene in 2004 [24–26].

To comprehend graphene, envision it as an ultra-thin layer of graphite; thus, its properties, albeit remarkable, might not appear novel, with historical roots extending back centuries, as graphite has been utilized since medieval times for the production of writing tools such as pencils. The extraordinary qualities of graphite, such as its thermal conductivity (about 3000 W/mK), in-plane electrical conductivity, and mechanical rigidity of the hexagonal network (1060 GPa), make it possible for it to be used in a variety of industrial

applications, with 1 million tonne annual demand worldwide [27,28].

Scientists have capitalized on the anisotropic properties of graphite materials in diverse scientific domains, owing to the 120° angle of the sp^2 atomic orbitals, incorporating s, p_x, and p_y electrons, forming C–C–C bonds resembling interconnected sheets akin to chicken wire. Due to each carbon atom forming bonds with three neighbouring carbons using its four valence electrons, the remaining p_z orbitals are positioned below the three adjacent carbon atoms, constituting the valence band (occupied π* orbitals) and conduction band (unoccupied π* orbitals) as distinct bands; three of these orbitals contribute to the sigma bond (σ), while one orbital contributes to a third of the bond. The absence of a chemical bond between adjacent planes leads to relatively weak inter-plane interactions, resulting in a honeycomb-like structure. This unique arrangement imparts anisotropic mechanical and physicochemical properties to graphite, where characteristics significantly vary both out-of-plane and in-plane, contingent upon the specific direction [29,30].

Graphene consists of both primary derivatives Graphene oxide (GO) and reduced-graphene oxide (rGO). Graphite may be used to create GO by oxidizing it, and GO could be reduced to produce rGO. A broad range of functional groups on GO, including hydroxyl, epoxy groups, and carboxyl, facilitates its conjugation with a variety of molecules, particularly in the realm of biological applications; its heightened bioactivity and mechanical properties make it an intriguing and enhanced alternative for bio-applications. The degree of the reduction reaction in the rGO may control the ratio of groups that include oxygen to surface defects, resulting in a structure that is very similar to that of pure and filtered graphene [31–33]. The critical milestone in development of graphene has been shown tabulated in Table 1.

1.2. Graphene morphology and its Analogous

Graphene and its derivatives, all falling within the category of 2D materials, share structural resemblances, yet minor alterations significantly influence their physicochemical properties. This section discusses the composition of graphene and its two main variants in detail [40].

1.2.1. Graphene

Since carbon constitutes the fundamental element in graphene and its derivatives, a comprehensive understanding of carbon and its bonding is imperative, wherein covalent interactions enable two carbon atoms to mutually share four valence electrons, potentially leading to hybridization into three distinct states: sp, sp^2, and sp^3. Graphenes 2D structure results from sharing one carbon atom sp^2 hybridized orbitals with its three nearby carbon atoms. The in-plane bonding in graphene is facilitated by sp^2 hybridized orbitals, comprising the s, p_x, and p_y orbitals, while the p_z orbital remains unutilized and parallel to the plane [29–31]. Formation of the sigma bond in the 2D plane has a shallow interatomic distance (approximately 1.42 Å), making it more cogent than sp^3 hybridized orbital in diamond [32,33].

The surface-functionalizing carbon nanotubes procedures are remarkably similar to those used for surface-functionalizing graphene sheets, involving the separation of graphene layers and preparation for subsequent surface modifications. Ultrasound treatment appears to be necessary before any functionalization. Ultrasound treatment is a crucial step preceding functionalization. "Hot" reagents, such as radicals, diazonium salts, nitrenes, and fluorine, are employed due to their preferential reactivity with inactive sp^2 carbons; however, controlling the reaction extent can be challenging.

In contrast, less reactive cycloaddition procedures based on azomethine ylides or benzyne could be used to produce a controlled reaction rate. A mild Friedel-Crafts acylation method could similarly functionalize edges [41,42]. In covalent functionalization of graphene, the transformation of sp^2 orbitals to sp^3 hybridized orbitals is a fundamental process that alters the local symmetry and electronic structure. In bio applications, particularly in the synthesis of polymer nanocomposites, non-covalent functionalization is of significant interest alongside covalent functionalization [43]. The interaction between polymeric molecules and graphene involves various forces, including chemical binding, van der Waals forces, π-π interactions, and electrostatic interactions. Due to graphene's distinctive structure, van der Waals forces and supplementary interactions are consistently employed to interface graphene with polymers.

Additionally, the polymers possessing π-bonds, such as PVA and PMMA, can engage with graphene through π-π interactions, bolstering graphene's stability and augmenting its mechanical, electrical, and thermal attributes. Similarly, integrating graphene nanosheets into metallic or bio-ceramic structures fortifies their mechanical, thermal, and bioactive properties. Given the single-layered carbon structure of graphene nanosheets, precise characterization methods are crucial, albeit challenging. Scientists

Table 1
Critical milestone in development of graphene.

Year	Progression of graphene	References
1848–1958	Brodie, Hummer, and others prepared GO.	[34]
1962	GO is reduced chemically and thermally to create rGO.	[35]
1970	Carbon was separated from nickel surface to create monolayer graphite.	[36]
1986	The term "Graphene" is proposed by Boehm et al. to describe single layers of graphite – like carbon.	[37]
1997	According to IUPAC, graphene should be utilized when discussing individual layer reactions, structural relationships, and other characteristics.	[37]
1999	Rouff et al. isolate multiple layers of graphene by micromechanical exfoliation.	[38]
1999	Geim and Novoselov are awarded with the Nobel Prize.	[39]
2004	Single-layer of Graphene are isolated by Geim and Novoselov via mechanical exfoliation	[39]

typically employ diverse techniques to identify and distinguish graphene nanosheets, many based on standard methods, including optical microscopy, AFM, TEM, SEM, and Raman spectroscopy [44,45].

1.2.2. Graphene oxide

GO is a complex entity arising from the oxidation of graphene, and a definitive model for its structure remains elusive due to the lack of analytical tools capable of precisely characterizing this amorphous material with its nonstoichiometric atomic composition and unique berthollide features [46]. Despite the complexities, several researchers have proposed diverse models, with recent alternatives emphasizing an amorphous and nonstoichiometric approach, differing from traditionally established models such as Hofmann, Scholz-Boehm, Ruess, and Nakajima-Matsuo, which are typically rooted in lattice-based evaluations. The model devised by Anton Lerf and Jacek Klinowski proposes that GO sheets can form hydrogen bonds with each other, and is supported by experimental data obtained from solid-state NMR and various X-ray analysis techniques [47]. Dékány et al. have introduced an improved model, building upon the Scholz-Boehm model, portraying GO as having a corrugated quinoidal structure interspersed with *trans*-linked cyclohexyl segments. Experimental data from FTIR and DRIFT spectroscopy, revealing a characteristic peak at 1714 cm^{-1}, suggests the presence of single ketones and quinones instead of carboxylic groups. This underscores the diverse functional groups in GO, predominantly carboxyl, hydroxyl, or epoxy, which can be comprehensively examined through various analytical techniques, including FTIR and XPS

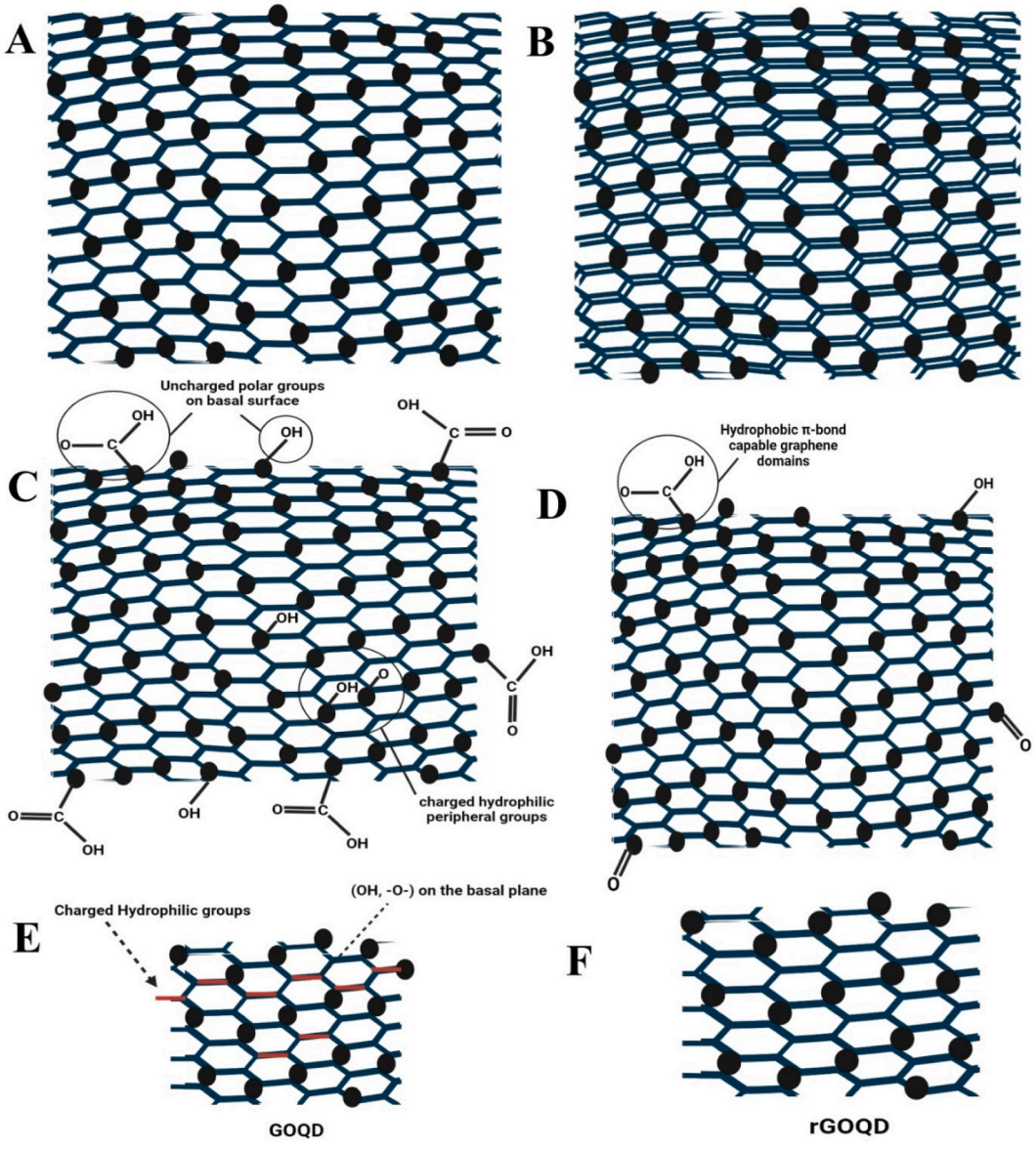

Fig. 1. Graphene in various forms and its Analogs: (A) single-layer graphene, (B) multilayer graphene, (C) GO, (D) rGO, (E) GOQD, (F) rGQD.

[48,49].

Additionally, it should be noted that variations in the raw materials used for the synthesis of GO or in the manner and intensity of the oxidation process may result in a product with different functional groups and structures, which may be the cause of the current uncertainty regarding the precise model structure for GO. However, alternative methods, such as thermal gravimetric analysis (TGA) at a meager rate of temperature change (1 °C/min), could be employed to characterize the thermal stability of GO in addition to the NMR-based characterization. The labile oxygen-containing functional groups within GO potentially contribute to the rapid degradation observed in the TGA curve at 226 °C. Discrepancies in the TGA curve and its slope across different samples can arise due to variances in the chemical structure of GO obtained through different synthesis techniques [50–52].

2. Synthesis of graphene

The production of Graphene and its analogs, the shape, size, and functional groups connected to the material surface significantly affect the desired structure and attributes. The most desirable form is single-layer graphene, which has a fully sp^2 hybridized carbon structure that is only one atom thick and has few imperfections (Fig. 1A). Nevertheless, the advantageous stacking of graphene sheets can lead to the formation of a multi-layered graphene structure. (Fig. 1B). It has proven difficult to synthesize these structures using a bottom-up approach for industrial purposes. Therefore, employing a top-down approach facilitates the production of the extensively oxidized form of graphene oxide. It is characterized by plentiful oxygen groups in both the sp^2 and sp^3 carbon configurations. (Fig. 1C), which can then be reduced (rGO) to produce a material that is more likely to graphene and has significantly better properties (Fig. 1D). The top-down method can then be used to process GO and rGO to form quantum dots that are both rGQD and GOQD (Fig. 1E and F). The following sections will cover the methods currently used to synthesize the materials mentioned above [53–55].

2.1. Synthesis of GO

The production of pristine graphene can be accomplished in two ways: "top-down" techniques that include extracting layers of graphene analogs from a carbon source, usually graphite. While the "bottom-up" approaches that use simple carbon molecules to create pristine graphene. Methods of bottom-up synthesis, like chemical vapor deposition (CVD) and epitaxial growth on silicon carbide wafers have shown to be laborious and difficult to scale up. As a result, top-down methods that produce GO and rGO are more frequently used to produce graphene derivatives, mostly for use in nanocomposite materials. The initial development of GO is often attributed to Staudenmaier, Brodie, Hummers, and Offeman, who independently oxidized graphite using distinct methodologies. The original two approaches by Hummers and Offeman are improved by employing sodium nitrate to create nitric acid in situ rather than using nitric acid as a solvent and by employing $KMnO_4$ as an oxidizer rather than $KClO_3$, which produces poisonous ClO_2 gas. The Hummers approach is typically utilized to create GO due to its safety and scalability [56–59]. The term "modified Hummers' method" refers to a technique that modifies or enhances the synthesis process outlined by Hummers, although its specific definition lacks standardization. Typically, this method involves the addition of a protonated solvent (such as sulfuric acid, phosphoric acid, or a mixture) to a carbon source (usually graphite flakes or powders). Subsequently, a powerful oxidizing agent, commonly $KMnO_4$, is introduced to the mixture. Post-dilution, the resulting mixture is treated with H_2O_2 to eliminate metal ions from the oxidizer and leading to the production of a yellow bubbling solution and ultimately turning into a yellow-brownish liquid. The solution undergoes

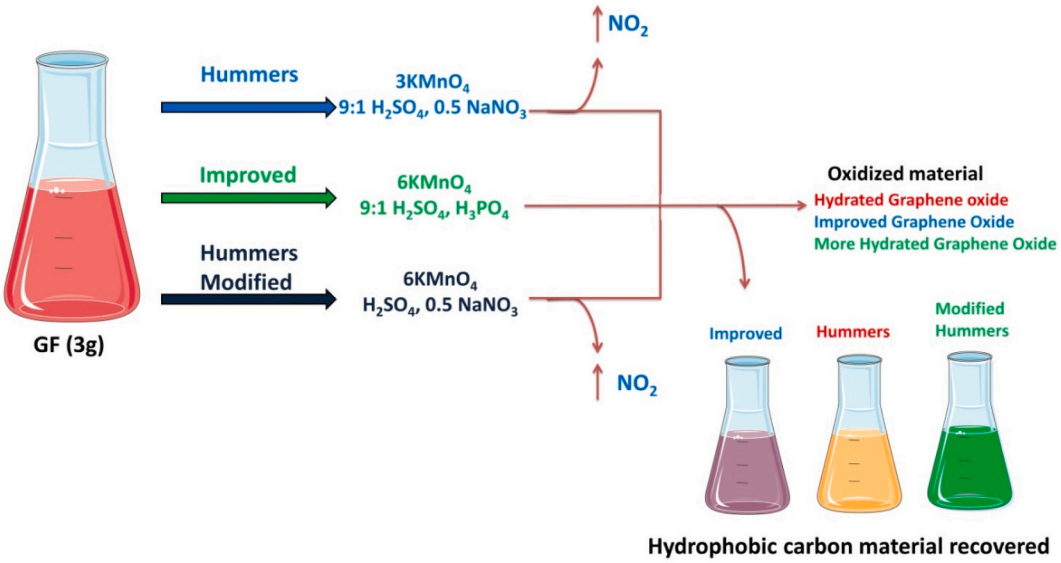

Fig. 2. Most common GO synthesis methods are shown schematically.

multiple rinsing and centrifugation cycles with water until achieving a nearly neutral pH. The resulting particles are then isolated and subjected to treatment with diluted hydrochloric acid to eliminate metal species. The general synthesis route can be changed to suit the requirements of a specific researcher. For instance, it is essential to remember that a modified version of Hummer's approach will allow the shape and size of the carbon source to be used to predict the shape and size of the producing GO. This typically indicates that the although alternative carbon sources can be employed, the average lateral dimension of the resulting GO sheets will depend on the average diameter of the graphite powders used in the synthesis [60].

