International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: https://doi.org/10.7439/ijbar
CODEN: IJBABN **Review Article**

Review on green synthesis and characterization of graphene supported metal nanocomposite for biomedical application

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Abstract

In this review different graphene supported metal nanocomposite particle, their green synthesis techniques, characterization and application are discussed. Currently green synthesis of nanoparticles has attained much interest because of their safe nature, environmentally benign, ease in manufacturing, and low production cost. It is a reliable process for developing a wide array of nanostructures such as metal salts from plants/fungal/bacterial extract and hybrid materials. The Synthesized nanocomposite particles can be characterized for some properties; such as optical analysis (UV-Vis, FTIR), chemical composition analysis (EDX, XAS), morphology and size analysis (SEM, TEM, AFM), and structural analysis (XRD). Graphene supported metal nanocomposite present high potential towards medical and biological applications, including drug delivery, anti-cancer, antibacterial and bioimaging, due to their exceptional properties such as thermal conductivity and high specific surface area. The main focus of this work is to review the current development of graphene supported metal nanocomposite green synthesis of different noble metal composite like silver, gold, platinum and copper for bioimaging, anti-cancer, antibacterial and drug delivery applications.

Keywords: Green synthesis, characterization, biomedical, nanocomposite, nanostructure.

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***Article History:**
Received: 07/06/2022
Revised: 27/06/2022
Accepted: 30/06/2022
DOI: https://doi.org/10.7439/ijbar.v13i6.5767

QR Code

How to cite: Yirga B. Review on green synthesis and characterization of graphene supported metal nanocomposite for biomedical application. *International Journal of Biomedical and Advance Research* 2022; 13(06): e5767. Doi: 10.7439/ijbar.v13i6.5767 Available from: https://ssjournals.com/index.php/ijbar/article/view/5767

1. Introduction

The nanoscience field has emerged into a wide range of efficient varieties over the last two decades and the emphasis of nanotechnology/nanomaterial is circumfluence in various potential areas, like sensor, biomedical, and plenty of useful applications. The progressions in related fields are absolutely due to the ability of synthesizing into nanoparticles from various materials, structures, as well as transforming the samples into complex Nano-architectures [1]. Due to this reason, the monolayer of two-dimensional graphene material with honeycomb lattice formation, which involves the inter-carbon bond length of approximately 0.142 nm is broadly applied for biomedical or nanomedicine applications due to its high conductivity properties. The graphene-based materials could definitely help in biomedical applications that allows for the proliferation and maturation of cells, e.g. drug delivery and anticancer therapies [2].

Graphene is the nanomaterial's consisting of a single layer of carbon arranged in an allotropic form. Graphene is chemically, mechanically, and thermally stable compound. It has various other properties such as large specific surface area and high flexibility. Graphene oxide, reduced graphene oxide, graphene nanosheets, graphene nanocrystal, graphene nanocubes, graphene nanotubes, graphene quantum dots, and others are also widely studied due to its exceptional properties (Fig. 1). Different methods are carried out to prepare graphene such as chemical vapor deposition, chemical or electrochemical reduction, and mechanical exfoliation [3]. The graphene layer is formed by chemical vapor deposition. Graphene oxide (GO) is prepared by oxidation of purified graphite by Hummer's

method and reduced graphene oxide (rGO) is prepared by chemical reduction of reduced graphene oxide [3].

Graphene is stronger than many other metals and it is highly flexible. It is the strongest material than any other material known. Graphene has many such notable features that allow the inorganic nanoparticle to accumulate in it to form nanocomposites. Graphene nanoparticles are decorated with other nanoparticles on its surface for more beneficial property. Graphene is mixed with several other nanoparticles such as metals (Ag, Au, Cu, Pt), non-metals (chitosan, polymers, epoxy), or metal oxides (TiO_2, ZnO) in order to produce nanocomposites [4] (Fig. 2).

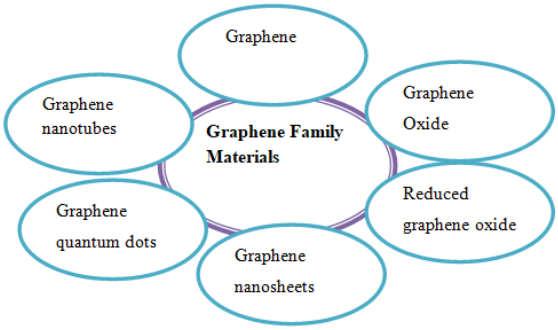

Figure 1: Graphene materials

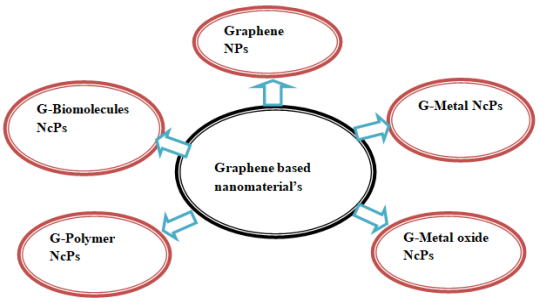

Figure 2: Graphene-based nanocomposites

Graphene nanocomposites have tremendous application in various fields such as antimicrobial, anticancer, biosensing, bioimaging, and drug delivery [5] (Fig. 3).

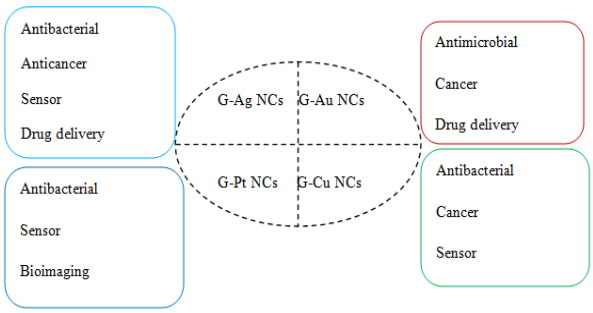

Figure 3: Graphene based nanocomposite application

In this review, we focused and discussed the green synthesis, characterization and major application of graphene-metal supported nanocomposites such as graphene-gold, graphene-silver, graphene-platinum, and graphene-copper in various biomedical aspects such as a biosensor, bioimaging, antibacterial, anticancer and drug delivery, etc. (Fig. 4).

