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Review

Graphene Production and Biomedical Applications: A Review

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Abstract: Graphene is a two-dimensional nanomaterial composed of carbon atoms with sp^2 hybrid orbitals. Both graphene and graphene-based composite have gained broad interest among researchers because of their outstanding physiochemical, mechanical, and biological properties. Graphene production techniques are divided into top-down and bottom-up synthesis methods, of which chemical vapor deposition (CVD) is the most popular. The biomedical applications of graphene and its composite include its use in sensors, implantology, and gene and drug delivery. They can be used for tissue engineering, anticancer therapies, and as antimicrobial agents in implant application. The biocompatibility of graphene-based nanomaterials enables their use in the field of biomedicine. This article reviews the properties of graphene, the methods used to produce it, the challenges associated with its use, and the potential applications of this material in biomedicine, regenerative medicine, and drug delivery systems.

Keywords: graphene; biosensors; tissue engineering

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1. Introduction

Carbon nanostructures have attracted a lot of attention, especially single-walled carbon nanotubes, multi-walled carbon nanotubes, graphene, and reduced graphene oxide (rGO) [1]. Graphene is a two-dimensional material composed of carbon (C) atoms with an sp^2 hybrid orbital [2–5], displaying a single π orbital and three σ bonds perpendicular to the plane [6]. It is a honeycomb like structure formed with a single thick planer carbon sheet [7]. Graphene can be wrapped up into zero-dimensional fullerenes, rolled into one-dimensional carbon nanotubes, or piled together with an inter-planar spacing of 0.335 nm to form three-dimensional graphite [6,8]. Due to its outstanding physical, mechanical, electrical, and chemical properties, materials based on graphene have attracted a lot of interest. These include excellent thermal and electrical conductivity, high specific surface area, elastic moduli (about 1 TPa [9]), an intrinsic strength of 130 GPa [5], adaptability to both flat and irregular surfaces, flexibility in chemical and biological functionalization, and simplicity in mass production, [10–15]. Sensors, energy harvesting, and storage devices such as solar cells and supercapacitors can all be improved by the use of graphene, as can lightweight polymer composites, membranes, and actuators. Graphene derivatives could also be used in biomedical applications such as drug delivery systems, gene therapies, photothermal therapies, antibacterial agents, and bioimaging tools [5,10,16–18]. However, scientists still face numerous challenges when attempting to utilize graphene such as cytotoxicity, biodistribution, and immunological responses.

Currently, there are many ways to produce graphene. Those include chemical vapor deposition, the mechanical cleavage of highly ordered pyrolytic graphite (HOPG), the chemical reduction of chemically exfoliated graphene oxide, epitaxial growth, and chemical synthesis [11,14]. Graphene oxide (GO), reduced graphene oxide (rGO), graphene, and graphene quantum dots (GQDs) are all included in the graphene family [19]. The molecular structures are shown in Figure 1 [20]. Chemical modification can reduce GO to rGO [18]. GQDs, also known as nano-graphene (NG), are nanomaterials that can be used in a range

of applications. To create NG, top-down and bottom-up techniques are used [18,21]. Furthermore, there is a separate kind of carbon-based nanomaterial called nano-graphene oxide (NGO). NGO is created by top-down methods. Graphene oxide can be oxidized to form nano-graphene oxide, which has hydrophobic properties and the qualities suitable for creating very stable aqueous colloidal suspensions [18].

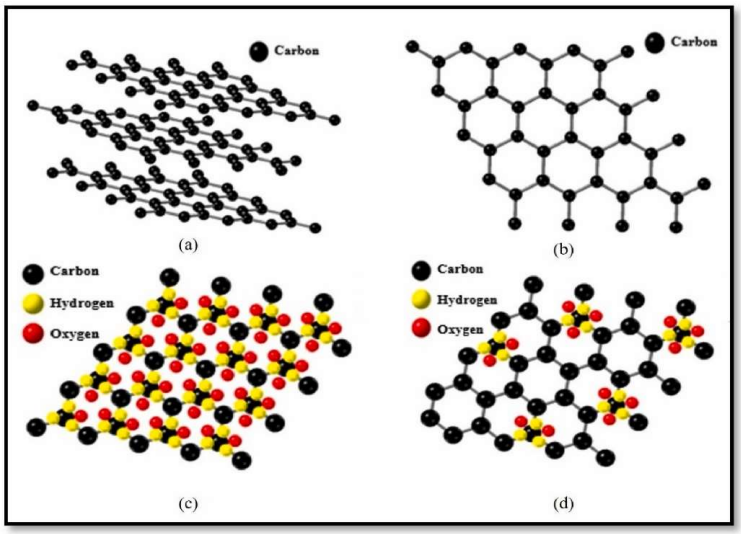

Figure 1. Molecular structure of (**a**) graphite, (**b**) graphene, (**c**) graphene oxide, (**d**) reduced graphene oxide (reprinted from [20], with permission from Elsevier, license number: 5595830853941).

Both GO and rGO are well recognized for their lower dispersion in water but also easy aggregation. This can reduce their surface areas, which would limit their usability and ability to be recycled from treated water. Additionally, graphene composites have significant limitations because of their hydrophobic qualities, which tend to result in the aggregation of sheets, along with curled, folded, and corrugated formations on base materials [19]. However, because of their enormous clinical potential in tissue regeneration therapy, GO and rGO-based scaffolds are particularly promising. The proliferation and differentiation of applied stem cells have been strongly influenced by both GO and rGO, but there are still some challenges to overcome, e.g., cytotoxicity, biodistribution, biotransformation, and immune response [17]. Graphene-reinforced polymer composites are also of great interest. The use of graphene as a reinforcing agent in the polymer matrix improves the composites' properties (for example, adding carbon nanotubes and graphene to metals reduces the coefficient of friction and the wear rate while increasing tensile strength) [5,22,23].

In this review, we will describe the properties of graphene and graphene composites, and the methods used to manufacture them with a particular emphasis on the challenges and potential applications of these materials in biomedicine, tissue engineering, and drug carrier systems.

2. Graphene Production Techniques

Several methods including pyrolysis, epitaxial growth, the chemical and electrochemical exfoliation of graphite, physical vapor deposition, and chemical vapor deposition have been used to produce graphene [6,11,14]. We divide these techniques into top-down (mechanical exfoliation, chemical exfoliation, chemical synthesis) and bottom-up synthesis methods (epitaxial growth, pyrolysis, CVD, etc.) [6]. To obtain graphene, it is possible to use the thermal expansion of graphite/graphite oxide [8,24], which can lead to its formation. However, this process rarely leads to results in the complete exfoliation of graphene to the atomic level of individual sheets of graphene [8]. There are also known methods such as liquid phase exfoliation of graphite employing ultrasonication, chemical exfoliation methods, or the electrochemical exfoliation method [8,25–27]. The disadvantages of graphene

obtained by liquid phase exfoliation of graphite employing ultrasonication include the graphene sheet size because it is typically smaller than 1 μm^2, and the graphene yield is too low to be utilized in technological applications. Chemical exfoliation methods involve the oxidation of graphite to graphene oxide and then reduction chemically or thermally. These methods have attracted a lot of interest due to their potentially low cost and easy production, but to obtain the graphene structure, the thermal reduction of graphite oxide is carried out at high temperatures. However, the oxidized forms of graphite oxide cannot be completely removed with a chemical reducing agent, which may cause the degradation of the electronic properties [8].

