ABSTRACT
Diamond and carbon nanostructures possess outstanding advantages, such as chemical inertness, stable fluorescence, tunable surface characteristics and excellent biocompatibility. In particular, diamond has extremely strong mechanical properties, and therefore the nanostructures have been developed for unique applications. Herein, we systematically review the very recent applications of these structures in drug delivery, bioimaging and biosensing, followed by discussion of their advantages, limitations and challenges in translation to potential clinical applications and presentation of our insights of their future development.

1. Introduction
As one of the earliest elements recognised by human beings, the use of carbon can be traced back to 3750 BC, when charcoal was used to smelt copper, zinc, and tin ores by the Egyptians and Sumerians [1]. As the fifteenth most abundant element in the earth's crust, carbon can exist in nature in the form of diamond and graphite [2]. The carbon atom has four valence electrons in its outermost shell, which occupy the 2s and 2p orbitals. Due to the small energy difference between the 2s orbital and 2p orbitals, the wave function of these four electrons is easy to mix and thus enables carbon atoms to form chemical bonds with neighbouring atoms. Because of the unique arrangement of outer electrons and small atomic radius, carbon can readily form long-chain compounds. Therefore, carbon is present in 95% of known compounds, from small molecule compound to large chain polymers [3]. For a long time, diamond and graphite were the only known allotropes of carbon [4]. These two carbon allotropes have similar chemical properties but have very different physical properties. Diamond crystal is composed of four tetrahedral sp³-hybridised carbon atoms and is the hardest known substance in nature, while graphite is composed of sp²-hybridised carbon atoms where one 2s and two 2p orbitals (2px and 2py) take part in bond formation, resulting in stacked layers of hexagonal sheets, and a substance with low hardness [5]. In diamond, all outer shell electrons are involved in bond formation, thus there are no free electrons in diamond. In the graphite, the px orbital electron is not involved in bond formation and form a delocalized π electron. This causes the electron cloud to expand over all carbon atoms. The delocalized π electrons overlap and interweave providing van der Waals interactions between individual layers of graphite [6]. Therefore, diamond is an insulator while graphite is a good conductor of electricity and heat. The age of synthetic carbon allotropes began in 1985 marked by the discovery of fullerenes [7].

With nearly four decades of studies, synthetic carbon materials have been developed at an astonishing rate. Nowadays, carbon nanomaterials with a variety of physical and chemical properties have been synthesised from 0D carbon to 3D nanomaterials [8–11]. Recent findings indicate that these carbon materials possess exceptional biocompatibility, stability and mechanical properties [12–14]. In this review, we will systematically discuss the properties and applications of diamond, graphene and amorphous carbon nanostructures, discuss the advantages, disadvantages and challenges of each type of material, and provide our insights into the future development of technologies using these nanostructures for biomedical applications.

2. Applications of nanodiamonds in biomedicine
Natural diamonds are generally formed in subcontinental lithospheric mantle or even deeper where element carbon is deposited by oxidation-reduction reactions and recrystallised to diamond at extreme temperatures and pressures [15]. Through crustal movements over...
long time, diamond is brought to the surface by volcanic activity. However, natural diamond is scarce and expensive and cannot meet the demand of practical applications. The history of synthetic nanodiamond can be traced to 1950s [16]. In early researches, nanodiamonds were synthesised from controlled detonation by using carbon-containing explosives such as trinitrotoluene (TNT) and cyclotrimethylene-trinitramine (RDX) [17]. Since then, artificial nanodiamond has made a rapid development and progress. Figure 1 shows the images of nanodiamonds synthesised from carbon films after laser pulse [18]. Today, one could easily synthesis nanodiamond with different dimensions and shapes through detonation technique, high pressure, high temperature (HPHT) synthesis, chemical vapour deposition (CVD) and laser ablation (Table 1) [27–29].

These nanodiamonds possess excellent chemical stability, optical property and less toxicity among nanostructures and exhibit great potential in drug delivery, bioimaging and biodetection [17, 30].

2.1. Nanodiamonds in biodetection

In recent years, the use of nanodiamonds for biodetection has attracted increasing attention because of their unique properties attributed to the nanoscale feature sizes. For example, Huang et al. developed a nonenzymatic electrochemical biosensor for glucose detection [31]. In this study, the electrochemical biosensor was composed of oxygen-doped nitrogen incorporated nanodiamond (NOND) and copper oxide modified silicon wafer electrode. A Si (100) wafer was first etched in alkaline solution forming pyramid structures. Next, NOND powder and titanium powder were deposited on the pyramid structures through microwave plasma enhanced CVD method. Finally, copper oxide was decorated on the Si wafer through sputter deposition method. The quantitative analysis of glucose was determined by cyclic voltammetry in the potential range from 0 to 1 V. As shown in Figure 2, in the absence of glucose, the anodic and cathodic peaks represent the redox current of CuO/Cu(II) and Cu(II)/Cu(III) while in the existence of glucose, glucose will be oxidised to gluconolactone under the action of Cu(II)/Cu(III) and generate an anodic peak. The biosensor exhibited attractive sensitivity, selectivity and stability. The concentration of glucose in the range of 100 to 700 μM can be determined through the current response and the signal is independent of interfering species such as uric acid, NaCl and ascorbic acid. Compared with several reported copper and nickel electrodes for glucose detection, NOND decorated Si electrode possesses better response speed and detection limit. The mechanism can be interpreted as the synergistic effect of CuO and nanodiamond. The deposited nanodiamond offered extra electrons during glucose measurement which increased the signal response.

Figure 1. (a) Scanning electron microscopy of nanodiamonds. (b) Mechanism of nanodiamond formation from Q-Carbon film. (c) Formation of nanodiamonds during initial stages and electron backscatter diffraction pattern (from red dot), showing characteristic diamond Kikuchi pattern. (d) Nanodiamonds covering the entire area with inset showing twins [18]. Reprinted with permission of Creative Commons license.
Meanwhile, the nanodiamond had a stimulating effect to the oxidation of glucose and thereby promoted the redox current response.

Nanodiamonds have been reported to possess chemical catalytic activity and thus can be used to develop biosensors to replace the use of enzymes [32, 33]. A study by Chen et al. revealed that nanodiamonds had pH dependent peroxidase-like and oxidase-like activities [34]. With this unique property, they developed a nanodiamond-based enzyme-linked immunosorbent assay (ELISA) for mouse superoxide dismutase (SOD) detection. Under acidic conditions, nanodiamonds could catalyse the oxidation reaction of 3,3,5,5-tetramethylbenzidine (TMB) and O₂ or H₂O₂ to generate a blue compound. In contrast, at alkaline conditions, nanodiamonds could play a role like horseradish peroxidase (HRP), which converts H₂O₂ into oxygen and water. These catalytic activities can be attributed to the nanoscale effects of nanodiamonds. It was reported that peroxides can be effectively adsorbed on nanodiamonds and nanodiamond can accelerate the electron transfer between TMB and peroxidases [34, 35]. Therefore, nanodiamonds can be used in place of HRP in traditional HRP-based ELISA. By conjugating nanodiamonds to

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### Table 1. Reported synthesis process of nanodiamonds.