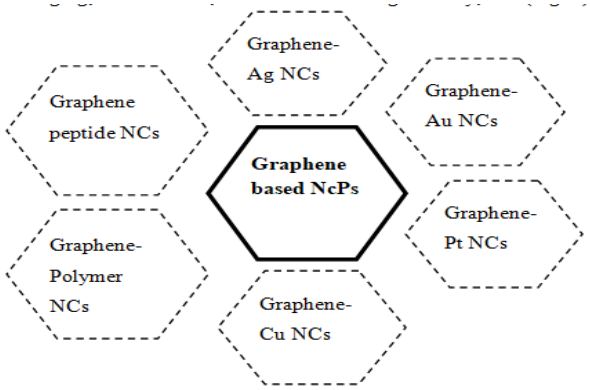

Figure 4: widely used graphene based nanocomposite

2. Green synthesis of graphene supported metal nanocomposite

Recently, biological approaches for synthesizing NPs have been increasingly considered. These methods represent a green technology aimed at minimizing negative environmental impacts. The synthesis of NPs using a chemical approach requires three main ingredients: a metal salt, a reducing agent, and a stabilizer or capping agent. One possible way to develop a "green" processes is to adapt benign synthesis approaches involving the utilization of nontoxic capping agents, less hazardous reducing agents, and the selection of environmentally benign solvents. In fact, biosynthetic routes provide nanostructures with better defined sizes and morphologies than some of the physicochemical techniques of production [6]. In the biological and green approach, many interesting biological tools, such as bacteria, yeasts, fungi, algae, and plants, offer accessible alternatives to chemical procedures and physical methods for the large-scale production of green NPs having dual functions (reducing and capping agents). However, the methods based on microorganisms are readily environmentally benign, scalable, and compatible for use in products with medical applications. However, the preparation of microorganisms is generally more expensive than the preparation of plant extracts. Among the organisms reported viable for the green synthesis of NPs, the best candidates are plants, owing to their diversity and sustainability. Plants can be used as an economic and valuable alternative for the large-scale biosynthesis of NPs. Among the existing biological methods for NP synthesis,

methods based on plant extracts have been widely reported in the literature [6, 7].

The capability of plant extracts to reduce metal ions has been known since the early 1900s. However, it is only over the past few years that the simple use of plant extracts and plant tissues for reducing metal ions to NPs has attracted considerable attention. Biomolecules present in plant extracts can be utilized to reduce metal ions to NPs in a single-step green synthesis approach. Among the MNPs, silver (Ag) and gold (Au) have been the specific focus for plant-based synthesis via the biogenic reduction of metal salts due to their potential applications in a number of fields [8]. This approach is particularly well suited to preparing NPs that need to be free of toxic contaminants, such as those used in therapeutic applications.

Although the precise mechanisms underlying the green synthesis of MNPs using plant extracts have not been thoroughly investigated yet, a bottom-up mechanism has been proposed [9]. This proposed mechanism involves four major steps; (i) initial activation step (bio-reduction) where the metal ions are reduced into their zero-oxidation states, (ii) subsequent growth and agglomeration of the small nanoparticles into more substantial and more thermodynamically stable particles, (iii) termination, where stabilization and capping of the MNPs are performed to form nanoparticles of diverse morphologies and average sizes and (iv) purification and washing of the MNPs usually by centrifugation [10, 11].

2.1 Plant sources used in the synthesis of nanostructures

Plants have long been known to demonstrate the ability to absorb, hyper accumulate, and degrade inorganic metallic and metallic oxide ions from their nearby environment. More recently, is has been shown that many organic entities present in plants can act as effective biological factories to remarkably reduce environmental contamination as well as being capable of retrieving metals from industrial waste. On the other hand, the true challenge to synthesizing MNPs is finding the balance between price, scalability, and applicability. In addition, reagent suitability plays an important role in the large-scale synthesis of NPs. Plants are the best candidates for use in the large-scale biosynthesis of NPs. Plant extracts can be used as reducing agents in the production of NPs and provide a cost-effective and environmentally friendly alternative production source [12]. Extracts from various parts of plants including their stems, gum, leaves, seeds, and fruits are efficaciously used for the preparation of NPs [13-15] (Fig. 5). The source of a plant extract is known to influence the characteristics of a nanostructure.

This is because various extracts include different concentrations and combinations of organic reducing agents. Reducing agents include various water-soluble plant metabolites (e.g., alkaloids, terpenoids, and phenolic compounds) and coenzymes. Phytochemicals that exist in plant extracts can be used as reducing and stabilizing agents for NPs synthesis. Because of the number of different chemicals involved, the biogenic reduction process is comparatively complex. Phenolic compounds possess hydroxyl and ketone groups that are capable of binding to metals and showing chelation. Preparation of NPs using these approaches is environmentally friendly and provides NPs with better defined sizes, morphologies, and stability [14, 15].

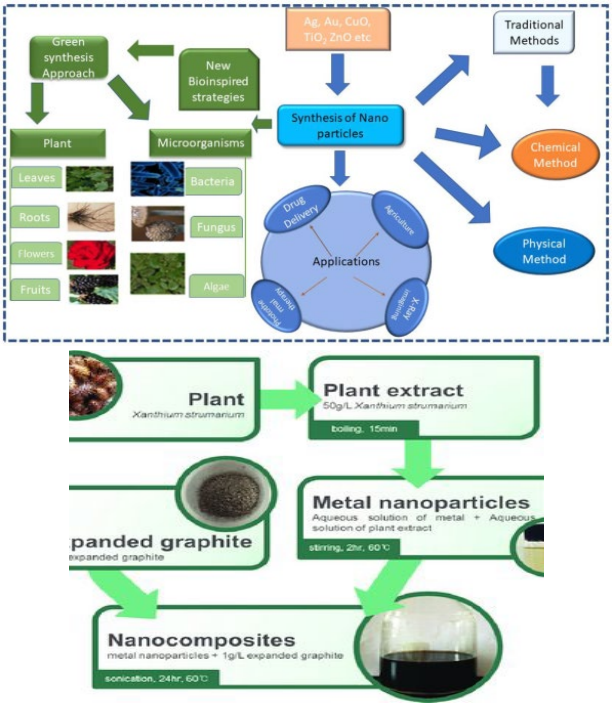

Figure 5: Green synthesis of graphene supported metal nanocomposite and plant sources

2.2 Green and economical synthesis of graphene–silver nanocomposite

Green chemistry methods involve the use of simple, economic, environmental-friendly materials and processes that reduce the generation of hazardous waste and harsh reaction conditions. The use of leaf extract as stabilizing and reducing agent is the most attractive approach for this purpose owing to its ease of preparation, simple handling and low cost availability. A variety of these reducing agents such as ascorbic acid, tea polyphenols, amino acids, carbohydrates, fenugreek extract and aloe vera extract have been utilized for reduction of GO [16].

