REVIEW ON SYNTHESIS, CHARACTERIZATION & BIOMEDICAL APPLICATIONS OF GRAPHENE

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ABSTRACT

Graphene is a wonder material that attracts great interests in material science and condensed matter physics. It is the thinnest material and also the strongest material ever measured. It's distinctive band structure and physical properties determine it's bright application prospects. Among various applications, biomedical applications of graphene have attracted ever-increasing interest over many years. In this review, we present an overview of various synthesis and characterization techniques of graphene and current advances in application of graphene in biomedicine with focus on drug delivery, cancer therapy and biological imaging.

Keywords:- Graphene, synthesis, characterization, biomedical applications, drug delivery, biosensing, bioimaging.

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1. Introduction:-

Graphene is an exciting material. It has attracted great interests because of its outstanding electrical and mechanical and optical properties[1-5]. Graphene has a well defined two dimensional crystal structure Fig. 1(a), and it is regarded as the elementary structure of carbon-based materials, such as carbon nanotubes, fullerene and graphite[6]. Graphene also has many special characteristics, such as bipolar supercurrent, chiral tunnelling of relativistic particles, absence of Anderson location and anomalous Quantum Hall Effect[7]. These unique characteristics of graphene provide a research platform for quantum mechanics and condensed matter physics. Many phenomena are easily observed in graphene, which were only observed in black holes or heavy ion accelerators earlier.

Graphene can be used as transparent electrode, which maybe widely used in liquid crystal display (LCD), organic light emitting display (OLED), or organic solar cells[8]. Graphene has an optical transmission rate over 98%, significantly higher than that 82-85% of the standard indium tin oxide (ITO) film. Established on large specific area, graphene is also used as gas detector and biological system. Schedin et al.[9] showed that the micrometer –sized sensors made from graphene are capable of detecting individual events when a gas molecule is attached or detached from graphene surface. Mohanty et al.[10] demonstrated the interfacing of chemically modified graphene with biological systems to build a novel live-bacteria-hybrid device and a DNA-hybridization device with excellent senstivity.

Due to excellent electrical, thermal and mechanical properties, graphene is expected to be used in high performance nanoelectronic devices., composite materials, field emission materials, gas sensors, energy storage areas etc. It is possible using graphene for ballistic field effect transistor at room temperature. The nanoribbon transistors with large on-off current ratios at room temperature[11] in Fig.1(b) . Graphene makes it possible for this relatively simple framework to implement complex and new circuit and becomes the basic electronic material beyond the silicon age. As a robust yet flexible membrane, graphene provides essentially infinite possibilities for the modification or functionalization of its carbon backbone[12].

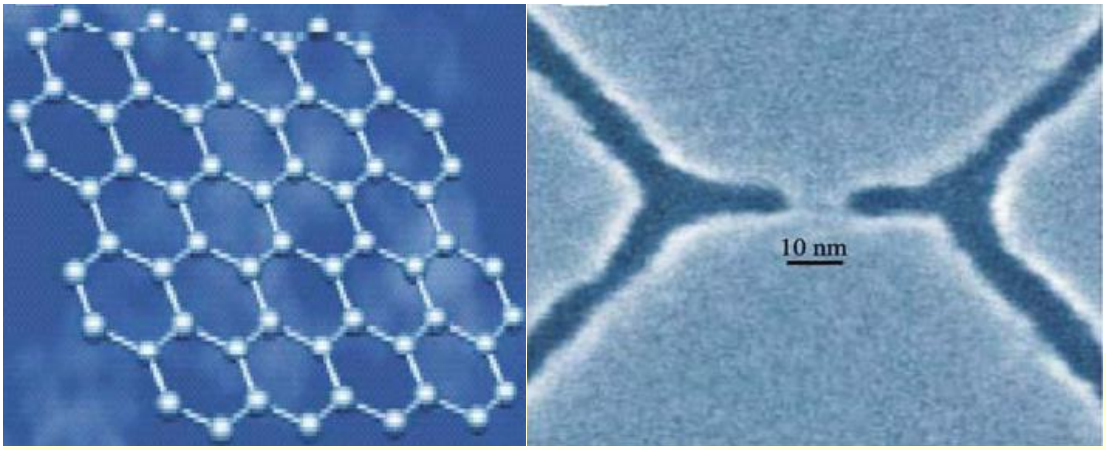

Figure 1 (a) The structure of graphene; **(b)** Graphene nanoribbons exhibit the transistor action with large on-off ratios (Reprinted)

Graphene is one layer of atomic carbon. It's honeycomb lattice is composed of two equivalent sub-lattices of carbon atoms bonded together with σ bonds Fig. 2, then each carbon atom in the lattice has a π orbital that contributes to a delocalized network of electrons. Ripples can be introduced[13], suggesting that the local electrical and optical properties of graphene could be altered for possible application in devices. Apart from intrinsic corrugations, graphene in real 3D space can have other 'defects', including topological defects, vacancies, adatoms, edges/cracks, adsorbed impurities and so on. Experiments have demonstrated that defects in a graphene layer found in the material containing single wall carbon nanotubes (CNTs), such as topological defects, vacancies and adatoms, could be induced locally by electron irradiation[14]. Graphene has been used as a TEM support membrane for the study of light atoms like carbon and hydrogen[15] and Au nanoparticles[16]. Individual atoms on graphene and carbon chains and vacancies, generated by knock-on by the electron beam in TEM could be investigated dynamically, providing insights into generation of defects and their evolution[15].