In an intriguing study, Huang et al. generated elongated GO strips by unzipping multi-walled carbon nanotubes, denoting them as "GO nanoribbons," and to diminish the final product's carbon-to-oxygen ratio, additional oxidation of graphite can be performed before the production of GO [61]. Many laboratories employ the Kovtyukhova et al. approach, which involves pre-treating graphite particles in $K_2S_2O_8$ and P_2O_5 before using them in the Hummers' procedure. Other researchers improve the interlayer distance of the carbon source by thermally treating or subjecting expanded graphite to powerful oxidizers, which facilitates the breakdown of the graphite oxide layers [62]. The "improved Hummers' method," a prominent modification, involves the exclusion of sodium nitride and the addition of phosphoric acid along with increased $KMnO_4$. This refinement results in GO powders with a heightened degree of oxidation and eliminates the emission of harmful gases, simplifying temperature control. Researchers, in adapting the Hummer's procedure for their specific applications, need to carefully consider factors such as the choice of carbon source, pre-treatment methods, oxidizing agent, and selection of protonated solvent, as these parameters significantly influence the carbon-to-oxygen (C/O) ratio of the final product. Fig. 2 depicts an overview of the most often used techniques [63]. Even more innovative methods for GO synthesis have recently been put forth. Using a stationary oxidation mechanism, Zhang et al. created bigger GO sheets which are around 108 m on average and 256 m at their most significant. The standard Hummer's approach yields GO that is significantly smaller than its graphite source and the splitting of GO during oxidation is caused by vigorous mixing during the mixing procedure as well as the elastic strain produced by adding oxygen groups to the flake surface. They added H_2SO_4, $KMnO_4$, and then H_2O_2 ″without external mechanical agitations" (without stirring), resulting in a 3D structure that is well-ordered and ready for "mild agitation" (either manual shaking or mechanical mixing) to exfoliate into extremely big GO. The resultant GO flakes resembled the parent graphite's in terms of size and shape [64–66].

The enlargement of GO sheets bears substantial implications for composite materials, and an alternative approach proposed by Dong et al. involves creating concentrated GO slurries that can be stored and exfoliated into flakes as required, addressing the challenge of poor dispersibility of GO in conventional solvents; while individual GO sheets tend to stack, ion adsorption hinders their agglomeration. Electrostatic repulsion energy defeats van der Waal attractive forces when the graphite precursor is pre-oxidized and suspended in a strongly alkaline aqueous environment (pH = 14). As a result, exfoliation results in low-viscosity slurry rather than a GO dispersion. This slurry demonstrated a potentially crucial method for storing and transporting GO flakes because it could be re-dispersed in N-methyl-2-pyrrolidone (NMP) or alkaline water and could be stored at a 23 wt % solid content. These two instances demonstrate how a greater comprehension of GO synthesis significantly affects scalability, characteristics, and uses [46,67].

2.2. Reduction of GO to rGO

Considerable effort has been dedicated to developing methods for removing oxygen functional groups from GO to achieve materials approaching the purity of graphene, with reduction achievable through diverse means such as chemical, thermal, and electrochemical processes, each imparting distinct shapes and electrical properties to the resulting materials. Key considerations in the reduction of GO involve preserving or enhancing the desired physical and chemical properties, addressing surface oxidation defects, and selecting eco-friendly reducing agents. Thermal treatment relies on the high-temperature decomposition of oxygen groups into CO and CO_2 gases, with the rapid gas evolution demonstrated to exfoliate specific GO nanosheets. Thermal annealing at high temperatures in an oxygen-free atmosphere is one way to do a thermal reduction, as is using less traditional techniques such as microwaving GO powders or flashing GO films with high-intensity light [68,69]. Numerous methods involving chemical-reducing agents in GO solutions are documented in the literature and widely utilized, utilizing agents such as hydrazine, hydrohalic acids, or metal hydrides. Photo-catalyzed reactions offer an alternative route. Williams et al. achieved a successful reduction of GO through UV radiation and a TiO_2 catalyst. In electrochemical reduction, where the transfer of electrons between GO and the electrodes occurs in a standard electrochemical cell, additional chemical agents are unnecessary. Notably, amino acids, sugars, and even microbes have emerged as effective "green" reducing agents for the synthesis of rGO in recent years. Various processes offer varying benefits energy consumption, scalability, and the amount of chemical waste produced [70,71].

2.3. Graphene quantum dots (GQDs)

The quantum confinement and edge effects observed in nanoscale graphene have led to the emergence of a new class of graphene derivatives, known as graphene quantum dots (GQDs), which are graphene nanosheets characterized by lateral dimensions not exceeding 100 nm and ideally consisting of one or a few stacked graphene layers. Since their band gap can be adjusted and their fluorescence is stable, GQDs have attracted much attention lately. Various bottom-up and top-down techniques, such as microwave-assisted thermal treatment, high-power ultrasonication, solvothermal, and hydrothermal are typically used in manufacturing GQDs [72,73].

Lu et al. developed a one-pot hydrothermal technique, which uses black carbon as the feedstock and manufactures GQDs in under 90 min. These GQDs have significant implications for bioimaging technology since they are highly photo-stable and biocompatible using only H_2O_2 as a reagent [74]. Similarly, Wang et al. synthesized GQDs from rice husk biomass through a one-step, one-pot

hydrothermal process, specifically tailored for Fe^3 detection utilizing luminescence quenching. The detailed production techniques, unique optical and electrical properties, and diverse applications including photocatalysis, drug delivery, and bioimaging are extensively covered in the existing literature. It's noteworthy that various methods exist for synthesizing graphene derivatives, and there is no universally standardized procedure for producing GO, rGO, or GQDs. However, there are a lot of novel options that can be implemented using this open-ended synthesis approach. These illustrations, along with the others covered in this review, highlight the capability of GO for surface functionalization and, consequently, the variety of uses for usage in nanocomposite materials [65].

3. Properties of graphene

The GO exhibits distinctive physicochemical properties attributed to the presence of ketones, hydroxyl groups, carboxylic acids, and epoxides, rendering it hydrophilic. These oxygenated groups significantly modify GO's characteristics, enabling biochemical and bioconjugation processes on both its basal plane and margins [75,76]. The hybridization stats (sp, sp^2, sp^3) of carbon-based materials are associated with their physical characteristics. While the diamond with sp^3 hybridization is a hard insulator, graphene with sp^2 hybridization is a fine, zero bandgap semiconductor. Graphene exhibits remarkable heat conductivity, chemical stability, and pliability. One of graphene's better qualities is that its charge carriers behave like massless particles and can move in an environment with minimal scattering. Graphene excellent electronic band structure accounts for both its charge transport and electrical characteristics. Graphene, in particular, has the largest surface area (2630 m^2g^{-1}) of any nanomaterial and may interact directly with a wide variety of biomolecules. It can be used with structural flaws by modifying chemicals using low-cost fabrication techniques. For extremely sensitive sensing applications, graphene's electrical resistance sensitivity to adsorption makes it a valuable material [77,78].

Graphene's magnetic, optical, and high elasticity are among its other special qualities that make it an ideal monolayer structure for the creation of various graphene-based nanocomposites. Graphene's remarkable mechanical qualities stem from its high Young's modulus, or the relationship between stress and strain, and one of the highest tensile strengths of any material. Chemical and biological sensors have been developed that use graphene in a number of sensing applications based on an electrochemical read-out. A combination of metal and proteins with increased sensitivity has been used to create a wide variety of graphene-based nanocomposites in biosensors using graphene derivatives like GO and rGO. GO's high surface-to-volume ratio and functional chemical groups give it a wide range of adsorption capabilities for biomolecules. GO is made up of graphene layers with functional groups like carboxyl, epoxy, and hydroxyl that are active and contain oxygen on their surface. GO possesses distinct optical and electrical characteristics, conductivity, and a tiny size ranging from 20 to 100 nm [79,80].

Moreover, graphene is hydrophobic and difficult to dissolve in water, but GO is hydrophilic and soluble in water. GO has several flaws and a severely disordered sp^2 carbon network; functional groups serve as its insulators. Compared to graphene, GO is less mechanically robust and non-conductive. As a result, GO must be converted into RGO in order to increase its conductivity. It is possible to functionalize graphene nanoparticles by covalent or non-covalent interactions. Oxidation, radicals, reduction, and nucleophilic/electrophilic additives are examples of common covalent reactions. Reduction is the process by which sp^3 carbons are changed into sp^2 carbons, and RGO is created by removing the functional groups from GO, which partially restores the mechanical and electrical conductivity qualities of the graphene layers. RGO and graphene share comparable mechanical, thermal, and electrical characteristics. Because of its superior electrochemical properties, including a reduced oxidation potential, RGO is a viable option for biosensor construction. RGO is clever for biological applications because of its affordability and the O_2 functional groups' controllability [81,82]. A relatively emerging field with enormous potential is the biomedical application of graphene, which goes beyond the applications indicated above. Many intriguing studies have been conducted to investigate the use of graphene for frequent biomedical uses, biological sensing and imaging, ranging from drug/gene delivery, and antibacterial materials, to biocompatible scaffold for cell culture. These studies date back to the 2008 seminal report on the use of graphene oxide (GO) as an efficient nanocarrier for drug delivery by Dai et al. Due to its many fascinating properties, including its high specific surface area (2630 m^2/g), thermal conductivity (5000 W/m/K), remarkable electrical conductivity (mobility of charge carriers, 200,000 $cm^2\ V^{-1}\ s^{-1}$), mechanical strength (Young's modulus, 1100 Gpa), intrinsic biocompatibility, low cost and scalable production, and easy biological/chemical functionalization of GO [79,83,84].

4. Cell Feasibility and toxicity

Nanomaterials based on graphene can either be biocompatible or harmful to living things. These nanoparticles' purity, dosage, surface chemistry, hydrophilicity, layer number, and lateral dimension significantly impact how living cells react to them. Because diverse synthesis techniques were used to create the graphene nanomaterials and different compounds or polymers were available for surface functionalization, the surface chemistries of these materials varied substantially. Numerous major cell lines are usually used for the in vitro assessment of nanomaterial toxicity, containing phagocytes and non-phagocytic cells [85,86]. Understanding the interactions between graphene nanoparticles and cells is crucial for their medical applications, as these nanoparticles can potentially disrupt cell membranes, posing harm to living organisms. Phospholipids are made up of two fatty acid chains and a phosphate head group, make up cell membranes. Variations in head groups, such as choline, phosphatic acid, inositol, ethanolamine, glycerol, and serine, give phospholipids unique characteristics. Cholesterol molecules are also present in cell membranes, playing a vital role in maintaining fluidity, stabilizing membrane structure, and regulating membrane-associated protein activities [87]. Pure graphene predominantly engages in hydrophobic interactions with lipid tails due to its lack of charges on the basal plane, preventing electrostatic binding with phospholipids. Additionally, the damage to the membrane is caused by hydrophobic interactions between the cholesterol tail and pure graphene, which can extract or remove molecules of cholesterol from the membrane. In a recent study,

Bernabo et al. reported that GO may interact with the swine spermatozoa cell, causing the membrane's cholesterol to be extracted. A computational simulation of biomembrane systems reveals that the removal of cholesterol molecules leads to cavities and membrane distortion due to strong forces exerted by the graphene sheet, compromising membrane stability. In a recent study by Duan et al., Surface perforations resulted from the observations that GO can extract phospholipids from the cell membranes of human alveolar epithelial A549 cells and mouse macrophage Raw 264.7 cells. This results in reduced cell viability, and ultimately leading to cell death. Molecular dynamics (MD) simulations explained this phenomenon by highlighting robust contacts between the hydrophobic domains of GO and the carbon atoms in the lipid tails [86,88,89]. The surface charge and chemistry of GO significantly influence cellular interactions. Due to the oxygenated functional groups that give GO its high electrostatic interactions, negative charge density between GO and membrane lipids might happen. Li et al. employed the Langmuir monolayer technique to study GO-lipid interactions using five lipids with comparable 18-carbon alkyl chains but varying head group charges. Their findings revealed that GO lacks electrostatic interactions with neutral or negatively charged lipids, displaying affinity solely for positively charged lipid head groups. Given that mammalian cell membranes predominantly feature negatively or neutrally charged phospholipids, the likelihood of negatively charged lipids attracting GO with similar charges is improbable [90]. According to Hu et al., negatively charged GO repels lipids with similar charges electrostatically, but hydrophobic interactions among negatively charged GO and lipids promote GO adsorption on the lipids. These results suggest that GOs can directly cause cell membrane damage without ever entering the cells through hydrophobic interactions. Recently, Xia and colleagues used hydrated GO (hGO), pristine GO, and rGO to study the effect of GO surface chemistry on the interactions between lipid membranes [91]. They claimed that hGO can cause the surface membrane's lipid peroxidation, resulting in membrane lysis and the breakdown of cell integrity. At 50 °C or 100 °C, sodium hydroxide solution reacted with GO to create hGO. By using the electron paramagnetic resonance method, carbon radicals (*C) and C–OH groups were produced as a result of the epoxy rings of GO reacting with nucleophiles in the solution during the hydration process. These radicals, bearing unpaired electrons, exhibited high reactivity, readily combining with oxygen to form superoxide radicals capable of oxidizing unsaturated lipids and protein thiol groups, leading to the production of lipid peroxides. In human bronchial epithelium BEAS-2B cells and human leukemic monocyte THP-1, this process led to membrane integrity breakdown and cell death. Pure GO's epoxy groups were also capable of generating carbon radicals, albeit to a lesser extent. Consequently, the order of cytotoxicity in both cell types is hGO > GO > rGO, reflecting the impact of different GO-based materials. Due to their small size and sharp edges, graphene-based nanomaterials can infiltrate the cytoplasm and interact with lipid membranes, disrupting cell membranes and causing cytoplasmic leakage. These materials, when present in living cells, induce toxicity by releasing lactate dehydrogenase (LDH), damaging cell membranes, and impairing mitochondrial function by reducing mitochondrial membrane potential (MMP). In the second scenario, membrane lipids' unsaturated fatty acids can react with Reactive Oxygen Species (ROS) to produce lipid peroxides like malondialdehyde (MDA), which