According to Chandu *et al.* research of photocatalytic materials, custard apple leaf extract-reduced graphene oxide-silver (CRG-Ag) nanocomposite was synthesized successfully reported as follows.

Figure 6: Schematic illustration of CRG-Ag nanocomposite formation

Graphene oxide (GO) was prepared using Hummers' method. To synthesize CRG-Ag nanocomposite, 40 mg of GO was dispersed in 100 mL of water using sonication for 4 h and then 10 wt% of $AgNO_3$ was added and further sonicated for 5 min to get a uniform solution. To this precursor solution, 100 mL of freshly prepared CLE was added and refuxed at 100 °C for 12 h. The air-cooled solution was then centrifuged at 10,000 rpm for 15 min and vacuum dried at room temperature for 1 h to fetch the final nanocomposite. A general schematic depiction of reaction methodology is shown in Fig. 6.

Additionally Maryami *et al.* have synthesized Ag/RGO nanocomposite using *Abutilon hirtum* leaf extract [17]. Vizuete *et al.* have reported Ag/RGO photocatalyst with Mortino berry extract. Maham *et al.* have reported the synthesis of Ag/RGO/Fe_3O_4 nanocomposite using *Lotus garcinii* leaf extract [18]. Wang *et al.* have also reported the synthesis of Ag/RGO nanocomposite by employing green tea extract. [19]

2.3Green synthesis of graphene-gold NPs nanocomposite

Gold nanoparticles (Au NPs) have been widely adapted in various potential applications such as electrocatalysis, antibacterial and biosensor due to their excellent conductivity, specific large surface area and good biocompatibility [20]. Li *et al.* studied the dispersion ability of reduced graphene oxide at room temperature using GA as a reducing and stabilizing agent with excellent dispersion ability both in water and organic solvents [21] Xu *et al.* was prepared reduced graphene oxide by GO was deoxygenated with GA for Li-storage applications with satisfactory storage performance [22]. Here, GA acts as a functionalization and reducing as well as stabilizing agent for the preparation of GA-RGO/Au NPs.

Thirumalraj *et al.,* report a one-pot facile green synthesis of GA-RGO/Au NPs composite for sensitive and selective electrochemical detection of DA for the first time. The functionalization of GA-RGO was achieved by the bond formation between the carboxylic group of graphite oxide and the phenolic group of GA. They as prepared GA-RGO/Au NPs composite were highly stable on the electrode surface and possessed excellent electrocatalytic activity and good sensing performance towards the detection of DA. The GA-RGO/Au NPs modified electrode was successfully applied for the determination of DA via differential pulse voltammetry (DPV), with excellent analytical performance such as good linear response, lower detection limit (LOD) and better sensitivity. The sensor was achieved a good recovery for the determination of DA in human serum and urine samples. The overall procedure of the preparation of GA-RGO/AuNPs composite was shown in Fig. 7.

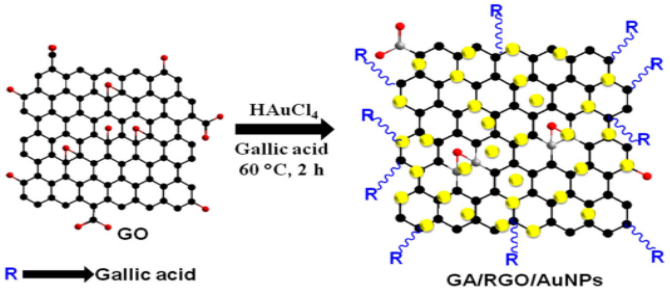

Figure 7, Schematic representation of the preparation of GA-RGO/AuNPs composite

2.4 Green synthesis of Graphene-Copper Nanocomposites

In order to produce copper/graphene based material nanocomposites, the interaction of copper ions and graphene based materials occur through physisorption, cation-π interaction and electrostatic force interaction [23]. Rios *et al.,* produced copper/reduce graphene oxide nanocomposites (Cu/RGO-NcPs) as catalyst for ammonium perchlorate decomposition by in situ reduction and they also studied the interaction between the materials using *ab initio* calculation to reveal the important role of the functional groups in stabilizing metal nanoparticles [24].

Fahiminia *et al.,* reported a green synthesis of Cu/RGO-NCs using *Euphorbia cheiradenia* Boiss extract and it was used for reduction of organic dyes and 4-nitrophenol. The presence of phenolic compounds in the plant extract such as quercetin, kaemferol and rutin with sugar moieties acts as bio-reducer for the nanostructure formation. The size of the Cu-NPs synthesized was in the average size of 30 nm and spherical shape dispersed on the reduce graphene oxide sheet layer [25].

2.5 Green synthesis of graphene-platinum nanocomposite

Plant extracts have been considered a green route and a reliable approach for safe, eco-friendly, and biocompatible Pt NPs. In one study, *Azadirachta indica* leaf broth was incubated with Pt (IV) ions for 1 h at 100 ◦C. The terpenoids of the *Azadirachta indica* leaf acted as the reducing and capping agents. The generated Pt NPs were sonicated for 30 min to enhance the monodispersity of the NPs [26]. A recent study reported on the phytosynthesis of Pt NPs using Nigella sativa (black cumin) seed extract. Pt (IV) ions were stirred with the black cumin extract for two days at 200 rpm and 75 ◦C [27]. Kumar *et al.* created a single and simple step for the biosynthesis of Pt NPs, employing the fruit extract of *Terminalia chebula*. Many researchers have considered using *Terminalia chebula* in the biosynthesis of MNPs due to its abundance in nature and polyphenolic content. The reaction temperature was maintained at 100^0C for 10 min. The reduction of Pt (IV) ions was mediated by the polyphenols present in the fruit extract [28].

Alshatwi *et al.* reported on the biosynthesis of Pt NPs using tea polyphenols that act as natural reducing agents with graphene oxide. Besides, their potential to form chelating complexes with various metal ions allowed them to be used as effective capping agents. The tea polyphenols were mixed with Pt (IV) in a ratio of 1:5 via magnetic stirring and then incubated for 1 h at room temperature [29].

