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Biomedical Application of Graphenes

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ABSTRACT

Graphene is one of the crystalline forms of carbon, along with diamond, graphite, carbon nanotubes and fullerenes where in carbon atoms are arranged in a regular hexagonal pattern. Graphene can be described as a one-atom thick layer of the layered mineral graphite. High-quality graphene is very strong, light, nearly transparent, and excellent conductor of heat and electricity. Its interaction with other materials and with light, and its inherently two-dimensional nature, produce unique properties Owing to its interesting electrical, optical, mechanical and chemical properties, graphene has found potential applications in a wide range of areas, including biomedicine. Graphene has a number of properties which make it potentially promising for bioapplications. Its large surface area, chemical purity and the possibility of easy functionalization provide opportunities for drug delivery. Its unique mechanical properties suggest its application in tissue- engineering and regenerative medicine. Its combination of ultimate thinness, conductivity and strength make it an ideal support for imaging biomolecules in transmission electron microscopy. Also, chemically functionalized graphene might lead to fast and ultrasensitive measurement devices, capable of detecting a range of biological molecules including glucose, cholesterol, hemoglobin and DNA. This review summarize the latest progress of using graphene for various biomedical applications, including drug delivery, cancer therapies and biosensing, and discuss the opportunities and challenges in this emerging field.

Keywords: Graphenes , Biomedical Application, drug delivery, Biosensing

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INTRODUCTION

Graphene is a nanomaterial consisting of single layer sheets of carbon atoms in a hexagonal arrangement. The media refer graphene as the "miracle material of the 21st Century. Its public profile was boosted in 2010 when the Nobel Prize in Physics was awarded to Andre Geim and Konstantin Novoselov of the University of Manchester "for groundbreaking experiments regarding the new two-dimensional material graphene. This new 2D nanomaterial has been a shining star in the material science in the past few years[1].

Definition of graphene:

A collective dentition of graphenes does not exist yet . Moreover, related terms are often confused (e.g., graphite oxide and graphene oxide). Most authors describe (rather than define) graphene as a material ''which consists of a single atomic sheet of conjugated sp2 carbon atoms arranged in a honeycomb lattice, which is the basic building block of other carbon allotropes including 0D fullerenes, 1D carbon nanotubes, and 3D graphite[2]. Graphene and graphene oxide (GO) have become a hotspot so far and have been vigorously explored to build new compound materials .This novel nanomaterial has great potential in applications such as electrochemical devices , energy storage ,catalysis , adsorption of enzyme , cell imaging and drug delivery , as well as biosensors.

Properties of Graphene

Graphene is a single layer of carbon packed in a hexagonal (honeycomb) lattice. A few properties of graphene which makes it special are[3],[4,5,6]

It is the purest form of carbon

Large theoretical specific area (2360 m2/g)

High intrinsic mobility (200,000 cm2 $v^{-1}s_{-1}$)

Extremely high Young's modulus (~1.0 TPa)

Thermal conductivity (~5000$Wm^{-1}K^{-1}$)

Optical transmittance (~97.7%)

Due to its robust and flexible membrane, graphenes has essentially infinite possibilities for the modification or functionalization of its carbon backbone.

Methods of preparation of Graphene

One reason that graphene research has advanced so fast is that the laboratory procedures to obtain high-quality graphene are relatively simple and cheap. Several methods of preparation of graphenes are available. The choice of method depends on several factors like the quality and level of purity required , which is defined by the lack of intrinsic defects and the size of the obtained

flakes or layers , the quantity of graphene which can be produced simultaneously (Amount) or the complexity such as the requirement of labor or the need for specially designed machines. One last attribute is how reproducible the method is. The two major methods used are 1) Exfoliation and 2) Surface Growth

Exfoliation

Basically there are two major approaches to preparing graphene. Graphene can be simply detached from an already existing graphite crystal, the so-called exfoliation methods, or the graphene layer can be grown directly on a substrate surface. Andre K. Geim and Konstantin S. Novoselov[6] first used a regular Scotch tape to exfoliate fine layers of graphite from pyrolitic graphite and then transferred these layers to a silicon substrate. Since then, this technique is referred as "mechanical exfoliation", which so far provides the best quality graphene in terms of structural integrity.