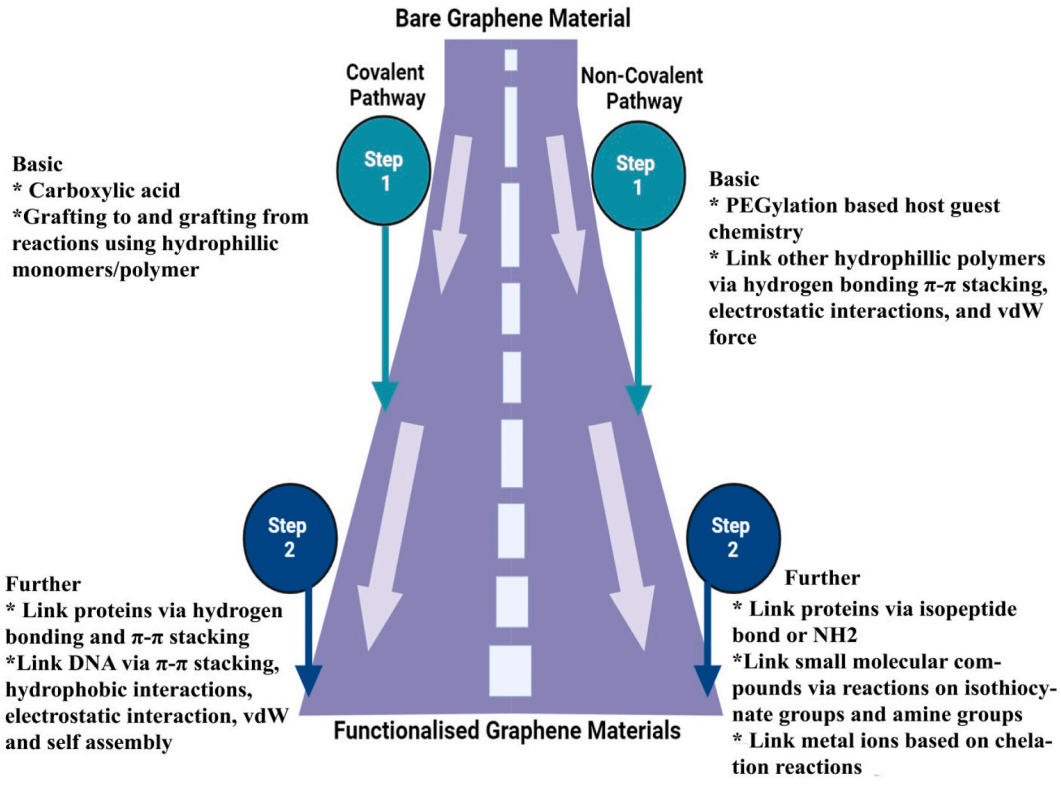

Fig. 3. Common functionalization techniques for materials made of graphene.

can then cause lipid peroxidation. These findings show that GO can cause extracellular and intracellular ROS production in a dose- and time-dependent manner, even at low concentrations. As seen in human HaCaT skin keratinocytes, ROS generation, mitochondrial malfunction, and LDH leakage are thus the main contributing causes of cell death. The nanomaterials might directly interact with DNA, resulting in genotoxicity if they can enter the nucleus. Furthermore, the biocompatibility and hazardous effects of graphene nanoparticles can be affected by in vitro and in vivo circumstances, including graphene dose, exposure time, cell and the technique employed to measure cell viability [92,93].

5. Functionalization of graphene

When used in vivo, bare graphene materials exhibit disadvantages, including dose, size, and time-related toxicity, low biocompatibility, poor dispersity, and inadequate biodegradation. Basic functionalization strategies involve the use of hydrophilic polymers or carboxylic acids through non-covalent and covalent processes to enhance circulation time, improve dispersion and stability, and reduce cytotoxicity. For more specialized applications, subsequent functionalization using proteins, DNA, etc., proves beneficial. Non-covalent methods rely on surfactants' stabilization effects, such as hydrogen bonding, van der Waals forces, electrostatic interactions, and stacking, which adsorb at the graphene surface, ensuring stability. In contrast, covalent bonds form between functionalized materials and graphene based on specific functional groups (-NH_2/-COOH). Covalent approaches offer superior stability and robust mechanical properties, while non-covalent modifications can maintain electrical conductivity and provide a broader graphene surface. Fig 3 illustrates the fundamental functionalization and further functionalization using covalent and non-covalent approaches in this section [94,95].

5.1. Surface functionalization

Bare p-G possesses few functional groups and a hydrophobic outer layer, in contrast to the sharp edges and negatively charged surfaces of bare GO or rGO. Additionally, plain graphene materials are produced utilizing a limited number of thickness or size controls, which results in dose, size, and time-related toxicity and poor dispersity, poor biodegradation, and low biocompatibility. Therefore, bare graphene characteristics must be altered using fundamental functionalization techniques that use carboxylic acids or hydrophilic polymers. After functionalization, graphene materials have improved stability and dispersion, are less toxic, and can support additional functionalization with other compounds [96].

5.1.1. Non-covalent surface functionalization

Non-covalent functionalization techniques necessitate ultrasonication and precise temperature control of the components, utilizing hydrogen bonding interactions, electrostatic interactions, π–π stacking, and van der Waals forces to functionalize graphene with various substances; among these, PEGylation stands out as the most prevalent and established method for graphene modification. According to various studies, rGO has a propensity to restack into graphite or to agglomerate irreversibly as a result of $\boldsymbol{\pi}$–$\boldsymbol{\pi}$ stacking interactions. In addition, because of the growing steric barrier, the non-covalent bonding of PEG with rGO might significantly enhance dispersity and stability while preventing aggregation [97,98].

With a concentration of less than 10 μg mL^{-1}, PEGylation also reduced rGO toxicity, and the presence of ROS had little impact on the level of toxicity. Further amide bonding between functional molecules could be accomplished using amino-terminated PEG as a bridge. Other hydrophilic polymers than PEG have also reportedly been shown to increase stability, dispersity, and lower toxicity. Several groups functionalized GO through $\boldsymbol{\pi}$–$\boldsymbol{\pi}$ interactions using polystyrene sulfonate (PSS) and pyrene-terminated side-chain liquid crystalline polymers [14]. GO could be functionalized by natural polymer dextran using hydrogen bonds and $\boldsymbol{\pi}$–$\boldsymbol{\pi}$ stacking. Based on electrostatic interaction, a few groups have also functionalized GO using cationic polymers such as poly (amidoamine) (PAMAM), poly (ethyleneimine (PEI), and polypropylene imine (PPI) [99–101].

Similarly, Fan et al. explored the physical adsorption of FLG with the polysaccharide chitosan in an acidic solution to enhance biocompatibility. Non-covalent functionalization methods involve a straightforward process where materials interact with graphene without undergoing a chemical reaction, allowing modification of dispersity, stability, and toxicity while preserving graphene's unique features. However, non-covalent interactions lack the robustness to withstand intense external stressors, such as high-temperature exfoliation or NIR laser irradiation, and have limited impact on electronic transport characteristics when utilized in biosensing applications [102].

5.1.2. Covalent surface functionalization

Covalent functionalization techniques are adjusted to establish a more substantial contact between graphene and various other materials and improve electrical transport in applications where hydrophilic polymers and carboxylic acids are frequently utilized. Adding hydroxyl, epoxy, and carboxyl side groups to graphene made it more dispersible in the aqueous phase. Examples of carboxylic acids include tetraphenyl porphyrin with dilute nitric acid or sulfonic acid groups, but despite their straightforward application, carboxylic acid treatment might introduce imperfections to graphene sheets, leading to an inclination toward hydrophilic polymers usage. Previous research has defined grafting-from and grafting-to methods as ways to modify graphene using polymers or poly-carboxylic acids. Graphene serves as the foundation for grafting approaches, creating polymers from monomers, whereas end-tethered polymers are attached to graphene surfaces through grafting-to techniques. Methods such as the direct electrophilic transformation of polyether ketones to graphene, Ziegler Natta polymerization of polypropylene (PP) to GO using catalysts, and connecting PEI to GO via ring-opening polymerization have been employed as grafting-from techniques. In terms of grafting-to techniques, various chemical

processes were employed to produce covalent solid interactions) nitrene cycloaddition for functionalizing p-G/rGO with one molecule of polymers like PS and PEG) cross-linking by esterification/amine-induced ring-opening/nucleophilic substitution to produce functional cross-links. It isn't easy to properly select a covalent functionalization technique from various reactions [103,104]. In Table 2, the advantages and disadvantages of the grafting-to and grafting-from techniques have been listed.

5.2. Encapsulation functionalization

Basic functionalization provides limited advantages for biomedical applications; therefore, subsequent functionalization techniques involving proteins, nucleosides/nucleotides, or metal ions enhance the activity of graphene materials. These functionalized graphene materials exhibit unique features applicable in diverse fields such as biomedical imaging, tissue engineering, gene and drug delivery, immunotherapy, and bio-sensing [110,111].

5.2.1. Non-covalent Encapsulation functionalization

Like covalent functionalization, non-covalent functionalization involves hydrogen bonds, π–π stacking, electrostatic, van der Waals forces, hydrophobic interactions, and self-assembly-based interactions. Proteins, Pluronic F38 (F38), doxorubicin (Dox), maltodextrin (MD), and Tween 80 (T80) are commonly used to functionalize graphene materials. Hydrogen bonding and π–π stacking are also frequently involved. Graphitic domains of GO and Hydrophobic interactions among the bases of DNA were discovered to be responsible for the binding of DNA chains to GO sheets. Hydrogen bonding and Electrostatic interactions were also found between the oxygen-containing GO groups and the bases' primary amines. Several protein functionalizations also required additional interactions.

In a recent study, steroid hormones were adsorbed onto GO nanosheets, displaying long-range hydrophobic and electrostatic contacts extending up to 10 nm, while short-range interactions within 2–5 nm involved hydrogen bonding, van der Waals forces, and π–π stacking. Several research teams utilized self-assembly techniques to bind DNA, polyoxyethylene sorbitan laurate (TWEEN 20), the SS-GrBP5 mutant, and photoreceptor proteins to graphene. It is noteworthy that non-covalent functionalization of proteins and nucleotides/nucleosides always relies on the synergistic interaction of multiple factors, including the distance between graphene and the molecules. When the regulated release of medications or DNA is required, non-covalent interaction is helpful [112–114].

5.2.2. Covalent Encapsulation functionalization

Covalent functionalization, essential for applications in tissue engineering, PTT, and biomedical imaging, ensures stronger interactions facilitated through chemical processes involving small molecules, proteins, and metal ions, as outlined in this section. The connection of BLUF proteins via reactive N-hydroxysuccinimide ester groups, the SBA-15 antibody through $-NH_2$ and reactive functional groups on graphene, and the formation of an isopeptide bond between graphene and SpyTag are all examples of connecting proteins that have been documented. The covalent functionalization of proteins enables precise targeting of graphene complexes to specific cells or tissues, exemplified by reactions such as thionyl chloride and octadecyl amine forming covalent bonds, chemical linking of isothiocyanate groups with amine groups for rhodamine B isothiocyanate, and amide formation for binding Cypate through carbodiimide-catalyzed reactions. PTT and fluorescence imaging can use these tiny molecules with a stable bond to graphene [115].

Regarding metal ions, chelated metal ions in graphene sheets such as Cu^{2+} and Gd(III) created a complex system resembling a metal compound with an extended planar ligand. Such complexes could be used to improve PTT or as contrast agents for imaging. However, the chemistry behind the covalent functionalization of graphene with nucleosides and nucleotides is not entirely understood. Novel covalent functionalization strategies aim to impart specific biomedical capabilities to graphene, enhancing interactions between medications, proteins, graphene sheets, and metal ions, resulting in relatively robust bonds. These specific drugs, proteins, and metal ions have a minimal impact on the fundamental characteristics of graphene due to their low concentration [116–118].

Table 2
Comparison of the grafting-to and grafting-from techniques.

Methods	Advantages	Drawbacks	References
Grafting-to methods	The grafting method covers more surface than the grafting-from method, which may help prevent graphene-cell interactions.	Large graphene sheets may induce steric hindrance, affecting functionalization.	[105,106]
	Allows graphene to blend with many polymers, including some that cannot be polymerized on the surface.	Hard to manage externally because strategy sequences and steric hindrance determine the compound's graphene content.	[106]
Grafting-from methods	The absence of huge graphitic sheets and steric hindrance produced a high-molecular-weight polymer.	On graphene sheets, some polymers perform poorly or are not suited for polymerization.	[107]
	The polymer/initiator ratio controls the graphene material combination, and the grafting percentage is usually higher than the grafting-to method.	The molecule has less graphene than with the grafting-to procedure, making molecular weight and polymeric dispersion difficult to regulate.	[108]
	By adjusting the position of the graft at either the graphene's basal planes or edges, the grafting intensity can be changed.		[101,102,102, 102–105,105–107, 107–109]

6. Application of graphene in Healthcare

6.1. Neural regeneration

Due to the intricate structure and function of the nervous system, restoring the whole process of wounded nerves and healing damaged nerves is still tricky compared to treating other tissues. The neural system provides an ideal model for exploring graphene's biological applications due to the electrical activity inherent in neural cells, which underpins the functioning of the nervous system. Clinical diagnoses and therapies often necessitate neuronal stimulation and monitoring, areas where graphene's distinctive electrical properties offer potential. Additionally, graphene can be tailored to establish a cellular electrical interface for efficient charge transport. Additionally, the chemical stability of graphene's characteristics helps it integrate with neural tissues [119–123].

6.1.1. Enhancing neuronal differentiation from stem cells

The NSCs control multilineage development into oligodendrocytes, neurons, and astrocytes and are self-renewing and multipotent cells. They exhibit promising potential for brain regeneration and are the most often employed stem cell type in neural tissue regeneration. Table 3 provides an overview of recent studies that mix different stem cells with materials composed of graphene for neural regeneration. Promoting the development of human neural stem cells (hNSCs) more toward neurons than glial cells for neural regeneration and brain repair is essential. However, numerous earlier investigations found that hNSCs were more likely to develop into glial cells than neurons without co-culturing or biochemical patterns [124,125]. To investigate how graphene affects NSC behavior, Park et al. produced graphene on an enormous scale and seeded hNSCs on the substrate [126]. It is demonstrated that graphene could cause hNSCs to differentiate more towards neurons than glial cells. Li et al. created 3D porous graphene foams (3D-GFs) in a different study. They discovered that the 3D-GFs might encourage the differentiation of mouse neural stem cells (mNSC) into Neurons and astrocytes [127].

In a recent study, Akhavan et al. investigated the differentiation of hNSCs on GO, hydrazine-rGO, and ginseng-rGO films, finding that the hydrazine-rGO and particularly the ginseng-rGO films showed a higher proportion of hNSCs developed into neurons than the GO films did. The accelerated differentiation observed on rGO films can be attributed to their enhanced electron transfer efficiency. Furthermore, the heightened hydrophilicity, enhanced biocompatibility, and attachment of ginsenoside molecules on the surface of these sheets contributed to more pronounced differentiation in the ginseng-rGO films. Moreover, when cells were stimulated on graphene films using pulsed laser light, hNSCs demonstrated effective and efficient differentiation into neurons [128,129]. It is intriguing to think about functionalized graphene, such as fluorinated graphene (FG), because the carbon-fluorine bond's high polarity is predicted to cause biological reactions. For instance, Wang et al. employed FG sheets as the scaffold for forming Human Bone Marrow Mesenchymal Stem Cells (hBMSCs), mesenchymal stem cells generated from human bone marrow. Morphological alterations suggested that FG could improve hBMSCs' ability to differentiate into neurons and that adding a neuron inducer could strengthen this impact under controlled conditions, stem cells can be patterned on fluorinated graphene, promoting neuronal lineage without the need for a chemical inducer, making it a potential treatment in numerous investigations involving dopamine neuron transplantation [130].