3. Characterization of graphene-metal supported nanocomposite

Nanotechnology is based on the recognition that materials possessing nanometer-scale dimensions (nanostructures) have unique properties compared with their bulk materials. These nanostructures can be engineered using various elements and in many different forms, such as nanoparticles, nanocrystal, nanotubes, nanofibers, and nanorods. Prior to using NPs in any application it is essential to have an overview of the various detection/characterization methods. In addition, choosing the appropriate techniques for characterization is important.

3.1 Nanoparticle Formation Analysis

Graphene supported metal nanocomposite have been prepared by adding aqueous plant extracts to metal salt solutions within graphene oxide and observing a color change that represents the first signal for MNP and formation of composite. The bioactive molecules present in the extract are responsible for metal ion reduction to MNPs and graphene oxide to reduced graphene oxide. The progress of this reaction can be monitored using UV-visible spectroscopy. A UV-visible spectrum of green synthesized MNPs showed an absorption peak related to the surface Plasmon resonance (SPR) and collective oscillations of conduction band electrons in response to electromagnetic waves, indicating the reduction process and formation of metallic NPs and its composite [30]. It also provided data about the progression of the reaction and the formation, structure, size, aggregation, and stability of NPs because variations in the shape, position, and symmetry of the absorption peak with time provides information about the stability of the product.

3.2 Morphology and Size Analysis

The size and morphology of nanoparticles plays a vital role in determining the various properties that are essential parameters for NPs applications. In regard to the importance of morphology, nanowires can be considered as an example: because these nanostructures show electrical properties they have an application in optical, electronic, and nanoelectromechanical devices, additionally, they offer advantages in terms of their use as field-emitters due to their needle-like shape and as sensors or as leads for biomolecular (nano) sensors [31]. Considering the importance of nanoparticle size, it has been observed that size influences the ability of NPs to be used for drug release and drug targeting [32]. The characterization techniques used to analyze NP composite size and morphology are given below and discussed subsequently:

3.2.1 Scanning electron microscopy (SEM);- is a technique that is used to characterize the morphology of NPs. SEM is a type of electron microscopy that produces images of a sample by scanning the surface with a focused beam of electrons. The electron beam interacts with atoms at various depths within the sample, producing various signals that contain information about the sample's surface topography and composition.

3.2.2 Transmission electron microscopy and high resolution (TEM): is the best technique to determine the morphology of NPs. It is a microscopy technique in which a beam of energetic electrons is transmitted through a sample and the interaction of electrons with the sample forms an image. The image is then magnified and focused onto an imaging device, such as a fluorescent screen, a layer of photographic film, or a sensor like a charge-coupled device.

3.2.3 Atomic force microscopy (AFM): - is used for quantitative and qualitative data based on different properties like morphology, size, surface roughness, and texture [33]. It is used for the 3D characterization of NPs as compare with other techniques like electron microscopy, dynamic light scattering, and optical characterization methods. It also provides details about the different geometries of NPs, an analysis of hydrated NPs, and physical properties like magnetic behavior. It is also used for the study of soft and hard synthetic materials regardless of their conductivity and opaqueness.

3.3 Optical Characterization

The green approach to synthesis of nanoparticles is to use plant extracts as reducing and stabilizing agents. Flavonoid and phenolic compounds present in plant extracts play a key role in the synthesis of MNPs composites and their stabilization. The presence of polyphenolic compounds and the preparation of NPs can be demonstrated with UV-visible and FT-IR spectra from plant extracts and obtained NPs. In addition, as NPs have extensive uses in the field of optoelectronics it is therefore necessary to characterize them in terms of their optical properties prior to their use [34]. The following tools are used to investigate the optical properties of NPs composite:

3.2.1 UV-visible spectroscopy: - is a case in point that is based on the Beer-Lambert-Bouguer law. In spectroscopy, absorbing is a process in which a chemical species in a transparent environment selectively reduces the specific frequencies of electromagnetic radiation (decreases its intensity). UV-visible spectroscopy is a technique used in order to acquire absorption measurements for different materials or fluids. By measuring the absorption of different wavelengths by a sample, a spectrum is obtained by plotting a graph between the wavelength of the whole region and the absorption of each wavelength.

3.2.2 Fourier transforms infrared spectroscopy (FT-IR): - is used in order to identify different functional groups associated with NPs. The principle behind this technique is that functional groups present in the matrix of NPs absorb particular frequencies known as resonant frequencies, i.e., if the incident light has the same frequency as the vibration frequency of the bond or group then it will be absorbed. The mass of the atoms, the shape of the molecular potential energy, and associated vibrancy coupling are responsible for the absorption of a particular energy.

In the green synthesis of nanostructures using plants, FT-IR analysis can be used to identify the possible biomolecules responsible for the reduction and capping of metal NPs. The FT-IR spectrum of a plant extract will show some peaks that demonstrate the functional groups inside the structures of its polyphenolic that are probably responsible for the reduction of metal ions and the formation of nanocomposites.

3.4 Chemical Composition Analysis

3.4.1 Energy-Dispersive X-Ray Spectroscopy

Energy-dispersive X-ray spectroscopy (EDS, EDX, EDXS), sometimes called energy-dispersive X-ray analysis (EDXA) or energy-dispersive X-ray microanalysis (EDXMA), is an analytical technique which is used for the identification of compositions of different elements in a specific sample. It relies on an interaction between some source of X-ray excitation and a sample. EDS can be used to determine which chemical elements are present in a sample (qualitative analysis), and can be used to estimate their relative abundance (quantitative analysis) [35]. In quantitative analysis, the concentration of a specific element present in a sample is measured by the intensities of peaks.

3.4.2 X-Ray Absorption Spectroscopy

X-ray absorption spectrometry (XAS) is a widely used technique for analyzing the chemical environment of elements in an unknown material, i.e., it is used to determine the local geometric and/or electronic structure of any material. XAS analysis is also used to determine the energy dependent structure of a specific sample to X-ray absorption coefficient in edge regions [36]. The graph of absorption coefficient against wavelength of incident X-ray is known as an X-ray absorption spectrum. This technique is used to define the oxidation state of any metal atoms.