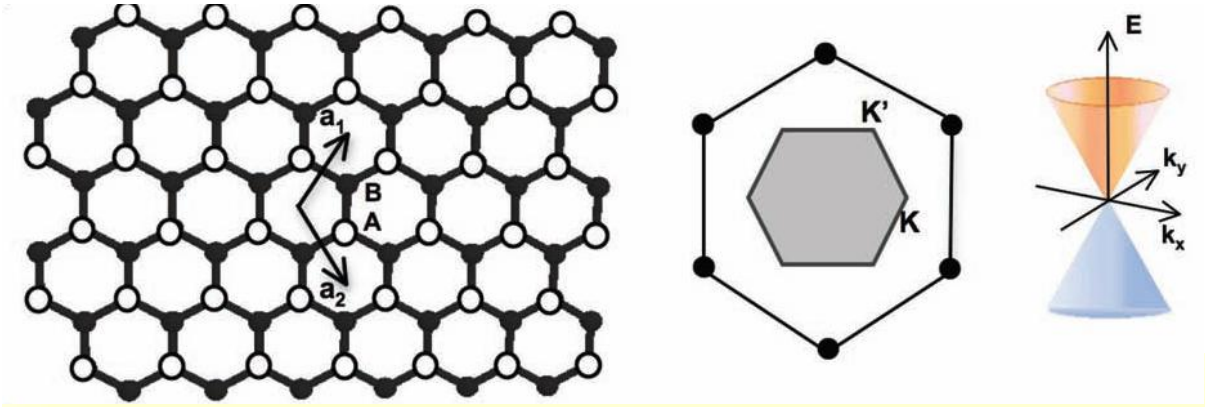

Figure 2 Schematics of the crystal structure, Brillouin zone and dispersion spectrum of graphene. (Copyright: 2007 Nature Publishing Group)

2. Synthesis of Graphene:-

At first graphene was fabricated from graphite by micromechanical cleavage known as scotch-tape technique. In this technique one destroys the Van-der Waals forces between graphite layers. Geim's group first isolated graphene from platelets of highly oriented pyrolytic graphite (HOPG)[1]. They first prepared 5mm-deep mesas on top of the platelets using dry etching in oxygen plasma. The structured surface was then pressed against a 1mm thick layer of a fresh wet photoresist spun over a glass substrate. After baking, the mesas became attached to the photoresist layer. Then using scotch tape, they started repeatedly peeling flakes of graphite off the mesas. Thin flakes left in the photoresist were released in acetone. When a Si wafer was dipped in the solution, some flakes became captured on the wafer surface. Though this technique[17] provided crystals of high structural and electronic quality but only in millimetre size and could not be used for large scale preparation.

The second method for graphene fabrication is chemical cleavage from graphite, which is an improvement of micromechanical cleavage. This method can lead to larger quantities. The single sheet is fabricated from graphite oxide. Due to the existence of oxide groups, atomic planes of graphite are partially detached by intercalation. After ultrasonic cleaning for several hours, these samples are exfoliated to create stable aqueous dispersions of individual sheets. After deposition, graphene oxide can be reduced to graphene either by means of chemical or by thermal annealing[18]. The small scale size, in tens of microns however, hinders the application of graphene by this method.

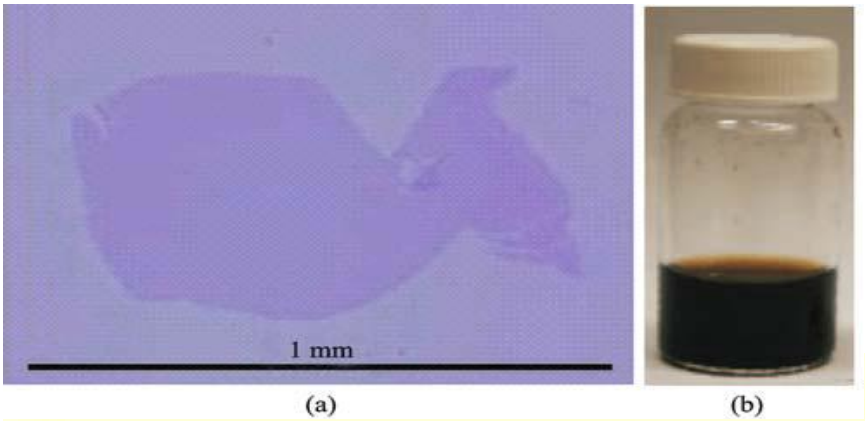

Figure 2 (a) Large graphene crystal prepared on an oxidized Si wafer by the scotch-tape technique (Reprinted); **(b)** Graphene made from chemical cleavage from graphite(Reprinted).