Among these technologies, CVD appears to be the most promising process for industrial development and scale-up production of graphene. Nearly all of the transition metals included in the periodic table may be used as catalysts for the production of graphene, according to recent developments in the CVD process for growing graphene [28]. Most commonly, CVD of graphene involves injecting a precursor in the gas phase into a reaction chamber. In the reaction chamber at elevated temperature, the precursor reacts with the catalyst, and graphene is produced on the surface of the catalyst. The precursor may be a hydrocarbon (for example, methane or ethylene) or also low-molecular-weight alcohols. The temperatures of growth range from a few hundred degrees Celsius to the melting point of the catalyst metal [29]. Nickel (Ni) and copper (Cu) are two catalysts mostly used to prepare homogeneous and high-quality graphene. Nickel-catalyzed graphene, consisting of a large proportion of few-layer or multilayer, could therefore be widely used as stretchable transparent electrodes. In contrast, graphene grown on Cu is a monolayer with a large area that has great potential in high-mobility field-effect transistors. The catalyst surface is where graphene grains often form during the CVD growth of graphene on metals. During graphene growth, the grains coalesce and eventually lead to the formation of grain boundaries, which are mainly dispersed in the as-grown graphene layer. As a result, CVD-grown graphene is frequently polycrystalline and composed of a patchwork of grains with different sizes and orientations. Two methods can be used to obtain monocrystalline graphene without grain boundaries using chemical vapor deposition. The first method involves complete control of the number of nucleation centers, as reducing the nucleation number to one will finally result in the formation of individual graphene crystals. The second method, on the other hand, involves controlling the orientation of the graphene grains. In the absence of grain boundaries in the stitching areas, grains with aligned crystal lattices would merge neatly, resulting in large-sized graphene crystals [28].

The catalyst-assisted CVD approach is an intriguing synthetic method for producing wafer-sized graphene. When the nucleation and growth of crystallized graphene domains take place in an atmospheric pressure CVD process, the average size of graphene domains raises with increasing temperature and CH_4 partial pressure, but the domain density decreases (but it is independent of the CH_4 partial pressure). In addition, research by Liu L. et al. [30] indicates that the nucleation of graphene domains on Cu depends on the initial annealing temperature [30]. Plasma-enhanced chemical vapor deposition (PECVD) has great potential for graphene production due to its low-temperature growth and fast reaction rate. The high temperature provides the energy needed for graphene nucleation and growth in the thermal CVD process. However, in the PECVD process, the growth of multilayer graphene is dominated mostly by radicals generated in the plasma, so it does not need to use high temperatures. Notwithstanding, the disadvantages of this method include poor controllability and quality of obtained graphene after the graphene is grown. Due to these limitations, this method is not widely used [11,31]. Li N. et al. [11] studied the nucleation and growth of graphene at different temperatures by PECVD. They noted that graphene could not be grown at temperatures under 600 °C. In the temperature range of 650–800 °C, they obtained a high nucleation density of nanoscale graphene. In the temperature range from 850 °C to 900 °C, they formed bigger grains of graphene, and over 950 °C, the coexistence of large and small grains was produced [11].

Electrochemical exfoliation graphene is a popular top-down graphene recovery method. It is composed of oxidizing, expanding, and exfoliating graphite to produce graphene with a lower oxygen content. The process is schematically shown in Figure 2. The electrochemical exfoliation method is an environmentally friendly technique to fabricate graphene and enables production at room temperature [8,32,33]. This technique uses many types of graphite, including graphite foils, rods, plates, and powders, as electrodes in an aqueous or non-aqueous electrolyte, together with an electric current to cause electrode expansion [6]. The surface shows high roughness after electrolytic exfoliation, which is associated with a large surface area. The highest surface area was obtained after 1 h of exfoliation, and after that it declined rapidly [8]. Applying a voltage of 10 V resulted in the formation of multilayer graphene platelets with high defect concentration. Awasthi G. et al. [33] produced multi-layered (i.e., 1–5), high-quality graphene nanoplatelets using low voltage (3–4 V) during the process. For this purpose, they used potassium hydroxide solution as an electrolyte. The stirring of an electrolyte during electrolysis affected the properties of graphene, i.e., the average size of graphene nanoplatelets increased in that case, but also a reduction in the defect concentration was observed with an increase in several layers of C atoms in graphene [33]. To create high-yield solution-processable graphene, Zhang Y. et al. [34] used an electrochemical anode and cathode co-exfoliation technique. They obtained graphene with an ultralow defect and oxygen content on electrodes. The flexible supercapacitor exhibits great electrochemical performance and can be potentially used in wearable electronics [34].

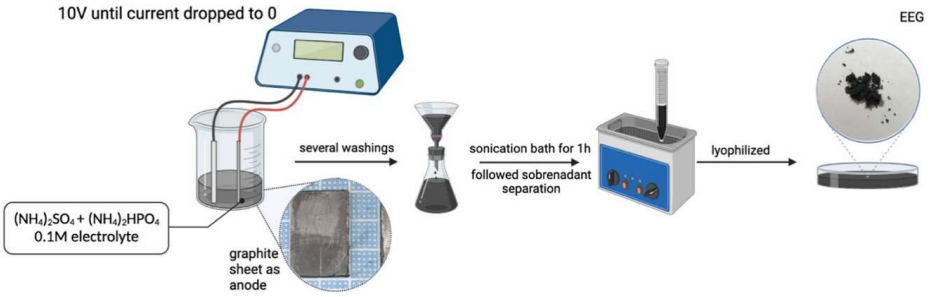

Figure 2. Electrochemical exfoliation method (reprinted from [32], with permission from Elsevier, license number: 5595791108264).

3. Application of Graphene

Graphene has received a lot of attention in a variety of sectors, including energy storage, electrochemical sensors, nanoelectronics, optical biosensors, membranes, composites, drug delivery systems, etc. [5,9,10].

3.1. Biosensors

A biosensor is a device that detects chemical or biological reactions by producing signals proportional to the concentration of an analyte in the reaction. Applications for biosensors include the detection of pollutants, disease-causing microorganisms, and markers that are indicators of disease in bodily fluids like blood, saliva urine, or even sweat. Biosensors are also used in the discovery of new medicines and the monitoring of diseases. A typical biosensor consists of a bioreceptor, transducer, electronics, and display. A bioreceptor is a molecule that recognizes the analyte specifically [35]. A transducer turns biological interactions into physical signals (for example, optical, chemical, electrical, or thermal signals) [36]. Electronics is a part that processes the transduced signal and prepares it for display. On the display, the output signal is presented in numerical, graphical, tabular, or pictorial form, depending on the requirements of the user [35].

Due to its excellent charge transfer, high specific surface area (2620 m^2/g), thermal conductivity of 500 W/mK, optical properties (visible and infrared light transmittance of 98%), and ability to immobilize molecules, graphene has become a widely used material in sensor

manufacturing [7,9]. Graphene can be modified, for example, with gold or silver nanoparticles. It has been proven that the addition of nanogold resulted in greater surface area, enhanced electrochemical reactions, and interaction between elements or nanoparticles, as well as simplified charge transfer for better robustness, sensitivity, and specificity [9,37]. Narayanan J. et al. [38] successfully developed an electrochemical immunosensor for the detection of botulinum neurotoxin-E, where glassy carbon electrodes were modified using graphene nanosheets-aryldiazonium salt as a sensing platform and enzyme induced silver nanoparticles, which were deposited on gold nanoparticles as a signal amplifier [38].

Graphene-based electrochemical sensors are used for the detection of cancer biomarkers and neurotransmitters, but also as immunosensors and for the detection various other bioanalytes [39]. Graphene's self-assembling characteristics are easily monitored, allowing it to be used in highly sensitive biosensors for DNA detection. Furthermore, due to their large surface area, superior electrical conductivity, and ability to load analytes, GO-based electrochemical biosensors have shown promising results in detecting cancers [18].

Ansari G. et al. [40] prepared a nanosensor using zinc oxide nanowires (ZnO NWs) and graphene. It can detect changes in hemoglobin content in vivo. ZnO NWs were produced using vapor-liquid–solid fabrication. The graphene layer can improve the sensitivity and response time of the biosensor, while the ZnO NWs can offer a significant surface area for immobilizing the biological molecules. The biosensor's sensitivity is improved by graphene's changed chemical potential. Furthermore, combining these two materials raises the selectivity of this device [40]. Soman G. et al. [41] designed a GO-based molecularly imprinted polymer for selective uric acid detection in blood serum. The main advantages of the manufactured sensor include the possibility of direct analysis of blood samples without requiring any pre-treatment, as well as extraordinary stability and reproducibility. The findings showed the potential of the developed sensor in uric acid analysis [41].