The "Scotch Tape Method"

It is a type of micromechanical exfoliation, where in graphene is detached from a graphite crystal using adhesive tape. After peeling off the graphite, multiple-layer graphene remains on the tape. By repeated peeling the multiple-layer graphene is cleaved into various flakes of few-layer / single layer graphene. The tape is then attached to the substrate and glue solved, e.g. by acetone, in order to detach the tape. Finally one last peeling with an unused tape is performed. Flakes differing in size and thickness are obtained. The sizes range from nanometers to several tens of micrometers for single-layer graphene. Even though it is a simple method however, it is difficult to obtain larger amounts of graphene by this method, also the process is difficult to control but, the quality of the prepared graphene is very high with almost no defects[6].

Solution Based Exfoliation

Another graphene synthesis method involves solution based exfoliation of graphene oxide (GO)[7] Particularly for large scale applications, such as super capacitors, composite materials, gas sensors and flexible electronic materials[8]. The principle of liquid-phase exfoliation can also be used to exfoliate graphite oxide. Due to several functional groups like epoxide or hyroxyl, graphene oxide is hydrophilic and can be solved in water by sonication or stirring. In general, simple graphite powders are used as a starting material. They are oxidized by chemical modification, the so-called Hummers' method to produce water dispersible Graphene oxide (GO). GO can then be substantially reduced or restored to graphene network by thermal annealing or chemical reducing agents treatment. This "chemically derived graphene" is usually referred as reduced graphene oxide (rGO). The synthesis process can easily be scaled-up to produce gram quantities of rGO in solution. However, the graphite materials undergo serious alteration during the process of

oxidation and reduction. It has been recognized that solution based production of graphene contributes significant structural defects and uncompleted reduction process, leaving oxygen functional groups on graphene flakes[9].

Growth on Surfaces

A new approach to obtaining graphene is to grow it directly on a surface. The growth can occur in two different ways. Either the carbon already exists in the substrate or it has to be added by chemical vapor deposition (CVD)

Epitaxial Growth[9]

Another relatively "straight forward" way to produce graphene is a conversion of Silicon carbide to graphene via sublimation of silicon atoms at high temperature (usually at ~1300^0C in ultrahigh vacuum condition)[10]. It has been shown that graphene via this method exhibits high mobility and remarkable 2D electron gas (2DEG) behavior[11]. This method has the potential for large scale integration of nano electronic devices. However, the requirement of ultrahigh vacuum and high temperature may limit the accessibility of this method, due to a higher cost.

Chemical Vapor Deposition

Chemical vapor deposition is a well known process in which a substrate is exposed to gaseous compounds. These compounds decompose on the surface in order to grow a thin film, whereas the by-products evaporate. (CVD) using transition metal substrates has been considered the most promising, inexpensive and feasible method to produce single layer or multi-layers graphene. Graphene grown on Ni , Pd , Ru , Ir and Cu have been demonstrated in the past few years[9].

Biofunctionalization of graphene and graphene-based nanomaterials

Parallel with the advancement of nanomaterial science and biotechnology, various nano/bio interfaces have been realized in the areas of biological device design, biomolecule detection, bioassays, and molecular medicine[12].The large 2D aromatic surface of graphene makes it an ideal substrate for adsorption of certain biomolecules. Graphene has been employed as a substrate to be interfaced with various biomolecules and cells Biological modification in turn benefits graphene by improving its biocompatibility, solubility and selectivity. Hence, several studies have focused on graphene modification and functionalization.

Biofunctionalization with DNA

There is immense demand for complex nanoarchitectures based on graphene nanostructures in the fields of biosensing or nanoelectronics. DNA molecules represent the most flexible and programmable recognition element and can provide a unique assembly for graphene nanomaterial. Bonanni A et al [13] covalently linked single stranded DNA (ssDNA) to graphene using

carbodiimide chemistry and applied it to genosensing. Another elegant approach of graphene tethering with DNA includes formation of nanoparticles (NPs) decorated with graphene biosystems. The DNA- graphenes complex produced can be used for the development of novel devices that can be used as alternatives to classical techniques for sensitive and fast DNA analysis.

Biofunctionalization with proteins

As a result of their varied surface functional groups and secondary structure, proteins can exfoliate and modify graphene through physical adsorption or chemical bonding. Graphene and GO have been tethered with various proteins, which results in biosystems with unique properties. Shao et al[14] reported enzyme immobilization matrix based on GO. It has been found that horseradish peroxidase (HRP) and lysozyme can be spontaneously immobilized on GO because the individual GO sheet is enriched with oxygen-containing groups, which makes it possible to immobilize enzymes without any surface modifications or coupling reagents[15]. Thus graphenes can be biofunctionalized with proteins and used for biosensing and preparation of biodevices.

