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Review

Graphene nanoribbons: A state-of-the-art in health care

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ABSTRACT

Graphene nanoribbons are thin strips of single sheet graphene used in diagnoses and treatments of cancer, inflammation and Alzheimer's disease and considered as a good nanocarrier in gene, photo-thermal, anti-microbial therapies, etc. This review article focuses on the overview of bio-conjugation and molecular interaction of graphene nanoribbons with different biomolecules present in body like enzymes and peptides. The use of graphene nanoribbons as biosensor, artificial receptor and cellular device extends their applications in theranostic and drug delivery. The relationship between graphene and biological molecules like RNA, DNA, etc. using molecular dynamics related to the electronic properties are discussed for site-specific action. The biodegradation and use of graphene nanoribbons in safe concentration are important aspects for the prevention of toxicity in living cells and body environment. Graphene nanoribbons display various applications in bio-imaging, green chemistry and material sciences due to electro-mechanical properties such as higher surface area, greater loading capacity, elevated thermal capacity, etc. The functionalized graphene nanoribbons demonstrated better adsorption and adhesive binding properties to mammalian cells which make them ideal bio-carrier for gene transfection and nucleic acid delivery. Further, research and development of graphene nanoribbons for novel drug delivery is currently necessary to overcome barriers like environmental toxicity and extensive cost.

1. Introduction

Graphene is made up of one sheet planar atoms of sp^2 carbon atoms from a naturally occurring mineral known as graphite wherein the carbon atoms are closely compressed into a honeycomb-like crystal lattice (Allen et al., 2010). It shows physical properties like high mechanical strength, thermal conductivity, elasticity, carrier loading capacity and electrical mobility due to the presence of π electrons conjugated by the sheet structure prevailing an ideal candidate for various applications in gene therapy, drug delivery, biosensors, etc. (Fig. 1) (Shende et al., 2020; Faccio et al., 2009; Jing et al., 2013. The interaction of graphene with various inorganic pollutants and oxygen species due to the formation of covalent, non-covalent or ionic bonds expands the applications by utilizing graphene as an absorbent in green chemistry (Zhang et al., 2016). Moreover, for broader applications in health care, graphene-based substances are utilized in different forms like carbon nanotubes (CNTs), fullerenes, nanorods and graphene nanoribbons (GNRs). The graphene nanoribbons are synthesized mainly by bottom-up (G. Li et al., 2016) and peeling methods wherein carbon nanotubes are unzipped to form nanoribbons (Hirsch, 2009). The in-solution bottom-up method of synthesis provides an easy functionalization of GNRs while the chemical vapor deposition produces low-cost GNR films for industrial scale-up. The on-surface ultrahigh method of bottom-up synthesis is expensive process although shows formation of zig-zag GNRs, considered as promising material (Panwar et al., 2019). The GNRs are repetitive hexagonal structures (Yang et al., 2008) with dimensions of 0.4–0.7 μm of length and 1–1000 nm of width and classified as narrow, wide and shortened according to their sizes, as shown in Fig. 2. The narrow GNRs show width less than 10 nm, the wide GNR width ranging from 10 to 100 nm and the shortened GNRs show no specific size due to shortening by cutting from the oxidized or reduced GNRs (Higginbotham et al., 2014). The GNRs are also classified as zigzag graphene nanoribbons (ZGNR) or chiral GNRs (Li et al., 2008) and armchair graphene nanoribbons (AGNR) according to their edge shape,

Abbreviations: GNRs, Graphene Nanoribbons; ZGNR, Zigzag Graphene Nanoribbon; AGNR, Armchair Graphene Nanoribbon; GO, Graphene oxide; rGNR, Reduced Graphene oxide; nGNR, Nanoscale Graphene Nanoribbon; LiP, Lignin Peroxidase; hMPO, Myeloperoxidase; PEG, Polyethylene Glycol; PEI-g-GNR, Polyetyleneimine Graphene Nanoribbons; PEI, Polyethylenimine; mAlb, Microalbuminuria; EGFR, Epidermal Growth Factor Receptor; HCC, Hepatocellular Carcinoma Cells; AFP, Alpha Fetoprotein; ChT, α-Chymotrypsin; 2AP, 2-Aminophenol; 4AP, 4-Aminophenol; TNF, Tumor Necrosis Factor; MoS_2, Molybdenum Disulfide; BSA, Bovine Serum Albumin; Tf, Transferrin; BGF, Bovine Fibrinogen.

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position and width of the hexagonal rings in the nanoribbons (Prezzi et al., 2008). The asymmetrical ZGNRs demonstrate higher electrical mobility and flow of current than symmetrical ZGNRs due to distorted structure (Kiran and Gangadharappa, 2019; Ezawa, 2008). The GNRs show various advantages due to the functionalization with many drugs molecules, diagnostic agents and genes like siRNA, mRNA and DNA. The attachment of GNRs to several ligands, antibodies and other biological compounds like DNA displays a stable interface and extends the use for site-specific targeted delivery into the cells for chemotherapeutic action and cell imaging (Rahman et al., 2019). The detection of the gene into the cytoplasm of the cell is performed using GNRs as the cellular device in gene therapy as non-viral vectors for diagnosis and treatment of gene-related disorders like breast cancer, defective microRNA, etc. (Dong et al., 2011). The electronic and structural abilities of the GNRs play a significant role in understanding the interaction between the GNRs with biomolecules such as proteins, DNA, amino acids, etc. The doping of amino-functionalized GNRs with boron due to higher dipole moment offered long-term stability, better electrical property and chemical reactivity, and larger attachment of biomolecules to the GNRs (Janani and Thiruvadigal, 2017). The GNRs are also used in medical devices for biosensing of various proteins like IgG, DNA, RNA, etc. by coating the plasmonic device. The plasmomic resonances (1450–1800 cm^{-1}) tuned on the GNR surface helped in the detection of human IgG and expand application in cell analysis by transcription, translation and genetic engineering (Panwar et al., 2019). Also, the different functional groups on the GNR provide attachments for different antibodies due to bio-conjugation property of the material. This property is used for recognition of certain viruses and bacteria such as *E. coli, Salmonella typhimurium,* Zika virus, rotavirus and dengue virus by development of biosensor and serve as a potential material for the detection of pathogens (Peña-Bahamonde et al., 2018).