Additionally, Mubarakali A. et al. [42] worked on a sensor to detect glucose. They obtained that using colloidal Cu nanoparticles modified with graphene coated on indium-coated tin oxide glass substrate as a working electrode. The results indicate that the bioelectrode shows adequate stability and repeatability with a shorter response time. Because of those properties, the electrode may be one option for a non-enzymatic glucose biosensor [42]. Li B. et al. [43] also studied biosensors for glucose detection. A flexible glucose oxidase/chitosan/graphene sponge/Prussian blue biosensor for sweat glucose detection was designed (fabrication process is presented in Figure 3). The immobilization of glucose oxidase with chitosan offers glucose oxidase good isolation from the environment and protection, which increases glucose oxidase's stability, reusability, and activity. The gaphene sponge (graphene aerogel) is characterized by a large specific surface area, high porosity, excellent electrical conductivity, and biocompatibility. As an electron transport medium, Prussian blue was electrochemically deposited on the working electrode. The results presented that the biosensor has a high selectivity for glucose and responds well to glucose in human sweat. In addition, the many binding sites of the graphene sponge allow for further composites with other functional materials to improve the flexible sweat glucose biosensors [43].

However, Pareek S. et al. [44] fabricated an electrochemical DNA biosensor for human papillomavirus-16 (HPV-16) detection. Cervical cancer is caused by the HPV-16 virus. GO and silver-coated gold nanoparticles were used to modify an indium tin oxide-coated glass electrode. The results indicate that the biosensor has a high sensitivity for the detection of HPV-16, and it is possible to detect it in the early stage, which can be crucial in developing point-of-care devices [44]. Bao J. et al. [45] developed an electrochemical biosensor for methylated DNA detection. DNA methylation is related to cell proliferation and differentiation. More and more often, there is information that abnormal methylation contributes to the occurrence of diseases, especially in tumorigenesis. It can also be used as biomarkers to predict response to chemotherapy strategies [45,46]. They proposed the biosensor, which included gold electrodes and nanocomposite based on gold nanoparticles, rGO, and graphite carbon nitride. The nanocomposite showed excellent electrochemical properties by the

synergistic effects of each component. The proposed biosensor also displayed features of high specificity, stability, and reproducibility. Because of the synergistic effect of each component, the nanocomposite demonstrated good electrochemical characteristics [45].

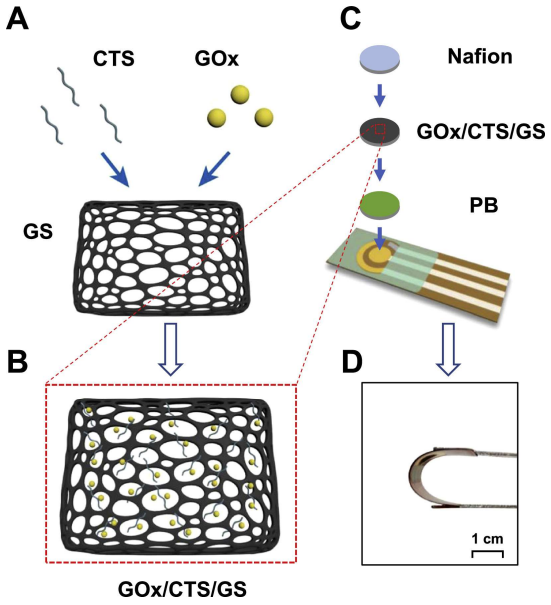

Figure 3. Fabrication process flexible glucose oxidase/chitosan/graphene sponge/Prussian blue biosensor for sweat glucose detection. (**A**) Graphene sponge (GS) mixed with chitosan (CTS) and glucose oxidase (GOx); (**B**) obtained GOx/CTS/GS composite; (**C**) Prussian blue (PB), composite and Nafion solution were added to the gold three-electrode; (**D**) flexible biosensor (reprinted from [43], with permission from Elsevier, license number: 5611890451239).

3.2. Tissue Engineering

Tissue engineering is "an interdisciplinary field that applies the principles of engineering and the life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function" [47]. Tissue engineering is often known as "regenerative medicine". It offers a variety of solutions, from building new organs for transplant surgery to repairing damaged tissues. Tissue engineering, apart from cell culture and stem cell differentiation, also uses biodegradable and biologically safe materials to create structures called "scaffolds" [48]. Due to different design restrictions related to tissue engineering, features including mass transport, scaffold degradation, and biocompatibility must be taken into consideration while designing scaffolds [49]. For successful tissue engineering, it is essential to produce a biocompatible porous scaffold to support the cells, delivering bioactive molecules (for example, growth factors); as a result, they can produce physical and chemical cues that will ultimately determine the cell fate and cellular organization [50]. The cell microenvironment has an impact on the adhesion, migration, differentiation, communication, and proliferation of cells on the surface, biomaterials, or inside the extracellular matrix (ECM). A perfect biomaterial for tissue engineering and regenerative medicine often aims to imitate the corresponding ECM, providing cells with the right microenvironment [51].

The commonly used materials for tissue engineering are presented in Table 1. In addition, 2D nanomaterials have recently attracted great interest. 2D nanomaterials have a thickness of a few nanometers but a large lateral size. The unique energy level structure and optical properties, controllable thickness, simplicity in modification and doping, inherent bioactivity, high biocompatibility, and biodegradability are just a few of the distinctive properties of 2D nanomaterials. Additionally, 2D nanomaterials' large specific surface area makes them good carriers of nanoparticles or medicines [52]. In addition to graphene, scientists in recent years have proposed the following: 2D oxide and hydroxide

nanosheets [53], black phosphorus nanoparticles, nanodots, nanosheets [54], non-spherical metal nanomaterials (noble metal or transition metal dichalcogenides) [55], or hexagonal boron nitride (hBN) nanoparticle [56]. Tarhan T. et al. [56] used hexagonal boron nitride nanoparticles, silver nanoparticles, and polydopamine to stimulate wound healing. Silver nanoparticles were chosen because of their anti-inflammatory properties and hBN for induced proliferation and migration of cells. Even the low concentration of the obtained agent enhances wound healing besides it reduces ROS production, promotes wound closure, and reorganizes tube formation in cells [56]. Whereas, non-spherical metal nanoparticles are used in biomedicine, including regenerative medicine and cancer therapy. What is important, the architecture of metal-based nanomaterials influences their interaction with biological systems, i.e., particle geometry is an important factor and can affect their biodistribution, interactions with blood vessels, tumor penetration, transport across endothelial cells, etc. [56,57].

Table 1. Examples of materials used for tissue engineering [49,51,58].

Natural Materials	Synthetic Polymers	Inorganic Material
collagens; hyaluronic acid; chondroitin sulfate; chitosan (CS); silk fibroin	polyethylene glycol (PEG); poly(lactic-co-glycolic acid) (PLGA); polycaprolactone (PCL); ultra-high molecular weight polyethylene (UHMWPE)	hydroxyapatite (HAp); bioglass; calcium phosphate cement (CPC)

Sharifi S. et al. [59] proposed magnesium–zinc–graphene oxide nanocomposite scaffolds for bone tissue engineering. Scaffolds were fabricated by using powder metallurgy method. Because of their biodegradability, adequate mechanical qualities, and required biocompatibility, magnesium (Mg) scaffolds are an appropriate option for bone tissue regeneration in several studies. The addition of Zn to Mg increases its corrosion resistance and mechanical qualities. Furthermore, zinc is an element crucial for the human organism; therefore, the released products should not be toxic to the body. Among the known alloys, Mg-6Zn is interesting because of its corrosion resistance, mechanical properties, and cell compatibility. However, GO indicates a positive effect on the adhesion and differentiation of osteoblasts and stimulates biomineralization [59–62]. The addition of graphene oxide made the mechanical strength and corrosion resistance significantly improved compared to Mg-Zn scaffolds. Additionally, Mg-Zn-GO scaffolds were biocompatible and not cytotoxic in contact with L-929 cells [59].

