Research Article

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

ISSN Print 2231 – 3648
Online 2231 – 3656

Pharmaceutical and Biomedical Applications of Graphene based Nanoparticles

Kokkilagadda Vinod Kumar, Tunuguntla Bhavani Ramesh, Kamadi Chandra Sekhar Varma, Mudavath Hanumanaik, Balaji Maddiboyina

Department of Pharmacy, Vishwabharathi College of Pharmaceutical Sciences, Guntur, Andhra Pradesh, India

ABSTRACT

Graphene and graphene based nanomaterials have gained broad interests in research because of their unique physiochemical properties. The biomedical applications of graphene and its composite include its use in gene and small molecular drug delivery. The biocompatibility of the newly synthesized nanomaterials allows its substantial use in medicine and biology. The current review summarizes the chemical structure and biological application of graphene in various fields. Although a large amount of researches have been conducted on these novel nanomaterials, limited comprehensive reviews are published on their biomedical applications and potential environmental and human health effects. It also discussed the perspectives and challenges associated with the biomedical applications of Graphene based Nanoparticles.

Keywords: Graphene, Nanoparticles, Tissue Engineering, Gene Therapy

DRUG DELIVERY

Over the past few years, motivated by the successes of carbon nanotubes in biomedicine, functionalized graphene has been explored in drug delivery. Advantages of graphene-based nanomaterials for drug delivery arise from their ability to cross cell membranes easily and their high specific surface area, which provides multiple attachment sites for drug targeting. Graphene based nanomaterials can load drugs noncovalently via p–p stacking interactions, hydrogen bonding, or hydrophobic interactions. Initial studies using graphene-based nanomaterials for drug delivery were conducted by Dai et al. Who loaded a camptothecin (CPT) analogue, SN38 to GO-PEG via p–p stacking. The complex exhibited good water solubility, retaining a high efficiency of SN38 delivery, as well as high cytotoxicity in cells. Inspired by this pioneer work, many researchers have published studies exploring grapheme based materials for drug delivery. For instance, the same group reported the targeted

Author for Correspondence:
Kokkilagadda Vinod Kumar
Department of Pharmacy,
Vishwabharathi College of Pharmaceutical Sciences,
Guntur, Andhra Pradesh, India.

delivery of rituxan using go-peg [1]. Jin et al. Prepared hematin-conjugated dextran-functionalized go hybrids via p–p interactions to deliver doxorubicin for killing drug-resistant mcf-7/ADR cells [2]. Improved physiological stability was obtained and this hybrid had a loading capacity as high as 3.4 mg/mg nGo. The authors demonstrated that the release profile was PH dependent, with more released at lower PH approximately 30% of the DOX was released over 6 days and the DOX-loaded hybrids accumulated effectively in the cytoplasm and nucleus to kill the cells.

A similar PH dependent DOX release profile was obtained when GO-PEG/ZnS:Mn bi) [3]. Doxorubicin was added on the composite particles noncovalently and the loading capacity was dependent on the concentration of GO-PEG/ZnS:Mn, because more GO surface was available for DOX binding .When 1000 mg/ ml of a GO-PEG/ZnS:Mn solution was mixed with 300 mg/ml of a DOX solution, the loading capacity reached 100%.The DOX- loaded composite particles killed approximately 85% of HeLa cancer cells (Figure 1).To further control the drug release, PEGylated Fe2O3@Au core-shell NP-rGO nanocomposites were prepared to control the loaded DOX via synergistic interactions between photothermal therapy and chemotherapy [4].The maximal DOX loading capacity was approximately 1.0 mg/mg nanocomposites via p–p stacking interactions. When the DOX-loaded composites were put in PBS of pH 7.4, 17% of DOX was released in 2 h, although a burst release occurred following irradiation under an 808-nm NIR laser. In addition, via magnetic field guidance, DOX–rGO–Fe2O3@Au NPs killed cells close to the magnetic field.