Another method is the graphene grown epitaxially on SiC substrates and patterned via standard lithographic procedures has been proposed as a platform for carbon-based nanoelectronics and molecular electronics in recent studies[19,20]. The epitaxial graphene was produced on the Si terminated (0001) facet of single –crystal 6H-SiC by thermal desorption of Si. After the surface is oxidized or reduced in H_2, samples are heated by electron bombardment in ultra-high vaccum and remove the oxide. Then the samples are heated again to temperatures in the range of 1250^{o}C to 1450 ^{o}C for 1-20 min. After these processes, thin graphene layers are formed and the layer thickness is determined predominantly by the temperature[19]. The shortcoming of the epitaxial graphene on SiC is a lot of defects in the layer besides it hard to transfer to other substrates. Therefore this method needs further improvement.

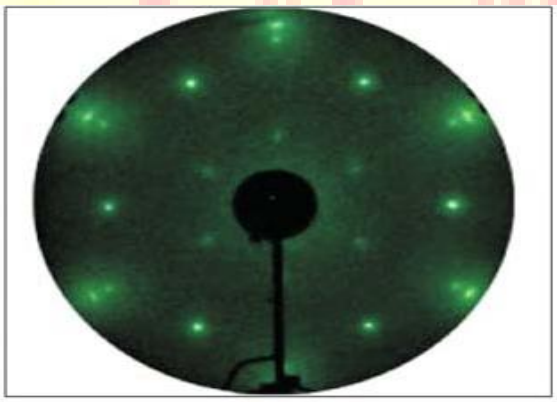

Figure 3 Low-energy electron diffraction (LEED) pattern (of three monolayers of epitaxial graphene on 4H-SiC(0001) (C-terminated face) (Reprinted)

The last and the best method to fabricate graphene is chemical vapour deposition (CVD). This method has the potential for large-scale production. Graphene made by CVD on silicon wafer with nickel layer as catalyst has achieved centimetre in size [21,22]. The mechanism is based on precipitation, Ni and C atoms form a solid solution in CVD after heating. Since the solubility of C in Ni is temperature-dependent, C atoms precipitate and form graphene layer on the Ni surface upon sharply cooling of the sample. The layer of graphene depends on the concentration of C precipitated in Ni. However, Li et al.[23] recently fabricated graphene films on copper foils. They concluded that the thickness of graphene films were independent on exploring time, and the low solubility of carbon in copper made this growth process self limiting. The precipitation mechanism did not work on copper substrate. Therefore, the mechanism of CVD growth graphene needs additional experiments. Anyway this method demonstrates the potential for large area production, and the graphene can be easily transferred to other substrates[24]. Therefore, the CVD technology has attracted wide attention for large-scale preparation of graphene. However graphene obtained by CVD technique usually is a mixture of single and multilayer and the main concern is to improve the quality of graphene.

In practical applications of graphene-based circuits, various types of graphene are needed. Thus, modulation of its electrical properties is of great technological interest. Hence doping it with other elements is a promising way to achieve this goal. Doped graphene may lead to promising fascinating properties with widespread applications.

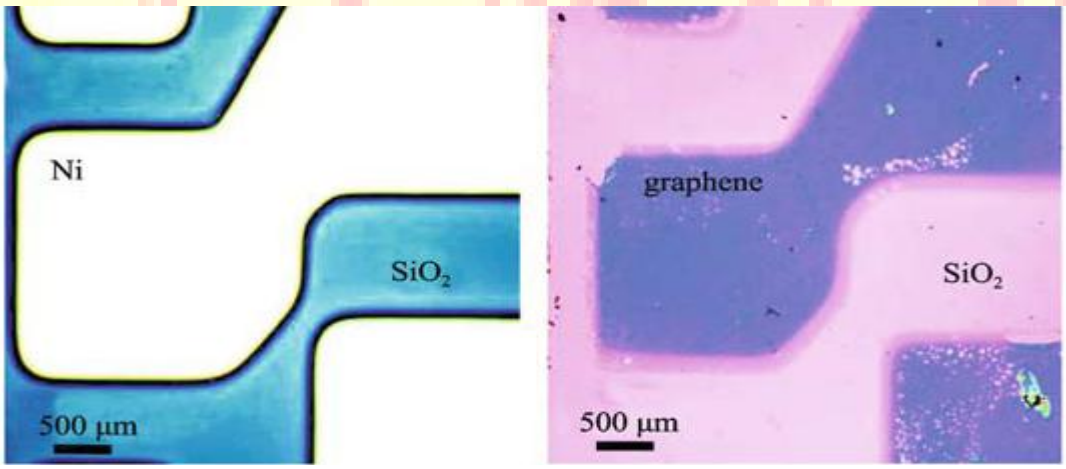

Figure 4 Optical image of Ni film on SiO2/Si, and CVD graphene is grown on the surface of the Ni pattern (Reprinted)

3. Characterization of Graphene:-

Graphene is only one layer of atomic carbon and very transparent, so the characterization method is very important and difficult. The usual methods include optical microscope, SEM, TEM, AFM, Raman spectroscopy etc.