Chitosan is an amino polysaccharide that is largely found in the cell walls of fungi, plants, insects, and marine invertebrates. Due to its chemical stability, high biocompatibility, antibacterial activity, biodegradability, ability to adsorb proteins, and to accelerate wound healing, CS is used in implantology, as a drug delivery system or scaffold [58,63,64]. Valencia A.M. [65] proposed to use chitosan functionalized GO by a covalent bond (CS-GO). The results show the potential application of the CS-GO compound in tissue engineering because of a low inflammatory response in vivo test and advanced resorption at 60 days of implantation [65]. Nakagawa de Arruda M. et al. [32] designed a few-layer graphene using the electrochemical exfoliation of graphite. It was supplemented with chitosan to form a homogeneous composite with low oxygen content. In addition to increasing surface area and enhancing chemical interactions, chitosan creates homogenous coatings with graphitic materials that improve the percolation of charges and electrical signals, increasing conductivity or capacitance. Because of its higher dispersion and resonance active groups, the graphene composite's electrical conductivity increased. Furthermore, it is environmentally friendly because it uses less water and produces less toxic waste [32].

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a commonly used material because of its similarity with natural bone in terms of chemistry and structure. It has high biocompatibility, bioactivity, and osteoconductivity. HAp also shows osteoinductive properties and it pro-

motes bone regeneration [66–69]. However, HAp's limited mechanical strength and fracture toughness limit its clinical applicability because they could cause the propagation of cracks and an increase in corrosion rate [66,67,69,70]. To improve mechanical properties, some materials are used as reinforcements of Hap, i.e., polyethylene, Al_2O_3, TiO_2, and carbon nanotubes. Unfortunately, these reinforcements may reduce the biological properties of hydroxyapatite and affect adjacent tissues [66]. However, HAp/GO composite showed improved in vitro osteoblast adhesion and apatite mineralization [71].

Liu Y. et al. [72] proposed HAp and HAp–graphene nanosheet (GN) composites synthesized using a liquid precipitation approach and deposited using vacuum cold spraying. The results demonstrated that the osteoblast cells spread and proliferated more readily on the GN-containing coatings [72]. Ramadas M. et al. [71] proposed hydroxyapatite nanorods grown on a graphene oxide sheet using a hydrothermal process. To assess cytotoxicity at various concentrations of material, they used human skin cancer cells. The nanocomposites presented no cytotoxicity effects on cancer cell lines. Although HAp/GO provides excellent biocompatibility [71]. However, Sánchez-Campos D. et al. [73] prepared a composite containing HAp, GO, and silver nanoparticles using a microwave-assisted hydrothermal method, a modified Hummer's synthesis, and by using dietary quercetin for silver nanoparticles formation and deposition. A dose-dependent toxic response was found in eukaryotic cells. It depended on the presence of GO, but superior cytocompatibility was observed for the samples with HAp. Furthermore, the composite increased the inhibition zone for *S. aureus* and *E. coli* compared to silver nanoparticles. The results of the antibacterial study are presented in Figure 4. These indicate that the material can be applied as a bactericidal agent for tissue replacement and surface coating [73].

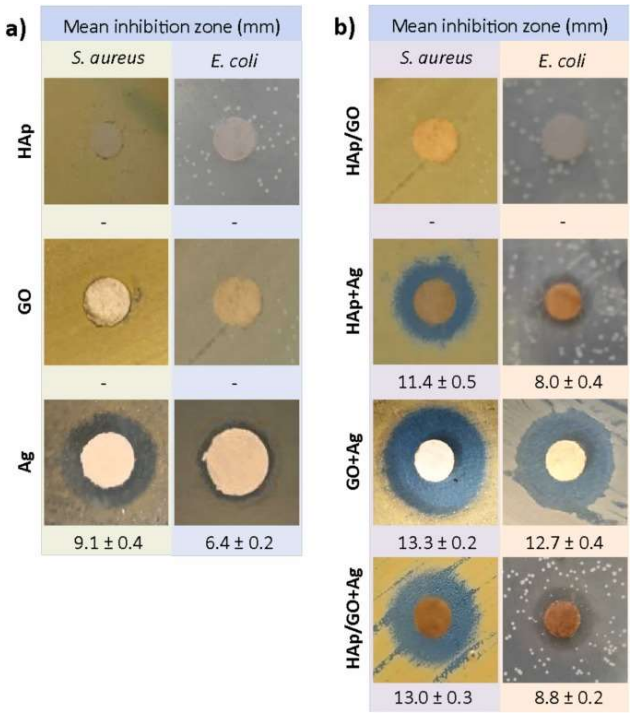

Figure 4. Pictures present inhibition zones (mm) of materials against *S. aureus* and *E. coli*: (**a**) the pure HAp, GO and silver nanoparticles (AgNPs); (**b**) HAp/GO, HAp + Ag, GO + Ag and HAp/GO + Ag composites (reprinted from [73], with permission from Elsevier, license number: 5611920074326).

Han W. et al. [66] prepared graphene oxide/hydroxyapatite composite coatings on a titanium substrate using the electrophoretic deposition method. GO/HAp composite coatings outperformed HAp coatings in terms of adhesion strength and corrosion resistance. An appropriate amount of graphene oxide promoted the proliferation of the mouse fibroblast cells and enhanced the mineralization properties. The results showed that 5 wt%

GO exhibited optimal mechanical properties, bioactivity, corrosion resistance, and good biocompatibility [66]. Fardi S.R. et al. [67] deposited hydroxyapatite–graphene oxide nanocomposites on titanium using an ultrasound-assisted electrophoretic technique. Ti sheets were anodized and then coated with HAp-GO with various compositions (0, 1, and 3 wt% of GO). They improved the mechanical properties with the best reported adhesion strength demonstrated by coating with 1 wt% GO. This coating showed excellent bioactivity and corrosion resistance [67]. Daulbayev C. et al. [74] fabricated a graphene oxide/calcium hydroxyapatite/polycaprolactone composite using an electrospun technique. Graphene oxide/hydroxyapatite composite was dispersed in biodegradable PLC. The results showed good antimicrobial activity against Gram-positive and Gram-negative bacterial strains, which depended on the GO loading. Furthermore, the composite had no cytotoxic effect on the preosteoblast MC3T3-E1 cell line. This indicates that this composite scaffold can be used for potential bone tissue regeneration [74]. Zhao H. et al. [75] fabricated porous graphene oxide/hydroxyapatite composite ceramic scaffolds using digital light processing technology. They demonstrated that adding a small amount of GO (0.1–0.4 wt%) to composite ceramics can improve their mechanical properties. The findings revealed that no scaffolds are cytotoxic. The composite scaffold increased cell adhesion, proliferation, and the expression of osteogenesis-related genes, while GO (0.1–0.2 wt%)/HAp scaffolds demonstrated superior alkaline phosphatase activity and more effective bone mineralization, as well as osteoinductivity [75].

3.3. Drug Delivery

Most conventional therapies and drug delivery systems have disadvantages such as rapid metabolism and excretion of drugs before reaching the target, poor water solubility, non-specificity to the target site, and opposing impacts on normal tissues. Therefore, nanotechnology and nanomaterials are becoming more and more popular in the development of innovative drug transport mechanisms. The efficiency of intelligent drug delivery systems is continually being improved. This is to maximize therapeutic activity and reduce side effects [76–78].

Li W. et al. [79] constructed a rectal delivery system using temperature-sensitive hydroxybutyl chitosan gel cross-linked with GO with pingyangmycin as a model drug. The temperature-sensitive gels are mostly based on polyisopropyl acrylamide, poloxamer, and CS. Because of their good biocompatibility, high permeability, and low drug toxicity, they are frequently used for injectable drug administration, oral drug delivery, rectal drug delivery, or mucosal drug delivery. The obtained material presented an effectively prolonged action time of the drug in vivo with rectal administration, and a sustained release effect [79]. Whereas, pH-responsive chitosan-based hydrogel can also be used as a drug delivery system. Hydrogel was efficiently fabricated by varying amounts of GO for controlled cephradine release. The content of GO proportionally affected the thermal stability. The material was most effective against Escherichia coli and Pseudomonas aeruginosa. Furthermore, pH-responsive swelling (schematic diagram presented in Figure 5) and biodegradability were confirmed in phosphate-buffered saline and proteinase K solutions [80].