Biofunctionalization with other biomolecules

Other biomolecules, such as peptides and cellulose, have also been used to functionalize graphene. Biologically compatible and biodegradable natural polymeric dispersants, such as lignin and cellulose derivatives, have been employed to fabricate stable aqueous suspensions of graphene nanosheets . Such nanosheets can be used as the loading platform for enzymes or biomarkers in biosensors, or the carriers of drugs in therapy studies.

Biomedial Applications of Graphenes

Biosensors

A biosensor is an analytical device that helps in understanding the bio-composition, structure and function of analytes by biological reactions. A biosensor mainly consists of a recognition layer, a transducer and an electronic component. The recognition layer determines the biological response which is further converted into an electrical signal with the help of the transducer. This electrical signal is then amplified and processed by the external electronic system. Biomolecules play indispensable roles in all life processes including disease development, so the accurate detection of biomolecules is critical to disease diagnosis and therapy. The graphene based materials have been used to construct various biosensors based on different sensing mechanisms including optical and electrochemical signaling[9]. Because of its interesting properties, graphene has found its way into a wide variety of biosensing schemes. The excellent electrochemical properties of graphene make it a promising electrode material to improve the detection of biomolecules.

Detection of H2O2 and small biomolecules

As an essential mediator in biological processes and clinical analyses, rapid identification and detection of hydrogen peroxide (H2O2) is of great importance. However, the detection of H2O2 is easily interfered with by other electro-active substances, resulting in its high over-potential. A key point in developing electrochemical H2O2 sensors is to decrease the oxidation/reduction over-potentials. Ezekiel Dixon Dikio et al[16] studied the Electrochemical Detection of Hydrogen Peroxide Based on Graphene Oxide (GO)/Prussian Blue (PB) Modified Glassy Carbon Electrode . On the basis of the high electrocatalytic activity of graphene toward H2O2, graphene could be an excellent electrode material for oxidase-based biosensors. An extremely important example is in the determination of glucose, which plays a crucial role in the diagnosis and therapy of diabetes. Several graphene-based glucose biosensors have been reported. Kang et al[17]. Employed thermally split graphene to construct a glucose oxidase (GOD)-graphene- chitosan nanocomposite modified electrode. The direct electro- chemistry of GOD was observed and the immobilized enzyme exhibited a surface confined and reversible two-proton/two-electron transfer reaction. They demonstrated that the resulting biosensor not only retained the bioactivity of GOD, but also exhibited a wider linear range with a detection limit of 0.02 mM and much higher sensitivity compared with other nano structured supports. Yeon Hwa Kwak et al[18] developed a flexible glucose sensor using a Chemical vapor deposition grown graphene-based field-effect-transistor (FET). The CVD-grown graphene was functionalized with linker molecules in order to immobilize the enzymes that induce the catalytic response of glucose. Polyethylene terephthalate (PET) was employed as the substrate material. , the fabricated FET sensor could detect glucose levels in the range of 3.3–10.9 mM, which mostly covers the reference range of medical examination or screen test for diabetes diagnostic. The excellent performance of graphene-based materials for detecting glucose indicates that graphene is a promising material for future generation glucometers.

Dopamine Detection[19]:

Dopamine (DA) is a monoamine neurotransmitter and hormone that is widely distributed in the central nervous system of mammals. Rapid and sensitive detection of DA is of urgency because changes in the concentration of DA are closely linked to a human's health. Due to its electrochemical activity, DA can be detected directly. Unfortunately, ascorbic acid (AA), which is always coexists with DA in organisms, may disturb the detection because it shares a similar oxidation potential with DA. To resolve this problem, ying wang et al. fabricated graphene modified electrode for selective detection of dopamine in presence of ascorbic acid. Graphene synthesized chemically by Hummers method was applied in selective determination of dopamine with a linear range from 5 μM to 200 μM in presence of excess of ascorbic acid. Selective

detection was realized by completely eliminating ascorbic acid, different from the methods based on the potential separations. π–π stacking interaction between dopamine and graphene surface .The resulted graphene-modified electrode also showed a better performance than multi-walled carbon nanotube -modified electrode.