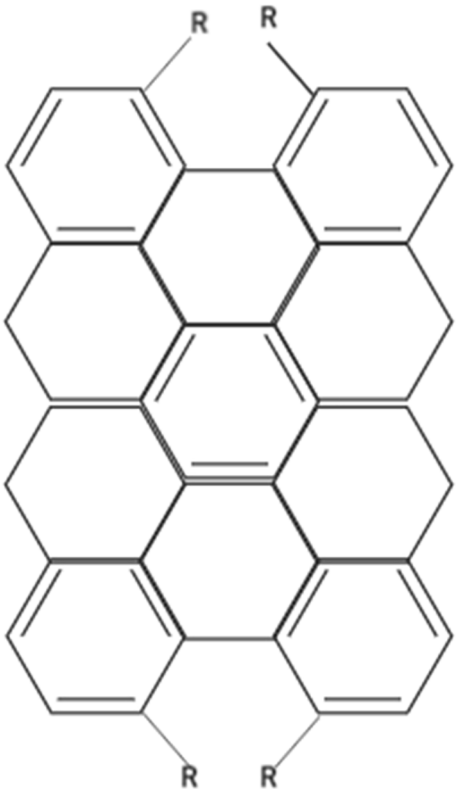

Fig. 2. Structure of graphene nanoribbon.

2. Bioconjugation

The carbon nanomaterials like graphene nanoribbons pass through several changes like de-functionalization, modification in the surface, bio-coronation, bio-distribution and targeting to the biodegradation by interacting with the proteins and lipids present in the body (Bhattacharya et al., 2015). The attachment of dextran with GNRs display the ability to form stable bioconjugates and enhances the *in-vitro* biocompatibility of the graphene oxide in the physiological solutions (Hu and Zhou, 2013). Another method of bioconjugation established by cellulose nanowhiskers- functionalized graphene sheets by using covalent cross-linking agents via nitrene chemistry to demonstrate an increase in the absorbing capacity of GNRs (Soleimani et al., 2018). In a study performed by Imani *et al.* using octa-arginine peptide sequence functionalized graphene nanosheets illustrated the applications for conjugation

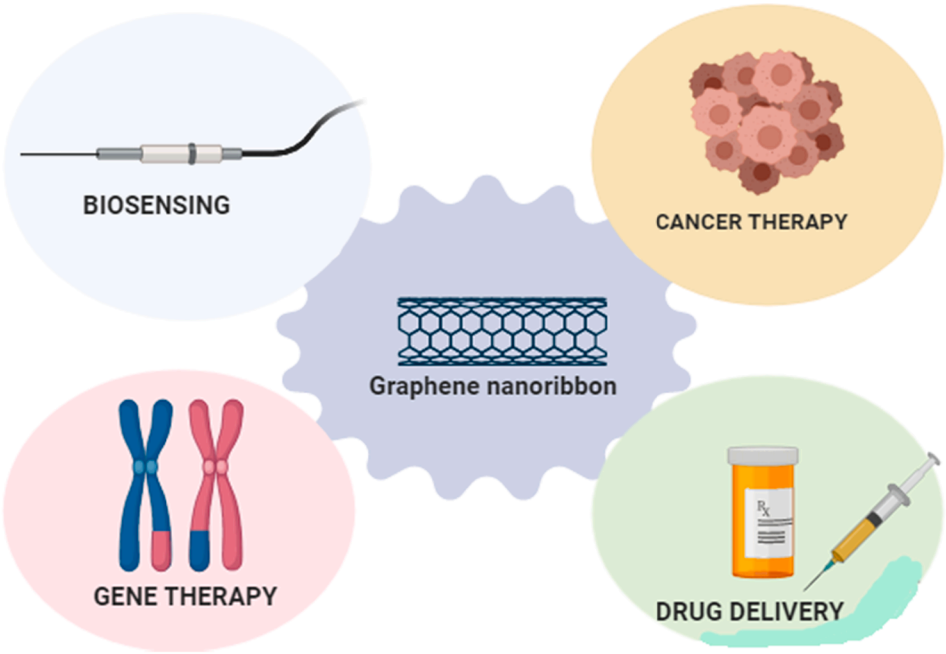

Fig. 1. Applications of graphene nanoribbons in health care.

with proteins, peptides, nucleic acids, and other biomolecules present in the body due to various surface functional groups like carboxyl, carbonyl, etc. (Eylu, 2018). The PEG one of the biocompatible polymer was used to achieve better physiological stability, reduction in cytotoxicity and higher gene transfection efficiency for bioconjugation by functionalizing with polyethyleneimine graphene oxide and showed several applications in *in-vitro* plasmid, DNA and siRNA delivery systems (Sun et al., 2008). The conjugation of minerals found in the body like calcium, magnesium, sodium and potassium with GNRs using L-glutamine, to help in generating electronic stability, higher values of charge transmission, ionic character and Gibb's free energy of solvation. The adsorption of such minerals induces an increase in energy levels by altering the chemical potential. The conjugation of calcium with l-glutamine was found to be highly effective compared to other metals for generating nanocarriers in drug delivery applications (Chowdhury et al., 2015). The bioconjugation of gamma-globulins from bovine blood and polyclonal antibody IgG–FITC from human serum to the graphene nanoparticles using iron nanoparticles was established by polyethyleneimine (cationic polymer) by covalently immobilizing sulphydrylated groups on the surface (Kasprzak et al., 2016). The improvement in hemolytic properties of graphene oxide was performed by utilizing bioconjugation of bovine serum albumin (BSA) with graphene oxide. The resistance on surface of RBC due to the charges on the BSA increased the hemocompatibility of graphene oxide and decreased the hemolysis ratio less than 0.2% (Cai et al., 2015). Similarly, the attachment of biodegradable polymers derived from marine, plant and bacterial sources like chitosan, cellulose, cyclodextrin, starch, alginate, etc. to graphene nanoribbons serve as a reinforcing nanomaterial in biomedical field for tissue engineering (Shende and Pathan, 2020). The functionalization of graphene nanoribbons by bioconjugation with different molecules are given in Table 1.

3. Degradation and toxicity

The biodegradation is of utmost importance for reducing the high amount of GNRs from the environment and to prevent toxicity in living cells. The attachment and entrance of GNRs into the cells of several micro-organisms including fungi, bacteria, viruses occur by endocytosis (Chen et al., 2017). A ligninolytic enzyme, known as lignin peroxidase (LiP) releases from white-rot fungus showed the degradation of oxidized and reduced forms of GNRs by forming large holes into the sheets (Lalwani et al., 2014). Moreover, the addition of hydrogen peroxide and veratryl alcohol illustrated faster and complete degradation of the GNRs in the environment within 96 h (Akhavan, 2012). Another enzyme derived from human neutrophils known as myeloperoxidase (hMPO) showed the biodegradation property of GNRs by perforation and degradation into smaller fragments. The hydrophilic nature and colloidal stability in the aqueous media of graphene oxide play an essential part in enhancing the degradation process by enzyme peroxidase (Fielden et al., 2018). The GNRs establish an advantage for biomedical use as they adhere to the cells by attaching to the phospholipid membrane, translocating into the membrane, or by persisting between the bilipid layers of the membrane (Mullick et al., 2014). Moreover, using *in-vitro* and *in-vivo* studies, it was observed that the functionalized graphene showed 85% more stability and less toxicity in the body (Kostarelos and Novoselov, 2014). Another study reported that the cytotoxicity of GNRs to neuroblastoma cells and were successful in producing autophagy by generating ROS molecules and enter into the cytoplasm of the cell by disrupting the phospholipid bilayer. Therefore, used in the treatment of cancer and brain delivery without affecting healthy cells. The oxidized GNRs below 100 μg/mL of concentration displayed no toxicity and alterations in gene expression on osteoblast cells (Ricci et al., 2017). Similarly, the reduced form of GNRs below the concentration of 10 μg/mL showed no cytotoxic effect on human mesenchymal stem cells while the exposure of reduced GNRs to the enzymes above the duration of 1 h disrupted cell membrane. Thus, the penetration of GNRs into the cell formed DNA fragmentation and chromosomal disruption (Mbeh et al., 2014). The bovine and human serum albumin functionalized GNRs below the concentration of 50 μg/mL displayed no toxicity on the human epithelium cells, but caused inhibition of cell proliferation and activated cell apoptosis above the concentration of 100 μg/mL, as mentioned in Table 2 (Zhang et al., 2016). In a study, the results of *in-vitro* studies showed that above the concentrations of 20 μg/mL, the cell metabolism and cellular proliferation were significantly decreased by structurally disruption of the GNRs (Mullick et al., 2014).