The identification of graphene sheets, down to one layer in thickness, is possibly realized through optical microscopy via the color contrast caused by the light interference effect on the SiO_2 substrate, which is modulated by the graphene layer[25,26]. Gao et al.[25] developed a total color difference (TCD) method to make it possible for characterization of large-area graphene samples. This method, based on a combination of reflection spectrum and international commission on illumination color space, provides accurate and reliable layer identification for rapid evaluation of the layer range of graphene by different color bands. It opens up the possibility for the non-destructive identification and physical property measurements of graphene with an optical microscope.

SEM is also used for observation of graphene. This method is similar to optical microscope. Usually, it needs to transfer graphene to silicon wafer with a specific thickness of SiO_2. From the color depth, the layer of graphene can be estimated. The graphene films give a more clear contrast in SEM.

AFM is an effective way for the characterization of graphene. Though the thickness of graphene is very thin, it can easily get morphological feature using AFM. From the step of graphene on substrate, it is possible to estimate the number of graphene layer. Due to differences in tip attraction/repulsion between the insulating substrate and semi-metallic graphene and under ambient conditions by the preferential absorption of a thin layer of water on graphene, it is hard to get the theoretical thickness of 0.34nm. Therefore, the AFM method for measurement of the graphene layer number is not accurate, and it has very low throughput[27].

TEM can not only observe the morphological feature of graphene but also count the number of graphene layers accurately. It is also known that the edges of the graphene films always fold back, which allows a cross sectional view of the films. The observation of these edges by TEM provides an accurate way to account the number of layers at multiple locations on the films. Besides, TEM is often assisted with electron diffraction pattern, which shows a hexagonal pattern of the graphene crystal structure.