Graphene Based Fret Biosensors:

Making use of super efficient fluorescence quenching ability of graphene, some novel fluorescence resonance energy transfer (FRET) based biosensors have been developed[20] FRET involves the transfer of energy from a donor fluorophore to an acceptor fluorophore, and is one of the advanced tools available for measuring nanometer-scale distance and changes, both *in vivo* and *in vitro*[20]. Graphene and GO have been reported to interact strongly with nucleic acids (NAs) through π–π stacking interactions between the ring structures in the NA bases and the hexagonal cells of graphene and GO; whereas double-stranded DNA (dsDNA) cannot be stably adsorbed onto the surface because of efficient shielding of nucleobases within the negatively charged dsDNA phosphate backbone . The development of graphene based FRET biosensors has been motivated greatly by reliance on this particular principle and integrating it with the advantages of graphene[21]. Song E et al[22] developed a graphene oxide-based FRET sensor for rapid and sensitive detection of matrix metalloproteinase 2 in human serum sample[23]. Fluorescein isothiocyanate-labeled peptide (Pep-FITC) was assembled onto the GO surface through covalent binding to construct a GO-Pep-FITC FRET sensor for sensitive, rapid, and accurate detection of MMP-2 in complex serum samples (detection limit: 2.5ng/mL).

DNA Detection

Electrochemical activity of nucleic acids was first reported by Palećek[24]. Since the discovery of electroactive behaviour of nucleic acids, a lot of research has been devoted to their quantification via electrochemistry. The aim is to use the nucleic acid recognition layers immobilized over a signal transducer (electrochemical, optical or piezoelectric), to fabricate a DNA biosensor for detecting target analytes. The first graphene-based FRET biosensor included a fluorescein amidite (FAM)-labeled ssDNA adsorbed onto GO[25]. As a result of the FRET effect between FAM and GO, fluorescence is quenched rapidly; however, binding between probe ssDNA and a complementary ssDNA alters the conformation, and consequently, releases FAM–ssDNA from the GO surface and results in fluorescence recovery. Detection of cDNA is therefore possible. Lu et al[26] reported the first graphene-based biosensor consisting of a dye-labeled ssDNA probe that could be bound and quenched by graphene oxide (GO), resulting from FRET effect between the dye and GO .Jang et al [27]developed a novel GO-related assay to investigate the helicase-mediated duplex DNA

unwinding activity. Balapanuru et al[28] fabricated a charge-transfer complex based graphene oxide (GO) and pyrene dye PNPB has by a simple ion-exchange process which specifically interacts with DNA compared to other biomolecules and also allows selective and rapid detection of DNA in biological mixtures.

Drug delivery

The major advantage of graphene over other nanomaterial is its unique structural features, such as large and planar sp2 hybridized carbon domain, high specific surface area (2630 m2/g), and enriched oxygen-containing groups, render GO excellent biocompatibility, and physiological solubility and stability and capability of loading of drugs or genes via chemical conjugation or physisorption approaches which make it an efficient drug carrier to load large amount of drug molecules on both sides of the single atom layer sheet[29]. Moreover, the reactive COOH and OH groups of GO facilitate conjugation with various systems, such as polymers[30], biomolecules (biotargeting ligand, DNA, protein, quantum dots, Fe3O4 nanoparticles and others, imparting GO with multi-functionalities and multi-modalities for diverse biological and medical applications[31]. GO, produced by vigorous oxidation of graphite by Hummers method, is an ideal nanocarrier for efficient drug and gene delivery. GO used for drug delivery is usually 1-3 layers (1-2 nm thick), with size ranging from a few nanometers to several hundred nanometers[32]. Due to their large surface area and delocalized π electrons, graphene derivatives can solubilize and bind drug molecules and thus have the potential to be drug delivery vehicles in their own right if sufficiently high drug loading and suitable *in vivo* drug distribution and release profiles can be achieved. Graphene is also lipophilic, which might help in solving another challenge in drug delivery membrane barrier penetration[33].

Cancer therapy

Liu et al[34] functionalized nano graphene oxide (NGO) a novel graphitic material with branched polyethyleneglycol (PEG) to obtain a NGO-PEG conjugate and used them for attaching hydrophobic aromatic molecules like camptothecin (CPT) analogue, SN38 noncovalently via π– π stacking. The PEG-functionalized NGO loaded with SN38 exhibited high cytotoxicity for HCT-116 cells, 1000-fold more potent than CPT-11. Multidrug therapy has always been one of the major approaches in treatment of cancer to avoid drug resistance. Liu et al[33]. further investigated targeted delivery of mixed anticancer drugs by functional GO, they control loaded two anticancer drugs, DOX and camptothecin (CPT), onto the folic acid-conjugated NGO (FA-NGO) via π–π stacking and hydrophobic interactions and specially transported it to MCF-7 cells. Results

demonstrated that FA-NGO loaded with the two anticancer drugs showed remarkably higher cytotoxicity against target cells compared to NGO loaded with only a single drug.