A powerful technique for understanding the genetic profile underpinning dopamine neuron maturation and source for transplantation is the differentiation of embryonic stem cells (ESCs) into dopamine neurons in vitro. The effective differentiation of ESCs into dopamine neurons is still a problem [131,132].

6.1.2. Increasing neuronal outgrowth and maintaining neuronal survival

The biocompatibility of graphene and its interaction with targeted cells present emerging research challenges. While extensive studies have focused on the nervous system, there is limited research on graphene's cellular interactions. Li et al. investigated graphene's effects on neurites in a cultured mouse hippocampus model, revealing significant stimulation of neurite sprouting and expansion during early development. Bendali et al. studied mature retinal neurons in direct contact with bare graphene, demonstrating the survival and neurite development of adult neurons. Understanding charge transport at cell membrane interfaces, crucial for physiological processes, including neuronal stimulation, is a key aspect of this research. For the regeneration of brain tissue,

Table 3
The most current approaches to brain regeneration usually blend different stem cells and graphene-based materials.

S. No.	Materials	Synthesis method	Test species	Key findings of the research	Reference
1.	Graphene (glass substrate)	CVD	hNSCs	Graphene may encourage hNSCs to differentiate more into neurons than glial cells.	[126]
2.	Fluorinated graphene	Reduction of GO to rGO	hBMSCs	FG may improve MSC neurogenesis, cell proliferation, and adhesion.	[115]
3.	Hydrazine-rGO films, Ginseng-rGO films, and GO films	Hummers method	hNSCs	In comparison to the GO film, the hydrazine-rGO as well as the ginseng-rGO films shows a greater differentiation of hNSCs into neurons.	[133]
4.	G and GO	Reduction of GO to rGO	hNSCs	The hNSCs differentiation into neurons was hastened by the stimulation of the rGO sheets by pulsed laser irradiation.	[134]
5.	3D-GFs	CVD	mNSCs	In particular, 3D-GFs may encourage the differentiation of mNSCs into astrocytes and neurons.	[130]
6.	CNTs, GO, and graphene	CVD	mESCs	GO could significantly promote dopamine neuron differentiation	[22]

electroactive scaffolds that can transfer applied electrical stimuli are crucial. Aqueous colloidal graphene is self-assembled onto 2D surfaces and 3D electrospun nanofibers to form graphene-poly-L-lysine/heparin polyelectrolytes. The layer-by-layer (LBL) coating approach utilizes electro and biofunctionalized scaffolds with intricate internal structures at the nano- and microscale. The in-vitro outcomes showed that 2D and 3D graphene-polyelectrolyte multilayers enhanced neurite development and neuron cell adhesion [119,135–137].

6.1.3. Neural electrical performance improving after electrical stimulation

A recent development is the creation of conductive platforms that subject NSCs to external electrical stimuli since electrical stimulation can influence NSC proliferation, and differentiation. Combining conductive polymers with carbon-based materials like graphite, CNTs, and graphene can create electrically conductive scaffolds. Because of its remarkable qualities, including electrical conductivity, an electrochemical potential window, and an extensive specific surface area, graphene is one of these conductive materials that is gaining popularity. Graphene-based substrates exhibit biocompatibility and promote brain cell proliferation. In the realm of tissue regeneration, graphene's effects on the electrical activity of neural networks have unveiled intriguing findings. In a study led by Park et al., graphene films were employed as stimulating electrodes to assess the electrical neuronal activity of cells differentiated from NSCs. Remarkably, these cells exhibited a fluorescence intensity increase of 60–70 % following electrical stimulation, indicating elevated intracellular calcium levels. This research highlights the potential of graphene filaments as electrodes for neurological stimulation [138–141].

The 3D architecture of GFs can offer a significant interface and 3D multiplexing, which can significantly enhance the electrical stimulation performance of conductive scaffold. According to Li et al. studies on the 3D GFs framework as a conductive device for electrical stimulation of cells. The cells should maintain their standard or even improved activity after stem cell differentiation and develop functional connections from one another when graphene is employed for stem cell-based therapy. Tang et al. examined the impact of graphene on neuronal activity and active development during the construction of neural networks in NSCs culture. Graphene was used as an electrode to track how cells react to electrical stimulation. The cells' approximately 30 % rise in fluorescence intensity in response to electrical stimulation strongly suggested that conductive graphene might transmit the electrical stimulation to the neurons [115,142–144].

6.1.4. Graphene substrates with patterns for neuronal growth

Numerous studies have attempted to cultivate neurons in an organised manner in order to determine the in vivo neural circuitry for both fundamental neurophysiology and prosthetic applications. By focusing brain activity at specific locations, multi-electrode arrays can be built using pattern-overlapping neurons and customised conductive materials. Interestingly, it is shown that patterned graphene can effectively replace the current range of biocompatible conductive materials [145,146].

In contrast to the unstructured differentiations observed on quartz and rGO substrates, the reduced graphene oxide nanoribbon (rGONR) grid exhibited organized proliferation of hNSCs and more pronounced neuronal differentiation. Utilizing arrays of silica microbeads, nano topographical features have been shown to enhance axonal development in hippocampal neurons. Solanki et al. engineered hybrids of graphene and silica nanoparticles by coating GO nanosheets on the surface of 300 nm silica nanoparticles (SiNPs). The modified nanotopographical features provided by GO encouraged hNSC neuronal development, leading to significant axonal alignment. Lorenzoni et al. provided a simple fabrication technique to produce patterned surfaces to promote ordered neuron development in a distinct work. They used the vast area fabrication process to arrange single-layer graphene on technologically intriguing substrates, which produced noticeably higher alignment for neuron attachment and development [147,148].

6.1.5. Graphene for neural interfaces

Recent research has inspired a great deal of interest in creating neural interfaces among external objects and neurons to replace or repair neurological system function that has been lost as a result of disease or damage. Because ionic potentials activate biological cells, the electrical impulses between a dry, rigid electrode and a wet, soft tissue should be transmitted by the neural interfaces. Graphene stands out as a promising candidate for bioelectronics applications due to its exceptional physical and chemical properties. Its high charge carrier mobility surpasses that of many semiconductors in field-effect transistors (FETs). Graphene's robust chemical stability and biocompatibility make it highly compatible with biological systems. When integrated with spontaneously beating cardiomyocytes, extracellular graphene FET conductance signals exhibited a signal-to-noise ratio frequently exceeding 4, outperforming comparable values observed in other planar devices. Similarly, Hess et al. reported on graphene-based solution-gated field effect transistor (G-SGFET) arrays for monitoring electrogenic cell activity. They could decode and follow the action potentials of HL-1 cells that resembled cardiomyocytes across the transistor array. Consequently, graphene SGFETs may have a higher signal-to-noise ratio than most known devices due to their low noise and high transductive sensitivity. For the purpose of altering the electrode site of implantable bioelectronic devices, Tian et al. doped GO into PEDOT to create a composite film by electrochemical deposition that gave the devices exceptional electrochemical properties [149].

As a result, the PEDOT/GO films' superior electrochemical performance was influenced by the development of impedance, charge injection, and charge storage capacity limit, in addition to the expansion of the efficient surface area. Thus, as electrodes and tissues contact, graphene-based materials open up new possibilities for tissue engineering and implantable electrophysiological devices [150,151].

6.2. Bone regeneration

Bone, a remarkably adaptable, intricate, and highly vascularized tissue, possesses the unique ability to heal and regenerate without scarring; however, severe bone deformities resulting from tumours, trauma, non-union fractures, and congenital anomalies often exhibit limited remodelling capacity [152]. A promising new method for clinical use is bone tissue engineering, which co-cultures autologous cells with biomaterial scaffolds. It is important to comprehend the common cell types in the biological setting of human bone before exploring the function and possible interactions of graphene in bone tissue regeneration. Mesenchymal stem cells (MSCs), a key cell type, significantly contribute to bone regeneration by transforming into osteoblasts. However, controlling the differentiation of stem cells into the osteoblast lineage remains a challenging aspect of bone repair. An alternative cell type called an osteoblast adheres to the surface of the extracellular matrix (ECM) to initiate the formation of bones, then spreads and proliferates to cover the ECM surface. Two more cells that can contribute to bone healing are fibroblasts and sarcoma cells [153–157].

6.2.1. Mesenchymal stem cell support for In vitro osteogenic differentiation

Various biomaterials and cells are used in current tissue regeneration techniques to provide biological alternatives that may repair and restore tissue functions. Different biomaterials have been developed for the precise differentiation of stem cells into bones, muscles, and cartilage after transplantation. Controlling stem cell proliferation and promoting their differentiation through the use of growth factors and osteogenic inducers is one of the most crucial objectives of bone regeneration. One of the main problems in this field is how to effectively promote the osteogenic differentiation of MSCs to repair and regenerate bone tissue using graphene-based stem cells and materials [158–161]. Table 4 includes a summary of some examples of bone regeneration. Large single-layer graphene supported by hMSC growth showed spindle-shaped morphology. Because they were evenly distributed throughout the surface, it was possible that graphene could be effective at causing hMSCs to differentiate into the osteoblast lineage (Fig 4). Likewise, Lee and colleagues noted enhanced osteogenic behaviour and increased mineral deposition of MSCs cultured on graphene substrates compared to GO and polydimethylsiloxane (PDMS) substrates. Their study also revealed graphene's unique ability to serve as a pre-concentration platform for β-glycerophosphate and dexamethasone, potentially influencing the onset of osteogenic differentiation [162–164]. Graphene nanostructures, particularly graphene nanoribbons, served as efficient 2D templates, promoting rapid proliferation and differentiation hMSCs. Within a brief 7-day period, the rGONR grid exhibited the fastest osteogenic development in hMSCs, with a 2.2-fold difference observed on rGO sheets after the same duration. This accelerated differentiation on rGONR grids was attributed to the surface topography features inducing physical stresses and the efficient adsorption of chemical inducers. Additionally, a self-supporting graphene hydrogel (SGH) sheet was utilized as a versatile platform for studying graphene's intrinsic properties both in

Table 4
The most recent approaches for bone regeneration often combine different types of stem cells with materials based on graphene.

S. No.	Constituents		Cell type	Highlights of the research	Reference
1.	Single-layer graphene produced by CVD	CVD	hMSCs	With a higher chance of stimulating MSC development into the osteoblast lineage, graphene is promising for bone transplant surgery.	[184]
2.	Graphene coated with PMMA	CVD	hMSCs	Graphene can potentially expedite hMSC development at a pace similar to that of differentiation induced by BMP-2.	[161]
3.	Graphene and GO films	CVD	hMSCs	It is shown that GO and graphene are efficient pre-concentration platforms for promoting the proliferation and differentiation of stem cells.	[185]
4.	GO-coated Ti substrate	modified Hummers method	hMSCs	Ti/GO substrates adsorbed with BMP-2 have the potential to dramatically improve hMSCs' in vitro osteogenic differentiation and promote more robust bone production.	[163]
5.	Graphene nanogrids	modified Hummers method	hMSCs	When chemical inducers were present, rGONR grids demonstrated the quickest osteogenic development of hMSCs in 7 days.	[186]
6.	Self-supporting graphene hydrogel films	CVD	rBMSCs	The SGH film on its own can induce stem cell osteogenic differentiation without the need for any other inducer.	[165]
7.	Graphene-coated plates	Reduction of GO to rGO	gMSCs	GO encourages gMSC proliferation and osteogenic differentiation without glucocorticoids or other added growth hormones.	[166]
8.	3D graphene foams	CVD	hMSCs	3D graphene foams can encourage spontaneous osteogenic differentiation and enhance the adhesion and survival of hMSCs.	[168]
9.	Graphene-incorporated CS substrate	CVD	hMSCs	The substrata's nanotopographic cues encouraged hMSC attachment and differentiation.	[169]
10.	GO/PLL composite films	Electropolymerization	rBMSCs	Not only did GO/PLL composite film promote the growth of MSCs with a high rate of proliferation, but it could also hasten the differentiation of MSCs into osteogenic cells.	[170]
11.	PLLA nanofibrous scaffolds containing CNT and graphene	Thermal-induced phase separation	mBMSCs	When it came to encouraging BMSCs to differentiate into osteoblasts and triggering osteogenesis in vivo, graphene outperformed CNT.	[171]
12.	GO-CaP nanocomposites	Double reverse microemulsion	hMSCs	The osteogenesis of hMSCs was considerably aided by GO-CaP nanocomposites, which also increased calcium deposition.	[172]

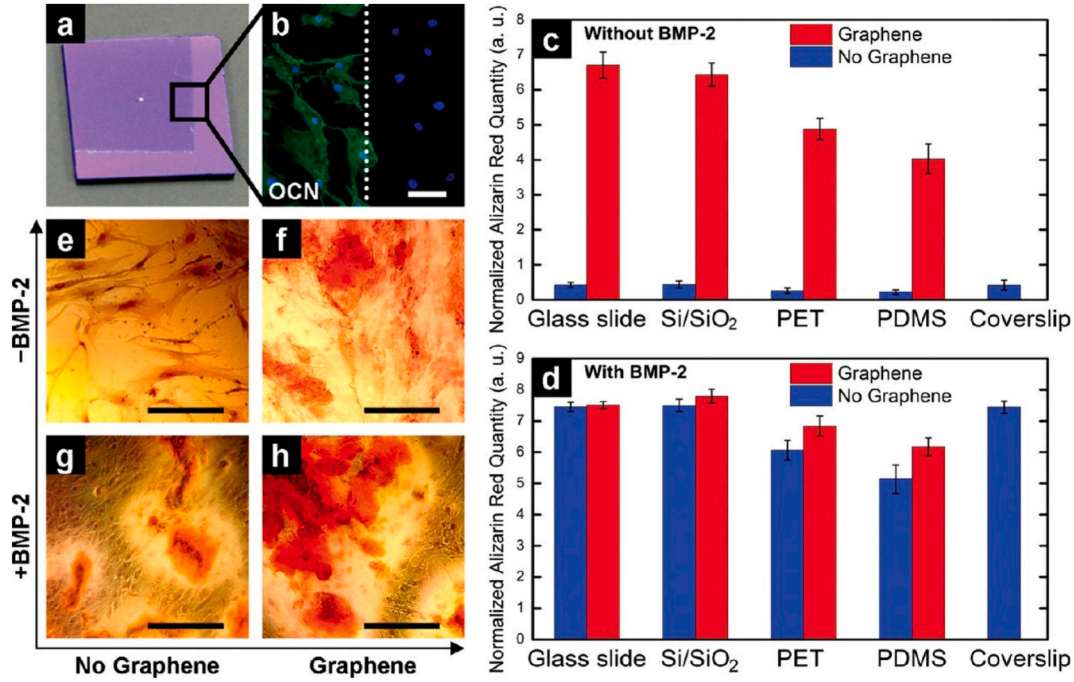

Fig. 4. The differentiation of osteoblasts is hastened by graphene. (A) An optical view of 1 × 1 cm a Si/SiO_2 device that has been partially coated with graphene and demonstrates the graphene border. (b) The graphene barrier is also clearly visible in this image of the osteocalcin (OCN) marker, which only shows bone cell growth on the identical chip in the graphene-coated area. (c, d) Quantification of alizarin red derived from 15-day growth of hMSCs on substrates containing or lacking graphene. (c) Cells expanded without the presence of BMP-2 coverslips is displayed as a source. (d) Cells that have been cultured using BMP-2. Standard plain coverslips were utilized as a successful control. PET substrate with alizarin red staining, displaying calcium deposits brought on by osteogenesis. PET without (e) PET without BMP-2 and with graphene; BMP-2 and without graphene (f) Graphene-only PET, (g) BMP-2-only PET, (h) and BMP-2-only PET are all examples of PET. 100 m scale bars are used (Adapted with permission [18]). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

vitro and in vivo. Remarkably, rat bone marrow stromal stem cells (rBMSCs) were stimulated to differentiate into osteoblasts solely by the SGH film, without requiring additional inducers. A related study also examined the impact of graphene on the development and differentiation of goat adult mesenchymal stem cells (gMSCs). 3D graphene foams have recently been created and used in bone regeneration [165–167].