Table 1
Applications and bioconjugation of GNRs with various compounds.

Sr. no.	Bioconjugation of GNRs	Advantage	Application	Reference
1	Cellulose	Increased biocompatibility	Drug delivery	(Soleimani et al., 2018)
2	Dextran	Higher absorption capacity	Bio-sensing and gene delivery	(Hu and Zhou, 2013)
3	Octa-arginine peptide	Greater conjugation with biomolecules	Attachment of proteins, peptides	(Eylu, 2018)
4	PEG, polyethyleneimine	higher gene transfection efficiency	Targeted human therapy, siRNA delivery	(Kasprzak et al., 2016) (Sun et al., 2008)
5	Minerals	Enhanced electronic ability	Nanocarrier for drug delivery	(Chowdhury et al., 2015)
6	Albumin	Decreased hemolysis ratio < 0.2%	Potential for biomedical use	(Cai et al., 2015)
7	Biopolymers	Increase in biocompatibility, mechanical strength and thermal stability	Drug delivery for cancer therapy	(Shende and Pathan, 2020)

4. Molecular interaction with biomolecules

The molecular interactions of graphene with biomolecules such as proteins and peptides using various computational methods like molecular dynamics simulations and density functional theory (DFT) were conducted using peptides non-covalently conjugated with GNRs in the aqueous environment. The peptides displayed planar residues adsorbed on the surface of graphene and hydrophilic residues exposed away from the graphene surface (Zou et al., 2017). Moreover, certain non-aromatic amino acids like arginine and isoleucine were adsorbed by the AGNR and established prolong conductance, higher charge transfer and dipole moment due to the electronic property by placing the formulation between gold electrodes. Therefore, GNRs are used in electrochemical enantiomeric bio-sensing of several amino acids like tyramine, methionine, etc. (Martín et al., 2015). A study conducted on the interfacial bonding characteristics on GNRs functionalized with collagen using molecular dynamics revealed the collagen-graphene nanocomposites

Table 2
Safe concentration of GNRs in different cells.

Sr. no.	Cells	Safe concentration (μg/mL)	References
1	Neuroblastoma cells	10–100	(Zhang et al., 2016)
2	Osteoblast cells	Below 100	(Ricci et al., 2017)
3	Human mesenchymal stem cells	Below 10	(Mbeh et al., 2014)
4	Human epithelium cells	50–100	(Zhang et al., 2016)
5	Chicken embryo	Below 20	(Mullick et al., 2014)

density to be 0.5 g/cm^3, that is very close to the density of tissues in the body (0.51 g/cm^3) (Ebrahimi et al., 2013). The adsorption of four serum proteins like bovine fibrinogen (BFG), immunoglobulin (Ig), transferrin (Tf) and bovine serum albumin (BSA) was demonstrated on the rGO and GO using molecular dynamics wherein it illustrates the binding affinities in the order of BFG > Ig > Tf > BSA, as shown in Fig. 3. The GO displays greater binding affinity to aromatic protein residues due to the π-π stacking interactions and hydrophobic bonding which are necessary for the adsorption of protein. The protein coated GO displayed thicker membrane surface, lower surface area and lower the transportation through cell membranes leading to reduction in cytotoxicity by suppression of redox activity and lysosomal disruption (Chong et al., 2015).

4.1. Enzymes and GNR

Graphene shows enzyme modulating property by reverse binding to the protein without changing the receptor conformation and effectively suppressing the activity of the enzyme as depicted in Fig. 4. The α-chymotrypsin (ChT), a serine protease enzyme binds to the graphene oxide due to the electrostatic contact to inhibit the activity of the enzyme and therefore used as synthetic receptors (De et al., 2011). The carbon nanotubes and graphene oxide are the most potent inhibitors of VIM-2 Metallo-β-Lactamase enzyme wherein it degrades the broad-spectrum antibiotics like carbapenems and establishes enzyme inhibition by non-competitive binding to the active site of enzymes due to hydrophobic interactions (Huang et al., 2015). The functionalized three-dimensional graphene (monolithic and porous) is used as an enzymatic biosensor for the detection of redox reactions like oxidation of glucose with a lower detection limit, wider linear range and better stability (Liu et al., 2014).

4.2. DNA and GNR

The bioconjugation of graphene nanoribbons due to the optical and electrical properties shows wider applications in gene delivery, theranostic, biosensing and biomedical imaging (Chepyala et al., 2019). Several electronic sensing devices like DNA sensors are formulated for sensing of biological molecules by using the π-π stacking property of graphene to the ssDNA (Feng et al., 2018). The graphene biosensors are used for enhancing human cell therapy and showed various applications in early diagnosis of cancer and stem cell therapy. The nucleobase functionalized GNRs such as cytosine demonstrated higher speed and accuracy for DNA sequencing up to 90% at the rate of 66 million nucleobases per second during the translocation process on ssDNA without any advanced data processing or highly restrictive operational conditions like strain flexibility, microscopy, etc. (Paulechka et al., 2016). Another study demonstrated the electrochemical sensing of targeted DNA in human serum by using gold nanoparticles with graphene nanoribbons for better sensitivity, higher porosity, and larger surface area with greater stable immobilization of DNA. Moreover, improved sensitivity and specific capacitance of the formulation was achieved due to the electronic property and the charge transfer between the gold nanocomposites and the GNRs (D. Li et al., 2016). The graphene nanoribbons form similar double helix structures like dsDNA and conformation similar to those of biopolymers like DNA and proteins, indicating the biocompatibility with body cells due to similarity in shape and physical appearance. The similarity between the tunability and rigidity of biomolecules and GNRs extends the applications in the biomedical field like gene delivery, photothermal therapy, drug delivery, etc. (Wijeratne et al., 2016). According to the computational study, the binding energy of nucleobases to the graphene displays in the order of G > A > T > C > U where G shows the highest because of the NH···π interactions in combination with the π − π stacking interactions (Umadevi et al., 2014).