There- fore, the loaded DOX could be released at specific sites and at a controlled rate. Zhou et al. also prepared graphene/Fe3O4compo- sites using graphite oxide and FeCl3 6H2O as starting materials and used the composites to load DOX [5]. Given the large specific surface area provided by graphene and the p–p interactions between graphene and DOX, a 65% loading capacity was obtained, which was higher than GO with the same Fe3O4. Mean - while, the use of Fe3O4 NPs also led to better bioimaging because of the enhanced cell-to-background contrast [6]. In the delivery of paclitaxel (PTX), graphene-based nanomaterial-loaded PTX provided better cytotoxicity against cancer cells. Xu et al. functionalized GO with PEG to improve its stability in physiological solution [7]. Then loaded PTX onto the GO-PEG composites via p–p stacking and hydrophobic interactions, resulting in a relatively high loading capacity of 11.2 wt % In a cellular uptake experiment, GO-PEG/PTX entered A549 and MCF-7 cells within 1 h. Similar results were also obtained in Zhang and colleagues using PEGylated nGO to co-deliver PTX and indocyanine green (ICG) [8]. Low cytotoxicity was observed of nGO-PEG on MG-63 cells even at a concentration of 200 mg/m. Meanwhile, lower cell viability was obtained with nGO-PEG-ICG/PTX compared with free PTX. Angelopoulou et al. modified GO with poly(lactide)–poly-(ethylene glycol) (PLA–PEG) copolymers for stability in an aqueous solution [9]. The PTX release profile was regulated by the molecular weight of PLA or PEG in the copolymer and their proportion in the composite, with a smaller molecular weight and larger proportion leading to faster release. The PTX-loaded composites demonstrated cytotoxicity against A549 cells with an increasing incubation time. Other drugs have also been delivered using graphene-based nanomaterials in addition to those described above, and selected examples are detailed in Table 1.

Carriers	Drugs	Applications	Ref
nGO-PEG	Cisplatin	Inhibition of cell proliferation and morphology	[10]
nGO-folic acid	Doxorubicin and camptothecin	Targeted delivery of mixed anticancer drugs	[11]
PNIPAM-GS	Camptothecin	Temperature-dependent drug release with high CPT-loading	[12]
GO-PEG	Ce6 photosensitizer	Photodynamic therapy	[13]

Chitosan-GO	Ibuprofen, 5-fluorouracil	Controlled release of chemically diverse drugs	[14]
GN-CNT-Fe3O4	5-fluorouracil	pH-dependent drug release	[15]
GO	Hypocrellin	Photodynamic therapy	[16]

GENE THERAPY

Gene therapy is a technique that uses genes to treat a genetic disease, and this approach has attracted intensive research attention. A key factor for gene therapy is to find efficient and safe gene vectors that protect DNA from nuclease degradation and facilitate its uptake with high transfection efficiency [17, 18]. Graphene-based nanomaterials are appropriate candidates for gene delivery because of their high loading efficiency and increased gene transfection. To decrease their cytotoxicity and obtain cationic surface properties to allow their interaction with anionic oligonucleotides electrostatically, graphene derivatives have to be modified by polymers, such as chitosan [19], polyamidoamine (PAMAM) [20], polyethylenimine (PEI) [21], and so on. Zhang et al. reported using PEI-conjugated GO to sequentially deliver siRNA and DOX [22], exhibiting a synergistic effect that led to significantly improved therapy efficacy. Liu et al. explored using PEI-functionalized GO for gene delivery using different molecular weights of PEI [23], and showed significantly low cytotoxicity of the PEI-GO complex and successful use of GO as a novel nano gene delivery vector with high transfection efficiency. Lactosylated chitosan oligosaccharide (LCO)-functionalized graphene oxides (GO-LCO) was prepared for the targeted delivery of DNA sequences to human hepatic carcinoma cells (QGY-7703) [24]. The loading capacity of FAM-DNA was as high as 4 m mole/g and it was delivered specifically QGY-7703 within 0.5 h. In addition, no obvious toxicity was observed even concentrations of 100 mg/ml. Hu et al. prepared folate-conjugated trimethyl chitosan (FTMC)/GO nanocomplexes (FG NCs) via electrostatic self-assembly to realize the targeted delivery of plasmid DNA (pDNA) [25]. Compared with A549 cells, Hela cells with folate receptors exhibited higher accumulation of FG NCs. The existence of FTMC in FG NCs could retard the migration of pDNA and facilitate pDNA condensation. It was reported that 31.1% of pDNA could be released within 72 h in vitro in PBS, highlighting FG NCs as a promising candidate for gene delivery. Liu et al. synthesized graphene-oleate-PAMAM dendrimer hybrids via oleic acid adsorption followed by covalent linkage of PAMAM dendrimers as gene delivery vectors [26].The graphene-oleate-PAMAM exhibited good dispersity in aqueous solutions and good biocompatibility with HeLa cells, but showed cytotoxicity to MG-63 cells at concentrations >20 mg/ml. The graphene-oleate-PAMAM loaded with a quarter of pEGFP-N1 exhibited a GFP gene transfection efficiency of 18.3% to HeLa cells. PAMAM dendrimer- grafted gadolinium-functionalized GO [27] and mPEGylated GO/ poly (2-dimethylaminoethyl methacrylate) (PDMAEMA) nanohybrids [28] were also synthesized for RNA delivery to obtain improved gene transfection efficiency and good biocompatibility. Teimouri et al. prepared three different GO-based nanocarriers for gene delivery based on the conjugation of GO with cationic polymers of PEI, polypropylenimine (PPI), and PAMAM to com- pare their cytotoxicity and transfection efficiency [29]. GFP was used to evaluate the transfection efficiency and the results showed that PEI-GO conjugate was nine fold more effective in the EGFP- transfected cells .Choi et al. also prepared GO-PEI complexes to effectively load mRNA for clinical applications [30]. After treatment with GO-PEI/RNA complexes, the cells increased their reprogramming efficiency and rat and human induced pluripotent stem cells (iPSCs) were generated successfully from adult adipose tissue-derived fibroblasts without repetitive daily transfection. In addition to DNA, proteins can also be delivered using graphene-based materials for gene therapy. Zhang et al. reported the co-delivery of ribonuclease A and protein kinase A to the cell cytoplasm without enzymatic hydrolysis and loss of biological activity intracellularly, by loading proteins onto GO-PEG with a high payload via noncovalent interactions [31]. Hong and co-