By growing graphene on a three-dimensional nickel scaffold, Crowder et al. created 3D porous graphene foams, which they then used to cultivate hMSCs. These findings showed that graphene materials might encourage MSC adherence and survival and trigger naturally occurring osteogenic differentiation. In conjunction with MSCs, composite materials based on graphene have also been used to regenerate bone tissue. For instance, Kim et al. created a Chitosan (CS) substrate with graphene incorporation. They discovered that graphene's distinct nanoscale topographical cue and downstream effects, such as stiffness and roughness, encouraged hMSC adherence and differentiation. A novel class of composite films, combining GO with poly L-lysine (PLL) through LBL assembly, was developed to enhance MSC adhesion, proliferation, and differentiation. Specifically, the GO/PLL composite film demonstrated significant improvements in osteogenic differentiation and accelerated MSC growth, indicating a high proliferation rate. These outcomes suggested that the enhanced osteogenic differentiation of MSCs could be attributed to the GO/PLL films' robust pre-concentration ability for osteogenic inducers. Similarly, Duan et al. created poly(L-lactide) (PLLA) nanofibrous scaffolds containing carbon nanomaterials like Carbon Nanotubes and graphene. In a recent study, Tatavarty et al. created nanocomposites using GO micro flakes and calcium phosphate (CaP) nanoparticles with an elliptical, ultrathin plate structure. The GO-CaP nanocomposites greatly accelerated calcium deposition and hMSCs' osteogenesis. Research on graphene's impact is scarce, and investigations on its role in bone tissue regeneration remain in their early stages. Within the parameters of the present study, we believe that the applications of graphene-based materials for bone tissue regeneration may have a promising future; additional research will fulfil its promise [168–173].

6.2.2. Controlling osteoblast activity and Directional growth

Numerous applications in the field of bone regeneration rely on understanding the influence of graphene-based materials on cellular responses. Nevertheless, regulating cellular behavior on graphene and its derivatives remains challenging due to the complex interplay between graphene surface properties and cell responses. Research conducted by Mahmood et al. revealed that graphene did not induce toxic effects on osteoblasts, and substrates coated with graphene exhibited enhanced osteoblast adhesion compared to uncoated substrates. These results supported the advantageous effects of graphitic nanomaterials with different structures (graphene sheets and MWCNTs) on the process of bone cell mineralization [174,175].

Kanayama and associates added GO and chemically produced rGO films on a safe collagen scaffold and evaluated the films' biological effects. Alkaline phosphatase (ALP) activity and calcium absorption were markedly increased by rGO, suggesting that rGO was efficient for osteogenic differentiation. In a different study, graphene nanoplatelets were electrostatically sprayed onto UHMWPE to act as reinforcement. This study showed that particle aggregation and dosage both affected the cytotoxicity of nanoplatelets made of graphene to osteoblasts [176–179]. For instance, Kim et al. developed self-supporting GO/graphene-$CaCO_3$ composites consisting of vaterite microspheres, the most brittle polymorph of $CaCO_3$, enveloped in GO (or graphene) networks, facilitating the survival of osteoblast cells with elongated morphology. Baradaran et al. engineered rGO-reinforced hydroxyapatite nanotube composites, demonstrating enhanced osteoblast adhesion and proliferation in vitro. Additionally, a practical one-pot hydrothermal method was devised to synthesize composites comprising graphene nanosheets and HA nanorods for similar applications. Li et al. successfully synthesized GO-HA and CS-GO-HA nanocomposites by developing GO and CS functionalized GO for the first time as templates to fabricate HA using an easy solution-based in situ synthesis process. Both nanocomposites demonstrated a high rate of cell proliferation. However, the CS-GO-HA composite outperformed GO-HA regarding cell viability and ALP activity. Similarly, a solution casting technique created a genipincross-linked CS/GO composite film. In vitro, studies revealed that the composite film was suitable for MC3T3-E1 cell growth and adhesion [180–183].

6.3. Cartilage regeneration

The sparsely dispersed chondroblasts that comprise articular cartilage, non-nervous, an avascular, and elastic tissue, are surrounded by a dense extracellular matrix (ECM) of collagen fibres and an abundant solid substance rich in proteoglycan and elastin fibres. In traditional practices, stem cells are cultured in pellets to facilitate chondrogenic differentiation. However, this method has limitations such as poor cell-extracellular matrix interaction and the potential diffusion constraints of Transforming growth factor - beta (TGF-β) protein within the pellet structure. To address these challenges in the chondrogenic differentiation of human adipose-derived stem cells (hASCs) within pellets, GO sheets were utilized to adsorb fibronectin (FN) and TGF-β3, enhancing cell-FN interactions and ensuring effective delivery of TGF-β3. The hybrid pellets of hASCs and GO significantly expedited the differentiation of hASCs into chondrogenic cells by improving the cell-matrix interaction and growth factor delivery (Fig. 5). The application of GO to speed up stem cell chondrogenic differentiation may pave the way for new developments in cartilage regeneration [187–189].

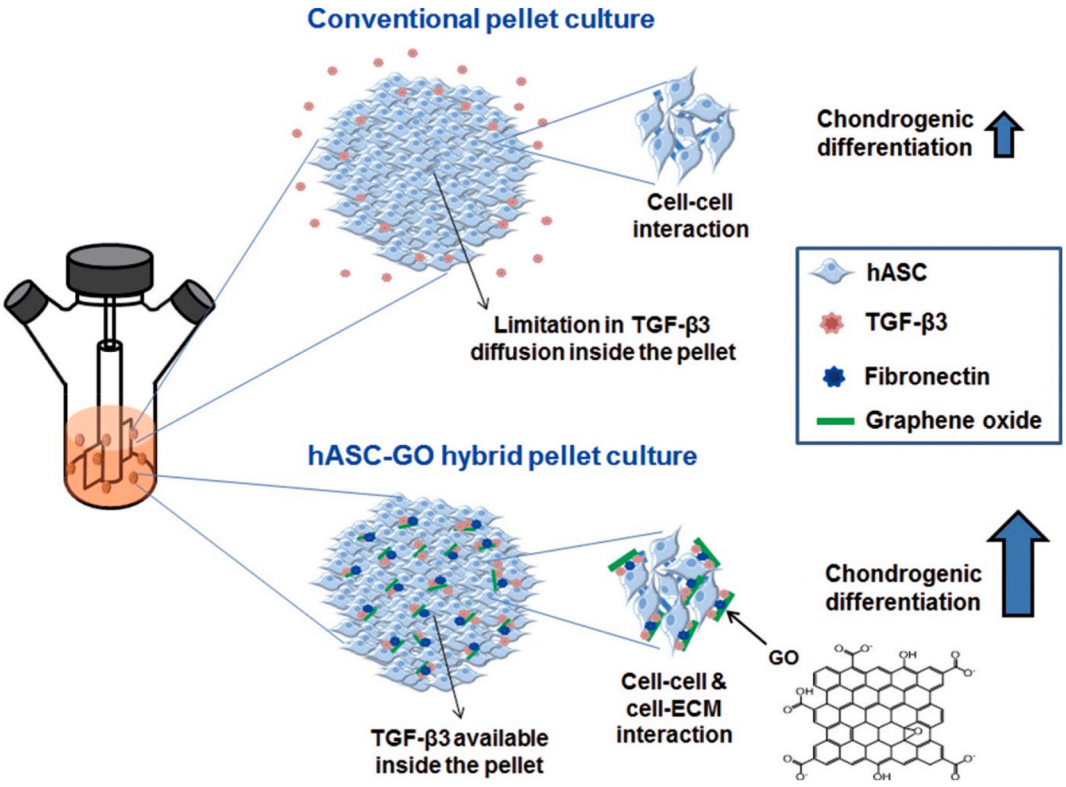

Fig. 5. A schematic showing how the use of GO improved hASCs' ability to differentiate into cartilage. The traditional pellet culture only allows for cell-cell interaction, and TGF-3 diffusion within the pellets is frequently constrained; both of these variables prevent stem cells from differentiating into chondroblasts. In hybrid pellets of hASCs and GO, stem cells can be grown to enhance chondrogenic development. TGF-3 and cell-adhesion proteins, such as FN, are adsorbed on GO sheets and disseminated in hASC pellets, promoting cell-ECM interactions and hASC chondrogenic development. (Adapted with permission [189]).

6.4. Skeletal muscle regeneration

Skeletal muscles, characterized by dense and well-aligned muscular fiber bundles, can experience functional impairment due to factors such as tumor removal, trauma, or myopathies. Muscle transplantations have been developed to restore partial muscle function in such cases, but skeletal muscle tissue engineering remains a challenging scientific endeavor. In vitro studies were conducted to assess myotube development on GO and rGO substrates. Significantly enhanced myogenic differentiation was observed on GO, attributed to its surface roughness, which influenced serum protein adsorption, and the presence of abundant oxygen functional groups. The findings indicated potential uses in skeletal muscle regeneration by suggesting GO could speed up myogenic differentiation [190,191].

6.5. Cardiac regeneration

MSCs possess significant therapeutic potential for cardiac conditions, although their in vivo differentiation into cardiomyocytes is rare, leading to limited published clinical trial data on MSCs as cardiac treatments. It has been demonstrated that the transplantation of cardiomyogenically differentiated MSCs significantly enhances cardiac contractility. Thus, many scientists attempt to use 5-azacytidine to steer MSCs in the direction of the cardiomyogenic lineage. However, because of the inhibition of deoxyribonucleic acid methylation, which interferes with regular cell activity, 5-azacytidine-treated MSCs cannot be therapeutically acceptable. Therefore, new techniques for MSC maturation into the cardiomyogenic lineage must be created to use stem cells clinically to treat myocardial infarction. The first hypothesis put forth by Park et al. was that graphene could increase the expression of MSCs' cardiomyogenic genes. They later discovered graphene-enhanced MSC differentiation into the cardiomyogenic lineage without exogenous chemical inducers. The increased expression of ECM protein genes and cell signalling molecules may cause MSCs' improved cardiomyogenic differentiation in graphene-based cultures. This result showed that graphene-based materials might be a cutting-edge platform for differentiating MSCs into cardiomyocytes [192–194].

6.6. Skin regeneration

The skin, the body's largest organ, provides protection against external elements, and extensive efforts have been made to develop skin substitutes resembling human skin, employing various organic and synthetic materials [195]. A study by Lu et al. involved depositing graphene sheets onto electrospun CS-PVA nanofibers to leverage the advantageous properties of graphene and CS for wound healing applications. In vivo experiments using rabbit and mouse models demonstrated that the graphene-infused scaffolds facilitated faster wound healing compared to other groups. The presence of graphene's free electrons was theorized to hinder bacterial cell growth without affecting eukaryotic cell division, potentially preventing pathogen spread and promoting wound healing. First, this discovery makes it possible to directly use graphene for wound healing, which may further the development of graphene's biological applications. Collagen-fibrin (CF) biocomposite films combined with GO (CFGO) were created for wound healing in a similar application. The presence of GO increased the mechanical strength of films made of collagen and fibrin. Rats treated with CFGO showed faster wound healing than rats treated with GO, suggesting that graphene-based materials could be employed on more clinical wounds in various animal models before being applied to people [196–199].

6.7. Adipose tissue regeneration

The most crucial element required for soft tissue repair is adipose tissue. Adipose tissue restoration is highly clinically necessary since shape flaws affect patients visually and functionally. Lee et al. looked into the impact of graphene and GO surfaces on the adipogenic differentiation of MSCs. They found that graphene dramatically reduced the differentiation to adipocytes because insulin was denatured upon its adsorption on graphene. Conversely, GO's strong affinity for insulin significantly increased adipogenic differentiation. These results revealed that GO was the efficient pre-concentration platform for molecular interactions that promoted stem cell differentiation into adipocytes [18,200].

6.8. Blood vessel

Blood is carried from the heart by a network of blood vessels that are arranged hierarchically, starting with the arteries and moving through the arterioles and capillary network. This forms a small, closed loop that permeates most bodily tissues and makes it easier for waste materials, nutrients, and oxygen to exchange. The outer membrane provides vascularization and autonomic control; the arteries and veins are further connected to the second layer of the median membrane. Which is responsible for mechanical strength; and the arteries, capillaries, and veins have an inner membrane composed of endothelial cells (EC), which is responsible for antithrombotic properties. These three types of blood vessels are susceptible to damage that impairs their ability to operate. Atherosclerotic cardiovascular disease (CVD) and other ischemic disorders are likely to occur in the big vascular system (BV inner diameter >1 mm), which can lead to BV occlusion. Occlusion in the microvascular (BV inner diameter <50 μm) and intermediate vessel (50 μm $<$ BV inner diameter <1 mm) can result in tissue ischemia and, in extreme situations, total tissue failure necessitating organ replacement. Autologous transplantation is the method used for traditional vascular repair; however, there are very few possibilities for grafted vascular replacements, and further associated operations may result in high incidence and rates of failure at donor locations [201,202]. Vascular tissue engineering has proven to be a useful method for creating a range of possibly functional vascular substitutes in order to

get around the drawbacks of these existing treatments. It creates multi-materials that resemble the extracellular matrix of blood vessels in order to recreate the structure and function of extravasal blood vessels prior to transplantation. The following essential criteria for current vascular tissue engineering should be fulfilled: The requirements for tubular tubing are as follows: (1) its lumen diameter, length, and application must match; (2) the vessel and graft's mechanical characteristics must coincide, at least within the range of the graft's maximum tensile strength; (3) the graft's thrombotic capacity must be low (for improved hemocompatibility); and (4) regenerative/integration potential to guarantee the graft's longevity. Due to their distinct physicochemical characteristics, GBNs have piqued attention among researchers who want to use them in vascular tissue engineering [203,204].

Polyvinyl alcohol (PVA) flat and tubular nanocomposite scaffolds with 1, 2, and 3 wt percent graphene were prepared by Alavije et al. using an electrospinning method. When graphene content was raised to 3 wt percent, the scaffold's elongation at break improved, the water contact angle raised to 69°, and endothelial cells propensity to adhere to and multiply on the scaffold surface. Additionally, tubular stents have better electrical function when compared to flat stents. In addition to encouraging cell proliferation, GO enhances the polymer matrix's thermomechanical characteristics [205]. RGD-GO-PLGA, a poly (lactide-*co*-glycolide, PLGA) nanofiber membrane co-functionalized with RGD peptide, was developed by Shin et al. [130]. This membrane can offer the perfect conditions for the development of vascular smooth muscle cells [206]. Due to rGO's distinct two-dimensional structure and large specific surface area, it may be shaped into an appropriate shape for use as a catalyst carrier. Huo et al. were able to successfully create rGO enzyme-coated small-bore tissue-engineered vasculatures (TEBV) that have endothelial and antiplatelet properties. The antithrombotic effect and patency of TEBV were confirmed when its patency rate reached 90 % after seven days of transplantation [207].