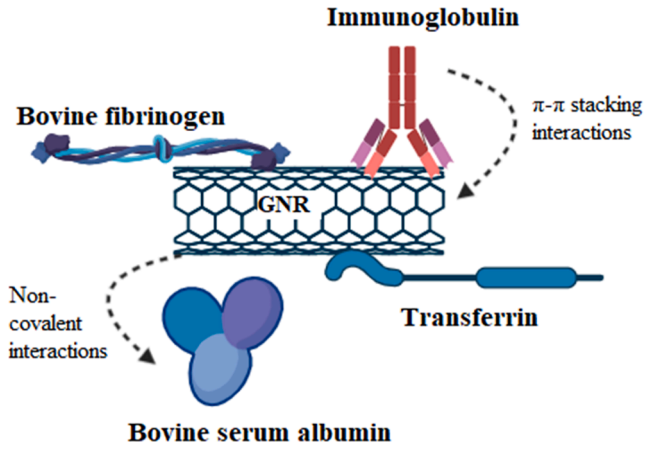

Fig. 3. Adsorption of serum proteins on graphene nanoribbon.

5. Applications

5.1. Biosensor

The detection of inflammatory cytokine, tumor necrosis alpha (TNF-α) established by Ag@Pt nanoparticles functionalized rGO nanosheets as an immunosensor used for exploring atherosclerosis and various other diseases related to the immunoglobulin malfunction such as influenza A virus, abnormal prothrombin, carcino-embryonic antigen, etc. (Vlăsceanu et al., 2018). The detection of galectin-3 biomarker for heart failure by using electrochemical immunosensing wherein bioconjugation of graphene nanoribbons in biosensing was carried out by attaching antibodies of galectin-3 to the silver nanoparticles with GNRs from both the sides to form sandwich immobilized GNRs using iron-based metal–organic framework deposits to establish long-term sensitivity with 3.79% RSD (Table 3) (Tang et al., 2018). Another study reported the detection of microalbuminuria (mAlb) for the diagnosis and treatment of nephrotic and hypoproteinemia patients. The mAlb functionalized GNRs with gold nanoreactor formulated for quantitative analysis of microalbuminuria in urine using immunoreaction by Surface-Enhanced Raman Scattering (SERS) with RSD 0.49%–2.28%, 96.9%–109.8% recovery, higher sensitivity and detection limit of 0.02 ng/mL (Wen et al., 2018). The electrochemically reduced nanoplatelets and electrochemically reduced nanoribbons formed by graphene oxide were compared for the recognition of biomarkers like uric acid, ascorbic acid, dopamine, NADH and DNA bases such as guanine wherein the GNRs were found to be more sensitive to dopamine and uric acid than nanoplatelets (Lim et al., 2014). The hybrid GNRs are chemically reduced form of GNRs and serve as biosensors due to higher sensitivity and low detectability property for the detection of ascorbic acid in presence of interference like uric acid, dopamine, etc. (Lavanya and Gomathi, 2015). Another study displays the extended application of GNRs in the diagnostic approach in clinical biology by the detection of neurotransmitters like dopamine and glutathione using silver nanoparticles functionalized GNRs where silver nanoparticles enhanced the sensitivity for the detection of neurotransmitters from the serum samples (Rostami et al., 2020). The attachment of gold nanoparticles to graphene is exponentially used in the immune-sensing and geno-sensing because gold to provide the immobilization of biological molecules and graphene helps in providing a large network of active sites with higher sensitivity. Therefore, GNRs are used for the detection of disease-causing pathogens such as HIV, papillomavirus and DNA sensing of specific sequences (Kumar et al., 2015). The doping of graphene nanoribbons by nitrogen with amine aptamer is used for detection of aflatoxin B1 using chitosan polymer in presence of visible light. The toxic substance aflatoxin B1 causes cancer and is present in many foods like peanuts, corns used by the food and agriculture industries for the detection of toxic compound (Chen et al., 2019). Another example of GNRs as artificial receptors is

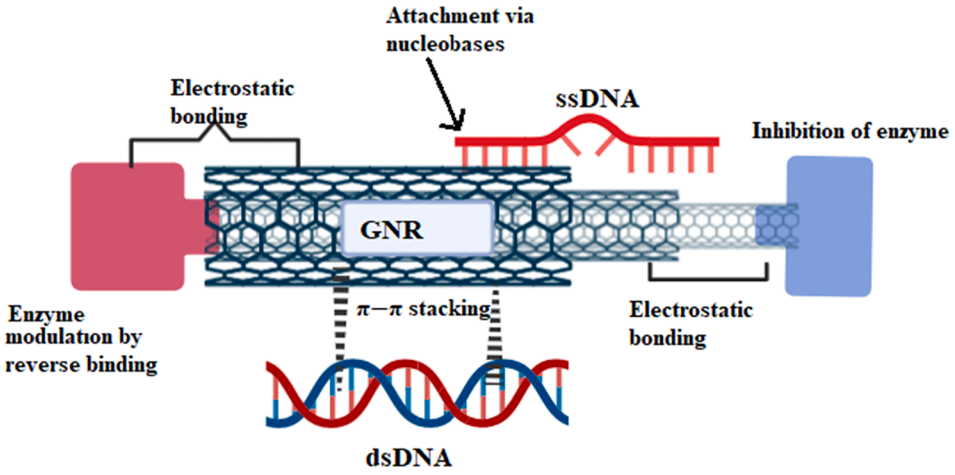

Fig. 4. Molecular interaction of biomolecules with graphene nanoribbon.

Table 3
Detection of different compounds by graphene nanoribbons.

Sr. no	Detection	Functionalization	Detection limit	Detection method	Sensitivity range	Reference
1	TNF-α	Silver nanoparticles	1.64 pg/mL	Electro-chemical	–	(Vlăsceanu et al., 2018)
2	Galactin-3	Silver nanoparticles	33.33 pg mL-1	Signal amplification-electrochemical	100 fg mL-1 to 50 ng mL-1	(Tang et al., 2018)
3	Microalbuminuria	Gold nanoparticles	0.02 ng/mL	SERS quantitative analysis	0.065 to 2.62 ng/mL	(Wen et al., 2018)
4	Ascorbic acid	–	230 nM	Colorimetric	22nA μM-1 cm-2	(Lavanya and Gomathi, 2015)
5	Aflatoxin B1	Amine aptamer	1.7pgmL	UPLC-MS and ELISA	$5pgmL^{-1}$ to $15ngmL^{-1}$	(Li et al., 2014)
6	Quercetin	molybdenum disulfide	8.2 × 10–10 M	differential pulse voltammetry	2.0 × 10–9 – 1.6 × 10–6 M	(Zhao et al., 2019)
7	Aminophenols	l-arginine and cyclodextrin	6.2 and 3.5 nM	differential pulse voltammetry	25.0–1300.0 nM	(Yi et al., 2016)
8	Bisphenols	Gold + copper nanoparticles	0.004 μmol/L	Electro-chemical	0.01 to 2.0 and 2.0 to 70 μmol/L	(Zhao et al., 2019)
9	Dopamine and glutathione	Silver nanoparticles	0.46 and 1.2 μM	Colorimetric	2.5–25 μM and 5–75 μM	(Rostami et al., 2020)