| Synthetic technique | Starting materials | Size   | Yield  | Advantages                                      | Disadvantages                    | References |
|---------------------|-------------------|--------|--------|-------------------------------------------------|----------------------------------|------------|
| Detonation technique | TNT, RDX        | 2–5 nm | 1%–8%  | Low cost, ease for mass production, narrow size  | Strong agglomeration, low purity | [17, 19, 20] |
| Laser ablation      | Graphite, coal    | 3–70 nm| 5–10%  | Facile, mild conditions                         | Difficult to industrialize       | [21, 22]   |
| CVD                 | Methane, hydrogen, nitrogen | 30–400 nm | N/A    | Ease for mass production                        | Could be Contaminated            | [23, 24]   |
| HPHT                | Graphite, adamantane | 1–55 nm | 50–62% | High purity, high yield, low cost, outstanding optical property | Polydisperse and irregular shape, harsh reaction conditions | [25, 26]   |

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Figure 2. (a) Cyclic voltammetry test results of glucose sensing. (b) Amperometric performance of different electrodes at 0.5V in 0.1 M NaOH at a scan rate of 50 mV s⁻¹. (c) Calibration curves of glucose detection from Si, Pyr-Si, CuO and NOND electrodes. (d) Amperometric response of CuO/NOND/Pyr-Si electrodes at 0.5V in 0.1 M NaOH [31]. Reprinted with permission. Copyright © 2018, American Chemical Society.
secondary antibodies and utilising TMB as a chromogenic substrate, nanodiamond-based ELISA can be used to detect multiple antigens.

### 2.2. Nanodiamond in drug delivery

Nanodiamond possesses high surface-area-to-volume ratio, and good biocompatibility and bioactivity [36–38]. It is chemically inert but still can be surface modified to meet the demands of different applications [39]. Drug molecules can bind to nanodiamond either covalently or noncovalently [40]. Nanodiamond has been demonstrated to boost the effectiveness of small molecule drugs through altering the pharmacokinetics behaviour [41]. Therefore, nanodiamond can serve as a versatile drug delivery platform for delivering therapeutic agents. Gu et al. reported a nanodiamond based drug delivery system to overcome the hurdles of the existing chemotherapeutic drug, UNC0646, to increase in vivo efficiency [42]. In this study, the hydrophobic drug interacted with the carboxylic group on the surface of nanodiamond through polar interactions, electrostatic interactions and hydrogen bonding in basic conditions. The drug molecules could then be released in acidic environments. Approximately 60.3% of drug was released at pH 4 within 12 h and 71.7% of drug was released at pH 2 within 6 h. Nanodiamond particles also had stronger affinity to charged proteins, which could further promote drug release in the cellular environment. In 10% FBS-supplemented DMEM culture media, 78.8% drug release was observed after 24 h, while in 0.15% FBS supplemented DMEM, only 42.6% of the drug was released. Binding of hydrophobic drugs with nanodiamond improved the water dispersibility of these drugs while retaining their pharmacological activities. Drugs with nanodiamond had longer blood circulation half-lives than their free molecule counterparts and could accumulate in tumour tissue. In addition to functioning as a drug delivery platform for insoluble drugs, nanodiamonds can also act as a targeted drug delivery platform to realise high bioavailability and minimise adverse side effects [43]. In a study carried out by Chan et al., nanodiamonds functionalised by folic acid and mitochondrial localising sequence (MLS-FA-PEGylated-NDs) were demonstrated to have cancer cell and mitochondrial targeting ability [44]. Under fluorescence microscopy, MLS-FA-PEGylated-NDs were recognised by cancer cell lines through specific folate–folate receptor interactions followed by transportation into the cytoplasm via endocytosis. After that, MLS-modified nanodiamonds were localised in the mitochondria while the unmodified nanodiamonds were concentrated in lysosomes. With this strategy, loading the anticancer drug doxorubicin (DOX) to nanodiamonds led to the drug’s accumulation in the mitochondria, resulting in impairment of the components in the respiration chain.

Colloidal stability and biocompatibility are two important requirements for drug delivery systems. Coating nanodiamond with polymer ligands aids improved stability, safety, and prolonged blood circulation times [45, 46]. Merz et al. used zwitterionic ligands to modify nanodiamond and obtained protein repulsive and colloidal stable nanodiamond particles [47]. These nanodiamond particles were then grafted with different conjugates with a benzoic acid head as a linker. By adjusting the conjugates, nanodiamond particles exhibited excellent colloidal stability in different environments and protein repulsion, which could prevent nonspecific binding to protein.

Madamsetty et al. described a multi-drug delivery platform based on PEGylated nanodiamonds for pancreatic cancer treatment [48]. Two anticancer drugs, irinotecan (IRT) and curcumin (CUR), were conjugated to nanodiamonds by physical adsorption. Through simulation, drug molecules could interact with nanodiamonds via van der Waals and π–π cloud interactions and on average, each nanodiamond particle could bind 46 drug molecules. The drug-loaded nanodiamonds could keep colloidal stability after incubation in serum-containing medium for 72 h. Fluorescence images showed that upon injection, drug-loaded nanodiamonds concentrated in tumour tissue. After treatment, the tumour volume in the nanodiamonds group decreased significantly and few liver metastases were observed. Furthermore, the nanodiamonds exhibited immunomodulatory function, which downregulated the secretion of IL-9 and IL-10 and modulated the inflammatory response. This research demonstrated that nanodiamonds might have great applications in both drug delivery and immunotherapy.

In addition to delivering small molecule drugs, nanodiamonds can also deliver macromolecular drugs including peptides, antibodies, and DNA. Liao et al. utilised nanodiamonds as a drug carrier to deliver cetuximab and paclitaxel (PTX) to enhance therapeutic efficiency in breast cancer [49]. In this study, PTX was covalently bonded to nanodiamonds, and cetuximab was bonded through physical adsorption. The treatment with these drug-loaded nanodiamonds considerably reduced the tumour size in a nude mice model and induced apoptosis. The anti-tumour effect could be attributed to the synergistic effect of the chemotherapy drug and antibody. As one of most common biomarkers in triple-negative breast cancer, epidermal growth factor receptor (EGFR) is highly expressed in the cancer cell membrane. Therefore, cetuximab labelled nanodiamonds can target cancer cells and trigger receptor mediated endocytosis. Upon uptake, the ester linkage between PTX and nanodiamonds was hydrolysed in lysosomes resulting in the generation of free PTX. Finally, the free PTX disrupted the function of tubulin leading to mitotic catastrophe. The use of nanodiamonds enabled not only efficient delivery and stabilisation of hydrophobic drug but also reduced side effects. Due to improved stability, targeting, and anticancer activities, drug loaded nanodiamond could fulfil its anticancer therapeutic role at a low dose to inhibit tumour growth.
Besides using free standing nanodiamond particles for enhanced drug delivery, diamond can also be used to create 1D nanoneedles to mechanically disrupt cell membranes for highly efficient intracellular delivery [38, 50–52]. Zhu et al. reported a diamond nanoneedle array for intracellular delivery of drugs. In this study, diamond nanoneedle arrays were synthesised through a top-down method [53]. The synthesised conical nanodiamond needles were about 50 nm in diameter and 2 μm in height with a density of $1 \times 10^6 \text{mm}^{-2}$. It was reported that the sharp tip of diamond nanoneedles can puncture cell membranes and facilitate drug penetration [54]. In the presence of the same concentration of anticancer drug, the viability of cancer cells decreased over 60% with the aid of nanodiamond nanoneedle array, while no significant reduction of cell viability was observed in the control group. Administration methods did not cause noticeable damage to cell membranes, which could recover integrity shortly after nanoneedle disruption. Compared with other reported nanoneedle arrays with different materials such as aluminium oxide and silicon, diamond nanoneedle arrays possess superior mechanical properties and biocompatibility [53].