3.4.3 X-Ray Photoelectron Spectroscopy

X-ray photoelectron spectroscopy (XPS) is one of the most sensitive, informative, and accessible surface analysis techniques. XPS deals with elemental composition at the parts per thousand range, empirical formula, and chemical and electronic states of elements existing within a material [38]. XPS analysis generally is more advantageous compared with FT-IR and perhaps even NMR. XPS spectra are obtained by irradiating a material with an X-ray beam and measuring the number of electrons and their kinetic energy released from the sample upon irradiation. XPS is a very surface-sensitive tool that can be used to determine the quantity and entity of elements present in a sample.

3.5 Characterizations for Structure Analysis

3.5.1 X-Ray Diffractometer

X-ray diffraction (XRD) is one of the most important nondestructive instruments used to analyze all kinds of matter ranging from fluids, to powders and crystals. XRD is a unique method for the determination of the crystallinity of different compounds. From research to fabrication and engineering, XRD is an indispensable technique for material characterization and quality control [37].

XRD is chiefly used for:

1) Identification of crystalline material (utilized for regulatory purposes or during development).
2) Identification of various polymorphic forms (fingerprints).
3) Distinguishing between amorphous and crystalline material.
4) Quantification of the percentage crystallinity of samples. XRD methods are superior at elucidating the 3D atomic structure of the crystalline phases of NPs.

4. Biomedical application of graphene supported metal nanocomposite

The majority of graphene-based nanocomposites are comprised of only two components, but multicomponent composites have been prepared for special applications as well. The following sections are organized in such a way that the most common applications of graphene/metallic NPs based nanocomposites, such as biomedical, energy storage, sensing, catalysis and environmental application [39,40]. Among those graphene metal supported nanocomposite have gained noticeable attention in the biomedical field such as anti-cancer, biosensor, drug delivery, antibacterial, are discuss in detail below.

4.1 Graphene supported metal nanoparticle composites for anticancer

Recently, a few research groups have reported the anticancer effect of GO–metal nanoparticle composites by themselves without any chemotherapeutic agent. Gurunathan *et al.* fabricated an rGO–Ag NPs composite using *Tilia amurensis* plant extract and explored its anticancer potential in ovarian cancer cells (A2780) [41]. The synthesized rGO–Ag sNP nanocomposites were highly stable and water-soluble and did not aggregate for 3 months. The rGO–Ag NPs composite exhibited a dose-dependent inhibition of viability with an IC50 value of ≈12.5 µg/mL. The composite resulted in the loss of cell membrane integrity, as evidenced by enhanced lactate dehydrogenates leakage. Further, the rGO–Ag NPs system increased ROS generation and DNA fragmentation in A2780 cells, demonstrating its potential in cancer treatment [41].

Kavinkumar *et al.* synthesized GO/rGO-Ag NPs nanocomposites and explored the anticancer effect of this conjugate system against the human lung cancer A549 cell line. The authors first synthesized GO and rGO following conventional Hummer's method, and Ag nanoparticles were synthesized using traditional methods using vitamin C as a reducing agent. Finally, negatively charged Ag nanoparticles were adsorbed onto the surface-modified positively charged GO/rGO via electrostatic attractions [42]. The Cytotoxicity of GO/Ag NPs, rGO/Ag NPs, and GO were evaluated against A549 cells by MTT assay. For GO only, even at a very high concentration (200 µg/mL) after 24 h, the cell viability was higher than 40%. This low Cytotoxicity of GO on lung cancer cells can be due to oxygen-containing functional groups, e.g., OH, -COOH, and epoxy groups on the GO surface. Cytotoxicity of rGO was slightly higher than GO, with cell viability IC_{50} values of 160 µg/mL and 180 µg/mL, respectively, after 24 h. Further, the rGO–Ag NPs nanohybrid system demonstrated better anticancer activity (IC50 of 30 µg/mL) than the GO–Ag NPs composite (IC50 of 100 µg/mL) against the A549 cell line. The authors suggested that the improved anticancer activity of the rGO–Ag NPs composite was a result of the synergistic effect of rGO and Ag NPs and enhanced intracellular delivery of rGO.

GO–Au NPs nanocomposites have been well studied for cancer therapy [43]. More recently, Lina *et al.* reported the synthesis of rGO–Curcumin (CUR)-capped gold (CAG) nanoparticle composite and investigated its efficiency as an antioxidant and anticancer agent [44]. CAG was synthesized using reducing properties of CUR and, alternatively, following conventional sodium citrate reduction as well. The activity of CUR-capped Au NPs–rGO composite was tested against two human colon cancer cell lines, namely, HT-29 (colon adenocarcinoma) and SW948 (Duke's C colorectal carcinoma). Cytotoxicity studies revealed that both cancer cells displayed a change in size and morphology upon CAG treatment compared to the control. Optical microscopy revealed cellular shrinkage and inhibition of proliferation in a dose and time dependent manner. The cytotoxic effect of CAG in the WST-8 assay showed cell viability IC50 of 100 µg/mL in HT-29 and SW-948, cells, while only very low inhibition to normal colon cell line (CCD-841) was observed. This composite also showed low inhibition in normal liver cells (WRL-68) as a RES organ. The 2,2-diphenylpicrylhydrazyl (DPPH) assay showed CAG's ability to inhibit free radicals and exert antioxidant effects to neutralize reactive oxygen species (ROS) and inflammatory intracellular tumor microenvironments [44].

Another composite hydrogel containing spinach extract (SE), rGO and gold nanocages (AuNCs) was prepared for the combination of localized drug release with multimodal therapy of malignant tumor (Figure 8). SE did not only form hydrogel but also played a role in the improvement of hydrogel biocompatibility as well as killing tumor cells as a natural photosensitizer under 660 nm laser radiation. Au NCs exhibit obvious photothermal properties and enhance $^1O_2$ generation. Upon loading with fluorouracil (5-FU), good drug retention ability and controllable release of 5-FU were observed in a neutral physiological environment and the acidic environment of tumor cells,

respectively. After 10 min of NIR irradiation, the survival rate of HeLa cells incubated with 5-FU loaded hydrogel sharply decreased to 1.2% due to the synergistic photothermal/photodynamic/chemo therapeutic effect, making it an excellent delivery carrier for combined PTT/PDT/Chemo cancer treatment [45].