the nanoscale graphene nanoribbons (nGNR) used for biosensing of enzymes and detecting the changes in photocurrent signals of the cells. The improvement in the sensitivity and affinity of nGNR to unknown proteins is achieved by a reduction in the size of GNRs below 20 nm due to biomolecular interaction to reveal the size-dependent sensing property of GNRs (Mbeh et al., 2014). The electrochemical sensing of certain phenolic compounds using β-cyclodextrin and L-arginine with the carbon nanotubes attached to the graphene nanoribbons displayed a synergistic effect due to the host-recognition ability and electrocatalytic property and helps in the detection of the phenolic compounds like 2-aminophenol (2-AP) and 4-aminophenol (4-AP) indicating higher toxic effect like mutagenic, genotoxic and hepatotoxic effects due to their easy penetration into the skin and cell membrane using the electropolymerization (Yi et al., 2016). Another study involved the detection of bisphenols at the surface by using GNRs in combination with Au-Cu bimetallic nanoclusters for determination of BSA at lower concentration levels in food storage containers, baby bottles, bottled water and tap water (Pino et al., 2017). The detection of quercetin by molybdenum disulfide (MoS_2) fabricated GNRs act as ultrasensitive electrochemical biosensors with better sensitivity (89 μA/μM) and with the recovery of 95.4–106.1% indicating improved accuracy for detection in actual samples like juice and honey (Zhao et al., 2019).

5.2. Cancer therapy

Graphene is used as theranostics for early diagnosis and treatment of cancer as targeted drug delivery in the treatment of multidrug resistance tumors in the body. The chemotherapeutic agents showed adverse effects like hepato-toxicity, respiratory-toxicity, etc., therefore the dose reduction is established by using GNRs which effectively reduces the tumor size and associated side effects (Rahman et al., 2019). The PEGylated GNRs are most commonly used in the formulation for the treatment of cancers due to higher hydrophilicity and pH modification property. The phospholipid-polyethylene glycol (PL-PEG) functionalized GNRs were investigated for the treatment of cancer on U87 glioma cells by using combinational therapy with a chemotherapeutic agent, photothermal therapy and doxorubicin (Lu et al., 2014). The detection of alpha-fetoprotein (AFP), a biomarker used for the detection of hepatocellular carcinoma (HCC) was loaded into porous GNRs fabricated by gold nanoparticles and established an electrochemical biosensor for quick diagnosis of cancer. The anti- AFP were immobilized on GNRs which served as excellent biosensor at pH 7.4 with higher sensitivity (99.9% and 102%), long-term stability with a low detection limit of 1 ng/mL (Jothi et al., 2020). A study demonstrated the PEG-DSPE (1–2,distearoyl-s-n-glycerol-3-phosphoethanolamine N{aminopolyethyleneglycerol}) functionalized oxidized GNRs utilized for the diagnosis of overexpression of epidermal growth factor receptor (EGFR) mediated by human papillomavirus E5 protein and established a 75% increase in drug efficacy with higher permeability and retention property with a detection limit of 8 pg/mL (Liu et al., 2016). The graphene oxide composites in combination with N-isopropylacrylamide elucidate the drug delivery of doxorubicin and paclitaxel due to their adsorbing property at pH 7.4 (plasma pH) and the drug release at acidic pH i.e. pH of cancerous tissue (pH). Furthermore, slower release of drug and faster adsorption rate on graphene successfully formulated for sustained release by increasing the half-life of the drug wherein the N-isopropylacrylamide polymer enhanced the water solubility

and protected the drug molecules from the outer environment (Rezaian et al., 2018).

5.3. Gene delivery

The detection of genes in the cytoplasm of the cells was established by polyethyleneimine graphene nanoribbons (PEI-g-GNR) demonstrated as a non-viral vector for gene therapy and were successful in effectively transferring the gene probes into the Hela cell lines (Dong et al., 2011). The improvement in the DNA binding capacity, condensation and transfection efficiency was achieved by conjugating low-molecular-weight branched polymer such as polyethyleneimine (PEI) to GO. Moreover, the functionalization of PEI-GO by polyethylene-glycol extends its application to siRNA delivery, drug delivery and photothermal therapy (Kim et al., 2011). The oxidized graphene nanoribbons serve as a DNA carrier by suspending the oxidized GNRs in the dsDNA containing solution inside the mammalian cells for long-term stability (Foreman et al., 2017).

5.4. Drug delivery

Metal functionalized graphene-based materials are used in drug transport systems by combining various polymers like β-cyclodextrin, dextran, poly-ethylene glycol, wherein conjugation of metals like iron enhances the applications by rendering its super-paramagnetic property (Goenka et al., 2014). The thioflavin-functionalized GO is used in the treatment of Alzheimer's disease in combination with hyperthermic radiation disrupting amyloid plaques. The unique functional GO displays the property of crossing BBB and aids in the treatment of AD with lesser side effects like bone marrow disruption, mutagenesis, etc. The monoclonal antibody anti-CD20 known as rituximab is widely used for the treatment of non-Hodgkin's lymphomas wherein PEG functionalized GO is formulated to adsorb rituximab on the surface with the addition of doxorubicin for a synergistic effect. The PEG-GO-DOX-rituximab was more effective in treating the lymphoma than a single drug (DOX) (Sao et al., 2015). The GNRs show a wider application in antimicrobial drug delivery in treating methicillin-resistant *Staphylococcus aureus* by using the silver nanocomposites functionalized graphene oxide. Similarly, zinc oxide nanorods-decorated graphene nanoplatelets were formulated for treating *Streptococcus* mutans in anti-microbial therapy. The capability of killing drug-resistant bacteria makes them suitable for disinfection, wound healing and biomedical coating of formulations (Joshi et al., 2018).