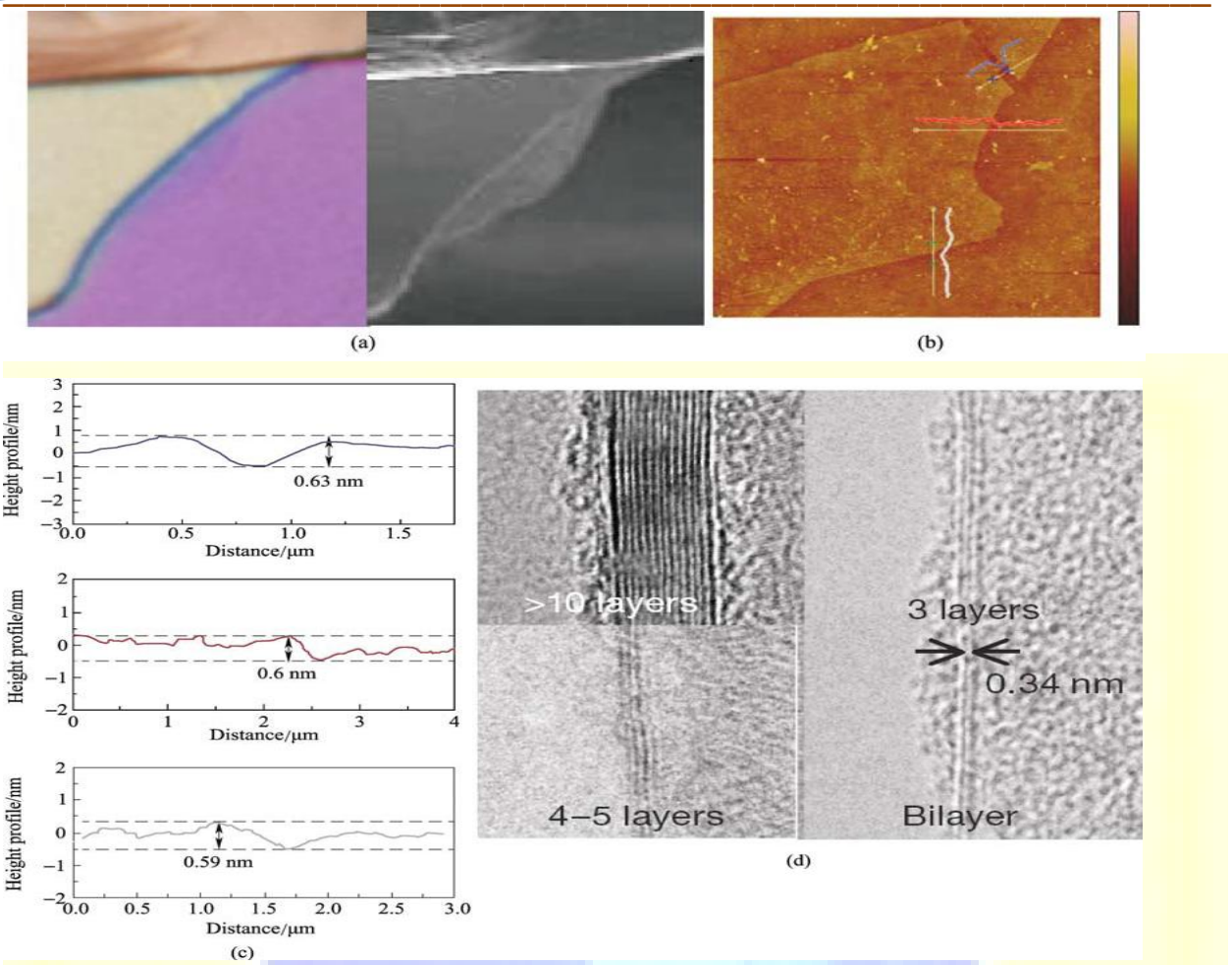

Figure 5 (a) Images of a thin graphitic flake in optical (left) and scanning electron (right) microscopes. Few-layer graphene is clearly visible in SEM (in the center) but not in optics (Reprinted); **(b)** AFM image of graphene on SiO2/Si substrate (Reprinted); **(c)** The height of graphene based on (b) (Reprinted); **(d)** High-magnification TEM image of graphene (Reprinted)

Raman spectroscopy can provide a quick and effective way for structure and quality characterization of graphene. The Raman spectra of graphene includes the G peak located at 1580 cm^{-1} and 2D peak at 2700 cm^{-1}, caused by the in-plane optical vibration and second-order zone boundary phonons, respectively. The D peak located at 1350 cm^{-1} due to first order zone boundary phonons, is absent from the defect free graphene, but exists in defected graphene. Hence the D band of graphene associates with the existence of defects; the lower intensity of D peak, the fewer defects of the graphene layer. It also associates with doping in grapheme[28]. Thus it was proposed that Raman spectra could be used to distinguish the 'quality' of graphene

and to determine the number of layers for n-layer graphene (for n upto 5) by the shape, width, and position of the 2D peak[29-32]. As shown in Fig. The 2D peak shifts to higher wave-number values and becomes broader for an increasing number of layers.

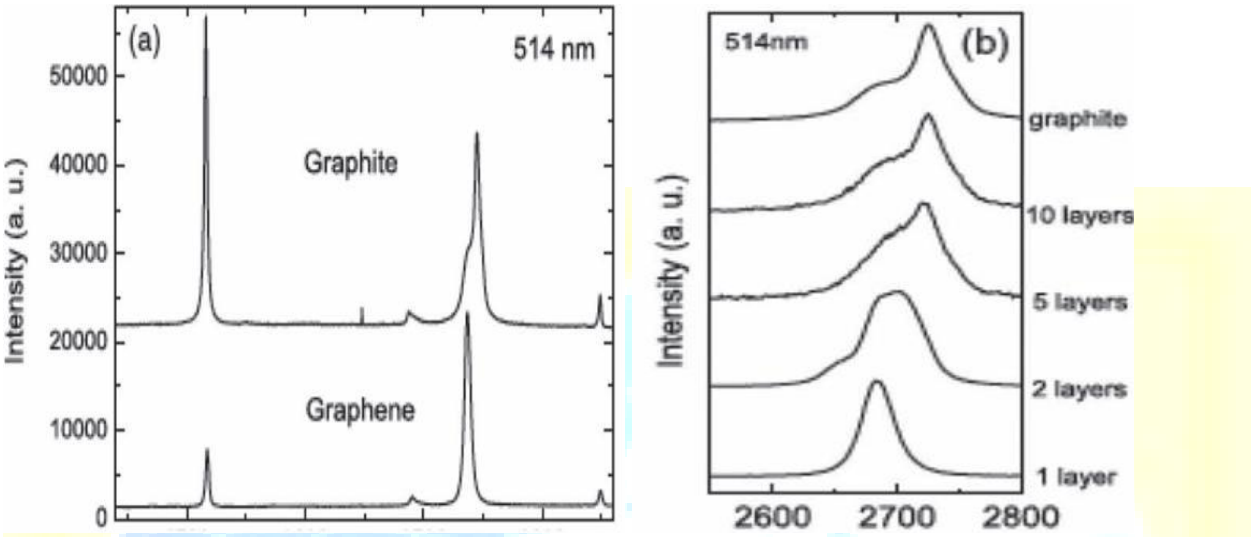

Figure 6 . (a) Comparison of Raman spectra at 514 nm for bulk graphite and graphene. They are scaled to have similar height of the 2D peak at 2700 cm − 1 ; **(b)** Evolution of the spectra at 514 nm with the number of layers.