TARGETED DRUG DELIVERY

Photothermal therapy

Intravenous administration of polyethylene glycol-modified graphene oxide, labeled with a near-infrared fluorescence dye but not carrying any drug, has shown passive tumour targeting in mouse xenograft models. The tumours were killed when irradiated with a low-power near-infrared laser, showing the potential of using graphene derivatives for photothermal cancer treatment However, given the high safety, clinical and regulatory hurdles and long timescales associated with drug development, which are exacerbated when new materials are involved, it is unlikely that products using graphene-based drug delivery technology will be near the market before 2030[35]. PDT is a novel and efficient technique to treat diseases including cancers in clinical practice because of its attractive traits with low toxicity and high stability under physiological conditions. In an experiment, GO was loaded with a chlorine photosensitizer with high efficiency via hydrophobic interactions and π-π stacking. Such system, significantly increases the accumulation of photosensitizers in tumor cells, leading to a remarkable concentration depended photodynamic effect on cancer cells under irradiation[35]. PEG- modified nanoscale graphene sheets (NGSs) have been prepared, and the strong optical absorbance of NGSs in the near-infrared region has been utilized to achieve ultra-high in vivo tumor uptake of anticancer drugs, which can be used for photothermal therapy of cancer[36]. Wen et al. developed a PEGylated nanographene oxide (NGO) with redox-responsive detachable PEG shell using disulphide linkages (NGO-SS-mPEG) .This complex rapidly released encapsulated payload at tumor-relevant glutathione (GSH) levels. The PEG shell selectively detached from NGO upon intracellular GSH stimulation due to disulphide linkages and released DOX 1.55 times faster than that in the absence of GSH[37].

pH dependent drug release

Liu et al studied targeted delivery of chemical drugs into cells by using a Rituxan (CD20+ antibody) conjugated NGO-PEG[38] it was further found out that drug release from GO surface is pH dependent suggesting a possibility of pH controlled drug delivery system with the use of GO. The pH-sensitive drug release behavior from many different GO-based drug delivery systems is beingh widely studied. Rana et al[39] reported the delivery of an anti-inflammatory drug, Ibuprofen, by using a chitosan-grafted GO. In this case, the loading rate of Ibuprofen on the GO sheet was determined to be 9.7%. Furthermore, the work demonstrates that controlled drug release can be achieved by adjustment of pH value. To enhance the anticancer effect, Yang and colleagues[40]

designed and prepared a magnetic- and biodual targeting drug delivery system based on GO-Fe3O4 nanoparticle hybrid. The *in vitro* experiments indicated specific targeting of the multifunctional drug carriers by SK3 human breast cancer cells. Clearly, in vivo study is desired to demonstrate the performance of this external magnetic field-guided and bio-targeted drug delivery system keeping in view the loading capacity and Biofunctionalization of GO it is a very promising material for drug delivery and cancer therapy.

Scaffolds for tissue engineering

Tissue engineering is a very challenging area of research and highly desirable to improve the well-being of mankind. Many three-dimensional scaffolds for bioengineered organs have been studied for their suitability for tissue growth for example carbon nanomaterials (nanotubes, nano-diamond and fullerene[41]). Tissue engineering is an interdisciplinary field that strives to develop biological substitutes to restore, maintain or improve function of a tissue or whole organ. Graphene has excellent mechanical properties (high elasticity, strength, flexibility) and ability to tailor various functionalities on flat surfaces. Hence, it can be potentially used as a reinforcement material in hydrogels, biodegradable films, electrospun fibers and other tissue engineering scaffolds. GO-chitosan hydrogel scaffolds prepared by covalent linkages of chitosan amino groups with carboxylate groups of graphene exhibited significant improvement in cell adhesion, differentiation proliferation and calcium phosphate deposition by mouse pre-osteoblast MC3T3-E1 cells. These hydrogel scaffolds have better mechanical properties and lower degradation rate. Further, these scaffolds retain size, shape under physiological and extreme pH conditions as compared to those of chitosan alone[34]. Lu et al. explored graphene-based composite materials for wound healing by preparing chitosan–PVA nanofibrous scaffolds containing graphene. Three groups, chitosan–PVA–graphene electrospun fibers, chitosan–PVA fibers without graphene and control (no scaffold), were studied to understand wound healing potential in mice and rabbit[42]. Another study revealed that chemical vapor deposition (CVD) grown graphene substrate is biocompatible for human osteoblasts as well as for hMSCs with higher proliferation of cell, compared to SiO2 substrate, and stimulates the growth and differentiation of cells[43]. In addition to its mechanical and electrical properties, graphene functionalization with protein/peptides can be useful for tissue engineering applications. Variousmicro/nanofabrication approaches can be employed to achieve spatial patterning of cells and/or proteins[44]. Recently, Kodali et al[45] functionalized graphene with pyrenebutanoic acid–succinimidyl ester (PYR–NHS) without disrupting graphene's electronic structure. PYR-functionalized graphene was further patterned at microscale resolution with fluorescently labeled proteins such as laminin using microcontact printing. Along similar lines,