7. Challenges and limitations of graphene in tissue engineering

The primary constraint of contemporary tissue engineering techniques is the compromised functionality of regenerate organs as a result of inadequate neural regeneration. Targeted medication delivery, functional neural networks, and the regeneration of injured nerve cells and connections are all aspects of nervous system tissue engineering. Furthermore, the next generation of biotechnology is being made possible by graphene because of its exceptional thinness and conductivity, which are being used to create customised scaffolds for neural-machine interfaces or neuroprosthetic devices. Currently, surgical repair to fuse two nerve terminals is the mainstay of treatment for damaged peripheral nerves. A mechanical anastomosis procedure, which frequently involves autografting, does not fully restore functionality. It also comes with a number of restrictions, such as the need that the nerve gap not exceed 4 cm, which occasionally prevents a full recovery of pre-injury capability. Because of the intricate structure including the conventional neuronal network and the uncontrollably formed glial scars, damage to the central nervous system (CNS) frequently results in more catastrophic repercussions and is therefore more difficult to repair. To address the issue of inadequate support for brain tissue regeneration, a biomaterial that provides the optimal conditions for cell proliferation and differentiation as well as acts as a site for attachment of cells must be developed [79,208,209].

Despite the fact that graphene is a great scaffold material, it functions as an impermeable barrier that prevents ions from moving freely between cells. Incomplete synaptic development could result in issues for neural networks. Consequently, following desired cell proliferation, graphene scaffolds must degrade. With the aid of a metal oxide photocatalyst, this can be accomplished by flash photostimulation. According to recent research, NIR laser irradiation can damage CVD produced nanomesh scaffolds. In the NIR degradation process, ascorbic acid functions as a hole-scavenger; in the flash photostimulus, this is not the case. Nevertheless, comprehensive findings on the biodegradability of graphene scaffolds have not yet been published in the literature. Graphene's carbon-based structure is expected to produce a biocompatible surface that promotes cell development and proliferation. However, graphene is poisonous on its own, which could be brought on by the material's smaller size and higher concentrations in graphene nanoplates and nanoribbons [210,211].

ROS production, aggregated graphene sheets encasing the cells in a solution, and physical harm from graphene particles touching cell membranes are some of the potential causes of graphene toxicity. Although these pathways may be involved in potential toxicities caused by graphene in the body, it is evident that in vitro investigations do not demonstrate these effects. When applied to humans, graphene-based nanomaterials breakdown into aromatic hydrocarbons and holey sheets. Additionally, biotransformed nanomaterials have been shown to be effective in delivering drugs and capable of killing tumour cells [212,213]. On the other hand, it was believed that the pure graphene family of nanomaterials was connected to secondary structural damage to proteins as well as disruptions in cellular metabolic pathways. Graphene excretion through urine has been demonstrated, but there is a special consideration about graphene uptake by various organs from the body's graphene sheets. Additionally, a study by Akhavan et al. that involved injecting male mice with nanoscale-GO (NGO) provided support for it. They saw how graphene was absorbed by the testis, which has a notable effect on DNA fragmentation and spermatozoa motility and viability. Furthermore, the transfer of these mice's semen which contains ROS and damaged spermatozoa into female mice has led to altered hormone output, reduced fertility, and impaired gestation [214]. However, after treating animals with polyethylene glycol (PEG) functionalized graphene for three months, Yang et al. detected no toxicities in the liver or kidney. Limited in vivo research has been conducted on the toxicity and biodistribution of graphene [215]. Wang et al. have shown persistent toxicity in the kidneys, spleen, lungs, and granuloma development in mice given an intravenous high dose of graphene (0.4 mg). Mice injected with low doses (0.1, 0.25 mg) did not exhibit these adverse effects. Furthermore, the kidneys were unable to filter the graphene at greater dosages [216]. In a different study, graphene oxide was injected directly into the mice's lungs by Dutch et al., which enhanced mitochondrial respiration and produced reactive oxygen species [217]. Polyethylene glycol-coated graphene oxide nanoparticles have been administered intravenously to mice by Yang et al. The study's findings indicate that the liver and spleen initially accumulated graphene. After three months, there was total clearance at a dosage of 20 mg/kg with no toxicity seen. Additionally, graphene exhibits bacterial toxicity [215]. The harmful effects of graphene and its constituents on strains of

S. Aureus and E. Coli have been investigated by Akhavan et al. and Liu et al. It was discovered that direct contact between bacteria and graphene nanosheets disrupted the bacteria's cell membrane, which is why graphene compounds have antibacterial properties. On the other hand, a few investigations have revealed that graphene is not harmful to bacteria and does not prevent bacterial growth [218–220].

Human embryonic kidney cells (HEK293) have demonstrated dose-dependent cytotoxicity towards graphene oxide. The production of ROS, the release of LDH, and the drop in reduced glutathione levels all of which contribute to oxidative stress were determined to be the causes of the cytotoxicity. Consequently, this leads to damage to DNA and a reduction in ATP synthesis and mitochondrial membrane potential. To promote future applications and minimise hazards, greater research on the toxicities (including geno-toxicity) of graphene nanoparticles is needed in order to use them in tissue engineering and therapeutic applications [221,222].

8. Conclusion and perspective

In recent years, graphene's applications in regenerative medicine have substantially expanded, marked by notable developments in this field. While these initial preclinical studies show promise, several challenges must be addressed before potential clinical applications can be actualized. Graphene-based materials exhibit superior mechanical and electrical properties. However, achieving a homogeneous distribution of graphene nanosheets within the matrix and preventing their aggregation in solution remain unresolved challenges. Innovative approaches are required to ensure uniform dispersion of graphene nanosheets throughout the matrix and prevent aggregation, thus advancing research in this area.

Research findings shows the advantages of graphene over other materials utilized in tissue regeneration. Research has demonstrated that, in comparison to conventional substrates, graphene's strong electrical conductivity can markedly improve brain and heart cell differentiation. It offers strong support for tissue integration and growth because of its mechanical strength and flexibility, which surpass those of many polymers and ceramics. Numerous in vitro and in vivo studies have proven graphene's biocompatibility, underscoring its promise for safe implantation with few side effects. Additionally, studies show that the enormous surface area of graphene facilitates faster tissue regeneration by more effectively promoting cellular adhesion and proliferation than traditional materials. Furthermore, during the healing process, the antibacterial qualities of graphene help to lower infection rates. These results confirm graphene's position as a prominent material in the growing field of regenerative medicine by highlighting its superiority in tissue regeneration.

Promising results in stem cell research with graphene-based materials are highlighted in this article. Nevertheless, there is still much to learn about the signalling routes and physiological processes controlling stem cell development. Since this subject is still in its infancy, more research is needed to fully understand the complex mechanisms driving stem cell differentiation into distinct lineages. Because it is conductive, graphene has the potential to improve brain interfaces for engineering uses. Graphene-based materials have not yet achieved therapeutic success in electrical stimulation applications, despite numerous in vitro experiments. Further research is required to fully comprehend and compare the effects of various forms of electrical stimulation on neurons.

In vivo assessments involving implanted conductive graphene-based materials are essential to evaluate the efficacy of neuron regeneration. In conclusion, despite several unresolved problems and difficulties, applying graphene-based materials may open the door for significant advancement in the next research on regenerative medicine.

Availability of data and materials

The data reported in this paper will be made available on request.

CRediT authorship contribution statement

Rajesh Singh: Writing – original draft. **Hemant Rawat:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Conceptualization. **Ashwani Kumar:** Writing – review & editing. **Yashika Gandhi:** Resources. **Vijay Kumar:** Visualization, Formal analysis. **Sujeet K. Mishra:** Visualization, Formal analysis. **Ch Venkata Narasimhaji:** Supervision.

Declaration of Competing interest

The authors report no conflicts of interest associated with this manuscript.

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References

[1] R. Ghosh, M.S. Siddiqui, H. Kalita, Fabrication of highly conductive graphene paper for supercapacitors with a one-step hydrothermal method, Carbon Trends 14 (2024) 100317.

[2] K. Ghosal, et al., Graphene family nanomaterials- opportunities and challenges in tissue engineering applications, FlatChem 30 (2021) 100315.
[3] M. Thangamuthu, et al., Graphene- and graphene oxide-based nanocomposite platforms for electrochemical biosensing applications, Int. J. Mol. Sci. 20 (12) (2019).
[4] R. Singh, R. Deshmukh, DNA nanobots – emerging customized nanomedicine in oncology, Curr. Drug Deliv. 20 (2) (2023) 111–126.
[5] R. Singh, R. Deshmukh, Carbon nanotube as an emerging theranostic tool for oncology, J. Drug Deliv. Sci. Technol. 74 (2022) 103586.
[6] G. Eda, M. Chhowalla, Chemically derived graphene oxide: towards large-area thin-film electronics and optoelectronics, Adv Mater 22 (22) (2010) 2392–2415.
[7] X. Li, et al., Graphene and related two-dimensional materials: structure-property relationships for electronics and optoelectronics, Appl. Phys. Rev. 4 (2017) 021306.
[8] G. Reina, et al., Promises, facts and challenges for graphene in biomedical applications, Chem. Soc. Rev. 46 (15) (2017) 4400–4416.
[9] D. Cohen-Tanugi, J.C. Grossman, Water desalination across nanoporous graphene, Nano Lett. 12 (7) (2012) 3602–3608.
[10] K. Hu, et al., Graphene-polymer nanocomposites for structural and functional applications, Prog. Polym. Sci. 39 (11) (2014) 1934–1972.
[11] M. Yi, Z. Shen, A review on mechanical exfoliation for the scalable production of graphene, J. Mater. Chem. A 3 (22) (2015) 11700–11715.
[12] X. Li, L. Colombo, R. Ruoff, Synthesis of graphene films on copper foils by chemical vapor deposition, Adv. Mater. 28 (2016) n/a-n/a.
[13] Y. Zhu, et al., Graphene and graphene oxide: synthesis, properties, and applications, Adv Mater 22 (35) (2010) 3906–3924.
[14] K.P. Loh, et al., Graphene oxide as a chemically tunable platform for optical applications, Nat. Chem. 2 (12) (2010) 1015–1024.
[15] R. Deshmukh, et al., A snapshot of selenium-enclosed nanoparticles for the management of cancer, Curr. Pharmaceut. Des. 30 (11) (2024) 841–858.
[16] S. Pina, et al., Scaffolding strategies for tissue engineering and regenerative medicine applications, Materials 12 (11) (2019).
[17] H.L. Tan, S.Y. Teow, J. Pushpamalar, Application of metal Nanoparticle-Hydrogel composites in tissue regeneration, Bioengineering (Basel) 6 (1) (2019).
[18] W.C. Lee, et al., Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide, ACS Nano 5 (9) (2011) 7334–7341.
[19] X. Chen, X. Hai, J. Wang, Graphene/graphene oxide and their derivatives in the separation/isolation and preconcentration of protein species: a review, Anal. Chim. Acta 922 (2016) 1–10.
[20] X. Ding, H. Liu, Y. Fan, Graphene-Based Materials in Regenerative Medicine 4 (10) (2015) 1451–1468.
[21] J. Kim, et al., Durable and water-floatable ionic polymer actuator with hydrophobic and asymmetrically laser-scribed reduced graphene oxide paper electrodes, ACS Nano 8 (3) (2014) 2986–2997.
[22] S.R. Shin, et al., Graphene-based materials for tissue engineering, Adv. Drug Deliv. Rev. 105 (Pt B) (2016) 255–274.
[23] R. Deshmukh, et al., Andrographis paniculata and andrographolide - a Snapshot on recent Advances in nano drug delivery Systems against cancer, Curr. Drug Deliv. 21 (5) (2024) 631–644.
[24] G. Hlawacek, C. Teichert, Nucleation and growth of thin films of rod-like conjugated molecules, J. Phys. Condens. Matter 25 (14) (2013) 143202.
[25] J.W. Evans, P.A. Thiel, M.C. Bartelt, Morphological evolution during epitaxial thin film growth: formation of 2D islands and 3D mounds, Surf. Sci. Rep. 61 (1) (2006) 1–128.
[26] K.S. Novoselov, et al., Two-dimensional atomic crystals, Proceedings of the National Academy of Sciences of the United States of Americas 102 (30) (2005) 10451–10453.
[27] M.J. Allen, V.C. Tung, R.B. Kaner, Honeycomb carbon: a review of graphene, Chem. Rev. 110 (1) (2010) 132–145.
[28] V. Rosa, et al., Graphene oxide-based substrate: physical and surface characterization, cytocompatibility and differentiation potential of dental pulp stem cells, Dent. Mater. 32 (8) (2016) 1019–1025.
[29] G. Yang, et al., Structure of graphene and its disorders: a review, Sci. Technol. Adv. Mater. 19 (1) (2018) 613–648.
[30] A. Sinitskii, et al., Kinetics of diazonium functionalization of chemically converted graphene nanoribbons, ACS Nano 4 (4) (2010) 1949–1954.
[31] R. Sharma, et al., Anomalously large reactivity of single graphene layers and edges toward electron transfer chemistries, Nano Lett. 10 (2) (2010) 398–405.
[32] J.R. Lomeda, et al., Diazonium functionalization of surfactant-wrapped chemically converted graphene sheets, J. Am. Chem. Soc. 130 (48) (2008) 16201–16206.
[33] H. Liu, et al., Photochemical reactivity of graphene, J. Am. Chem. Soc. 131 (47) (2009) 17099–17101.
[34] A. Talyzin, et al., Brodie vs Hummers graphite oxides for preparation of multi-layered materials, Carbon 115 (2017).
[35] A. Pandey, P. Chauhan, Chapter 1 - functionalized graphene nanomaterials: next-generation nanomedicine, in: S. Mallakpour, C.M. Hussain (Eds.), Functionalized Carbon Nanomaterials for Theranostic Applications, Elsevier, 2023, pp. 3–18.
[36] Q. Yu, et al., Graphene Segregated on Ni Surfaces and Transferred to Insulators, vol 93, Birck and NCN Publications, 2008.
[37] V.B. Mbayachi, et al., Graphene synthesis, characterization and its applications: a review, Results in Chemistry 3 (2021) 100163.
[38] D. Dikin, et al., Preparation and characterization of graphene oxide paper, Nature 448 (2007) 457–460.
[39] E. Gerstner, Nobel prize 2010: andre geim & konstantin novoselov, Nat. Phys. 6 (11) (2010) 836, 836.
[40] M. Tahriri, et al., Graphene and its derivatives: opportunities and challenges in dentistry, Mater. Sci. Eng. C 102 (2019) 171–185.
[41] R.R. Nair, et al., Fluorographene: a two-dimensional counterpart of teflon, Small 6 (24) (2010) 2877–2884.
[42] L.-H. Liu, M. Lerner, M. Yan, Derivitization of pristine graphene with well-defined chemical functionalities, Nano Lett. 10 (2010) 3754–3756.
[43] A. Wang, et al., A 1,3-dipolar cycloaddition protocol to porphyrin-functionalized reduced graphene oxide with a push-pull motif, Nano Res. 8 (3) (2015) 870–886.
[44] M. Quintana, et al., Functionalization of graphene via 1,3-dipolar cycloaddition, ACS Nano 4 (6) (2010) 3527–3533.
[45] V. Georgakilas, et al., Noncovalent functionalization of graphene and graphene oxide for energy materials, biosensing, catalytic, and biomedical applications, Chem. Rev. 116 (9) (2016) 5464–5519.
[46] M.U.A. Khan, et al., Graphene oxide-functionalized bacterial cellulose–gelatin hydrogel with curcumin release and kinetics: in vitro biological evaluation, ACS Omega 8 (43) (2023) 40024–40035.
[47] M.U.A. Khan, et al., Chitosan/poly vinyl alcohol/graphene oxide based pH-responsive composite hydrogel films: drug release, anti-microbial and cell viability studies, Polymers 13 (18) (2021) 3124.
[48] H.S. Low, M.N.M. Ansari, S. Begum, Model, synthesis and applications of graphene oxide: a review, Nanomater. Energy 3 (2014) 61–65.
[49] A.K. Mishra, et al., Nanoinformatics and nanotechnology in anti-inflammatory therapy: a review, J. Drug Deliv. Sci. Technol. 93 (2024) 105446.
[50] W. Gao, et al., New insights into the structure and reduction of graphite oxide, Nat. Chem. 1 (5) (2009) 403–408.
[51] D. Lee, et al., The structure of graphite oxide: investigation of its surface chemical groups, The journal of physical chemistry. B 114 (2010) 5723–5728.
[52] A. Bagri, et al., Structural evolution during the reduction of chemically derived graphene oxide, Nat. Chem. 2 (2010) 581–587.
[53] Z. Wang, et al., Aqueous phase preparation of graphene with low defect density and adjustable layers, Chem. Commun. 49 (92) (2013) 10835–10837.
[54] C.K. Chua, M. Pumera, Chemical reduction of graphene oxide: a synthetic chemistry viewpoint, Chem. Soc. Rev. 43 (1) (2014) 291–312.
[55] M.U.A. Khan, et al., Role of graphene oxide in bacterial Cellulose–Gelatin hydrogels for wound dressing applications, ACS Omega 8 (18) (2023) 15909–15919.
[56] X.-Y. Wang, A. Narita, K. Müllen, Precision synthesis versus bulk-scale fabrication of graphenes, Nat. Rev. Chem 2 (2017) 100.
[57] B.C. Brodie, XIII. On the atomic weight of graphite, Phil. Trans. Roy. Soc. Lond. 149 (149) (1859) 249–259.
[58] L. Staudenmaier, Verfahren zur darstellung der graphitsäure, Ber. Dtsch. Chem. Ges. 31 (2) (1898) 1481–1487.
[59] W.S. Hummers Jr., R.E. Offeman, Preparation of graphitic oxide, J. Am. Chem. Soc. 80 (6) (1958) 1339, 1339.
[60] F. Pendolino, N. Armata, *Graphene Oxide In Environmental Remediation Process.* Graphene Oxide in Environmental Remediation Process, 2017.
[61] N.-J. Huang, et al., Efficient interfacial interaction for improving mechanical properties of polydimethylsiloxane nanocomposites filled with low content of graphene oxide nanoribbons, RSC Adv. 7 (2017) 22045–22053.
[62] N.I. Kovtyukhova, et al., Layer-by-Layer assembly of ultrathin composite films from micron-sized graphite oxide sheets and polycations, Chem. Mater. 11 (3) (1999) 771–778.
[63] L. Sun, B. Fugetsu, Mass production of graphene oxide from expanded graphite, Mater. Lett. 109 (2013) 207–210.
[64] S. Stankovich, et al., Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide, Carbon 45 (7) (2007) 1558–1565.