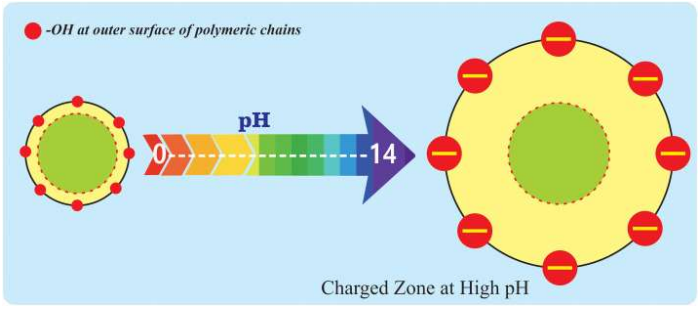

Figure 5. Schematic diagram for swelling of hydrogel due to charge zones at polymeric chains (reprinted from [80], with permission from Elsevier, license number: 5611930020590).

Breast cancer is one of the most common cancers in women and has a high mortality rate. Chemotherapy, immunotherapy, radiotherapy, and surgery are just a few of the methods that have been used to treat cancer, and combining these methods typically results in a more successful treatment [81–83]. However, scientists are still working on improving therapeutic methods. Matiyani M. et al. [84] developed a multi-functionalized GO-based novel drug nanocarrier for the delivery of the chemotherapeutic. They used quercetin and curcumin, which are potent anticancer drugs. The drug delivery system was created by combining graphene oxide with a hydrophilic polymer and metal oxides, zinc oxide and titanium dioxide. The results showed that the nanocarrier exhibits a pH-sensitive release of loaded drugs, besides demonstrating cytotoxicity towards breast cancer cells [84]. Whereas, Rajaei M. et al. [81] synthesized pH-sensitive hydrogel of chitosan/agarose/graphene oxide with glyoxal as the cross-linker. It was prepared using the water-in-oil-in-water emulsification technique. The results indicate that the loading and entrapment efficiencies of the drug are satisfactory. A highly effective and sustained medicine release profile was observed at pH 5.4; within 48 hours, almost the entire content of the drug model was released. In addition, effective cytotoxicity was observed against breast cancer cell lines (MCF-7) [81].

Quantum dots can be used to minimize the size of sheets to less than 100 nm. Moreover, quantum dots show better properties with more active groups on their surface than typical graphene and graphene oxide. They exhibit unique properties, i.e., water solubility, non-toxicity, high biocompatibility, a small size with a large surface area, high drug loading capacity, and better cellular uptake [85–87]. Mohammed-Ahmed H.K. et al. [85] examined graphene oxide quantum dots conjugated with glucosamine and boric acid with and without doxorubicin (DOX) for cancer therapy. The results showed that nanocomposite with boric acid improved the loading and release of DOX, with higher cellular internalization. Seyyedi Zadeh et al. [86] also worked on the production of a drug carrier based on quantum dots. They synthesized a magnetic graphene quantum dots-Fe_3O_4 nanocarrier targeted with folic acid (FA) and loaded with curcumin. pH-dependent drug release was observed for both GQDs-Fe_3O_4 and GQDs-Fe_3O_4-FA. The bare nanocarrier had no cytotoxic effect on normal cells. In addition, folic acid acted as a selective targeting agent in the nanocarriers [86].

4. Conclusions

Single-walled carbon nanotubes, multiwalled carbon nanotubes, graphene, and reduced graphene oxide are just a few of the carbon nanostructures that have drawn a lot of research interest. Graphene is a 2D sheet of sp^2-hybridized carbon atoms of atomic thickness that are organized in a honeycomb crystal lattice. Graphene is attracting a lot of interest due to its unique structural, optical, chemical, thermal, mechanical, electrical, and biological properties. Its derivatives are graphene oxide and reduced graphene oxide and graphene quantum dots. Graphene can be created using a variety of processes, including pyrolysis, epitaxial growth, chemical and electrochemical exfoliation of graphite, physical vapor deposition, and chemical vapor deposition. But the most promising method for industrial development and the production of graphene among those approaches is CVD.

Biomedical applications such as biosensing, medication delivery, regenerative medicine, and diagnostic tools are rapidly expanding. Graphene-based scaffolds are a particularly promising technology and have attracted interest due to their strong clinical potential in tissue regeneration therapies due to their substantial influence on the proliferation and differentiation of used stem cells. However, scientists still have many problems to solve, the biggest challenges are as follows: cytotoxicity, biodistribution, biotransformation, and immune response.

In conclusion, graphene nanomaterials have a bright future in biomedical applications. However, scientists still have many problems to solve, the biggest challenges being cytotoxicity, biotransformation, and immune response.

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References

1. Chakrabarty, N.; Chakraborty, A.K.; Kumar, H. Nickel-Hydroxide-Nanohexagon-Based High-Performance Electrodes for Supercapacitors: A Systematic Investigation on the Influence of Six Different Carbon Nanostructures. *J. Phys. Chem. C* **2019**, *123*, 29104–29115. [CrossRef]
2. Meng, X.; Hou, L.; Jin, H.; Li, W.; Wang, S.; Wang, Z.; An, J.; Wen, C.; Ji, G.; Xu, X.; et al. Study on Corrosion Protection Properties of PANI/ZnO/Zn/Graphene Coating on Aluminum Alloy. *Diam. Relat. Mater.* **2023**, *136*, 110067. [CrossRef]
3. Geim, A.K.; Novoselov, K.S. The Rise of Graphene. *Nat. Mater.* **2007**, *6*, 183–191. [CrossRef] [PubMed]
4. Abergel, D.S.L.; Apalkov, V.; Berashevich, J.; Ziegler, K.; Chakraborty, T. Properties of Graphene: A Theoretical Perspective. *Adv. Phys.* **2010**, *59*, 261–482. [CrossRef]
5. Mohan, V.B.; Lau, K.; Hui, D.; Bhattacharyya, D. Graphene-Based Materials and Their Composites: A Review on Production, Applications and Product Limitations. *Compos. Part B Eng.* **2018**, *142*, 200–220. [CrossRef]
6. Mbayachi, V.B.; Ndayiragije, E.; Sammani, T.; Taj, S.; Mbuta, E.R.; khan, A.U. Graphene Synthesis, Characterization and Its Applications: A Review. *Results Chem.* **2021**, *3*, 100163. [CrossRef]
7. Shahdeo, D.; Roberts, A.; Abbineni, N.; Gandhi, S. Chapter Eight—Graphene Based Sensors. In *Comprehensive Analytical Chemistry*; Hussain, C.M., Ed.; Elsevier: Amsterdam, The Netherlands, 2020; Volume 91, pp. 175–199.
8. Bose, S.; Kuila, T.; Kim, N.H.; Lee, J.H. 4—Graphene Produced by Electrochemical Exfoliation. In *Graphene: Properties, Preparation, Characterisation and Devices*; Skákalová, V., Kaiser, A.B., Eds.; Woodhead Publishing: Sawston, UK, 2014; pp. 81–98.
9. Lin, L.P.; Tan, M.T.T. Biosensors for the Detection of Lung Cancer Biomarkers: A Review on Biomarkers, Transducing Techniques and Recent Graphene-Based Implementations. *Biosens. Bioelectron.* **2023**, *237*, 115492. [CrossRef]
10. Kumar, Y.; Sahoo, S.; Chakraborty, A.K. Mechanical Properties of Graphene, Defective Graphene, Multilayer Graphene and SiC-Graphene Composites: A Molecular Dynamics Study. *Phys. B Condens. Matter* **2021**, *620*, 413250. [CrossRef]
11. Li, N.; Li, D.; Zhen, Z.; Zhang, R.; Mu, R.; Xu, Z.; He, L. Nucleation and Growth of Graphene at Different Temperatures by Plasma Enhanced Chemical Vapor Deposition. Mater. *Today Commun.* **2023**, *36*, 106568. [CrossRef]
12. Kenry; Lee, W.C.; Loh, K.P.; Lim, C.T. When Stem Cells Meet Graphene: Opportunities and Challenges in Regenerative Medicine. *Biomaterials* **2018**, *155*, 236–250. [CrossRef]
13. Sanchez, V.C.; Jachak, A.; Hurt, R.H.; Kane, A.B. Biological Interactions of Graphene-Family Nanomaterials: An Interdisciplinary Review. *Chem. Res. Toxicol.* **2012**, *25*, 15–34. [CrossRef]
14. Wang, Y.; Li, Z.; Wang, J.; Li, J.; Lin, Y. Graphene and Graphene Oxide: Biofunctionalization and Applications in Biotechnology. *Trends Biotechnol.* **2011**, *29*, 205–212. [CrossRef] [PubMed]
15. Balandin, A.A.; Ghosh, S.; Bao, W.; Calizo, I.; Teweldebrhan, D.; Miao, F.; Lau, C.N. Superior Thermal Conductivity of Single-Layer Graphene. *Nano Lett.* **2008**, *8*, 902–907. [CrossRef]
16. Tale, B.; Nemade, K.R.; Tekade, P. V Graphene Based Nano-Composites for Efficient Energy Conversion and Storage in Solar Cells and Supercapacitors: A Review. *Polym. Technol. Mater.* **2021**, *60*, 784–797. [CrossRef]
17. Raslan, A.; Saenz del Burgo, L.; Ciriza, J.; Pedraz, J.L. Graphene Oxide and Reduced Graphene Oxide-Based Scaffolds in Regenerative Medicine. *Int. J. Pharm.* **2020**, *580*, 119226. [CrossRef]
18. Arshad, F.; Nabi, F.; Iqbal, S.; Khan, R.H. Applications of Graphene-Based Electrochemical and Optical Biosensors in Early Detection of Cancer Biomarkers. *Colloids Surf. B Biointerfaces* **2022**, *212*, 112356. [CrossRef]
19. Ndagijimana, P.; Rong, H.; Ndokoye, P.; Mwizerwa, J.P.; Nkinahamira, F.; Luo, S.; Guo, D.; Cui, B. A Review on Activated Carbon/Graphene Composite-Based Materials: Synthesis and Applications. *J. Clean. Prod.* **2023**, *417*, 138006. [CrossRef]
20. Zhu, S.; Zhang, J.; Liu, X.; Li, B.; Wang, X.; Tang, S.; Meng, Q.; Li, Y.; Shi, C.; Hu, R.; et al. Graphene Quantum Dots with Controllable Surface Oxidation, Tunable Fluorescence and up-Conversion Emission. *RSC Adv.* **2012**, *2*, 2717–2720. [CrossRef]
21. Hulagabali, M.M.; Vesmawala, G.R.; Patil, Y.D. Synthesis, Characterization, and Application of Graphene Oxide and Reduced Graphene Oxide and Its Influence on Rheology, Microstructure, and Mechanical Strength of Cement Paste. *J. Build. Eng.* **2023**, *71*, 106586. [CrossRef]
22. Dorri Moghadam, A.; Omrani, E.; Menezes, P.L.; Rohatgi, P.K. Mechanical and Tribological Properties of Self-Lubricating Metal Matrix Nanocomposites Reinforced by Carbon Nanotubes (CNTs) and Graphene—A Review. *Compos. Part B Eng.* **2015**, *77*, 402–420. [CrossRef]