DNA origami structures were patterned using chemically modified GO and nitrogen rGO (NGO) with nanoscale resolution[46]. Such an approach may open up new avenues for mechanically flexible and electrically active scaffold surfaces with nanoscale precision for tissue engineering and/or bio-nanodevices. Ability of graphene materials to adsorb proteins/DNA due to its large surface area can be exploited in many therapeutic applications. Researchers at Clemson University have demonstrated a graphene coating for cardiovascular stent applications. Graphene exhibits unique properties and has been hailed as a material of the future. The research has effectively demonstrated that molecular coating of graphene on clinical grade nitinol is more bio- and hemo-compatible than uncoated nitinol due to its unique properties to form a smooth layer of atoms and electrochemical interactions with plasma proteins. Notably, the graphene-coated nitinol supports controlled smooth muscle and endothelial cell growth resulting in natural cell morphology and behavior. These advanced properties, in addition to high durability and chemical inertness, make graphene an ideal material candidate for coating bio-medical implants. Overall, mechanical and electrical properties of graphene materials can be useful in reinforcing tissue engineering scaffolds. Recent literature indicates that graphene-based composites interfaced with micro/nanofabrication technologies may lead to development of scaffolds with properties fine-tuned for target organ/tissues. However, along with detailed in vitro characterization of scaffolds, more emphasis should be placed on their evaluation in vivo with respect to inflammatory responses, biocompatibility and regenerative potential.

Prosthetic retina and nerve:

The artificial retina is composed by neuronal photoreceptors, known as cones and rods, which capture light signals and transform them in electrical signals, transferring them to the retinal ganglion cells and, through the optic nerve, to specific sites in the brain. Graphene oxide can serve as photovoltaic semiconductor which, unlike the metal or silicon-based materials used until now for such biotechnological interfaces, is soft, light and flexible and highly biocompatible and naturally sensitive to visible light. Its photovoltaic property also makes it a prosthesis which does not require an external electrical source to function. Lorenzoni et al. prepared Primary hippocampal embryonic neurons from E18 rat. Neurons were plated on the patterned graphene/glass substrates, which exhibited promising activity. Therefore graphenes can considered a valuable candidate to realize new generation of highly specialized prosthetic sensors[47].

CONCLUSION

Graphene is a cheap and multifunctional material with unique physical and chemical properties.

Graphene-based nanomaterials have been widely explored in non medical fields for at least a decade. Remarkable progress in synthesis and functionalization of graphene materials has opened new avenues exploring their use in drug/gene delivery, biosensors and tissue engineering. However, the merging of graphene and biotechnology is in its infancy, with many challenges remaining. Better understanding of physics and chemistry at the surface of graphene and interaction of chemicals and biomolecules at the interface of graphene will play an important role in applying graphene as nano scaffold in catalysis, chemical/biosensing, imaging and drug delivery. However, in spite of the considerable advances, substantial fundamental research is still necessary to provide a basic understanding of these materials to enable full exploitation of their nanoengineering potential.

REFERENCE

1. Yuqi Yang, Abdullah Mohamed Asiri, Zhiwen Tang, Dan Du, Yuehe Lin, Graphene based materials for biomedical applications , Materials today 2011 ,16 (10),365–37
2. Rao CNR, Sood AK, Subrahmanyam KS, Govindaraj A (2009) Graphene: the new two-dimensional nanomaterial. Angew. Chem. Int Ed.48:7752–7777.
3. Y. Zhu , S. Murali , W. Cai , X. Li, J.W Suk, J.R. Potts, R.S. Ruoff : Graphene and Graphene Oxide : Synthesis, Properties, and Applications: Advanced Materials (2010) 22, 3906–3924.
4. Q. Liu, J. Shi, M. Cheng, G. Li, D. Cao, G. Jiang: Preparation of graphene-encapsulated magnetic microspheres for protein/peptide enrichment and MALDI-TOF MS analysis : Chemical Communications (2012) 48,1874-1876.
5. H. Suna, Y. Yangb, Q. Huang : Preparation and Structural Variation of Graphite Oxide and Graphene Oxide : Integrated Ferroelectrics (2011) ,128,163-170
6. K. Novoselov et al : Electric field effect in Atomically Thin Carbon films : Science 306 [666-669] (2004).
7. tankovich S, Dikin DA, Dommett GHB, Kohlhaas KM, Zimney EJ, Stach EA, et al. Graphene-based composite materials. Nature (2006);442(7100): 282-286.
8. Fowler JD, Allen MJ, Tung VC, Yang Y, Kaner RB, Weiller BH. Practical Chemical Sensors from Chemically Derived Graphene. ACS Nano (2009); 3(2): 301-306.
9. Ajay Kumar and Chee Huei Lee. Synthesis and Biomedical Applications of Graphene: Present and Future Trends, Advances in Graphene Science, Dr. M. Aliofkhazraei (Ed.), (2013) ISBN: 978-953-51-1182-5, InTech, DOI: 10.5772/55728.
10. De Heer WA, Berger C, Wu X, First PN, Conrad EH, Li X, et al. Epitaxial graphene. Solid