[65] W. Wang, et al., One-pot facile synthesis of graphene quantum dots from rice husks for Fe3+ sensing, Ind. Eng. Chem. Res. 57 (28) (2018) 9144–9150.
[66] M.U.A. Khan, et al., Sodium alginate-f-GO composite hydrogels for tissue regeneration and antitumor applications, Int. J. Biol. Macromol. 208 (2022) 475–485.
[67] L. Dong, et al., A non-dispersion strategy for large-scale production of ultra-high concentration graphene slurries in water, Nat. Commun. 9 (1) (2018) 76.
[68] K.K.H. De Silva, et al., Chemical reduction of graphene oxide using green reductants, Carbon 119 (2017) 190–199.
[69] G. Williams, B. Seger, P.V. Kamat, TiO2-Graphene nanocomposites. UV-assisted photocatalytic reduction of graphene oxide, ACS Nano 2 (7) (2008) 1487–1491.
[70] S. Yang, et al., New-generation graphene from electrochemical approaches: production and applications, Adv. Mater. 28 (29) (2016) 6213–6221.
[71] S. Pei, H.-M. Cheng, The reduction of graphene oxide, Carbon 50 (9) (2012) 3210–3228.
[72] L. Li, et al., Focusing on luminescent graphene quantum dots: current status and future perspectives, Nanoscale 5 (10) (2013) 4015–4039.
[73] J.P. Melo, et al., Graphene oxide quantum dots as the support for the synthesis of gold nanoparticles and their applications as new catalysts for the decomposition of composite solid propellants, ACS Omega 3 (7) (2018) 7278–7287.
[74] Q. Lu, et al., A facile and simple method for synthesis of graphene oxide quantum dots from black carbon, Green Chem. 19 (4) (2017) 900–904.
[75] Rattana, et al., Preparation and characterization of graphene oxide nanosheets, Procedia Eng. 32 (2012) 759–764.
[76] S. Park, R.S. Ruoff, Chemical methods for the production of graphenes, Nat. Nanotechnol. 4 (4) (2009) 217–224.
[77] D.G. Papageorgiou, I.A. Kinloch, R.J. Young, Mechanical properties of graphene and graphene-based nanocomposites, Prog. Mater. Sci. 90 (2017) 75–127.
[78] R. Dong, P.X. Ma, B. Guo, Conductive biomaterials for muscle tissue engineering, Biomaterials 229 (2020) 119584.
[79] Z. Liu, et al., PEGylated nanographene oxide for delivery of water-insoluble cancer drugs, J. Am. Chem. Soc. 130 (33) (2008) 10876–10877.
[80] H. Yan, et al., Effects of the oxidation degree of graphene oxide on the adsorption of methylene blue, J. Hazard Mater. 268 (2014) 191–198.
[81] J. Kuchlyan, et al., Spectroscopy and fluorescence lifetime imaging microscopy to probe the interaction of bovine serum albumin with graphene oxide, Langmuir 31 (51) (2015) 13793–13801.
[82] S. Stankovich, et al., Synthesis of graphene-based nanosheets via chemical reduction of exfoliated graphite oxide, Carbon 45 (2007) 1558–1565.
[83] H. Jiang, Chemical Preparation of Graphene-Based Nanomaterials and Their Applications in Chemical and Biological Sensors 7 (17) (2011) 2413–2427.
[84] S. Guo, S. Dong, Graphene nanosheet: synthesis, molecular engineering, thin film, hybrids, and energy and analytical applications, Chem. Soc. Rev. 40 (5) (2011) 2644–2672.
[85] C. Liao, Y. Li, S.C. Tjong, Graphene nanomaterials: synthesis, biocompatibility, and cytotoxicity, Int. J. Mol. Sci. 19 (11) (2018).
[86] N. Bernabò, et al., Graphene oxide affects in vitro fertilization outcome by interacting with sperm membrane in an animal model, Carbon 129 (2017).
[87] C. Chen, Y. Xi, Y. Weng, Progress in the development of graphene-based biomaterials for tissue engineering and regeneration 15 (6) (2022) 2164.
[88] L. Zhang, B. Xu, X. Wang, Cholesterol extraction from cell membrane by graphene nanosheets: a computational study, J. Phys. Chem. B 120 (5) (2016) 957–964.
[89] G. Duan, et al., Graphene-induced pore formation on cell membranes, Sci. Rep. 7 (1) (2017) 42767.
[90] S. Li, et al., Head groups of lipids govern the interaction and orientation between graphene oxide and lipids, J. Phys. Chem. C 117 (31) (2013) 16150–16158.
[91] X. Hu, et al., Strong hydrophobic interaction between graphene oxide and supported lipid bilayers revealed by AFM, Microsc. Res. Tech. 79 (8) (2016) 721–726.
[92] R. Li, et al., Surface oxidation of graphene oxide determines membrane damage, lipid peroxidation, and cytotoxicity in macrophages in a pulmonary toxicity model, ACS Nano 12 (2) (2018) 1390–1402.
[93] M. Pelin, et al., Graphene and graphene oxide induce ROS production in human HaCaT skin keratinocytes: the role of xanthine oxidase and NADH dehydrogenase, Nanoscale 10 (25) (2018) 11820–11830.
[94] J. Chen, et al., One-step reduction and PEGylation of graphene oxide for photothermally controlled drug delivery, Biomaterials 35 (18) (2014) 4986–4995.
[95] Y. Xiao, et al., Synthesis and functionalization of graphene materials for biomedical applications: recent advances, challenges, and perspectives, Adv. Sci. 10 (9) (2023) 2205292.
[96] R. Lakshmanan, N. Maulik, Graphene-based drug delivery systems in tissue engineering and nanomedicine, Can. J. Physiol. Pharmacol. 96 (2018) 1–10.
[97] V. Georgakilas, et al., Functionalization of graphene: covalent and non-covalent approaches, derivatives and applications, Chem. Rev. 112 (11) (2012) 6156–6214.
[98] D.F. Báez, Graphene-based nanomaterials for photothermal therapy in cancer treatment, Pharmaceutics 15 (2023), https://doi.org/10.3390/pharmaceutics15092286.
[99] P. Huang, et al., Folic acid-conjugated graphene oxide loaded with photosensitizers for targeting photodynamic therapy, Theranostics 1 (2011) 240–250.
[100] Y. Wang, et al., Multifunctional mesoporous silica-coated graphene nanosheet used for chemo-photothermal synergistic targeted therapy of glioma, J. Am. Chem. Soc. 135 (12) (2013) 4799–4804.
[101] S. Shi, et al., Tumor vasculature targeting and imaging in living mice with reduced graphene oxide, Biomaterials 34 (12) (2013) 3002–3009.
[102] I. Imae, Reduction of graphene oxide using an environmentally friendly method and its application to energy-related materials, Coatings 11 (2021), https://doi.org/10.3390/coatings11030297.
[103] T. Zhou, et al., A simple and efficient method to prepare graphene by reduction of graphite oxide with sodium hydrosulfite, Nanotechnology 22 (4) (2010) 045704.
[104] Z. Liu, et al., Preparation of carbon nanotube bioconjugates for biomedical applications, Nat. Protoc. 4 (9) (2009) 1372–1381.
[105] M. Quintana, et al., Functionalization of graphene via 1,3-dipolar cycloaddition, ACS Nano 4 (6) (2010) 3527–3533.
[106] J. Park, M. Yan, Covalent functionalization of graphene with reactive intermediates, Accounts of Chemical Research 46 (1) (2013) 181–189.
[107] P. Tian, et al., Graphene quantum dots from chemistry to applications, Mater. Today Chem. 10 (2018) 221–258.
[108] D.X. Luong, et al., Gram-scale bottom-up flash graphene synthesis, Nature 577 (7792) (2020) 647–651.
[109] M.G. Stanford, et al., Flash graphene morphologies, ACS Nano 14 (10) (2020) 13691–13699.
[110] C.H. Lu, et al., A graphene platform for sensing biomolecules, Angew Chem. Int. Ed. Engl. 48 (26) (2009) 4785–4787.
[111] S. He, et al., A graphene nanoprobe for rapid, sensitive, and multicolor fluorescent DNA analysis, Adv. Funct. Mater. 20 (3) (2010) 453–459.
[112] L. Feng, Z. Liu, Graphene in biomedicine: opportunities and challenges, Nanomedicine (Lond) 6 (2) (2011) 317–324.
[113] K. Yang, et al., Behavior and toxicity of graphene and its functionalized derivatives in biological systems, Small 9 (9–10) (2013) 1492–1503.
[114] R. Singh, et al., A glance on gold nanoparticle: an emerging theranostic tool for oncology, J. Drug Deliv. Sci. Technol. 97 (2024) 105766.
[115] D. Bitounis, et al., Prospects and challenges of graphene in biomedical applications, Adv Mater 25 (16) (2013) 2258–2268.
[116] L. Feng, L. Wu, X. Qu, New horizons for diagnostics and therapeutic applications of graphene and graphene oxide, Adv. Mater. 25 (2) (2013) 168–186.
[117] K. Yang, et al., Nano-graphene in biomedicine: theranostic applications, Chem. Soc. Rev. 42 (2) (2013) 530–547.
[118] L. Zhang, et al., Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs, Small 6 (4) (2010) 537–544.
[119] A. Fraczek-Szczypta, Carbon nanomaterials for nerve tissue stimulation and regeneration, Mater. Sci. Eng. C 34 (2014) 35–49.
[120] N. Li, et al., The promotion of neurite sprouting and outgrowth of mouse hippocampal cells in culture by graphene substrates, Biomaterials 32 (35) (2011) 9374–9382.
[121] H. Chen, et al., Mechanically strong, electrically conductive, and biocompatible graphene paper, Adv. Mater. 20 (18) (2008) 3557–3561.
[122] J.D. Foley, et al., Cooperative modulation of neuritogenesis by PC12 cells by topography and nerve growth factor, Biomaterials 26 (17) (2005) 3639–3644.
[123] N. Gomez, et al., Micropatterned polypyrrole: a combination of electrical and topographical characteristics for the stimulation of cells, Adv. Funct. Mater. 17 (10) (2007) 1645–1653.
[124] F.H. Gage, Mammalian neural stem cells, Science 287 (5457) (2000) 1433–1438.
[125] L. Conti, E. Cattaneo, Neural stem cell systems: physiological players or in vitro entities? Nat. Rev. Neurosci. 11 (3) (2010) 176–187.
[126] A. Björklund, S.B. Dunnett, Dopamine neuron systems in the brain: an update, Trends Neurosci. 30 (5) (2007) 194–202.