2.3. Nanodiamond in bioimaging

Bioimaging is one of the most researched areas in biomedicine since it provides a non-invasive manner to visualise different biological processes in real time, from metabolic processes to pathological lesions, from subcellular structures to tissues and organs. In the past few decades, great progress has been made in the field of bioimaging with a wide range of probes having been developed [55, 56]. However, it is still a challenge to simultaneously achieve high sensitivity and high resolution. Nanodiamond, due to its outstanding biocompatibility and stability, has emerged as a potential imaging probe and contrast agent in bioimaging [57]. Nanodiamonds have low genotoxicity and cytotoxicity and thus can be used at higher dose levels without negative effects [58]. Additionally, due to the excellent chemical and physical stability, nanodiamonds can be tracked for weeks or even months within organisms, without much degradation or quenching [59]. Nanodiamonds were reported with a broad emission spectrum, from visible to near infrared [60]. The photoluminescence of nanodiamond is generally considered to be originated from radiative relaxation of electronic transitions by local defects, also known as colour centres [60]. Pure diamond is transparent and nonfluorescent [61]. However, during synthesis, some heteroatoms, such as silicon atoms and nitrogen atoms, can be doped into nanodiamond and replace the carbon atoms in the diamond lattice. For example, nanodiamonds synthesised from HPHT can natively contain 10–300 ppm substitutional nitrogen defects [62]. These substitutional heteroatoms bond to carbon atoms in diamond lattice and introduce localised electronic states. Upon light excitation, colour centres in nanodiamond can absorb photons and make the electrons transit to a higher energy level and then return to its ground state, accompanied by emission of photons (Figure 3) [63]. However, it should be noted that, sometimes, the observed fluorescence from nanodiamond may not originate from colour centres. In a recent study, Reineck found that in

![Figure 3](image_url)
functionalised detonation nanodiamonds (DNDs), there are two forms of fluorescence: (1) carbon-dot-like fluorescence that originates from the residual non-diamond carbon and (2) fluorescence from the colour centres in nanodiamond [61]. In these DNDs, the crystal structure of the outmost carbon atoms changed from diamond crystals to amorphous carbon and graphite structure during the functionalisation processes. Additionally, the introduction of nitrogen impurities from the surface functional groups further promoted the fluorescence from non-diamond carbon.

However, there are some limitations that hinder the application of nanodiamonds as contrast agents in bioimaging. Compared with traditional dyes, nanodiamonds have a wide fluorescence spectrum and the fluorescence property is non-uniform and is generally influenced by size, surface functional groups and heteroatom doping (Figure 4) [57, 61].

Recently, there have been reports of using modified nanodiamonds to overcome these disadvantages. Morita et al. designed a hybrid nanoparticle which is composed of fluorescent nanodiamonds and gold nanoparticles to enhance fluorescence property and to increase element contrast for electron microscopy analysis [64]. Specifically, nanodiamonds were first irradiated by He⁺ ions at 40 keV, repeatedly, followed by annealing at 800°C for 2 h and oxidizing in air at 500°C for 5 h. Then, the nanodiamonds were treated with strong acids for 4 h to remove any graphite components. Finally, gold nanoparticles were decorated on the nanodiamonds though an electron-beam reduction method (Figure 5). The Au-nanodiamonds had a mean particle size of about 16.3 nm. On average, approximately three gold nanoparticles were attached to each nanodiamond particle. Upon binding of gold nanoparticles, the fluorescence lifetime of the nanodiamond decreased from 28.6 ns to 10.3 ns and the fluorescence intensity decreased from 3.36 photons/ms to 1.80 photons/ms while there was no significant change of spectral shapes. This phenomenon could be explained as the nonradiative processes via energy transfer between nanodiamond and gold nanoparticles. The hybrid nanodiamond nanoparticles exhibited low toxicity. The physiological functions of mice and cells were not affected by the nanoparticles. Importantly, the hybrid nanodiamonds were found to be stable in very acidic conditions in cells without photobleaching. The hybrid nanodiamonds were imaged using two bioimaging modalities. Under conventional confocal microscopy, there was no difference between unmodified nanodiamonds and gold-decorated nanodiamonds. However, under the fluorescence lifetime mapping, it was easy to distinguish the cells which were labelled with hybrid nanodiamonds and unmodified diamonds. Therefore, it is possible to establish a high-throughput separation protocol based on nanodiamonds with different fluorescence lifetimes. For this, nanodiamonds can be sorted into several groups with different and narrow fluorescence lifetime distributions. After labelling the cells or biomolecules with these nanodiamonds, long term tracking of target biomolecules, drugs, or cells can be achieved through dual-modal multicolour imaging. Additionally, due to the doping with Au, the hybrid nanodiamonds can be used as an element-selective probe in transmission electron microscopes (TEM). Compared

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**Figure 4.** Fluorescence spectra of DNDs with different functional groups (hydrogen-, hydroxyl-, carboxyl-, ethylenediamine- and octadecylamine-functionalized DNDs) excited at different wavelengths between 400 and 700 nm [61]. Reprinted with permission. Copyright 2017, American Chemical Society.
with other element probes (e.g. C, Os, U and Pb), hybrid nanodiamonds can provide a sub-nm-scale spatial resolution.

The enhanced permeability and retention (EPR) effect is a concept wherein macromolecules and nanoparticles are expected to accumulate in tumour tissue in greater amounts that they would in normal tissues. The tumour tissues usually possess abnormal vasculature and show poor lymphatic drainage. Thus, even in the case that macromolecular drugs and nanoparticles may not have active targeting capability, they can still passively accumulate in tumour tissue by passing wide fenestrations in vessel walls. Meanwhile, due to the lack of effective lymphatic drainage in tumour tissue, the trapped nanoparticles tend to have longer retention time. Because of this kind of feature, nanoparticles may be used as contrast agents with unique advantages in tumour tissue imaging. In a recent study, Yoshino et al. reported the application of Cy7-labelled nanodiamonds in tumour imaging [65]. In this study, nanodiamonds were coated with a hydrophilic polymer, polyglycerol (PG), to enhance the colloidal stability. Then, the hydroxyl groups on PG were subjected to a substitution reaction by azido groups followed by a reduction reaction to amino groups. Finally, through esterification, Cy7 was fixed on the surface of nanodiamonds. Since PG has been reported to provide good stealth properties to protect nanoparticles from nonspecific adsorption of proteins and subsequent clearance by macrophages, the nanodiamonds exhibited an extended circulation time. The concentration of nanodiamonds plotting with time fitted with two-phase model. In the beginning α-phase, the decrease of concentration corresponded to free diffusion of nanodiamonds from the injection site to peripheral blood. In the following β-phase, the decrease of concentration was attributed to metabolism, capture, and excretion. The half-lives of nanodiamonds were 6 and 58 h at α-phase and β-phase, respectively. In comparison, most of other nanoparticles have a β-phase half-life less than 30 h [66–68]. BALB/c nude mice bearing GFP/HeLa tumours were used to evaluate in vivo and ex vivo fluorescence imaging of nanodiamonds. The fluorescence signal was concentrated at the tumour site and could last for over 8 days. The Cy7 fluorescence intensity at the tumour site was sharply increased within the first 24h and then gradually increased over 7 days indicating the preferential accumulation of nanodiamonds in the tumour tissue. The fluorescence intensity at the tumour site was much stronger than that in other tissues, such as liver and spleen which have more reticuloendothelial cells, showing the superior stealth property of nanodiamonds. In addition to the tumour tissue, there was gradual accumulation of nanodiamonds in superficial lymph nodes of head, neck, and deep axillary lymph nodes. This phenomenon can be explained by the stealth property because the nanodiamonds were not eliminated by liver and spleen, but entered into blood circulation and accumulated into lymph nodes. As a conclusion, the polymer-modified nanodiamonds had high stealth ability and thus had a long cyclic half-life. Meanwhile, due to the unique EPR effect of nanoparticles, the nanodiamonds were accumulated in the tumour tissue.