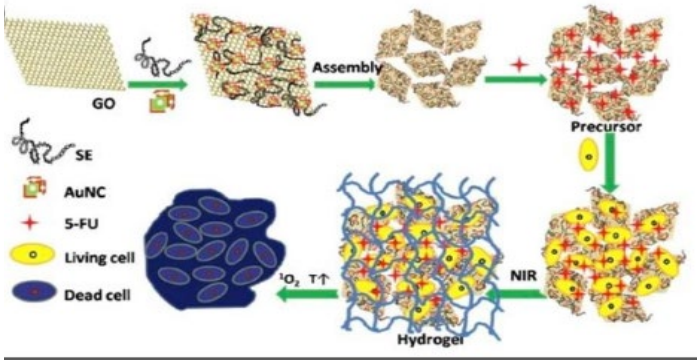

Figure 8: Formation process of rGO/Au/SE hydrogel synthesis, 5-FU drug loading and combined photothermal/ photodynamic/ chemo synergetic antitumor

4.2 Graphene supported metal nanoparticle Composite for Antibacterial application

Although the graphene family exhibits antibacterial properties, they tend to aggregate due to strong inter-plane interactions, which limit their surface area and modes of action [46]. Therefore, to reduce the aggregation and enhance the antibacterial activity, various functionalizations and surface modification with metal ions/oxides/sulfides NPs, polymers, antibiotics, and enzymes have been performed on graphene. Several metal ions/oxides/sulfides NPs such as Ag NPs, Cu NPs, titanium dioxide (TiO_2) NPs, zinc oxide (ZnO) NPs, manganese disulfide (MnS_2) NPs, and cadmium sulfide (CdS) NPs possess antibacterial properties with the generation of ROS via photocatalytic processes by irradiating the light on their surface with the energy greater than their band gap.

Silver and its compounds have been used since the age of ancient Egyptians and have been widely used in modern human medical science from the 20th century for their antibacterial activity [46]. The antibacterial and antiviral properties of Ag, Ag ions and Ag-based compounds have been thoroughly investigated. The Ag ions can penetrate the cells and destroy the membranes to inactivate bacteria. Ag NPs is a promising alternative to Ag salts and can exhibit the direct damage to bacterial cell membrane. Moreover, it can also generate ROS to fight against bacteria via photocatalytic activation using a light source. Several studies on antibacterial activity of Ag NPs have been reported [46, 47]. Ag NPs may exhibit the combined antibacterial effect with the release of Ag ions. However, when bare Ag NPs come in contact with bacteria, they aggregate and lose active surface area, which, thereby, show deceased antibacterial activity. To conquer this, several nanocomposites composed of Ag NPs with graphene have been prepared and studied against various bacterial strains [48, 49] and showed promising antibacterial activities. These promising results drove the research toward producing more efficient GO/rGO-Ag NPs based nanocomposites with improved antibacterial properties.

Zhu *et al.* used poly(diallyldimethyl ammonium chloride) (PDDA) as an adhesive agent for conjugating different densities, sizes, and forms of Ag NPs to GO sheets via electrostatic interactions. The synthesized GO-Ag NPs composites showed enhanced colloidal stability, photo-stability, and antibacterial activities against *E. coli* and *B. subtilis* compared to Ag NPs [50]. Moreover, GO modified with smaller Ag NPs showed higher antibacterial activity than GO modified with larger Ag NPs. The functionalization of GO-Ag NPs with polymers increases the antibacterial performance of the GO-Ag NPs composites. Chen *et al.* [51] developed a method for synthesizing four different particle sizes (10, 30, 50, and 80 nm) of polyoxyethylene bis (amine) (PEG) directed Ag NPs grown on the GO to form GO@PEG@Ag NPs composites. The antibacterial activities against *E. coli* and *S. aureus* demonstrated that GO@PEG@Ag NPs composites exhibited enhanced efficacy compared to Ag NPs alone and the smallest composite (10 nm) showed higher antibacterial activity than other large composites (30, 50, and 80 nm).

As shown in Figure 9, the Ag NPs were formed and anchored on the defects of the GO sheets, which was reduced to RGO at the same time by glucose [52]. Under a NIR irradiation, the photothermal effect of RGO was significantly enhanced compared with GO, which was not only used directly to kill bacteria, but also enhanced the release of Ag+ to generate ROS stress for breaking the integrity of bacterial cells. Therefore, the synergistic effect of RGO/ Ag nanocomposite satisfied the therapeutic requisites for destroying MDR bacteria under a NIR irradiation.

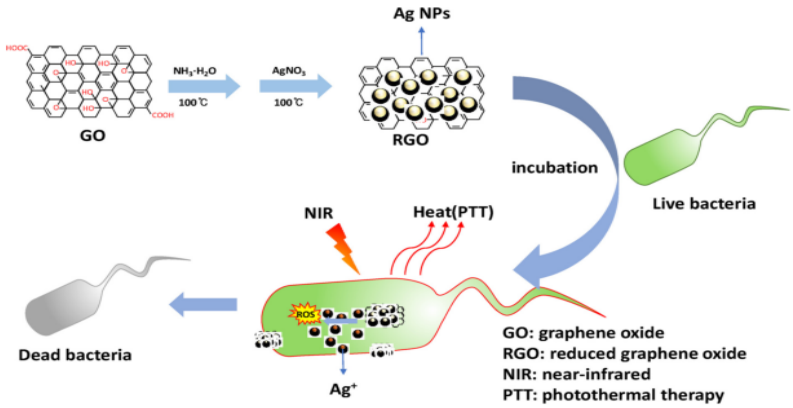

Figure 9: Schematic illustration of the synergistic antibacterial activity using the RGO/Ag nanocomposite under a NIR irradiation

Tu *et al.,* reported that antibacterial activity for copper/reduce graphene oxide (Cu/RGO-NCs) exhibit better antibacterial activity compared to the Cu-NPs alone [53]. Normally, the possible mechanism for copper/graphene based material nanocomposites is by the interaction of the positive charge of copper ions on RGO with the negatively charge surface of both gram positive and gram negative bacterial cells. The negative charge of gram positive bacteria is due to the presence of the teichoic acid with high amount of phospholipid groups that extend from the cell wall of the bacterial strain [54].