[127] N. Li, et al., Three-dimensional graphene foam as a biocompatible and conductive scaffold for neural stem cells, Sci. Rep. 3 (2013) 1604.
[128] J.T. Robinson, et al., Properties of fluorinated graphene films, Nano Lett. 10 (8) (2010) 3001–3005.
[129] M.P. Lutolf, J.A. Hubbell, Synthetic biomaterials as instructive extracellular microenvironments for morphogenesis in tissue engineering, Nat. Biotechnol. 23 (1) (2005) 47–55.
[130] Y. Wang, et al., Fluorinated graphene for promoting neuro-induction of stem cells, Adv. Mater. 24 (2012) 4285–4290.
[131] N.S. Roy, et al., Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes, Nat Med 12 (11) (2006) 1259–1268.
[132] N. Prakash, W. Wurst, Genetic networks controlling the development of midbrain dopaminergic neurons, J. Physiol. 575 (2) (2006) 403–410.
[133] O. Akhavan, et al., Accelerated differentiation of neural stem cells into neurons on ginseng-reduced graphene oxide sheets, Carbon 66 (2014) 395–406.
[134] O. Akhavan, E. Ghaderi, The use of graphene in the self-organized differentiation of human neural stem cells into neurons under pulsed laser stimulation, J. Mater. Chem. B 2 (34) (2014) 5602–5611.
[135] P. Fattahi, et al., A review of organic and inorganic biomaterials for neural interfaces, Adv Mater 26 (12) (2014) 1846–1885.
[136] Y. Kocakaplan, E. Kantar, M. Keskin, Hysteresis loops and compensation behavior of cylindrical transverse spin-1 Ising nanowire with the crystal field within effective-field theory based on a probability distribution technique, Eur. Phys. J. B 86 (10) (2013) 420.
[137] K. Zhou, et al., Method to impart electro- and biofunctionality to neural scaffolds using graphene–polyelectrolyte multilayers, ACS Appl. Mater. Interfaces 4 (9) (2012) 4524–4531.
[138] K.-A. Chang, et al., Biphasic electrical currents stimulation promotes both proliferation and differentiation of fetal neural stem cells, PLoS One 6 (4) (2011) e18738.
[139] S. Kumagai, et al., Development of plasma-on-chip: plasma treatment for individual cells cultured in media, 01af01, Jpn. J. Appl. Phys. 55 (1S) (2015).
[140] T. Cohen-Karni, et al., Graphene and nanowire transistors for cellular interfaces and electrical recording, Nano Lett. 10 (3) (2010) 1098–1102.
[141] B.-L. Sun, et al., Fabrication of electrochemical sensor modified with porous graphene for determination of trace calycosin, Chin. J. Anal. Chem. 47 (2) (2019) 271–279.
[142] S.Y. Park, et al., Enhanced differentiation of human neural stem cells into neurons on graphene, Adv Mater 23 (36) (2011) H263–H267.
[143] Z. Guo, et al., Synthesis and characterization of rGO-graft-poly(trimethylene carbonate) for nerve regeneration conduits, Biomedical Materials 14 (3) (2019) 034101.
[144] L.M.Y. Yu, N.D. Leipzig, M.S. Shoichet, Promoting neuron adhesion and growth, Mater. Today 11 (5) (2008) 36–43.
[145] E. Marconi, et al., Emergent functional properties of neuronal networks with controlled topology, PLoS One 7 (4) (2012) e34648.
[146] O. Akhavan, E. Ghaderi, Differentiation of human neural stem cells into neural networks on graphene nanogrids, J. Mater. Chem. B 1 (45) (2013) 6291–6301.
[147] M. Lorenzoni, et al., Simple and effective graphene laser processing for neuron patterning application, Sci. Rep. 3 (1) (2013) 1954.
[148] A. Solanki, et al., Axonal alignment and enhanced neuronal differentiation of neural stem cells on graphene-nanoparticle hybrid structures, Adv. Mater. 25 (38) (2013) 5477–5482.
[149] P. Fromherz, et al., A neuron-silicon junction: a Retzius cell of the leech on an insulated-gate field-effect transistor, Science 252 (5010) (1991) 1290–1293.
[150] K. Koczkur, et al., Electron transfer initiated formation of covalently bound organic layers on silicon surfaces, J. Phys. Chem. C 118 (2014) 20908–20915.
[151] C. Heo, et al., The control of neural cell-to-cell interactions through non-contact electrical field stimulation using graphene electrodes, Biomaterials 32 (1) (2011) 19–27.
[152] M.U.A. Khan, et al., Bioactive scaffold (sodium alginate)-g-(nHAp@SiO(2)@GO) for bone tissue engineering, Int. J. Biol. Macromol. 222 (Pt A) (2022) 462–472.
[153] M. Gu, et al., Is graphene a promising nano-material for promoting surface modification of implants or scaffold materials in bone tissue engineering? Tissue Eng Part B Rev 20 (5) (2014) 477–491.
[154] H. Petite, et al., Tissue-engineered bone regeneration, Nat. Biotechnol. 18 (9) (2000) 959–963.
[155] S.P. Bruder, et al., Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells, J. Orthop. Res. 16 (2) (1998) 155–162.
[156] M. Bhattacharya, et al., Bone formation on carbon nanotube composite, J. Biomed. Mater. Res. 96 (1) (2011) 75–82.
[157] M.U. Aslam Khan, et al., Development of biopolymeric hybrid scaffold-based on AAc/GO/nHAp/TiO(2) nanocomposite for bone tissue engineering: in-vitro analysis, Nanomaterials 11 (5) (2021).
[158] R. Ravichandran, et al., Precipitation of nanohydroxyapatite on PLLA/PBLG/Collagen nanofibrous structures for the differentiation of adipose derived stem cells to osteogenic lineage, Biomaterials 33 (3) (2012) 846–855.
[159] C. Correia, et al., Development of silk-based scaffolds for tissue engineering of bone from human adipose-derived stem cells, Acta Biomater. 8 (7) (2012) 2483–2492.
[160] M. Spoliti, et al., In vitro release and expansion of mesenchymal stem cells by a hyaluronic acid scaffold used in combination with bone marrow, Muscles Ligaments Tendons J 2 (4) (2012) 289–294.
[161] T.R. Nayak, et al., Graphene for controlled and accelerated osteogenic differentiation of human mesenchymal stem cells, ACS Nano 5 (6) (2011) 4670–4678.
[162] E.S. Kang, et al., Three-dimensional graphene-RGD peptide nanoisland composites that enhance the osteogenesis of human adipose-derived mesenchymal stem cells, Int. J. Mol. Sci. 19 (3) (2018).
[163] W.G. La, et al., Delivery of a therapeutic protein for bone regeneration from a substrate coated with graphene oxide, Small 9 (23) (2013) 4051–4060.
[164] M.U.A. Khan, et al., Multifunctional bioactive scaffolds from ARX-g-(Zn@rGO)-HAp for bone tissue engineering: in vitro antibacterial, antitumor, and biocompatibility evaluations, ACS Appl. Bio Mater. 5 (11) (2022) 5445–5456.
[165] J. Lu, et al., Self-supporting graphene hydrogel film as an experimental platform to evaluate the potential of graphene for bone regeneration, Adv. Funct. Mater. 23 (28) (2013) 3494–3502.
[166] S. Han, et al., The application of graphene-based biomaterials in biomedicine, Am J Transl Res 11 (6) (2019) 3246–3260.
[167] M.U.A. Khan, et al., Electroactive polymeric nanocomposite BC-g-(Fe3O4/GO) materials for bone tissue engineering: in vitro evaluations, J. Biomater. Sci. Polym. Ed. 33 (11) (2022) 1349–1368.
[168] S.W. Crowder, et al., Three-dimensional graphene foams promote osteogenic differentiation of human mesenchymal stem cells, Nanoscale 5 (10) (2013) 4171–4176.
[169] J. Kim, et al., Graphene-incorporated chitosan substrata for adhesion and differentiation of human mesenchymal stem cells, J. Mater. Chem. B 1 (7) (2013) 933–938.
[170] W. Qi, et al., Growth and accelerated differentiation of mesenchymal stem cells on graphene oxide/poly-l-lysine composite films, J. Mater. Chem. B 2 (33) (2014) 5461–5467.
[171] S. Duan, et al., Enhanced osteogenic differentiation of mesenchymal stem cells on poly(l-lactide) nanofibrous scaffolds containing carbon nanomaterials, J. Biomed. Mater. Res. 103 (4) (2015) 1424–1435.
[172] R. Tatavarty, et al., Synergistic acceleration in the osteogenesis of human mesenchymal stem cells by graphene oxide–calcium phosphate nanocomposites, Chem. Commun. 50 (62) (2014) 8484–8487.
[173] M. Umar Aslam Khan, et al., Development of porous, antibacterial and biocompatible GO/n-HAp/bacterial cellulose/β-glucan biocomposite scaffold for bone tissue engineering, Arab. J. Chem. 14 (2) (2021) 102924.
[174] M. Mahmood, et al., Role of carbonaceous nanomaterials in stimulating osteogenesis in mammalian bone cells, J. Mater. Chem. B 1 (25) (2013) 3220–3230.
[175] W.S. Al-Arjan, et al., Development of arabinoxylan-reinforced apple pectin/graphene oxide/nano-hydroxyapatite based nanocomposite scaffolds with controlled release of drug for bone tissue engineering: in-vitro evaluation of biocompatibility and cytotoxicity against mc3t3-E1, Coatings 10 (11) (2020) 1120.
[176] I. Kanayama, et al., Comparative study of bioactivity of collagen scaffolds coated with graphene oxide and reduced graphene oxide, Int J Nanomedicine 9 (2014) 3363–3373.

[177] D. Lahiri, et al., Graphene nanoplatelet-induced strengthening of UltraHigh molecular weight polyethylene and biocompatibility in vitro, ACS Appl. Mater. Interfaces 4 (4) (2012) 2234–2241.
[178] M.U.A. Khan, et al., Smart and pH-sensitive rGO/Arabinoxylan/chitosan composite for wound dressing: in-vitro drug delivery, antibacterial activity, and biological activities, Int. J. Biol. Macromol. 192 (2021) 820–831.
[179] M.U. Aslam Khan, et al., Arabinoxylan/graphene-oxide/nHAp-NPs/PVA bionano composite scaffolds for fractured bone healing, J Tissue Eng Regen Med 15 (4) (2021) 322–335.
[180] S. Kim, et al., Graphene-biomineral hybrid materials, Adv Mater 23 (17) (2011) 2009–2014.
[181] S. Baradaran, et al., Mechanical properties and biomedical applications of a nanotube hydroxyapatite-reduced graphene oxide composite, Carbon 69 (2014) 32–45.
[182] M. Li, et al., An overview of graphene-based hydroxyapatite composites for orthopedic applications, Bioact. Mater. 3 (1) (2018) 1–18.
[183] M. Li, et al., In situ synthesis and biocompatibility of nano hydroxyapatite on pristine and chitosan functionalized graphene oxide, J. Mater. Chem. B 1 (4) (2013) 475–484.
[184] M. Kalbacova, et al., Graphene substrates promote adherence of human osteoblasts and mesenchymal stromal cells, Carbon 48 (15) (2010) 4323–4329.
[185] S. Prasadh, S. Suresh, R. Wong, Osteogenic potential of graphene in bone tissue engineering scaffolds, Materials 11 (8) (2018).
[186] E. Akbari, I. Akbari, M.R. Ebrahimi, sp2/sp3 bonding ratio dependence of the band-gap in graphene oxide, Eur. Phys. J. B 92 (4) (2019) 71.
[187] C. Chung, J.A. Burdick, Engineering cartilage tissue, Adv. Drug Deliv. Rev. 60 (2) (2008) 243–262.
[188] O.S. Beane, E.M. Darling, Isolation, characterization, and differentiation of stem cells for cartilage regeneration, Ann. Biomed. Eng. 40 (10) (2012) 2079–2097.
[189] H.H. Yoon, et al., Dual roles of graphene oxide in chondrogenic differentiation of adult stem cells: cell-adhesion substrate and growth factor-delivery carrier, Adv. Funct. Mater. 24 (41) (2014) 6455–6464.
[190] A.D. Bach, et al., Skeletal muscle tissue engineering, J. Cell Mol. Med. 8 (4) (2004) 413–422.
[191] N. Kasoju, U. Bora, Silk fibroin in tissue engineering, Adv. Healthcare Mater. 1 (4) (2012) 393–412.
[192] P.J. Psaltis, et al., Concise review: mesenchymal stromal cells: potential for cardiovascular repair, Stem Cell. 26 (9) (2008) 2201–2210.
[193] H. Song, et al., Specific differentiation of mesenchymal stem cells by small molecules, Am J Stem Cells 1 (1) (2012) 22–30.
[194] P. Antonitsis, et al., In vitro cardiomyogenic differentiation of adult human bone marrow mesenchymal stem cells. The role of 5-azacytidine, Interact. Cardiovasc. Thorac. Surg. 6 (5) (2007) 593–597.
[195] M.U.A. Khan, et al., Multifunctional arabinoxylan-functionalized-graphene oxide based composite hydrogel for skin tissue engineering, Front. Bioeng. Biotechnol. 10 (2022) 865059.
[196] J. Park, et al., Graphene-regulated cardiomyogenic differentiation process of mesenchymal stem cells by enhancing the expression of extracellular matrix proteins and cell signaling molecules, Adv Healthc Mater 3 (2) (2014) 176–181.
[197] F. Groeber, et al., Skin tissue engineering–in vivo and in vitro applications, Adv. Drug Deliv. Rev. 63 (4–5) (2011) 352–366.
[198] B. Lu, et al., Graphene-based composite materials beneficial to wound healing, Nanoscale 4 (9) (2012) 2978–2982.
[199] W.S. Al-Arjan, et al., pH-responsive PVA/BC-f-GO dressing materials for burn and chronic wound healing with curcumin release kinetics, Polymers 14 (10) (2022).
[200] A. Casadei, et al., Adipose tissue regeneration: a state of the art, J. Biomed. Biotechnol. 2012 (2012) 462543.
[201] S. Dimitrievska, L.E. Niklason, Historical perspective and future direction of blood vessel developments, Cold Spring Harb Perspect Med 8 (2) (2018).
[202] H.G. Song, et al., Vascular tissue engineering: progress, challenges, and clinical promise, Cell Stem Cell 22 (3) (2018) 340–354.
[203] J. Chen, et al., Recent progress in vascular tissue-engineered blood vessels, Adv. Exp. Med. Biol. 1064 (2018) 123–144.
[204] M. Lovett, et al., Tubular silk scaffolds for small diameter vascular grafts, Organogenesis 6 (4) (2010) 217–224.
[205] S. Karimi Alavije, M. Kokabi, M. Soleimani, Endothelial cells performance on 3D electrospun PVA/graphene nanocomposite tubular scaffolds, Polym. Bull. 78 (9) (2021) 4797–4815.
[206] Y.C. Shin, et al., RGD peptide and graphene oxide co-functionalized PLGA nanofiber scaffolds for vascular tissue engineering, Regen Biomater 4 (3) (2017) 159–166.
[207] D. Huo, et al., Construction of antithrombotic tissue-engineered blood vessel via reduced graphene oxide based dual-enzyme biomimetic cascade, ACS Nano 11 (11) (2017) 10964–10973.
[208] W. Shang, et al., The uptake mechanism and biocompatibility of graphene quantum dots with human neural stem cells, Nanoscale 6 (11) (2014) 5799–5806.
[209] O. Akhavan, et al., Photodegradation of graphene oxide sheets by TiO2 nanoparticles after a photocatalytic reduction, J. Phys. Chem. C 114 (30) (2010) 12955–12959.
[210] J. Liu, et al., Toward a universal "adhesive nanosheet" for the assembly of multiple nanoparticles based on a protein-induced reduction/decoration of graphene oxide, J. Am. Chem. Soc. 132 (21) (2010) 7279–7281.
[211] O. Akhavan, E. Ghaderi, A. Akhavan, Size-dependent genotoxicity of graphene nanoplatelets in human stem cells, Biomaterials 33 (32) (2012) 8017–8025.
[212] D. Li, X. Hu, S. Zhang, Biodegradation of graphene-based nanomaterials in blood plasma affects their biocompatibility, drug delivery, targeted organs and antitumor ability, Biomaterials 202 (2019) 12–25.
[213] K. Yang, et al., Graphene in mice: ultrahigh in vivo tumor uptake and efficient photothermal therapy, Nano Lett. 10 (9) (2010) 3318–3323.
[214] O. Akhavan, et al., Dose-dependent effects of nanoscale graphene oxide on reproduction capability of mammals, Carbon 95 (2015).
[215] K. Yang, et al., In vivo pharmacokinetics, long-term biodistribution, and toxicology of PEGylated graphene in mice, ACS Nano 5 (1) (2011) 516–522.
[216] G. Wang, et al., Microbial reduction of graphene oxide by Shewanella, Nano Res. 4 (6) (2011) 563–570.
[217] M.C. Duch, et al., Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility of graphene in the lung, Nano Lett. 11 (12) (2011) 5201–5207.
[218] A.M. Jastrzębska, P. Kurtycz, A.R. Olszyna, Recent advances in graphene family materials toxicity investigations, J Nanopart Res 14 (12) (2012) 1320.
[219] S. Liu, et al., Antibacterial activity of graphite, graphite oxide, graphene oxide, and reduced graphene oxide: membrane and oxidative stress, ACS Nano 5 (9) (2011) 6971–6980.
[220] O. Akhavan, E. Ghaderi, Toxicity of graphene and graphene oxide nanowalls against bacteria, ACS Nano 4 (10) (2010) 5731–5736.
[221] S. Gurunathan, et al., Evaluation of graphene oxide induced cellular toxicity and transcriptome analysis in human embryonic kidney cells 9 (7) (2019) 969.
[222] O. Akhavan, Graphene scaffolds in progressive nanotechnology/stem cell-based tissue engineering of the nervous system, J. Mater. Chem. B 4 (19) (2016) 3169–3190.