State Communications (2007); 143 (1–2): 92-100.

11. Berger C, Song Z, Li X, Wu X, Brown N, Naud C, et al. Electronic Confinement and Coherence in Patterned Epitaxial Graphene. Science (2006); 312(5777):1191-1196.
12. Ying Wang , Zhaohui Li , Jun Wang , Jinghong Li and Yuehe Lin: Graphene and graphene oxide: biofunctionalization and applications in biotechnology :Trends in Biotechnology,(2011) 29,5-9.
13. Bonanni A, Ambrosi A, Pumera M. Nucleic acid functionalized graphene for biosensing. Chemistry. (2012) 6; 18(6):1668-1673.
14. Shao, Y.Y. et al. Graphene based electrochemical sensors and biosensors: a review. Electroanalysis (2010) 22, 1027–1036.
15. Zhang, J.L. et al. Graphene oxide as a matrix for enzyme immobilization. Langmuir (2010) 26, 6083–6085.
16. Abdullahi Mohamed Farah, Force Tefo Thema and Ezekiel Dixon Dikio, Electrochemical Detection of Hydrogen Peroxide Based on Graphene Oxide/Prussian Blue Modified Glassy Carbon Electrode Int. J. Electrochem. Sci (2012),7.
17. Xinhuang Kang, Jun Wang, Hong Wu, Ilhan A. Aksay, Jun Liu, Yuehe Lin ; Glucose Oxidase–graphene–chitosan modified electrode for direct electrochemistry and glucose sensing , Biosensors and Bioelectronics (2009), 25(4), 901-905
18. Flexible glucose sensor using CVD-grown graphene-based field effect transistor Yeon Hwa Kwaka, c, 1, Dong Soo Choib, 1, Ye Na Kimb, Hyeongkeun Kima, Dae Ho Yoonb, Sang-Sik Ahnc,Ji-Woon Yangc, Woo Seok Yanga, Sungkyu Seoc.
19. Application of graphene-modified electrode for selective detection of dopamine Ying Wang, Yueming Li, Longhua Tang, Jin Lu, Jinghong Li
20. Graphene fluorescence resonance energy transfer aptasensor for the thrombin detection. Chang H[1], Tang L, Wang Y, Jiang J, Li J.
21. A graphene platform for sensing biomolecules. Lu CH[1], Yang HH, Zhu CL, Chen X, Chen GN.
22. A graphene oxide-based FRET sensor for rapid and sensitive detection of matrix metalloproteinase 2 in human serum sample. Song E[1], Cheng D, Song Y, Jiang M, Yu J, Wang Y.
23. Biosens Bioelectron. 2013 Sep 15;47: 445-50. doi: 10.1016/j.bios.2013.03.030. Epub 2013 Mar 21.