2.4. Nanodiamond as additives in tissue engineering

As one of the hardest materials known, diamond has attracted extensive interest due to its extraordinary mechanical properties. For example, the Young’s modulus and Vickers hardness of diamond are 1.2 TPa and 115 GPa, which endow diamond with high stiffness [69]. Additionally, diamond was reported with low friction coefficient and high wear resistance, which make it an ideal material for protective layers and coatings. Recently, to exploit nanodiamonds as a reinforced phase to enhance the mechanical properties of scaffolds has been a hot topic in tissue engineering. In the case of Poly-L-lactic acid (PLLA) scaffolds, upon the addition of nanodiamond particles, the compressive strength, compressive modulus and Vickers hardness increased by 375%, 298% and 178%, respectively. Besides, compared with the unmodified scaffolds, the water contact angle of the nanodiamond modified PLLA scaffolds decreased significantly, which is beneficial to cell adhesion and spreading [70]. Similarly, Morimune-Moriya et al. reported that by adding 0.5 wt% of nanodiamond particles, the Young’s
modulus and tensile strength of the polyamide 66 nanocomposite increased by 140% and 39% [71]. Furthermore, with the introduction of nanodiamond particles, the mobility of the macromolecular chains is restrained, leading to a higher thermal property; the decomposition temperature of the nanodiamond modified polyamide 66 nanocomposite increased by 16°C.

Beyond improving the mechanical properties of materials, nanodiamond particles have been reported with positive effects on supporting cell proliferation and differentiation. Zhang et al. reported a novel biodegradable poly(lactic-co-glycolic acid) (PLGA) matrix that was modified with nanodiamonds for bone tissue engineering [72]. The nanodiamond particles were first modified with amphipathic phospholipids and then the diamond modified PLGA films were produced through a solution casting method. Similar to previous studies, the addition of nanodiamond enhanced the mechanical properties of the PLGA nanocomposite. Approximately 100% increase in the Young’s modulus and approximately 550% increase in hardness was observed in the PLGA samples with nanodiamond modification. Importantly, after culturing cells on the nanodiamond modified PLGA films, a quick proliferation of hFOB1.19 osteoblasts was observed and after one-week, some interconnected cell populations were formed with tight adherence to the PLGA films. However, compared with low diamond containing PLGA film, a lower cell viability was observed in PLGA films with high ratio of nanodiamond addition. This phenomenon could be interpreted as that with the degradation of PLGA polymers, the incorporated nanodiamond particles exposed and formed high density aggregates, interfering cell proliferation. After culturing osteoblast on the modified PLGA polymers for one-week, the expression of osteocalcin gene was significantly increased. The osteocalcin gene is an important osteogenesis marker, suggesting the potential of nanodiamond modified PLGA to support osteoblast proliferation and osteogenic differentiation. Similarly, in another study, Feng et al. incorporated nanodiamond particles and MoS2 nanosheets into poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) bone scaffold to enhance the mechanical properties [73]. The introduction of MoS2 nanosheets restrained the aggregation of nanodiamond particles while the addition of nanodiamonds also prevented the re-stacking of MoS2 nanosheets. The synergetic effect of MoS2 nanosheets and nanodiamond increased the compressive strength and modulus by 52% and 87%, respectively, compared with the original PHBV scaffold. The nanodiamond and MoS2 nanosheets modified PHBV scaffold also stimulated cell adhesion and proliferation. Through analysing the elements produced by the cells, more calcium and phosphorus signals were found on the modified scaffold, which suggests better cell adhesion and spreading.

3. Applications of nanographene in biomedicine

Graphite is another naturally occurring mineral form of carbon. Different from diamond, graphite was formed within the crust at lower temperatures and pressures. In graphite, carbon atoms arrange themselves in layers of hexagonal rings. There are one 2s and two 2p orbitals in each carbon atom in graphite and thus, those carbon atoms are sp2-hybridised. In this situation, σ bonds are formed when the orbitals are overlapped between two contiguous carbon atoms. Graphene is the 2D building block of natural graphite which is composed of only one layer of sp2-hybridised carbon atoms in a hexagonal framework. Graphene was first discovered in 2004 in a study of isolation of a single layer from graphite [74]. Since then, graphene has attracted extraordinary interest due to its fascinating physical and chemical properties, which include high electron mobility, high thermal conductivity, and flexibility, low density, and high elasticity. Such unique features generated huge interest in developing new graphene-based materials and devices. Graphene has shown great potential in the field of energy storage, thermally and electrically conductive reinforced composites, optical electronics, sensors, photovoltaics, and plasmonic circuits [75]. However, there are some limitations that restrict the applications of graphene. For example, the zero bandgap of graphene makes it difficult to be used as an electronic switch, the solubility is low in water and organic solvents, and the planar structure makes graphene tend to aggregate easily. According to recent studies, the physical and chemical properties can be adjusted by tuning the size of graphene [76,77]. Nanographene usually refers to the graphene sheets with the size of 1–100 nm [78]. At this size, nanographene features unique electronic, optical and mechanical properties. Scholl reaction is the most widely used synthetic method for the synthesis of nanographene [79]. Figure 6 illustrates the morphology of nanographene synthesised from Scholl reaction. Due to these characteristics, nanographene has attracted great attention in the field of biomedicine. Nanographene and its derivatives possess large surface area, easily modified surface, strong loading efficiency with drugs and DNA/RNA, and satisfactory safety. Thus nanographene can be used as a drug delivery platform. Meanwhile, due to the high adsorption ability to enzymes and proteins and its high conductivity, nanographene can be used to develop highly sensitive and selective biosensors. Here, we will introduce two types of optical biosensors: fluorescent biosensors and colorimetric biosensors; we will also review some applications of nanographene in biomedicine in the last few years.
3.1. Nanographene for biosensing