6. Conclusions

The graphene nanoribbons encompass many advantageous properties like large surface area, mechanical strength with higher elasticity, optical properties and absorbing capacity for treatment and diagnosis of various problems like cancers, lymphomas, Alzheimer's diseases, genetic disorders, etc. The GNRs display the ability to form conjugates with several biomolecules present in the body like enzymes, peptides, nucleobases, etc. and establish potential applications in bio-sensing and bio-imaging for the detection of amino acids and peptide sequences. The bio-conjugation occurs due to the molecular interactions like π-π stacking and the adsorption of biomolecules to the graphene nanoribbons. Also, the GNRs show no toxicity when used in limited concentration (<10–20 μg/ml), and degradation in the body as well as in the environment by several enzymes viz oxidases, peroxidases, etc. Therefore, the bioconjugation of graphene nanoribbons reveals exploring applications in health care and serves as ultimate nanocarrier for the early detection and treatment of cancer, gene therapy, drug delivery, etc. However, no studies are performed on toxicity behaviour, biocompatibility and other health related risks of graphene nanoribbons in human body. Also, very less information is available on underlying mechanism of graphene-related material in cells and requires additional research study. Further, the cellular distribution and uptake of graphene nanoribbons into the cells remain unclear. Although bottom-up method is widely used process for the development of GNR, there is essential need to study the synthesis technique for scale-up of graphene nanoribbons in cost-effective way. A significant work is required to ensure the long-term use of GNR in humans for usage in the biomedical field.

CRediT authorship contribution statement

Pravin Shende: Conceptualization, Writing - review & editing, Supervision. **Nazneen Pathan:** Visualization, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Akhavan, O., 2012. Genotoxicity of graphene nanoribbons in human mesenchymal stem cells. Carbon N. Y. 54, 419–431. https://doi.org/10.1016/j.carbon.2012.11.058.

Allen, J.M., Vincent, T.C., Richard, K.B., 2010. Honeycomb carbon: a review of graphene what is graphene? Chem. Rev. 110, 132–145.

Bhattacharya, K., Mukherjee, S.P., Gallud, A., Burkert, S.C., Bistarelli, S., Bellucci, S., Bottini, M., Star, A., Fadeel, B., 2015. Biological interactions of carbon-based nanomaterials: from coronation to degradation 1–19. https://doi.org/10.1016/j.nano.2015.11.011.

Cai, B., Hu, K., Li, C., Jin, J., Hu, Y., 2015. Bovine serum albumin bioconjugated graphene oxide: red blood cell adhesion and hemolysis studied by QCM-D. Appl. Surf. Sci. 356, 844–851. https://doi.org/10.1016/j.apsusc.2015.08.178.

Chen, M., Qin, X., Zeng, G., 2017. Biodegradation of carbon nanotubes, graphene, and their derivatives. Trends Biotechnol. 35, 836–846. https://doi.org/10.1016/j.tibtech.2016.12.001.

Chen, W., Zhu, M., Liu, Q., Guo, Y., Wang, H., Wang, K., 2019. Fabricating photoelectrochemical aptasensor for sensitive detection of aflatoxin B 1 with visible-light-driven BiOBr/nitrogen-doped graphene nanoribbons. J. Electroanal. Chem. 840, 67–73. https://doi.org/10.1016/j.jelechem.2019.03.033.

Chepyala, R., Badruddoza, A.Z.M., Azad, M., McCarthy, J.R., Nurunnabi, M., 2019. Graphene and its derivatives as biosensing platform for healthcare applications, Biomedical Applications of Graphene and 2D Nanomaterials. Elsevier Inc. https://doi.org/10.1016/B978-0-12-815889-0.00009-X.

Chong, Y., Ge, C., Yang, Z., Garate, J.A., Gu, Z., Weber, J.K., Liu, J., Zhou, R., Biology, Q., Medicine, R., Higher, J., Institutions, E., Thomas, I.B.M., Heights, Y., York, N., States, U., York, N., York, N., States, U., 2015. Reduced Cytotoxicity of Graphene Nanosheets Mediated by Blood-Protein Coating 5713–5724.

Chowdhury, S.M., Fang, J., Sitharaman, B., 2015. Interaction of graphene nanoribbons with components of the blood vascular system. Futur. Sci. OA 1. https://doi.org/10.4155/fso.15.17.

De, M., Chou, S.S., Dravid, V.P., 2011. Graphene oxide as an enzyme inhibitor: modulation of activity of α-chymotrypsin. J. Am. Chem. Soc. 133, 17524–17527. https://doi.org/10.1021/ja208427j.

Dong, H., Ding, L., Yan, F., Ji, H., Ju, H., 2011. The use of polyethylenimine-grafted graphene nanoribbon for cellular delivery of locked nucleic acid modified molecular beacon for recognition of microRNA. Biomaterials 32, 3875–3882. https://doi.org/10.1016/j.biomaterials.2011.02.001.

Ebrahimi, S., Montazeri, A., Rafii-tabar, H., 2013. Molecular dynamics study of the interfacial mechanical properties of the graphene – collagen biological nanocomposite. Comput. Mater. Sci. 69, 29–39. https://doi.org/10.1016/j.commatsci.2012.11.030.

Eylu, D., 2018. 9.1.1 definition. https://doi.org/10.1016/B978-0-12-813691-1.00009-9.

Ezawa, M., 2008. Graphene nanoribbon and graphene nanodisk. Phys. E Low-Dimension. Syst. Nanostruct.s 40, 1421–1423. https://doi.org/10.1016/j.physe.2007.09.031.

Faccio, R., Denis, P.A., Pardo, H., Goyenola, C., Mombrú, Á.W., 2009. Mechanical properties of graphene nanoribbons. J. Phys. Condens. Matter 21. https://doi.org/10.1088/0953-8984/21/28/285304.

Feng, Q., Zhao, X., Guo, Y., Liu, M., Wang, P., 2018. Stochastic DNA walker for electrochemical biosensing sensitized with gold nanocages@graphene nanoribbons. Biosens. Bioelectron. 108, 97–102. https://doi.org/10.1016/j.bios.2018.02.050.

Fielden, M., Vogt, C., Newman, L., Rodrigues, A.F., Shao, W., Fournier, P.M., Toprak, M. S., Star, A., 2018. The degradation products are non-genotoxic † 1180–1188. https://doi.org/10.1039/c7nr03552g.

Foreman, H.C.C., Lalwani, G., Kalra, J., Krug, L.T., Sitharaman, B., 2017. Gene delivery to mammalian cells using a graphene nanoribbon platform. J. Mater. Chem. B 5, 2347–2354. https://doi.org/10.1039/c6tb03010f.

Goenka, S., Sant, V., Sant, S., 2014. Graphene-based nanomaterials for drug delivery and tissue engineering. J. Control. Release 173, 75–88. https://doi.org/10.1016/j.jconrel.2013.10.017.

Higginbotham, A., Alamos, L., Us, N.M., Price, B.K., 2014. (12) United States Patent (10) Patent No .: 2.