4.3 Graphene supported metal nanoparticle composite for biosensor application

A large number of diagnostic devices termed biosensors have been designed for biological sensing or quantification of a specific target analyte or chemical constituents of analyte. Biosensors are basically composed of a receptor (organic or inorganic material capable of interacting with a target analyte) and a transducer (captures the recognition event between the analyte and the receptor), which further converts it into a measurable signal of many forms. Sensitivity, limit of detection (LOD), selectivity, reproducibility are the main determining factors on which biosensors are evaluated. Other factors like portability, ease of use, stability, reusability make an ideal biosensor [55]. As already mentioned graphene possesses excellent physical and chemical properties and can thus be efficiently used in different biosensing applications [56]. Likewise, NPs because of their distinctive physical and chemical characteristics such as quantum size and surface effect etc. are extensively explored in the field of biosensing. Combining NPs with graphene to form graphene-NPs hybrid gives much higher sensing properties to the hybrid materials and increases the existing surface area for analyte binding and improves the electron mobility as well as conductivity. In this section we try to present the works on the development of biosensors based on graphene metal hybrids.

Au or Ag NPs decorated graphene based biosensors exhibit pronounced encroachments in bio sensing field, due to strong NIR optical absorbance and enhanced conductivity exerted by both components. These nanocomposites showed unique character of augmented sensitivity with lower detection limits. In bio sensing field, there is a requirement for more sensitive, accurate and cost effective detection kits. Current detection methods for different important biomolecules include radioimmunoassay, chemiluminescence, protein chip technology and flow cytometry [57].

The graphene materials coated with gold and functionalized with probes showed excellent ultra-sensitive specificity for lower level molecular detection as an example, plasma miR-155 (breast cancer) and human papilloma virus DNA. Au and Ag NPs with reduced size have been widely explored in biosensing owing to their biocompatibility with different biomolecules. With a view of enhancing the properties and incorporating novel properties, Au and Ag NPs have been hybridized with GO/rGO resulting in composite materials suitable for biosensing, energy devices, SERS platform etc. As a bio-imaging and biosensing probe, SERS has been widely investigated because of its high sensitivity. Graphene nanosheets supported Au/Ag MNPs apart from acting as superior support with large surface area for immobilizing different target biomolecules; they are successfully applied as efficient electrochemical biosensors for the detection of folic acid, glucose, ascorbic acid, dopamine, uric acid, tryptophan, H_2O_2 etc. by promoting electron transfer between the electrode and analyte in solution [58]. For instance, an efficient glucose sensor based on rGO/PAMAM-Ag NPs nanocomposite was fabricated by Luo *et al*. rGO-PAMAM-Ag nanocomposite was used to modify a glassy carbon electrode (GCE) followed by immobilization of glucose oxidase (GOD). An efficient electrochemical behavior was exhibited by the electrode and direct electron transfer (DET) was easily obtained

between GOD and the modified electrode. The biosensor displayed good analytical performance with a high sensitivity of 75.72 µA mM^{-1} cm^{-2} and low detection limit of 4.5 µM at an applied potential of -0.25 V.

As GO/rGO with large surface area and low production cost, display target specific drug delivery, high electron mobility and synergy effect when combined with noble MNPs, they are widely explored for biosensing [59]. Depending on SERS signal, Ren *et al.* utilized GO/PDDA/Ag NPs (where PDDA stands for poly (diallyldimethyl ammonium chloride)) for the detection of folic acid. GO/PDDA/Ag NPs improved the SERS response of folic acid and the strongest SERS peak appeared at 1595 cm^{-1} where its intensity increased with increasing folic acid concentration. 9 nM was the minimum concentration of folic acid detected in serum with a linear response ranging from 9 to 180 nM [57].

Graphene oxide-gold NPs (AuNPs@GO) hybrids were synthesized by Sanchez *et al.* as SERS platforms for the detection of flavin adenine dinucleotide (FAD). FAD is a flavoprotein coenzyme with many important roles in oxidoreductase and reversible redox biochemical reactions. The Au NPs@GO displayed a detection limit of 10^{-9} M and enhancement factor of 1×10^{4} for FAD [60]. In one of the studies by Manikandan *et al.* Au nanohexagons on graphene templates were developed and used to enhance Raman scattering and also to distinguish between normal and cancer human breast, and cancer stem cells. The detection limit was enhanced by 5.4 fold towards detecting breast cancer cells (BCCs) at concentrations of 100 µg/1×10^{4} cells and the sensitivity was increased by 4.8 fold towards breast cancer stem cells (BCSCs); at the same time the differentiation between normal cells, cancer cells and cancer stem cells was also made possible. A hybrid nanomaterial consisting of Au, Ag and graphene was fabricated by Yi *et al.* where rGO was sandwiched by Ag and Au nanostructures. The nanohybrid material behaved as efficient SERS platform for the identification of tumour cells (BEL-7402 hepatocarcinoma cells) without using any biomarkers [61].

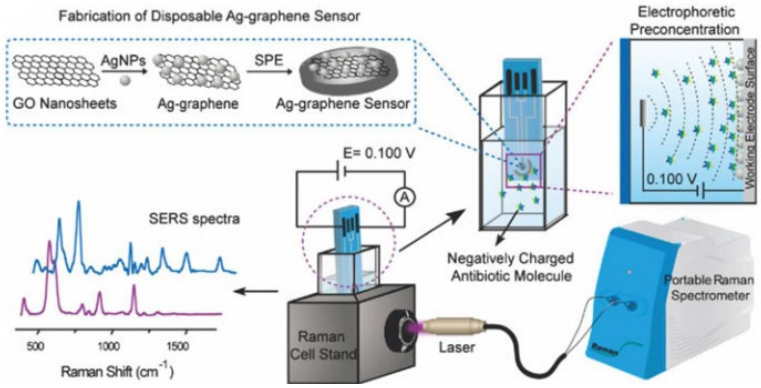

Figure 10: Graphene-Ag NPs sensor based on electrophoretic pre-concentration and SERS

Schematic representation of a disposable Ag-graphene sensor for the detection of polar antibiotics in water. The magnification insets show the fabrication of Ag-graphene sensors and the electrophoretic pre-concentration process of polar antibiotics. The distribution of antibiotics molecules is sketched for the case of a negatively charged analyte. At a given potential, most of the negatively charged antibiotics are concentrated onto the positively charged printed electrode, due to the generated electric field between the working electrode and the counter electrode. In SERS experiments, the laser comes vertically from the side view of the spectroelectrochemical cell and is focused on the Ag-graphene sensor [62].