Different from graphene, which has an almost infinite hexagonal honeycomb-like arrangement, nanographene has defined-size and various defects and edges \[81, 82\]. The properties of nanographene are somewhere between those of graphene and large polycyclic aromatic hydrocarbon (PAH) molecules. The physical properties of nanographene will change with size. For example, Manrique \textit{et al.} reported that the number of resonance peaks in the absorption spectra of nanographene increases with the size of nanographene \[83\]. Banerjee \textit{et al.} reported that the HOMO–LUMO band gap of nanographene decreases with increased the size of nanographene \[84\]. Based on these properties, Robertson \textit{et al.} designed an RNA detection platform to discriminate and quantify target RNA among similar RNA sequences \[85\]. In this study, nanographene oxide (nGO) was used to adsorb a dye-labelled single-stranded DNA probe (ss-DNA) using the action of aromatic stacking, hydrophobic interactions and Van der Waals forces. The dye labelled on oligonucleotide probes can be quenched on nanographene. However, the adsorption of DNA on graphene oxide (GO) could be reversed by adding complementary oligonucleotide strands, which can hybridise with the DNA sequences. Hence, such a biosensor could be used to detect miR-10b, an abnormally expressed gene involved in the development of cancer metastases. Specifically, a dye-labelled complementary unlocked nucleic acids (UNAs) probe targeting to miR-10b was first induced to the system followed by the addition of 2 μL of stock dsDNase. After incubation, different concentrations of the miR-10b and analogues, miR-10a and miR-10c, were added. In next step, miR-10b would hybridise with dye-labelled UNAs resulting in the release of miRNA-UNAs complex from the nanographene surface. Then the dsDNase could cleave the phosphodiester backbone of DNA molecules and release miR-10b. Finally, the released miR-10b triggered the next cycle of releasing and hydrolysing of UNA probes. From analysing the fluorescence intensity, the detection and quantification of miR-10b was possible. This biosensor exhibited high sensitivity and selectivity; the fluorescence signal generated from miR-10b was 70-fold higher.
than that of miR-10a and miR-10c and the detection limit could down to 10 nM. In another study, nGO was used as a nanoamplifier in a fluorescence polarisation assay [86]. Human Immunodeficiency Virus (HIV) is a retrovirus that attacks the body's immune system and causes acquired immune deficiency syndrome (AIDS). At present, there is no specific drugs for this kind of chronic disease. HIV-1 regulator of viral expression (Rev) protein plays an important role in HIV gene expression and virus replication by binding with Rev response element (RRE). Therefore, developing an antagonist which blocks the interaction between Rev protein and RRE can provide a new therapeutic strategy against HIV-1 infection. In a fluorescence polarisation assay, if the fluorescence dye is fixed, the emission light has a specific polarisation under the excitation of polarised light. However, as the molecules are constantly moving, the fluorescence dye will deflect at an angle during the fluorescence lifetime and the intensity of polarised emission light will decrease. As shown in Figure 7, proflavine is a fluorescent compound and can interact with RRE RNA. In the absence of RRE RNA, proflavine conforms to Brownian motion and has a high rotation speed in solution because of its small molecular weight. In this situation, the fluorescence polarisation of proflavine should be very small. In the presence of RRE RNA, proflavine can bind to RRE RNA and lead to the decrease in the rotation speed of proflavine. Hence, an increase in fluorescence polarisation was observed. In order to further amplify the signal, GO was introduced to the system. Since there was π-π stacking and electrostatic adsorption between RRE RNA and GO, RRE RNA can be adsorbed on the surface of GO. The binding of proflavine with RRE RNA on the graphene surface would generate a sharp increase in fluorescence polarisation. By adding antagonists, antagonists could competitively bind to RRE RNA and release proflavine resulting in decrease of fluorescence polarisation. Through analysing the change in fluorescence polarisation, the binding affinity of antagonists and RRE RNA could be calculated. Compared with traditional fluorescence polarisation assay, the GO-based fluorescence polarisation assay allows a label-free method that is immune from unnatural folding of biomacromolecules and can achieve higher sensitivity.

3.2. Nanographene for drug delivery

In recent years, research has demonstrated that nanographene can be used as a versatile drug delivery platform for delivering drugs, from small molecule drugs to macromolecular DNA and antibodies. Nanographene can load drugs through covalent bonding and noncovalent bonding. The surface area, layer number, lateral dimension, surface chemistry and purity could influence the interaction between nanographene and drug molecules and the biological effects of nanographene [87].

![Figure 7.](image-url) (a) Schematic of GO-based fluorescence polarisation biosensor for identification of Rev peptide antagonists; ΔFP, changes in FP. (b) Chemical structure of proflavine (Pro) and the sequence of the RRE RNA model nucleotide in the study [86]. Reprinted with permission. Copyright 2018, with permission from Elsevier.
As a 2D nanomaterial, atoms in nanographene are exposed on the surface, endowing it ultra-high surface area, and allowing loading of drugs and biomolecules with a high efficiency. In theory, the surface area of nanographene can reach 2600 m²/g [88]. Compared with other nanomaterials, the higher surface area of nanographene is expected to provide higher loading efficiency. However, sometimes, the high specific surface area of nanoparticles and the strong attractive interaction between particles may lead to aggregation [89]. Such a problem is particularly outstanding in nanographene. During aggregation, the plate-like nanographene tend to stack face-to-face, resulting in a sharp decrease of surface area while spherical nanoparticles tend to aggregate sphere-to-sphere, which can retain most of the surface area. Similarly, the layer number of nanographene influences the specific area and bending stiffness. With the decrease of the layer number of nanographene, the specific area increases, and the bending stiffness increase, and it is expected to load more drug molecules [90]. The lateral dimension influences the pharmacokinetic parameters; it influences the retention time, cell uptake, distribution, barrier transport, etc [87].

Structurally, nanographene can be considered as an extended planar aromatic macromolecule composed of a π-conjugated structure of six-atom rings. The original π bond at the centre of the six-membered carbon ring provides the possibility of drug delivery by π-π interaction [88]. The prerequisites of π–π interaction are (1) existence of π systems and (2) suitable geometries of the interacting species that support the overlap between two compounds. Nanographene possesses rich extended aromatic system and its geometry can be seen as a planar [91]. Thus, it is easy to form π–π interaction between nanographene and drug molecules, especially the interaction with small drug molecules. In fact, nearly all small molecules can be adsorbed on graphene [92]. Apart from π–π interaction, drug molecules can be loaded to nanographene through hydrophobic interaction. Due to the lack of hydrophilic functional groups, graphene is highly hydrophobic. Molecules with long linear carbon chain and aromatic compounds are more inclined to be adsorbed by nanographene [93]. However, it should be noted that due to the strong hydrophobicity, graphene may accumulate on cell membrane and causes cell death [94]. Therefore, scholars focused more on functionalised graphene rather than pristine graphene for drug delivery applications.

The surface of nanographene can be modified with different functional groups. GO is a kind of widely studied chemically modified graphene material that is synthesised by treating graphene with strong oxidant [95, 96]. Different from graphene, GO is hydrophilic and possesses better solubility. Through introduction of oxygen containing groups such as epoxy groups, carboxyl groups and hydroxyl groups, GO provides more types of interactions with drug molecules including covalent bonding, electrostatic bonding, and hydrogen bonding [97]. The systems with covalent conjugation with drugs are usually believed to possess advantages such as improved stability, long duration, higher specificity and avoiding pre-mature release. The covalent functionalisation of GO can be generally categorised into two categories: (1) the formation of covalent bonds with carbon skeleton and (2) the formation of covalent bonds with the surface functional groups [98]. In the case of carbon skeleton bonding, the C–C bond in the aromatic ring of GO can undergo addition reaction with drug molecules and form new C–C single bond [99]. For example, Wang et al. reported to link porphyrin on the carbon skeleton of GO through diazotisation reaction [98]. As to the covalent bonding with the surface functional group, the hydroxyl group on GO can undergo esterification; the carboxyl group can undergo esterification and carboxylic acylation; epoxy groups can undergo ring opening reaction [99, 100]. The hydrogen bonding between GO drug molecules can be formed when the hydrogen atom which is attached to an electronegative atom, known as H-bond donor, approaches a nearby electronegative atom, known as H-bond acceptor [101]. In GO, the carboxyl group and hydroxyl group can be both H-bond donor and acceptor and the epoxy group can be H-bond acceptor. Besides, with the ionisation of surface functional groups, the surface charge of GO varies, allowing GO to load drugs and biological macromolecules with opposite charge. Compared with covalent drug loading, noncovalent drug loading can be carried out in mild condition and is easier and the chemical structure of the drug molecule can remain unchanged.