Hirsch, A., 2009. Unzipping carbon nanotubes: A peeling method for the formation of graphene nanoribbons. Angew. Chemie - Int. Ed. 48, 6594–6596. https://doi.org/10.1002/anie.200902534.
Hu, X., Zhou, Q., 2013. Health and ecosystem risks of graphene. Chem. Rev. 113, 3815–3835. https://doi.org/10.1021/cr300045n.
Huang, P.J., Pautler, R., Shanmugaraj, J., Liu, J., 2015. Inhibiting the VIM - 2 Metallo- β -Lactamase by Graphene Oxide and Carbon Nanotubes. https://doi.org/10.1021/acsami.5b01954.
Janani, K., Thiruvadigal, D.J., 2017. SC. Appl. Surf. Sci. https://doi.org/10.1016/j.apsusc.2017.12.159.
Jing, Y., Sun, Y., Niu, H., Shen, J., 2013. Chirality and size dependent elastic properties of silicene nanoribbons under uniaxial tension. 13th Int. Conf. Fract. 2013, ICF 2013 7, 5663–5668.
Joshi, K., Mazumder, B., Chattopadhyay, P., Bora, N.S., Goyary, D., Karmakar, S., 2018. Graphene family of nanomaterials: reviewing advanced applications in drug delivery and medicine. Curr. Drug Deliv. 16, 195–214. https://doi.org/10.2174/1567201815666181031162208.
Jothi, L., Jaganathan, S.K., Nageswaran, G., 2020. An electrodeposited Au nanoparticle/porous graphene nanoribbon composite for electrochemical detection of alpha-fetoprotein. Mater. Chem. Phys. 242, 122514 https://doi.org/10.1016/j.matchemphys.2019.122514.
Kasprzak, A., Poplawska, M., Bystrzejewski, M., Grudzinski, I.P., 2016. Sulfhydrylated graphene-encapsulated iron nanoparticles directly aminated with polyethylenimine: A novel magnetic nanoplatform for bioconjugation of gamma globulins and polyclonal antibodiesf. J. Mater. Chem. B 4, 5593–5607. https://doi.org/10.1039/c6tb00838k.
Kim, H., Namgung, R., Singha, K., Oh, I., Kim, W.J., 2011. Graphene Oxide – Polyethylenimine Nanoconstruct as a Gene Delivery Vector and Bioimaging Tool. https://doi.org/10.1021/bc200397j.
Kiran, H.C., Gangadharappa, H.V., 2019. Reinforcing nanomedicine using graphene nanoribbons. J. Drug Deliv. Sci. Technol. 49, 334–344. https://doi.org/10.1016/j.jddst.2018.12.004.
Kostarelos, K., Novoselov, K.S., 2014. Exploring the interface of graphene and biology. Science (80-.). 344, 261–263. https://doi.org/10.1126/science.1246736.
Kumar, S., Ahlawat, W., Kumar, R., Dilbaghi, N., 2015. Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of biosensors for healthcare. Biosens. Bioelectron. 70, 498–503. https://doi.org/10.1016/j.bios.2015.03.062.
Lalwani, G., Xing, W., Sitharaman, B., 2014. Enzymatic degradation of oxidized and reduced graphene nanoribbons by lignin peroxidase. J. Mater. Chem. B 2, 6354–6362. https://doi.org/10.1039/c4tb00976b.
Lavanya, J., Gomathi, N., 2015. Author's accepted manuscript. Talanta. https://doi.org/10.1016/j.talanta.2015.07.018.
Li, D., Zhang, W., Yu, X., Wang, Z., Su, Z., Wei, G., 2016a. When biomolecules meet graphene: from molecular level interactions to material design and applications. Nanoscale 8, 19491–19509. https://doi.org/10.1039/c6nr07249f.
Li, G., Yoon, K., Zhong, X., Zhu, X., Dong, G., 2016b. Efficient bottom-up preparation of graphene nanoribbons by mild suzuki –. Miyaura Polymerization of Simple Triaryl Monomers 10027, 9116–9120. https://doi.org/10.1002/chem.201602007.
Li, S., Wang, H., Guo, L., Zhao, H., Ho, C.T., 2014. Chemistry and bioactivity of nobiletin and its metabolites. J. Funct. Foods 6, 2–10. https://doi.org/10.1016/j.jff.2013.12.011.
Li, Z., Qian, H., Wu, J., Gu, B.L., Duan, W., 2008. Role of symmetry in the transport properties of graphene nanoribbons under bias. Phys. Rev. Lett. 100, 21–24. https://doi.org/10.1103/PhysRevLett.100.206802.
Lim, C.S., Chua, C.K., Pumera, M., 2014. Detection of biomarkers with graphene nanoplatelets and nanoribbons. Analyst 139, 1072–1080. https://doi.org/10.1039/c3an01585h.
Liu, J., Wang, X., Wang, T., Li, D., Xi, F., Wang, J., Wang, E., 2014. Functionalization of Monolithic and Porous Three-Dimensional Graphene by One-Step Chitosan Electrodeposition for Enzymatic Biosensor.
Liu, W., Sun, C., Liao, C., Cui, L., Li, H., Qu, G., Yu, W., Song, N., Cui, Y., Wang, Z., Xie, W., Chen, H., Zhou, Q., 2016. Graphene enhances cellular proliferation through activating EGFR. https://doi.org/10.1021/acs.jafc.5b05923.
Lu, Y.J., Lin, C.W., Yang, H.W., Lin, K.J., Wey, S.P., Sun, C.L., Wei, K.C., Yen, T.C., Lin, C. I., Ma, C.C.M., Chen, J.P., 2014. Biodistribution of PEGylated graphene oxide nanoribbons and their application in cancer chemo-photothermal therapy. Carbon N. Y. 74, 83–95. https://doi.org/10.1016/j.carbon.2014.03.007.
Martín, A., Batalla, P., Hernández-Ferrer, J., Martínez, M.T., Escarpa, A., 2015. Graphene oxide nanoribbon-based sensors for the simultaneous bio-electrochemical enantiomeric resolution and analysis of amino acid biomarkers. Biosens. Bioelectron. 68, 163–167. https://doi.org/10.1016/j.bios.2014.12.030.
Mbeh, D.A., Akhavan, O., Javanbakht, T., Mahmoudi, M., Yahia, L.H., 2014. Ac ce p te d us cr t. Appl. Surf. Sci. https://doi.org/10.1016/j.apsusc.2014.09.155.
Mullick, S., Dasgupta, S., Mcelroy, A.E., 2014. Structural disruption increases toxicity of graphene nanoribbons 1235–1246. https://doi.org/10.1002/jat.3066.
Panwar, N., Soehartono, A.M., Chan, K.K., Zeng, S., Xu, G., Qu, J., Coquet, P., Yong, K.T., Chen, X., 2019. Nanocarbons for biology and medicine: sensing, imaging, and drug delivery. Chem. Rev. 119, 9559–9656. https://doi.org/10.1021/acs.chemrev.9b00099.
Paulechka, E., Wassenaar, T.A., Kroenlein, K., Kazakov, A., Smolyanitsky, A., 2016. Nucleobase-functionalized graphene nanoribbons for accurate high-speed DNA sequencing. Nanoscale 8, 1861–1867. https://doi.org/10.1039/c5nr07061a.