The shifting and splitting of Raman modes can be used to analyze mechanical strain in graphene. For example, Raman spectra of epitaxial graphene grown on SiC show a significant phonon 'hardening' (blue shift of the G & 2D peaks), mainly due to the compressive strain that occurs when the sample is cooled down after growth[33,34]. It has been stated that the substrates play a negligible role in the Raman spectrum of micromechanically cleaved graphene transferred onto them, indicating the weak interactions between the transferred graphene and such substrates[35]. The frequency of the G and 2D peaks can also be tuned by charge doping through electron-phonon coupling changes[36,37]. The Raman spectral signatures of epitaxial graphene grown on SiC, especially the width of the 2D peak, have been correlated to the carrier mobility of the grapheme[38]. The intensity ratio of the D and G peak has been used as a metric of disorder in graphene, such as arising from ripples, edges, charged impurities, presence of domain boundaries and others[39]. For edges, the intensity of the D peak depends on the edge structure, it is weak at the zigzag edge and strong at the armchair edge[40,41].

4. Biomedical Applications of Graphene:-

4.1 Drug/Gene Delivery -

Beyond the various applications of graphene, it's biomedical applications is a relatively new area with significant potential. GO, produced by vigorous oxidation of graphite by Hummers method[42], 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[43-45]. The unique structural features, such as large and planar sp^2 hybridized carbon domain, high specific area (2630 m^2/g) and enriched oxygen containing groups, render GO excellent biocompatibility, and physiological solubility and stability and capability of loading of drugs and genes via chemical conjugation or physisorption approaches. Moreover, the reactive COOH and OH groups GO bears facilitate conjugation with various systems, such as polymers[46], biomolecules[45], DNA[47], protein [48-50], quantum dots[51], Fe_3O_4 nanoparticles[52] and others[53], imparting GO with multi-functionalities and multi-modalities for diverse biological and medical applications.

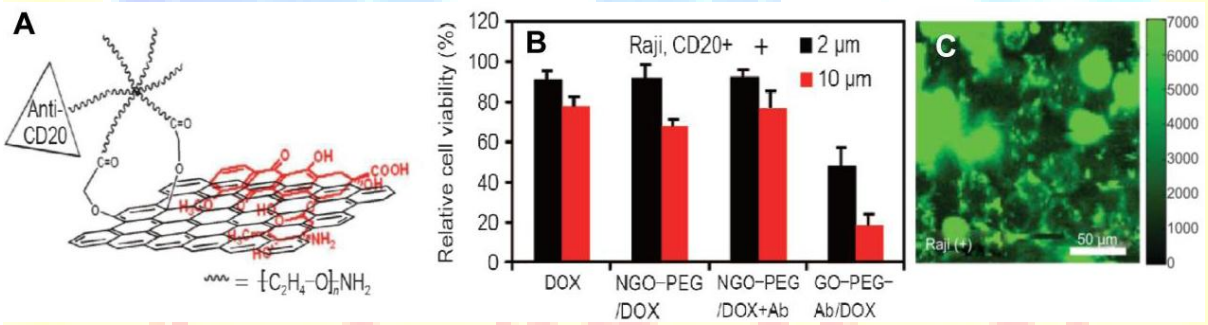

Figure 7 **(A)** Schematic illustration of DOX loading onto NGO-PEG conjugated with anti-CD20 antibody; **(B)** In vitro cytotoxicity at different DOX concentration showing targeted delivery of DOX into specific cells. **(C)** NIR fluorescence image of targeted cells treated with the NGO-PEG of Rituxan.[26] © 2008. Reproduced with kind permission from Springer Science+Business Media and Tsinghua Press.

Gene therapy is a novel and promising approach to treat various diseases caused by genetic disorders, including cystic fibrosis, Parkinson's disease and cancer[54]. Successful gene therapy requires a gene vector that protects DNA from nuclease degradation and facilitates cellular uptake of DNA with high transfection efficiency[55]. The major challenge facing the development of gene therapy is lack of efficient and safe gene vectors[56]. Recently Liu et

al.[57] and a group studied gene delivery using polyethylenimine (PEI) modified GO (PEI-GO). Their experiment indicates that grafting PEI to GO not only significantly lowered cytotoxicity of the cationic polymer, but also improved the transfection efficiency of the polymer. Thus Liu and other groups[58] suggested the PEI-GO is a promising candidate for efficient gene delivery. Also a group from Singapore has reported that chitosan-functionalized GO (GO-CS) sheets can be used as good drug/gene delivery.

4.2 Cancer Therapy-

Liu and others[59] have also reported application of GO for clinical cancer and other disease treatment. They observed very high tumor uptake of the PEG-modified GO due to highly efficient tumor passive targeting of GO caused by EPR effect. Moreover, under the low-power near-infrared (NIR) laser irradiation on the tumor, a highly efficient tumor destruction was achieved, taking use of strong absorbance of GO in the NIR region. Later, Markovic et al.[60] compared the photothermal anticancer activity of NIR-excited graphene and carbon nanotubes. Despite of its lower NIR- absorbing capacity, polyvinylpyrrolidone coated graphene nanoparticles display higher photothermal responsiveness and induce more photothermal cell death because of oxidative stress and mitochondrial depolarization and capase activation leaking cytochrome C of human glioma cells in vitro than single-walled CNT[61]. Zhang and co-workers[62] developed a NGO-PEG-DOX for the anti-tumor effect in vitro and in vivo by combination of photothermal and chemotherapies. The experiment revealed that the combined chem.-photothermal therapy exhibited synergistic effect that leads to better cancer killing effect than chemotherapy or photothermal therapy alone.