Functionalised nanographene with polymer can have further increased advantages in the field of drug delivery. For example, PEG modified nGO has enhanced hydrophilicity and biocompatibility. Liu et al. proved that insoluble aromatic drug SN38 loaded on PEG-nGO by π–π interaction presented good water solubility and showed 100-times higher cytotoxicity in a HCT-116 cancer cell line in comparison with its water-soluble prodrug. Meanwhile, the ultra-small size of the nGO applied in this case (about 5–50 nm) facilitated the active cellular uptake [102]. Mugnano et al. demonstrated that mild oxidation of graphene nanoparticles is characterised by highly efficient cellular uptake [103].

DOX, as a classic anti-cancer drug, can be loaded on the nGO; multiple benzene ring structure contributes to the binding between the DOX and the nGO. Additional, DOX is released from the nGO in a pH-triggered manner. Deoxidization of epoxide and hydroxyl groups on nGO under basic conditions facilitates the formation of hydrogen bonds between nGO and the hydroxyl groups of DOX [104]. Thus, the release rate of the DOX from nGO is 10% and 30% under pH 7.4 and pH 5.3,
respectively [105]. DOX and anti-CD20 antibody-loaded nGO with PEG modification showed higher cytotoxicity to B-cell lymphoma in vitro [106].

Delivery of genetic material is another application of nGO. Polyethylenimine (PEI), as a commonly used vector for gene delivery, is criticized for its excessive cytotoxicity. The cytotoxicity of PEI is deeply related to the concentration and structure (molecule weight and branch lengths) of PEI used in transfection [107, 108]. However, it is often difficult to avoid toxicity of PEI by adjusting the concentration and structure, since a certain concentration of PEI is necessary to keep PEI nanoparticle stable, which is critical to the cell transfection, and low molecule weight PEI is rarely used because of its low transfection efficiency [109, 110]. nGO-conjugated PEI may address the problems described [111]. Firstly, PEI can be bonded to nGO via electrostatic interactions, forming stable nGO-PEI, which can further load plasmid. It was reported that by binding PEI with nanoparticles, it could gain better stability than PEI nanoparticles, and it can also decrease the density of exposed cationic residues [111–114]. nGO-PEI with a diameter of less than 50 nm and a thickness of 3–4 nm is beneficial to the cellular uptake. Secondly, removal of free PEI polymer reduces the toxicity but without affecting the loading of plasmid. nGO-PEI showed an 80% cell viability at PEI concentrations of more than 10 mg/L, which was in a sharp contrast to the 20% cell viability in the case of PEI alone. It is worth noting that the amount of PEI bound to nGO may be limited, which explains why the cytotoxicity remains the same no matter how much the amount of PEI is increased. In addition, nGO-PEI conjugation was applied to delivery of siRNA as well, aiming to suppress the expression of the Bcl-2 [115]. nGO has also been used to modify other polymers, such as polyamidoamine (PAMAM), for delivery of nucleic acids [116,117].

### 3.3. Nanographene for tissue engineering

Tissue engineering is a comprehensive interdisciplinary technology that includes the frontier achievements of medicine, biology, biomaterials and stem cell engineering [118]. The primary objective of tissue engineering is to develop functional implanted scaffolds to restore, maintain or improve damaged tissues or whole organs. During the last several years, graphene and graphene-doped materials have made some achievements in wound healing, stem cell engineering, regenerative medicine and skin tissue engineering [119,120].

As a cheap, safe and easily available raw material, starch is one of the most preferred fillers and fibre-forming polymers used in electrospinning. However, the poor electrospinnability, inefficient mechanical strength and hydrophilicity make it challenging to apply starch as electrospun fibres. In order to circumvent this problem, Wu et al. doped nanographene in starch-derived bioactive nanofiber scaffolds for preparing artificial extracellular matrix (ECM) [121]. In the experiment, nanographene was doped in a mixture solution that was composed of maize starch and formic acid, followed by electrospinning. Formic acid acted as a solvent and an esterification reagent and nanographene acted as a nanoenhancer. Using electron microscopy, it was found that the prepared starch-formate nanofibers were small but stable. By increasing the ratio of nanographene, the size distribution of starch-formate nanofibers narrowed and the morphology turned homogeneous and intact. The addition of nanographene decreased the viscosity of starch solution and reduced water production during starch formate substitution. These factors offer starch-formate nanofibers improved morphology. In addition, nanographene also influenced the surface property and thermal stability of starch-formate nanofibers. Nanographene doped nanofibers possess a smoother surface and better wettability, which can help cell adhesion and differentiation. Additionally, due to stronger hydrogen bonding interactions between nanographene and nanofibers, these nanographene doped nanofibers exhibited excellent thermal stability.

In another study, Yadav utilised cellulose-derived nGO in high internal phase emulsions (HIPE) to enhance the performance of polymeric scaffolds [122]. HIPE templating is a widely applied technique used to create polymeric porous structures. However, it is still challenging to produce a stable HIPE using traditional surfactant. By adding nGO in continuous phase, the viscosity of continuous phase changed, resulting in the formation of smaller and more uniform droplets and these droplets finally transformed to porous scaffolds. Under TEM, the nGO particles were embedded within the polymer scaffold and thus the mechanical strength of the scaffold was enhanced. Through cyclic compression test, no visible fracture or deformation was observed.

In addition to enhancing mechanical properties, nGO was also reported to reduce cell toxicity and enhance cell differentiation. Jo et al. reported nGO-embedded polyurethanes fibres have great potential use in skeletal muscle engineering [123]. Specifically, different amounts of nGO were mixed with polyurethanes in chloroform. After electrospinning and solvent evaporation, nGO-embedded polyurethane fibre scaffolds were prepared. Compared with polyurethane fibre scaffolds, doping of nGO improved properties including wettability, elasticity and stress relaxation capacity while the flexibility was not affected. More importantly, nGO-embedded polyurethane fibres improved the adhesion, spreading, and proliferation of C2C12 cells, a murine skeletal myoblast cell line. With the increase in the ratio of nGO, expression of myosin heavy chain (MHC) and related mRNA increased, indicating the capacity to enhance myogenic differentiation.
3.4. Nanographene for wearable devices

Wearable devices, also known as wearables, are manufactured by a technology that integrates electronic devices into different items that consumers wear every day, such as clothing, wrist-worn accessories, glasses, ornaments and prosthesis. During the last decade, wearable devices and related investments were one of the fastest growing sectors in the world. According to predictions, the worldwide market value for wearable devices is expected to be $160 billion by 2026, powered by an impressive annual growth rate of 32% [124]. There is great interest in the role wearables can play in healthcare, as they have potential to integrate patient health monitoring and Internet of Things (IoT) to provide real-time medical monitoring, and facilitate data exchange and information access. Therefore, developing cheap, lightweight, high conductivity, high toughness and extensibility, flexible electronics is regarded as a precondition for the next generation of wearable devices. Nanographene and its derivatives possess high flexibility, conductivity and transparency, and are considered to be ideal materials for flexible electronics and wearables [125]. For instance, Wu et al. reported that heteroatom doping of nanographene films can be used to produce microscale supercapacitors with outstanding pseudocapacitive behavior [126]. Zhang et al. reported that Si anodes with spongy nanographene shells have excellent electrochemical properties and also possess elastic and sponge-like features that can change shape and volume, spontaneously [127]. Wu et al. also reported that doping of an appropriate amount of nanographene can modify the mechanical performance of polymer films [128].