24. Paleček E. Past, present and future of nucleic acids electrochemistry. Talanta 2002; 56(5): 809-19.
25. Graphene: Synthesis and Applications in Biotechnology - A Review Mohammad Hakimi and Paransa Alimard, World Applied Programming, Vol (2), No (6), June 2012.
26. Lu CH, Yang HH, Zhu CL, Chen X, Chen GN A graphene platform for sensing biomolecules. Angew Chem Int Ed Engl (2009); 48(26):4785-7. doi: 10.1002/anie.200901479
27. Jang, H., Y.K. Kim, H.M. Kwon, W.S. Yeo, D.E. Kim and D.H. Min. A graphene- based platform for the assay of duplex-DNA unwinding by helicase. Angew Chemie Int. Edn. (2010). 49: 5703-5707.
28. Janardhan Balapanuru, Jia-Xiang Yang, Si Xiao, Qiaoliang Bao, Maryam Jahan, Lakshminarayana Polavarapu, Qing- Hua Xu, Ji Wei, Kian Ping Loh "A Graphene OxideOrganic Dye Ionic Complex with DNA Sensing and Optical Limiting Properties", Angewandte Chemie, 2010, 49, 6549-6553.
29. Thomas Hirsch, Otto S. Wolfbeis, Graphenes in chemical sensors and biosensors Sven Kochmann, Trends in Analytical Chemistry, Vol. 39, 2012
30. Changsheng Shan, Huafeng Yang, Dongxue Han, Qixian Zhang, Ari Ivaska and Li Niu, Water-Soluble Graphene Covalently Functionalized by Biocompatible Poly-L-lysine , *angmuir*, 2009, *25* (20), pp 12030–12033DOI: 10.1021/la903265p.
31. Biomedical Applications of Graphene He Shen, Liming Zhang, Min Liu and Zhijun Zhang, T Thheranostic 2012; 2(3):283-294. doi: 10.7150/thno.3642.
32. Kovtyukhova NI, Ollivier PJ, Martin BR, et al. Layer-by-layer assembly of ultrathin composite films from micron-sized graphite oxide sheets and polycations. Chem Mater. 1999; 11: 771-778.
33. KS. Novoselov. A roadmap for graphene, *Nature* 2012;490:192–200.doi:10.1038/nature11458
34. Zhang, L., Xia, J., Zhao, Q., Liu, L. & Zhang, Z. Functional Graphene Oxide as a Nanocarrier for Controlled Loading & Targeted Delivery of Mixed Anticancer Drugs. Small 6, 537–544 (2010).
35. Huang Z, Xu HP, Meyers AD, et al. Photodynamic therapy for treatment of solid tumors-Potential and technical challenges. Technol Cancer Res T. 2008; 7(4): 309-20
36. Yang, K. et al. (2010) Graphene inmice: ultrahigh in vivo tumor uptake and efficient photothermal therapy. Nano Lett. 10, 3318–3323
37. Sahoo NG[1], Bao H, Pan Y, Pal M, Kakran M, Cheng HK, Li L, Tan LP. Functionalized carbon nanomaterials as nanocarriers for loading and delivery of a poorly water-soluble anticancer

drug: a comparative study. *Chem.Commun.*,(2011),47,5235-5237DOI: 10.1039/C1CC00075F

38. Sun XM, Liu Z, Welsher K, et al. Nano-graphene oxide for cellular imaging and drug delivery. Nano Res. 2008; 1(3): 203-212.
39. Rana VK, Choi MC, Kong JY, et al. Synthesis and drug-delivery behavior of chitosan-functionalized graphene oxide hybrid nanosheets. Macromol Mater Eng. 2011; 296(2): 131-140.
40. Yang XY, Wang YS, Huang X, et al. Multi-functionalized graphene oxide based anticancer drug-carrier with dual-targeting function and pH-sensitivity. J Mater Chem. 2011; 21(10): 3448-3454.
41. Lucea Bacakova et al , Adhesion and Growth of Human Osteoblast-Like Cell in Cultures on Nanocomposite Carbon-Based Materials. Nanoscience and Nanotechnology Letters 2011;3(1): 99-109.
42. B. Lu, T. Li, H. Zhao, X. Li, C. Gao, S. Zhang, E. Xie, Graphene-based composite materials beneficial to wound healing, Nanoscale 4 (2012) 2978–2982.
43. Kalbacova M, Broz A, Kong J, et al. Graphene substrates pro- mote adherence of human osteoblasts and mesenchymal stromal cells. Carbon. 2010; 48(15): 4323-4329.
44. S. Sant, S.L. Tao, O.Z. Fisher, Q. Xu, N.A. Peppas, A. Khademhosseini, Microfabrication technologies for oral drug delivery, Adv. Drug Deliv. Rev. 64 (2012)496–507.
45. V.K. Kodali, J. Scrimgeour, S. Kim, J.H. Hankinson, K.M. Carroll, W.A. de Heer, C. Berger, J.E. Curtis, Nonperturbative chemical modification of graphene for protein micropatterning, Langmuir 27 (2010) 863–865.
46. A. Kasry, A.A. Afzali, S. Oida, S.-J. Han, B. Menges, G.S. Tulevski, Detection of biomolecules via benign surface modification of graphene, Chem. Mater. 23 (2011) 4879–4881.
47. Matteo Lorenzoni, Fernando Brandi, Silvia Dante, Andrea Giugni, and Bruno Torre. Simple and effective graphene laser processing for neuron patterning application. Sci Rep. 2013; 3: 1954