23. Kuilla, T.; Bhadra, S.; Yao, D.; Kim, N.H.; Bose, S.; Lee, J.H. Recent Advances in Graphene Based Polymer Composites. *Prog. Polym. Sci.* **2010**, *35*, 1350–1375. [CrossRef]
24. Schniepp, H.C.; Li, J.-L.; McAllister, M.J.; Sai, H.; Herrera-Alonso, M.; Adamson, D.H.; Prud'homme, R.K.; Car, R.; Saville, D.A.; Aksay, I.A. Functionalized Single Graphene Sheets Derived from Splitting Graphite Oxide. *J. Phys. Chem. B* **2006**, *110*, 8535–8539. [CrossRef] [PubMed]
25. Hernandez, Y.; Nicolosi, V.; Lotya, M.; Blighe, F.M.; Sun, Z.; De, S.; McGovern, I.T.; Holland, B.; Byrne, M.; Gun'Ko, Y.K.; et al. High-Yield Production of Graphene by Liquid-Phase Exfoliation of Graphite. *Nat. Nanotechnol.* **2008**, *3*, 563–568. [CrossRef] [PubMed]
26. Stankovich, S.; Dikin, D.A.; Piner, R.D.; Kohlhaas, K.A.; Kleinhammes, A.; Jia, Y.; Wu, Y.; Nguyen, S.T.; Ruoff, R.S. Synthesis of Graphene-Based Nanosheets via Chemical Reduction of Exfoliated Graphite Oxide. *Carbon N. Y.* **2007**, *45*, 1558–1565. [CrossRef]
27. Liu, F.; Wang, C.; Sui, X.; Riaz, M.A.; Xu, M.; Wei, L.; Chen, Y. Synthesis of Graphene Materials by Electrochemical Exfoliation: Recent Progress and Future Potential. *Carbon Energy* **2019**, *1*, 173–199. [CrossRef]
28. Geng, D.; Wang, H.; Yu, G. Graphene Single Crystals: Size and Morphology Engineering. *Adv. Mater.* **2015**, *27*, 2821–2837. [CrossRef]
29. Frank, O.; Kalbac, M. 2—Chemical Vapor Deposition (CVD) Growth of Graphene Films. In *Graphene: Properties, Preparation, Characterisation and Devices*; Skákalová, V., Kaiser, A.B., Eds.; Woodhead Publishing: Sawston, UK, 2014; pp. 27–49.
30. Liu, L.; Zhou, H.; Cheng, R.; Chen, Y.; Lin, Y.-C.; Qu, Y.; Bai, J.; Ivanov, I.A.; Liu, G.; Huang, Y.; et al. A Systematic Study of Atmospheric Pressure Chemical Vapor Deposition Growth of Large-Area Monolayer Graphene. *J. Mater. Chem.* **2012**, *22*, 1498–1503. [CrossRef]
31. Terasawa, T.; Saiki, K. Growth of Graphene on Cu by Plasma Enhanced Chemical Vapor Deposition. *Carbon N. Y.* **2012**, *50*, 869–874. [CrossRef]
32. de Arruda, M.N.; Palley, B.F.; de Souza, G.F.; Lopez, L.; Pontes, M.A.P.; Fernandes, W.P.; Gandara, M.; Machado, F.B.C.; Ferrão, L.F.D.A.; Gonçalves, E.S. Physicochemical Characterization of a Low-Oxygen Composite Based on Chitosan and Electrochemically Exfoliated Graphene. *Mater. Chem. Phys.* **2023**, *306*, 128049. [CrossRef]
33. Awasthi, G.; Mistry, K.; Jamnapara, N.; Salot, M.; Santhy, K.; Mandal, D.; Chaudhury, S.K. Effect of Stirring on Characteristics of Electrochemically Exfoliated Graphene. *Materialia* **2023**, *30*, 101818. [CrossRef]
34. Zhang, Y.; Xu, Y.; Liu, R.; Niu, Y. Synthesis of High-Quality Graphene by Electrochemical Anodic and Cathodic Co-Exfoliation Method. *Chem. Eng. J.* **2023**, *461*, 141985. [CrossRef]
35. Bhalla, N.; Jolly, P.; Formisano, N.; Estrela, P. Introduction to Biosensors. *Essays Biochem.* **2016**, *60*, 1–8. [CrossRef] [PubMed]
36. Prasad, S.V.S.; Kumar, M.; Arulananth, T.S.; Ravi, B.; Kumar, B.; Kiran Kumar, B. Graphene/ZnO Nanocomposite Based Optical Biosensors. *Mater. Today Proc.* **2023**. [CrossRef]
37. O'Brien, C.; Varty, K.; Ignaszak, A. The Electrochemical Detection of Bioterrorism Agents: A Review of the Detection, Diagnostics, and Implementation of Sensors in Biosafety Programs for Class A Bioweapons. *Microsyst. Nanoeng.* **2021**, *7*, 16. [CrossRef]
38. Narayanan, J.; Sharma, M.K.; Ponmariappan, S.; Sarita; Shaik, M.; Upadhyay, S. Electrochemical Immunosensor for Botulinum Neurotoxin Type-E Using Covalently Ordered Graphene Nanosheets Modified Electrodes and Gold Nanoparticles-Enzyme Conjugate. *Biosens. Bioelectron.* **2015**, *69*, 249–256. [CrossRef]
39. Ashraf, G.; Aziz, A.; Iftikhar, T.; Zhong, Z.-T.; Asif, M.; Chen, W. The Roadmap of Graphene-Based Sensors: Electrochemical Methods for Bioanalytical Applications. *Biosensors* **2022**, *12*, 1183. [CrossRef] [PubMed]
40. Ansari, G.; Pal, A.; Srivastava, A.K.; Verma, G. Detection of Hemoglobin Concentration in Human Blood Samples Using a Zinc Oxide Nanowire and Graphene Layer Heterostructure Based Refractive Index Biosensor. *Opt. Laser Technol.* **2023**, *164*, 109495. [CrossRef]
41. Soman, G.; Molahalli, V.; Hegde, G. Molecularly Imprinted Graphene Based Biosensor as Effective Tool for Electrochemical Sensing of Uric Acid. *Sens. Int.* **2023**, *4*, 100243. [CrossRef]
42. Mubarakali, A.; Gopinath, S.; Parthasarathy, P.; Arun Kumar, U.; Alavudeen Basha, A. Highly Efficient and Sensitive Non-Enzymatic Glucose Biosensor Based on Flower-Shaped CuO-Colloid Nanoparticles Decorated with Graphene-Modified Nanocomposite Electrode. *Measurement* **2023**, *217*, 113145. [CrossRef]
43. Li, B.; Wu, X.; Shi, C.; Dai, Y.; Zhang, J.; Liu, W.; Wu, C.; Zhang, Y.; Huang, X.; Zeng, W. Flexible Enzymatic Biosensor Based on Graphene Sponge for Glucose Detection in Human Sweat. *Surf. Interfaces* **2023**, *36*, 102525. [CrossRef]
44. Pareek, S.; Jain, U.; Bharadwaj, M.; Saxena, K.; Roy, S.; Chauhan, N. An Ultrasensitive Electrochemical DNA Biosensor for Monitoring Human Papillomavirus-16 (HPV-16) Using Graphene Oxide/Ag/Au Nano-Biohybrids. *Anal. Biochem.* **2023**, *663*, 115015. [CrossRef]
45. Bao, J.; Ding, K.; Zhu, Y. An Electrochemical Biosensor for Detecting DNA Methylation Based on AuNPs/RGO/g-C_3N_4 Nanocomposite. *Anal. Biochem.* **2023**, *673*, 115180. [CrossRef]
46. Heyn, H.; Esteller, M. DNA Methylation Profiling in the Clinic: Applications and Challenges. *Nat. Rev. Genet.* **2012**, *13*, 679–692. [CrossRef] [PubMed]
47. Langer, R.; Vacanti, J. Advances in Tissue Engineering. *J. Pediatr. Surg.* **2016**, *51*, 8–12. [CrossRef] [PubMed]
48. Elitok, M.S.; Gunduz, E.; Gurses, H.E.; Gunduz, M. Chapter 20—Tissue Engineering: Towards Development of Regenerative and Transplant Medicine. In *Omics Technologies and Bio-Engineering: Towards Improving Quality of Life*; Barh, D., Azevedo, V., Eds.; Academic Press: Cambridge, MA, USA, 2018; pp. 471–495.