Based on these characteristics, researchers have been attempting to develop nanographene-based wearables. Liu et al. proposed a wearable strain sensor that is made from elastomer-filled graphene-woven fabric (E-GWF) for monitoring human physiological signals [129]. As shown in Figure 8, the design of wearables was inspired by the spider web architecture and the wearables were constructed with polydimethylsiloxane (PDMS) core. This design provided high reversibility and reliability, which allowed for long term use as a stretchable device. The graphene coating was composed of many polycrystalline graphene sheets, which were connected by several grain boundaries. When a stress was applied on the graphene coatings, the polycrystalline graphene sheets slipped, resulting in the change of contact area, and finally changing the resistance of graphene tube, which could vary from 6 to 8 orders of magnitude with the variation of tensile strain. Hence, such a device is suitable to monitor human motions. By connecting an external circuit with the wearable strain sensor, it can act as a switch to be worn on the wrist, which may be easily controlled by bending the wrist.

Romero et al. designed a flexible electrode for ubiquitous electrocardiogram monitoring [130]. In this study, laser-induced nanographene aggregates were deposited on flexible polyimide substrates through a one-step photothermal method, and a wire was connected to the graphene electrode by Ag-based conductive paint, providing a low-contact resistance (Figure 9). The electrode was attached to the arm using an adhesive patch with a polymer film. The synthesised flexible electrode has high porosity, which increases the contact area between the electrode and skin, and consequently leads to relatively small resistance and capacitance. Compared with the commonly used commercial electrodes, the nanographene electrode exhibited an enhanced performance. The nanographene electrode has a lower contact impedance, which is only one fifteenth of that of commercial electrodes at a low frequency range. Furthermore, the nanographene electrode is superior in frequency response (2.33 Ω Hz$^{-1}$ versus 66.52 and 37.84 Ω Hz$^{-1}$).

By combining with an algorithm, the nanographene-based wearables realised automatic analysis of electrocardiogram with high accuracy detection of R-peaks at a rate of up to 94.3%.

4. Applications of amorphous carbon in biomedicine

Amorphous carbon belongs to an allotrope of carbon that has no crystalline structure [131]. Compared with diamond and graphite, the atomic-scale structures of amorphous carbon are more complex. Amorphous carbon has sp$^2$ (graphite-like) and sp$^3$ (diamond-like) hybrid bonds. Unlike the three dimensionally cross-linked structure composed of sp$^3$-bonded carbon atoms found in diamond, and the benzene-like ring structure composed of sp$^2$-bonded carbon atoms found in graphite, various structures can be found in amorphous carbon [132, 133]. Hence, amorphous carbons with different structures usually have different properties. Amorphous carbon with high sp$^3$ content is referred to as diamond-like carbon (DLC). Conversely, higher sp$^2$ content yields materials with densities closer to that of graphite. By varying the sp$^2$/sp$^3$ ratio, Bhattarai et al. prepared models of amorphous carbon with densities spanning from 0.95 g/cm$^3$ to 3.5 g/cm$^3$ [132]. In amorphous carbon study, the properties of materials are primarily characterised by extrapolating the ratio of sp$^3$ bonded carbon and sp$^2$ bonded carbon atoms using spectroscopic methods [2]. With the development of modern amorphous carbon materials, different amorphous carbon nanocomposites have been synthesised, including amorphous carbon dots, amorphous carbon nanofoam, amorphous carbon nanofibers, and amorphous carbon films [134–138]. These amorphous carbon nanocomposites have offered many opportunities in biomedicine. Table 2 summarised some physical properties of diamond, graphene DLC and graphite-like carbon (GLC).
4.1. Amorphous carbon for biosensing

Due to the specific structure of amorphous carbon, the optical, electronic, and mechanical properties are dependent on the ratio of sp³ bonded carbon and sp² bonded carbon atoms [2]. This feature allows amorphous carbon to have variable advantageous material properties.

Tetrahedral amorphous carbon (ta-C) is a carbon structure that featured with sp³-bonded DLC and thus, it displays similar physical and chemical properties to...
diamond, and can be deposited on various substrate more easily [139]. Ta-C has been demonstrated to have excellent biocompatibility and resist bacterial adhesion. Palomäki et al. reported an in vivo electrochemical sensor that consists of ta-C films for dopamine detection [140]. In the beginning, a thin layer of ta-C film (15 nm and 50 nm) was coated on a Ti adhesion layer. Then, multi-walled carbon nanotubes (MWCNTs) with a diameter between 20 and 40 nm were deposited on the surface of ta-C films. As shown in Figure 10, uniform and intertwined MWCNTs were grown on ta-C films. Subsequently, cyclic voltammetry (CV) was used to detect dopamine in vivo. The limit of detection of dopamine was 84.3 ± 14 nM for 15 nm ta-C biosensor and 39.8 ± 5.9 nM for 50 nm ta-C biosensor. The superior sensitivity can be explained by the higher fraction of sp³-bonded DLC in 50 nm ta-C electrode. Compared with bare ta-C electrode, decoration of MWCNTs optimised the sensitivity of the biosensor, which can be attributed to the peak potential separation between dopamine and interferents. Deposition of MWCNTs enhance the mechanical integrity of the ta-C electrode. Similarly, in another study carried out by Peltola et al., a low detection limit of dopamine down to 10 nM was achieved with a nanodiamond-modified ta-C electrode [141].

GLC is another kind of amorphous carbon that resembles the morphology of classical graphite [142, 143]. These carbons are mainly sp²-hybridized carbons. However, unlike graphite, these carbons have higher hardness, higher corrosion-resistance and fast heterogenous electron transfer. Zhu et al. combined GLC in a voltametric pH sensor [144]. In this study, a GLC electrode was obtained through a CVD technique followed by modification with pH-sensitive quinone groups. In the measurement process, square wave voltammetry was used to analyse the reduction peak current of hydroquinone/quinone redox reaction in different pH solutions. The relationship between electrode potential shifts and pH conforms to Nernst equation. The prepared sensor exhibited a good linear range from pH 0 to pH 11 with an average slope of 63.3 ± 2.1 mV per pH.

In addition to its applications in electrochemical sensor, amorphous carbon can be also applied in an immune sensor. In a study by Zhang et al., amorphous carbon nanoparticles were applied in lateral flow immunoassays.

### Table 2. Physical properties of various forms of carbon allotropes.

|            | Diamond     | Graphene    | Diamond-like carbon | Graphite-like carbon |
|------------|-------------|-------------|---------------------|---------------------|
| Composition | sp³ bonded carbon | sp² bonded carbon | Mainly sp³ bonded carbon | Mainly sp² bonded carbon |
| Crystal structure | Diamond cubic | Hexagonal |                      |                     |
| Density    | 3.5 g/cm³ | 2.3 g/cm³ | ~3 g/cm³ | 2.5–2.9 g/cm³ |
| Electrical resistivity | 10¹⁵–10¹⁶ Ω cm | ~10⁴ Ω cm | 10⁵–10¹⁴ Ω cm | ~10⁻⁶ Ω cm |
| Band gap   | 5.45 eV | 0 eV | ~2.5 eV | ~1 eV |
| Elasticity module | ~1.2 Tpa | ~1 Tpa | ~0.9 Tpa | ~0.18 Tpa |

![Figure 9](image-url) (a) Schematic of the design of nanographene flexible electrode. (b) Images of nanographene flexible electrode attached arm. (c) SEM-image of the patterned area of the flexible polyimide substrate. (d) Raman spectrum of nanographene aggregates. (e) The resistance changes upon number of bending cycles [130]. Reprinted with permission of Creative Commons license.
for detection of mycotoxins, deoxynivalenol (DON), zearalenone (ZEN) and T-2 toxin, in maize samples [145]. In traditional lateral flow immunoassays, gold nanoparticles are most extensively used. However, this method faces the problem of sensitivity and poor quantitative discrimination, especially in trace analysis. To use amorphous carbon in the sensor, amorphous carbon dots were first prepared from candle soot. Subsequently, the T-2-toxin-targeting antibodies, zearalenone-targeting antibodies and fumonisins-targeting antibodies, were coated on the surface of the amorphous carbon dots. Then, samples flowed through the test strips and the capture of amorphous carbon dots at toxin binding sites caused a colour change. The results were analysed visually or using a strip reader (Figure 11). Compared with gold nanoparticles, the sensitivity of amorphous carbon dots was 8-times greater. On the white nitrocellulose strip, the black mark of aggregated amorphous carbon was clearer than that of colloid gold. In a recent study, Moyano et al. reported an improved lateral flow immunoassay consisting of amorphous-carbon-coated magnetic nanoparticles for extracellular vesicles detection [146]. The coating of amorphous carbon ensured adequate binding sites for bioconjugation. The detection limit of such an immune assay reached 4 × 10⁶ particles/μL.