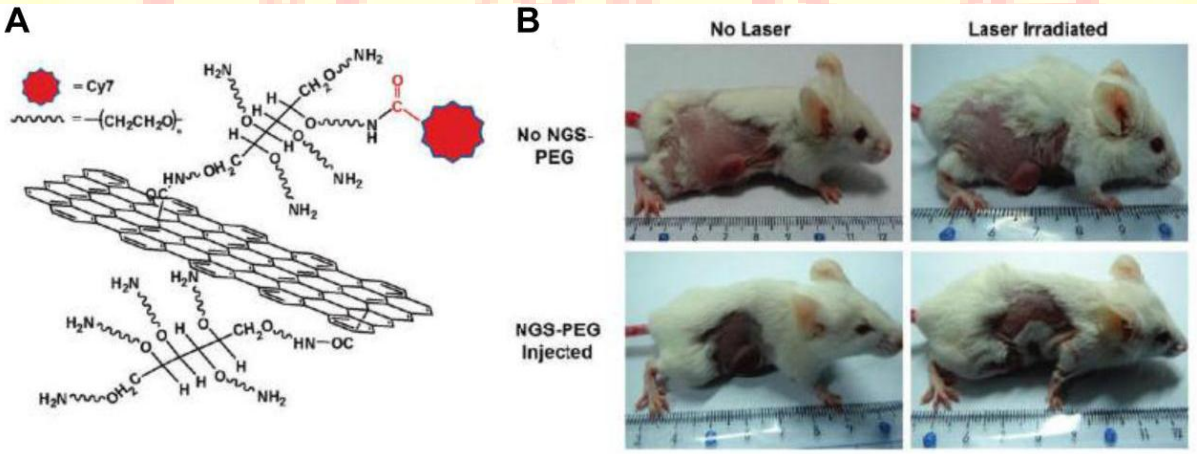

Figure 8 (A) Schematic illustration of PEG functionalized NGS and labeled by Cy7. (**B)** Photos of tumors on mice after various treatments indicated. The laser irradiated tumor on NGS injected mouse was completely destructed.(Reprinted) Copyright 2010 American Chemical Society.

Cui and his co-workers[63] reported application of folic acid and sulfonic acid conjugated GO loaded with porphyrin photosensitizers for targeting photodynamic therapy (PDT). In their experiment, GO was loaded witha a Chlorine e6 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. Liu and co-workers[64] further studied photothermally assisted PDT using GO loaded with a photosensitizer, chlorine e6, and demonstrated that such combined treatment yields remarkably improved cancer killing effect.

4.3 Biosensing-

Graphene derivatives, including pristine graphene, GO, chemically reduced GO (rGO)[65] and doped grapheme[66] have been intensively studied for their widespread applications in bio-sensing and detection of bio-molecules such as thrombin[67, ATP[68], oligonucleotide[69], amino acid[70] and dopamine[71]. Several types of GO based biosensors have been built, which include:- (1) Making use of super efficient fluroscence quenching ability of graphene, some novel florescence resonance energy transfer (FRET) based biosensors have been developed[67,68,72]. (2) Based on unique electronic property of graphene, FET type biosensors have been made[73], (3) Controllable self-assembling of graphene bio-molecules allows to build highly ultrasensitive biosensors for detection of DNA and other molecules[69,74-76] (4) As a matrix for detection of molecules, graphene based nano-platform for matrix assisted laser desorption/ionization time-of-flight mass spectroscopy has been reported[70,77] (5) GO-based novel biosensors via electrochemical principle have been constructed taking use of its huge surface area, good electrical conductivity and excellent capability of loading various bio-molecules via chemical or physical interactions[71,78].

4.4 Bioimaging-

Graphene has significant applications as biological imaging also[43,45,52,79]. Dai et al.[45] for the first time examined cellular uptake of PEG-modified GO loaded with chemical drugs using intrinsic fluorescence of GO in the NIR region. Another group[80] studied gelatine-grafted rGO labelled with a fluorescence dye for cellular imaging and drug delivery. Recently researchers