4.4 Graphene supported metal composite for Drug delivery and bioimaging application

Very recently, different scholars reported a hybrid system consisting of GO wrapped Mesoporous silica nanoparticles, aiming to protect sensitive dyes loaded within the nanoparticles for fluorescence bioimaging. Well-dispersed Au@NGO with nano-scale size is favorable for intracellular labeling and drug delivery, while Au/GO hybrids presented in a 2D morphology usually have relatively large size, up to hundreds of nanometers or even micrometers, which are unfavorable for biological applications in terms of cellular uptake and intracellular labeling. The experimental results indicate that nano-sized Au@NGO can enter into cancer cells through effective cellular uptake, acting as intracellular bioimaging tags by generating SERS signals inside the cancer cells [63]. In addition to the Raman imaging application, Au@NGO could also be used as an anticancer drug delivery carrier. In this case, anticancer drug doxorubicin (DOX) was attached onto the Au@NGO surface through non covalent interactions between NGO and DOX under neutral conditions. After entering into cancer cells, DOX can be gradually released from the nanoparticle surface, leading to apoptosis and cancer cell death.

In addition, GO has been considered as an excellent bioimaging tag based on its fluorescence 12–15 and Raman scattering signals, since it not only is benign to biological systems, but also can overcome many drawbacks of common fluorescent dyes, such as low stability in biological systems and photo-bleaching. On account of the special properties of both Au NPs and GO, rational combination of Au NPs and GO, i.e., Au NPs/GO hybrids, has attracted lots of attention. Au NP/GO hybrids have been employed in various applications; including SERS based chemical or bio-molecular detection, electric device fabrication, photothermal cancer therapy, and even catalysis for chemical reactions. Based on the promising superiority of SERS based biological imaging over conventional methods, such as its high sensitivity as well as stable and reproducible signal, the fabrication of Au/GO hybrids for SERS based bioimaging has been emerging. To the best of our knowledge, however, most of the current Au NPs/GO hybrids were prepared based on the 2-D morphology of GO sheets [64].

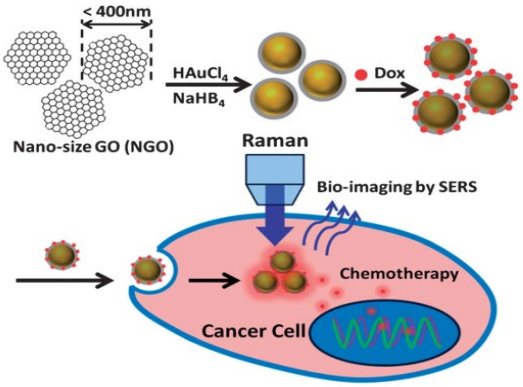

Figure 11: Illustrative mechanism of SERS based bioimaging and anticancer drug delivery by using Au@NGO

Au NPs/rGO composite was also applied for the delivery of monoclonal P-glycoprotein (P-gp) antibodies and anticancer drugs towards drug resistant leukemia cells in nude mice [62]. The composite material exhibited noteworthy targeting and binding towards leukemia cells (K562/A02; KA). Both in vitro and in vivo studies on tumour mounted nude mice indicated that combining P-gp antibodies and anticancer drug, daunorubicin (DNR), with graphene-gold nanocomposites (GGN) served as a competent drug delivery platform, with significant targeting and binding properties towards KA cell lines that are resistant to drug and also induced apoptosis of KA cells leading to inhibition of tumour growth in the nude mice.

Raman spectra of graphene-based nanomaterial have also been applied for bioimaging. Both graphene and GO exhibit unique intrinsic Raman signals that can be further enhanced by integrating metal NPs. Tan and Zhang *et al.* reported the facile synthesis of AgCu@graphene (ACG) NPs by growing several layers of graphene onto the surface of AgCu alloy NPs using the chemical vapor deposition method [65]. The ACG NPs have been utilized to enhance unique Raman signals from the graphitic shell, making ACG an ideal candidate for cell labeling, rapid Raman imaging, and surface-enhanced Raman spectroscopy (SERS) detection. In situ reduction of metal ions on GO results in stronger Raman signals from metal–GO hybrids compared with the pristine GO. Guo *et al.* developed GO-Ag NP composites by in situ reducing Ag+ using polyvinylpyrrolidone as a novel SERS label for fast cellular probing and imaging [66]. The authors demonstrated that, by utilizing GO-Ag NPs as a highly sensitive optical probe, fast SERS imaging of cancer cells was realized with a short integration time of approximately 0.06sec per pixel. Zhao and co-workers reported the one-step synthesis of nanosized GO-wrapped gold NPs. These GO-Au nanocomposites could be utilized for both intracellular bioimaging tags and drug delivery carriers. Non covalent attachment of Au NPs to GO via π–π stacking or other molecular interactions has also been used for Raman imaging. Zhang *et al.* reported that the presence of Au NPs on the GO sheet has an important role in the observation of enhanced Raman spectra of GO in live cells. In addition to these optical imaging methods, graphene-based nanomaterial's have also been used for other imaging modalities, including photo acoustic imaging, magnetic resonance imaging, computed tomography, as well as radionuclide-based imaging, such as positron emission tomography and single-photon emission computed tomography [66].

5. Conclusions

The nanoscience field has emerged into a wide range of efficient varieties over the last two decades and the emphasis of nanotechnology/nanomaterial is circumfluence in various potential areas, like sensor, biomedical, and plenty of useful applications. Graphene is the nanomaterial's consisting of a single layer of carbon arranged in an allotropic form. Graphene is chemically, mechanically, and thermally stable compound. It has various other properties such as large specific surface area and high flexibility. In this review article, we focused and discussed the green synthesis, characterization and major biomedical application of graphene-metal supported nanocomposites. In the biological and green approach synthesis, many interesting biological tools, such as bacteria, yeasts, fungi, algae, and plants, offer accessible alternatives to chemical procedures and physical methods for the large-scale production of green NPs composites having dual functions (reducing and capping agents). The synthesized materials are characterized using various

techniques like Uv-vis, FT-IR, EDX, SEM, TEM, AFM, and powder XRD, electronic microscopy, etc. that give insight into the morphology of the materials, particle size, impurities, roughness, doping level and chemical composition etc. The most common applications of graphene/metallic NPs based nanocomposites, such as biomedical, energy storage, sensing, catalysis and environmental application. Among those graphene metal supported nanocomposite have gained noticeable attention in the biomedical field such as anti-cancer, biosensor, drug delivery, antibacterial, are discuss in detail above.

Data Availability: No new data were created or analyzed in this study.

Conflict of Interest: The authors declare that have no conflict of interest.

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