4.2. Amorphous carbon for bioactive coating

In the past several decades, with the fast development of implants and medical equipment in healthcare industry, the implants comprising a single material are no longer able to fit all current desired functionalities. Hence,
Biocasting techniques are widely applied to improve or modify the surface properties of substrates. By creating a thin film on the surface of material, bioactive coating can improve the substrate's corrosion resistance, biocompatibility, wear resistance, wettability, antimicrobial bearing, durability and accelerated tissue regeneration [147–149]. In recent years, amorphous carbon coatings have been widely employed due to their attractive physical and chemical properties. Among them, DLC-based coatings are the most examined. DLC has excellent mechanical properties, such as a high flash point, a low coefficient of friction, good wear resistance, and high viscosity index, making DLC an ideal lubricating coating. Granek et al. evaluated the performance of DLC coatings [150]. After coating of DLC by CVD, the physical properties of Ti substrate altered significantly. In the nanohardness tests, the DLC coating led to an increase of hardness, accounting for more than a 3.5-fold increase in the hardness of Ti substrate. Meanwhile, the DLC contributed to the improvement of tribological performance, decreasing coefficient of friction for more than one order of magnitude. In another study, Hajduga et al. evaluated the biocompatibility of implants with different coatings [151]. Three different layers of TiN, ZrN and DLC were coated on a Ti substrate by physical vapour deposition (PVD). After that, the implants were placed in rabbits. No macroscopic changes and significant pathological changes were observed after implantation. It was found that there is no inflammatory reaction was caused by implantation, confirming the good biocompatibility of DLC coating.

However, a single layer of DLC coating has some disadvantages such as low toughness, high internal stress, and high sensitivity to ambient conditions. It was also regarded as the main reason why clinically used single-layer-DLC-coated orthopaedic bearing joints have high revision rates [152]. To address this problem, multilayer coatings of DLC may be an effective approach. Choudhury et al. evaluated the biotribological performance of orthopedic implants with different coating strategies [153]. A modified hip joint prosthesis consisting of three layers was proposed. Grade 5 Ti alloy was machined into a specific shape. Then, three different layers of coatings including a layer stack of Zr and ZrN (Zr:ZrN) sublayers, a Zr and DLC composite layer and a layer of N-doped DLC were coated on the Ti substrate successively (Figure 12). Each layer of the coating was mapped to different functions. The bottom Zr:ZrN layer is responsible for increasing load carrying capacity and corrosion resistance; the intermediate Zr and DLC layer is designed to enhance layer adhesion and to reduce fatigue residual stress; and the outermost N-doped DLC layer is to reduce friction resistance and enhance wearability. With this design, the DLC coating provided high hardness and low coefficient of friction, which increased the hardness to two to three times higher and decreased coefficient of friction by more than 50%. Moreover, the
design of multilayer coatings significantly reduced the wear. The wear track area of multilayer coated hip joints prosthesis is only 3.15% of that of the bare Ti substrate control group. The middle layer of Zr and DLC coating improved delamination strength and eliminated the delamination problem, which is very common in single DLC coating implants.

Another attraction of amorphous coatings is their antibacterial properties. Nosocomial infection is a thorny problem. It is one of the main reasons behind orthopedic and trauma surgery failure. According to a previous report, about 4–10% wards face the problem of nosocomial infection, making them the sixth leading cause of death [154]. Bacteria can attach to solid substrates forming biofilms allowing them to persist for extended periods. The contaminated items act as a vector for pathogens and multiply their pathways of transmission. Bacteria in biofilms have stronger resistance to antibiotics and external forces [155]. Therefore, development of antimicrobial coatings has become a research hotspot in health care. Amorphous carbon coatings have good antibacterial properties and excellent biocompatibility. Yonezawa et al. evaluated the antibacterial and cytotoxic properties of fluorinated DLC coating [156]. In comparison with non-coated Ti alloy discs, the fluorinated DLC coatings exhibited significantly improved antibacterial properties. After incubation with bacteria, S. aureus and E. coli counts decreased from $2.4 \times 10^4$ and $2.54 \times 10^4$, respectively, to less than 20, while the number of bacteria grew more than two order of magnitude in the control groups. As for cytotoxicity, no significant difference was observed between the groups, indicating the coating's high biocompatibility.

5. Conclusion and further outlook

To summarise, carbon-based nanomaterials are a new class of green materials with great potential in biomedicine. As one of the most familiar elements, carbon has a wide distribution in nature. In comparison with conventional biomaterials, carbon nanostructures have unique and outstanding features. The properties of carbon nanostructures vary with the change of the ratio of sp²/sp³ carbon atoms; from hard and low conductive nanodiamond to soft and conductive nanographene. Besides, the nanoscale size endows carbon nanostructures with unexpected advantages. Elegant design allows these nanostructures to possess a range of properties and functions including biocompatibility, chemical inertness, low friction coefficient and high conductivity. Indeed, these nanomaterials have been widely applied to drug delivery, bioimaging and biosensing. However, a number of significant challenges remain to be overcome. For example, although nanodiamond is famous for its chemical inertness and safety, there is still concern about the biodegradability of nanodiamonds in organisms. Some researchers proposed that nanodiamonds are difficult to degrade and may accumulate in body, causing long term safety issues [157]. Additionally, the current synthesis method could not successfully prepare monodisperse and homogeneous nanodiamonds. These irregular nanodiamond particles may have different defects and heteroatom doping, and thus, large variation in fluorescence emission. As to nanographene, the situation is similar to that of nanodiamonds. There is a lack of long-term monitoring data of the influence of nanographene to human body. However, different from nanodiamonds, nanographene do not possess excellent chemical inertness stability and may undergo chemical reaction in human body, which may cause oxidative stress injuries of cells [158]. Besides, the wearables that made from nanographene may suffer from durability issue because of the moisture absorption property of graphene. Last but not least, the most studies of carbon nanostructures are still in the stage of laboratory and there is a lack of relevant clinical data. The reliability of carbon nanostructures in clinic is not guaranteed. In recent years, some studies tried to solve these shortcomings and have brought us some encouraging results. It can be expected that with continuous development, carbon nanostructures may bring a revolutionary breakthrough in biomedicine.

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Author contributions

Conceptualization, Y.X. and X.C.; writing – original draft preparation, Y.X.; writing – review and editing, X.F., S.C.R., and X.C.; supervision, X.C.; project administration, X.C. All authors have read and agreed to the published version of the manuscript.

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