Peña-Bahamonde, J., Nguyen, H.N., Fanourakis, S.K., Rodrigues, D.F., 2018. Recent advances in graphene-based biosensor technology with applications in life sciences. J. Nanobiotechnology 16, 1–17. https://doi.org/10.1186/s12951-018-0400-z.
Pino, F., Mayorga-Martinez, C.C., Merkoçi, A., 2017. Nanomaterials-based platforms for environmental monitoring. Comprehens. Anal. Chem. Elsevier Ltd. https://doi.org/10.1016/bs.coac.2017.06.002.
Prezzi, D., Varsano, D., Ruini, A., Marini, A., Molinari, E., 2008. Optical properties of graphene nanoribbons: the role of many-body effects. Phys. Rev. B - Condens. Matter Mater. Phys. 77, 1–4. https://doi.org/10.1103/PhysRevB.77.041404.
Rahman, M., Kazmi, I., Beg, S., Hafeez, A., Afzal, M., Kumar, V., Anwar, F., Ahmad, F.J., 2019. Chapter 13. Functionalized graphene-based nanomaterials for drug delivery and biomedical applications in cancer chemotherapy, Nanoparticles in Pharmacotherapy. Elsevier Inc. https://doi.org/10.1016/B978-0-12-816504-1.00011-9.
Rezaian, M., Maleki, R., Dahroud, M.D., Alamdari, A., Alimohammadi, M., 2018. pH-sensitive co-adsorption/release of doxorubicin and paclitaxel by carbon nanotube, fullerene, and graphene oxide in combination with N-isopropylacrylamide: a molecular dynamics study. Biomolecules 8, 1–21. https://doi.org/10.3390/biom8040127.
Ricci, R., Leite, N.C.S., da-Silva, N.S., Pacheco-Soares, C., Canevari, R.A., Marciano, F.R., Webster, T.J., Lobo, A.O., 2017. Graphene oxide nanoribbons as nanomaterial for bone regeneration: Effects on cytotoxicity, gene expression and bactericidal effect. Mater. Sci. Eng. C 78, 341–348. https://doi.org/10.1016/j.msec.2017.03.278.
Rostami, S., Mehdinia, A., Niroumand, R., Jabbari, A., 2020. Enhanced LSPR performance of graphene nanoribbons-silver nanoparticles hybrid as a colorimetric sensor for sequential detection of dopamine and glutathione. Anal. Chim. Acta 1120, 11–23. https://doi.org/10.1016/j.aca.2020.04.060.
Sao, R., Vaish, R., Sinha, N., 2015. Multifunctional drug delivery systems using inorganic nanomaterials: a review. J. Nanosci. Nanotechnol. 15, 1960–1972. https://doi.org/10.1166/jnn.2015.9761.
Shende, P., Augustine, S., Prabhakar, B., 2020. A review on graphene nanoribbons for advanced biomedical applications. Carbon Lett. 30, 465–475. https://doi.org/10.1007/s42823-020-00125-1.
Shende, P., Pathan, N., 2020. Potential of carbohydrate-conjugated graphene assemblies in biomedical applications. Carbohydr. Polym. 117385 https://doi.org/10.1016/j.carbpol.2020.117385.
Soleimani, K., Tehrani, A.D., Adeli, M., 2018. Ecotoxicology and environmental safety bioconjugated graphene oxide hydrogel as an effective adsorbent for cationic dyes removal. Ecotoxicol. Environ. Saf. 147, 34–42. https://doi.org/10.1016/j.ecoenv.2017.08.021.
Sun, X., Liu, Z., Welsher, K., Robinson, J.T., Goodwin, A., Zaric, S., Dai, H., 2008. Nano-graphene oxide for cellular imaging and drug delivery. Nano Res. 1, 203–212. https://doi.org/10.1007/s12274-008-8021-8.
Tang, Z., He, J., Chen, J., Niu, Y., Zhao, Y., Zhang, Y., Yu, C., 2018. A sensitive sandwich-type immunosensor for the detection of galectin-3 based on N-GNRs-Fe-MOFs@AuNPs nanocomposites and a novel AuPt-methylene blue nanorod. Biosens. Bioelectron. 101, 253–259. https://doi.org/10.1016/j.bios.2017.10.026.
Umadevi, D., Panigrahi, S., Sastry, G.N., 2014. Noncovalent interaction of carbon nanostructures. Acc. Chem. Res. 47, 2574–2581. https://doi.org/10.1021/ar500168b.
Vlăsceanu, G.M., Amărandi, R., Ioniță, M., Tite, T., Iovu, H., Burns, J.S., 2018. Author ' s Accepted Manuscript Versatile Graphene Biosensors for Enhancing Human Cell Therapy. Biosens. Bioelectron. https://doi.org/10.1016/j.bios.2018.04.053.
Wen, G., Jing, Q., Liang, A., Jiang, Z., 2018. A new SERS strategy for quantitative analysis of trace microalbuminuria based on immunorecognition and graphene oxide nanoribbon catalysis. Int. J. Nanomedicine 13, 6099–6107. https://doi.org/10.2147/IJN.S174765.
Wijeratne, S.S., Penev, E.S., Lu, W., Li, J., Duque, A.L., Yakobson, B.I., Tour, J.M., Kiang, C.H., 2016. Detecting the biopolymer behavior of graphene nanoribbons in aqueous solution. Sci. Rep. 6, 1–6. https://doi.org/10.1038/srep31174.
Yang, X., Dou, X., Rouhanipour, A., Zhi, L., Räder, H.J., Müllen, K., 2008. Two-dimensional graphene nanoribbons. J. Am. Chem. Soc. 130, 4216–4217. https://doi.org/10.1021/ja710234t.
Yi, Y., Zhu, G., Wu, X., Wang, K., 2016. Highly sensitive and simultaneous electrochemical determination of 2-aminophenol and 4-aminophenol based on poly (l-arginine)-β-cyclodextrin/carbon nanotubes@graphene nanoribbons modified electrode. Biosens. Bioelectron. 77, 353–358. https://doi.org/10.1016/j.bios.2015.09.052.
Zhang, B., Wang, Y., Zhai, G., 2016. Biomedical applications of the graphene-based materials. Mater. Sci. Eng. C 61, 953–964. https://doi.org/10.1016/j.msec.2015.12.073.
Zhao, P., Ni, M., Xu, Y., Wang, C., Chen, C., Zhang, X., 2019. Sensors and Actuators B : Chemical A novel ultrasensitive electrochemical quercetin sensor based on MoS 2 - carbon nanotube @ graphene oxide nanoribbons / HS-cyclodextrin / graphene quantum dots composite fi lm. Sens. Actuat. B. Chem. 299, 126997 https://doi.org/10.1016/j.snb.2019.126997.
Zou, X., Wei, S., Jasensky, J., Xiao, M., Wang, Q., Brooks, C.L., Chen, Z., 2017. Molecular Interactions between Graphene and Biological Molecules Molecular Interactions between Graphene and Biological Molecules. https://doi.org/10.1021/jacs.6b11226.