49. Webber, M.J.; Khan, O.F.; Sydlik, S.A.; Tang, B.C.; Langer, R. A Perspective on the Clinical Translation of Scaffolds for Tissue Engineering. *Ann. Biomed. Eng.* **2015**, *43*, 641–656. [CrossRef]
50. Ghosal, K.; Mondal, P.; Bera, S.; Ghosh, S. Graphene Family Nanomaterials- Opportunities and Challenges in Tissue Engineering Applications. *FlatChem* **2021**, *30*, 100315. [CrossRef]
51. Gao, J.; Yu, X.; Wang, X.; He, Y.; Ding, J. Biomaterial–Related Cell Microenvironment in Tissue Engineering and Regenerative Medicine. *Engineering* **2022**, *13*, 31–45. [CrossRef]
52. Cao, Z.; Bian, Y.; Hu, T.; Yang, Y.; Cui, Z.; Wang, T.; Yang, S.; Weng, X.; Liang, R.; Tan, C. Recent Advances in Two-Dimensional Nanomaterials for Bone Tissue Engineering. *J. Mater.* **2023**, *9*, 930–958. [CrossRef]
53. Ma, R.; Sasaki, T. Two-Dimensional Oxide and Hydroxide Nanosheets: Controllable High-Quality Exfoliation, Molecular Assembly, and Exploration of Functionality. *Acc. Chem. Res.* **2015**, *48*, 136–143. [CrossRef]
54. Deng, L.; Xu, Y.; Sun, C.; Yun, B.; Sun, Q.; Zhao, C.; Li, Z. Functionalization of Small Black Phosphorus Nanoparticles for Targeted Imaging and Photothermal Therapy of Cancer. *Sci. Bull.* **2018**, *63*, 917–924. [CrossRef]
55. Makvandi, P.; Zarepour, A.; Zheng, X.; Agarwal, T.; Ghomi, M.; Sartorius, R.; Zare, E.N.; Zarrabi, A.; Wu, A.; Maiti, T.K.; et al. Non-Spherical Nanostructures in Nanomedicine: From Noble Metal Nanorods to Transition Metal Dichalcogenide Nanosheets. *Appl. Mater. Today* **2021**, *24*, 101107. [CrossRef]
56. Tarhan, T.; Şen, Ö.; Ciofani, M.E.; Yılmaz, D.; Çulha, M. Synthesis and Characterization of Silver Nanoparticles Decorated Polydopamine Coated Hexagonal Boron Nitride and Its Effect on Wound Healing. *J. Trace Elem. Med. Biol.* **2021**, *67*, 126774. [CrossRef]
57. Zhu, X.; Vo, C.; Taylor, M.; Smith, B.R. Non-Spherical Micro- and Nanoparticles in Nanomedicine. *Mater. Horiz.* **2019**, *6*, 1094–1121. [CrossRef]
58. Ma, P.; Li, M.; Hu, J.; Li, D.; Tao, Y.; Xu, L.; Zhao, H.; Da, J.; Li, L.; Zhao, G.; et al. New Skin Tissue Engineering Scaffold with Sulfated Silk Fibroin/Chitosan/Hydroxyapatite and Its Application. *Biochem. Biophys. Res. Commun.* **2023**, *640*, 117–124. [CrossRef]
59. Sharifi, S.; Ebrahimian-Hosseinabadi, M.; Dini, G.; Toghyani, S. Magnesium-Zinc-Graphene Oxide Nanocomposite Scaffolds for Bone Tissue Engineering. *Arab. J. Chem.* **2023**, *16*, 104715. [CrossRef]
60. Wen, C.; Zhan, X.; Huang, X.; Xu, F.; Luo, L.; Xia, C. Characterization and Corrosion Properties of Hydroxyapatite/Graphene Oxide Bio-Composite Coating on Magnesium Alloy by One-Step Micro-Arc Oxidation Method. *Surf. Coat. Technol.* **2017**, *317*, 125–133. [CrossRef]
61. Toghyani, S.; Khodaei, M. Fabrication and Characterization of Magnesium Scaffold Using Different Processing Parameters. *Mater. Res. Express* **2018**, *5*, 35407. [CrossRef]
62. Seyedraoufi, Z.S.; Mirdamadi, S. In Vitro Biodegradability and Biocompatibility of Porous Mg-Zn Scaffolds Coated with Nano Hydroxyapatite via Pulse Electrodeposition. *Trans. Nonferrous Met. Soc. China* **2015**, *25*, 4018–4027. [CrossRef]
63. Jugowiec, D.; Łukaszczyk, A.; Cieniek, Ł.; Kot, M.; Reczyńska, K.; Cholewa-Kowalska, K.; Pamuła, E.; Moskalewicz, T. Electrophoretic Deposition and Characterization of Composite Chitosan-Based Coatings Incorporating Bioglass and Sol-Gel Glass Particles on the Ti-13Nb-13Zr Alloy. *Surf. Coat. Technol.* **2017**, *319*, 33–46. [CrossRef]
64. Kumar, S.; Deepak, V.; Kumari, M.; Dutta, P.K. Antibacterial Activity of Diisocyanate-Modified Chitosan for Biomedical Applications. *Int. J. Biol. Macromol.* **2016**, *84*, 349–353. [CrossRef]
65. Valencia, A.M.; Valencia, C.H.; Zuluaga, F.; Grande-Tovar, C.D. Synthesis and Fabrication of Films Including Graphene Oxide Functionalized with Chitosan for Regenerative Medicine Applications. *Heliyon* **2021**, *7*, e07058. [CrossRef] [PubMed]
66. Han, W.; Liu, L.; Yu, B.; Zhu, Q. Electrodeposition of Graphene Oxide-Hydroxyapatite Composite Coating on Titanium Substrate. *Ceram. Int.* **2023**, *49*, 9647–9656. [CrossRef]
67. Fardi, S.R.; khorsand, H.; Askarnia, R.; Pardehkhorram, R.; Adabifiroozjaei, E. Improvement of Biomedical Functionality of Titanium by Ultrasound-Assisted Electrophoretic Deposition of Hydroxyapatite-Graphene Oxide Nanocomposites. *Ceram. Int.* **2020**, *46*, 18297–18307. [CrossRef]
68. Erakovic, S.; Jankovic, A.; Tsui, G.C.P.; Tang, C.-Y.; Miskovic-Stankovic, V.; Stevanovic, T. Novel Bioactive Antimicrobial Lignin Containing Coatings on Titanium Obtained by Electrophoretic Deposition. *Int. J. Mol. Sci.* **2014**, *15*, 12294–12322. [CrossRef] [PubMed]
69. Bensalem, A.; Kucukosman, O.K.; Raszkiewicz, J.; Topkaya, F. Synthesis, Characterization, Bactericidal Activity, and Mechanical Properties of Hydroxyapatite Nano Powders Impregnated with Silver and Zinc Oxide Nanoparticles (Ag-ZnO-Hap). *Ceram. Int.* **2021**, *47*, 21319–21324. [CrossRef]
70. Akao, M.; Aoki, H.; Kato, K. Mechanical Properties of Sintered Hydroxyapatite for Prosthetic Applications. *J. Mater. Sci.* **1981**, *16*, 809–812. [CrossRef]
71. Ramadas, M.; Bharath, G.; Ponpandian, N.; Ballamurugan, A.M. Investigation on Biophysical Properties of Hydroxyapatite/Graphene Oxide (HAp/GO) Based Binary Nanocomposite for Biomedical Applications. *Mater. Chem. Phys.* **2017**, *199*, 179–184. [CrossRef]
72. Liu, Y.; Dang, Z.; Wang, Y.; Huang, J.; Li, H. Hydroxyapatite/Graphene-Nanosheet Composite Coatings Deposited by Vacuum Cold Spraying for Biomedical Applications: Inherited Nanostructures and Enhanced Properties. *Carbon N. Y.* **2014**, *67*, 250–259. [CrossRef]