have begun to prepare smaller GO (size 10 nm or less) often referred as graphene quantum dots (GQDs), from chemical oxidation of graphite[81-83] and 'bottom-up approach[84]. These GQDs exhibit intrinsic fluorescence and can be used for bio-imaging purpose. Pan et al.[81] synthesized GQDs with blue emission color through hydrothermal cutting of GO. These as obtained GQDs are weakly fluorescent much lower than conventional organic dyes and II-VI type QDs, which hampers their practical bio-imaging applications. To overcome this problem, Eda et al.[82] treated the chemically produced GQDs by a brief exposure to hydrazine vapour and observed significantly improved fluorescence. Such functionalization of GQD with alkylamine also give rise to dramatically enhanced fluorescence[83]. Zhu et al.[85] and others[86] explored the possibility of GQDs for cellular imaging. Compared with CdSe and other II-VI type QDs, GQDs show excellent biocompatibility, physiological solubility and low cytotoxicity and can be used directly for intracellular imaging without the necessity for further surface processing or functionalization. In addition, GQDs allows them to be excited at NIR region, making both in vitro and in vivo bio-detection and imaging efficient, safe and without interference from auto-fluorescence from cells, organs, or tissues in this region[87].

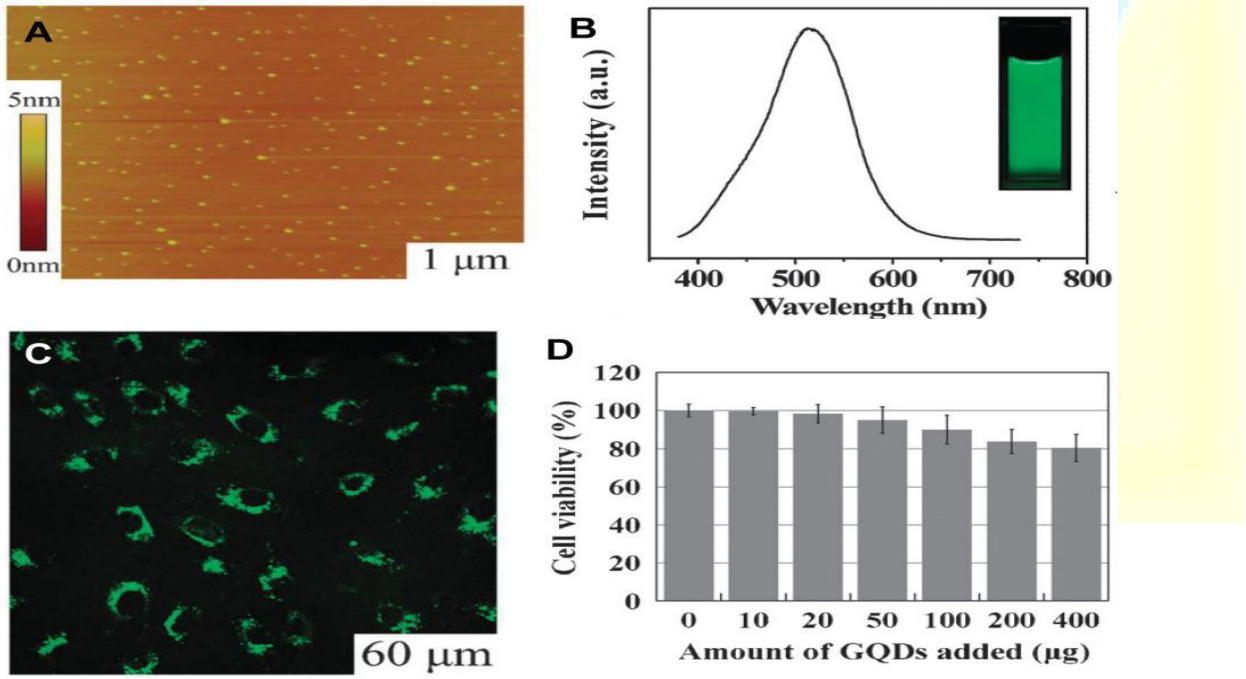

Figure 9 (A) AFM image of the GQDs; (**B)** Fluorescence spectra of aqueous solution of GQDs excited at 375 nm. Inset: aqueous solution of GQDs under UV light; (**C)** cellular imaging of

GQDs imaged under 405 nm; **(D)** Cytoxicity of GQDs. Reproduced by permission of The Royal Society of Chemistry.

4.5 GO-based antibacterial materials-

Fan et al.[88] prepared macroscopic freestanding GO and rGO paper from their suspension by vaccum filtration technique and found that these papers exhibit strong antibacterial effect. Considering the scalability and low cost of the graphene based antibacterial paper, it has opened up new opportunities for the use of GO in environmental and clinical applications. Later, Akhaven et al.[89] investigated anti-bacterial effect of graphene nanosheets in the form of nanowalls deposited on stainless steel substrates for both Gram-positive and Gram-negative models of bacteria. The rGO nanowalls are more toxic to bacteria than the GO nanowalls. The better antibacterial activity of the rGO nanowalls is due to the better charge transfer between the bacteria and more sharpened edges of the rGO nanowalls, during the contact interaction. Liu et al.[90] further probed the mechanism of antibacterial effect of 4 types of graphene derivatives, graphite (Gt), graphite oxide (GtO), GO and rGO. They found that the antibacterial activities decrease in the order of GO, rGO, Gt and GtO.

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