73. Sánchez-Campos, D.; Peña-Juárez, M.G.; Salado-Leza, D.; Mendoza-Anaya, D.; Rodríguez-Lugo, V.; González-Calderón, J.A.; Pérez, E. A Non-Toxic Synergistic Antibacterial Platform Based on Green Silver Nanoparticles Deposited on Hydroxyapatite/Graphene Oxide Composites. *Mater. Today Commun.* **2023**, *35*, 106097. [CrossRef]
74. Daulbayev, C.; Sultanov, F.; Korobeinyk, A.V.; Yeleuov, M.; Taurbekov, A.; Bakbolat, B.; Umirzakov, A.; Baimenov, A.; Daulbayev, O. Effect of Graphene Oxide/Hydroxyapatite Nanocomposite on Osteogenic Differentiation and Antimicrobial Activity. *Surf. Interfaces* **2022**, *28*, 101683. [CrossRef]
75. Zhao, H.; Xing, H.; Lai, Q.; Zhao, Y.; Chen, Q.; Zou, B. Additive Manufacturing of Graphene Oxide/Hydroxyapatite Bioceramic Scaffolds with Reinforced Osteoinductivity Based on Digital Light Processing Technology. *Mater. Des.* **2022**, *223*, 111231. [CrossRef]
76. Saharan, R.; Paliwal, S.K.; Tiwari, A.; Tiwari, V.; Singh, R.; Beniwal, S.K.; Dahiya, P.; Sagadevan, S. Exploring Graphene and Its Potential in Delivery of Drugs and Biomolecules. *J. Drug Deliv. Sci. Technol.* **2023**, *84*, 104446. [CrossRef]
77. Safari, J.; Zarnegar, Z. Advanced Drug Delivery Systems: Nanotechnology of Health Design A Review. *J. Saudi Chem. Soc.* **2014**, *18*, 85–99. [CrossRef]
78. Goodarzi, S.; Da Ros, T.; Conde, J.; Sefat, F.; Mozafari, M. Fullerene: Biomedical Engineers Get to Revisit an Old Friend. *Mater. Today* **2017**, *20*, 460–480. [CrossRef]
79. Li, X.; Chen, A.; Liu, Y.; Li, L. Preparation and Rectal Administration of Hydroxybutyl Chitosan/Graphene Oxide Composite Thermosensitive Hydrogel. *React. Funct. Polym.* **2023**, *189*, 105608. [CrossRef]
80. Qureshi, M.A.U.R.; Arshad, N.; Rasool, A. Graphene Oxide Reinforced Biopolymeric (Chitosan) Hydrogels for Controlled Cephradine Release. *Int. J. Biol. Macromol.* **2023**, *242*, 124948. [CrossRef] [PubMed]
81. Rajaei, M.; Rashedi, H.; Yazdian, F.; Navaei-Nigjeh, M.; Rahdar, A.; Díez-Pascual, A.M. Chitosan/Agarose/Graphene Oxide Nanohydrogel as Drug Delivery System of 5-Fluorouracil in Breast Cancer Therapy. *J. Drug Deliv. Sci. Technol.* **2023**, *82*, 104307. [CrossRef]
82. Dolatkhah, M.; Hashemzadeh, N.; Barar, J.; Adibkia, K.; Aghanejad, A.; Barzegar-Jalali, M.; Omidi, Y. Graphene-Based Multifunctional Nanosystems for Simultaneous Detection and Treatment of Breast Cancer. *Colloids Surf. B Biointerfaces* **2020**, *193*, 111104. [CrossRef]
83. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer Statistics, 2017. *CA. Cancer J. Clin.* **2017**, *67*, 7–30. [CrossRef]
84. Matiyani, M.; Rana, A.; Karki, N.; Garwal, K.; Pal, M.; Sahoo, N.G. Development of Multi-Functionalized Graphene Oxide Based Nanocarrier for the Delivery of Poorly Water Soluble Anticancer Drugs. *J. Drug Deliv. Sci. Technol.* **2023**, *83*, 104412. [CrossRef]
85. Mohammed-Ahmed, H.K.; Nakipoglu, M.; Tezcaner, A.; Keskin, D.; Evis, Z. Functionalization of Graphene Oxide Quantum Dots for Anticancer Drug Delivery. *J. Drug Deliv. Sci. Technol.* **2023**, *80*, 104199. [CrossRef]
86. Seyyedi Zadeh, E.; Ghanbari, N.; Salehi, Z.; Derakhti, S.; Amoabediny, G.; Akbari, M.; Asadi Tokmedash, M. Smart PH-Responsive Magnetic Graphene Quantum Dots Nanocarriers for Anticancer Drug Delivery of Curcumin. *Mater. Chem. Phys.* **2023**, *297*, 127336. [CrossRef]
87. Iannazzo, D.; Pistone, A.; Salamò, M.; Galvagno, S.; Romeo, R.; Giofré, S.V.; Branca, C.; Visalli, G.; Di Pietro, A. Graphene Quantum Dots for Cancer Targeted Drug Delivery. *Int. J. Pharm.* **2017**, *518*, 185–192. [CrossRef] [PubMed]