workers used multilayer GO-poly (b-amino ester) to entrap ovalbumin (OVA), a protein antigen [32], and demonstrated that the multilayer films blocked the initial burst release of OVA and could be precisely controlled to trigger its release upon the application of electrochemical potentials. Other examples include loading bone morphogenic protein-2 (BMP-2) to a GO-coated Ti substrate to enhance the differentiation of human mesenchymal stem cells (MSCs), which also showed robust new bone formation with this Ti-GO-BMP2 implant, as demonstrated by Char et al. [33] in addition, Geest and co-workers reported that intracellular protein vaccine delivery of GO-adsorbed proteins that could be efficiently internalized by dendritic cells and promoted antigen cross-presentation to CD8 T cells [34].

TISSUE ENGINEERING

Tissue engineering has emerged as a significant novel medical strategy to restore, maintain, or improve the function of a tissue or whole organ by using a combination of cells, engineering materials, and suitable biochemical factors [35]. A crucial parameter for tissue engineering is to develop suitable bio materials that can mimic the biological environment and provide surfaces to interface with living cells, enabling cell attachment, proliferation, and differentiation. Given their ease of functionalization combined with fascinating mechanical strength, stiffness, and electrical conductivity, graphene-based materials have attracted tremendous interest in the tissue-engineering field [36, 37]. One application of graphene-based materials in tissue engineering is as reinforcement materials in hydrogels, films, fibers, and other tissue-engineering scaffolds, to enhance their mechanical properties and stability by utilizing the mechanical strength and stiffness of graphene based materials.

Cerruti et al prepared a 3D GO/HA hydrogel for bone tissue engineering [38].The resulting highly porous hydrogel showed strong mechanical properties, high electrical conductivity, and good cell compatibility to MSCs, making them excellent candidates for bone tissue- engineering applications. GO nanosheets have also been shown to greatly improve the mechanical properties of poly (acrylic acid) PAA)/gelatin hydrogels and chitosan hydrogel scaffolds [39, 40]. Additionally, Ruoff and co-workers presented polyoxyethylene sorbitan laurate (TWEEN) and rGO hybrid films [41]. They demonstrated that the as-prepared hybrid films were mechanically robust, biocompatible with three different mammalian cell lines, and antimicrobial. Xie et al. prepared chitosan-poly (vinyl alcohol) nanofiber-containing graphene by using the electrospinning method [42]. They found that these reinforced fibers showed rapid wound healing in mice and rabbits, because of the presence of free electrons in graphene inhibiting prokaryotic cell multiplication but without affecting the proliferation of eukaryotic cells. In addition to mechanical enhancement, the electrical proper- ties of graphene-based materials enable researchers to use them for specific tissue-engineering applications.

Hong et al. reported that a graphene substrate promoted the adhesion and neural differentiation of human neural stem cells (hNSCs) [43]. The authors proposed that the mechanism of enhanced differentiation to neurons was electrical coupling between the hNSCs and the graphene. Cheng et al. demonstrated the promotion of neurite sprouting and outgrowth of mouse hippocampal cells on graphene substrates [44].They speculated that the high electrical conductivity of graphene led to better neurite outgrowth, indicating that graphene could be used as an implant material or neural chip for tissue engineering in the nervous system. Similar results were also reported by Liu et al. [45]. Recently, Wang and co-workers reported a hybrid conducting system of poly(3,4-ethylenedioxythiophene) (PEDOT)-rGO microfibers [46]. These hybrid micro- fibers exhibited enhanced cell proliferation and improved neural differentiation of MSCs with electrical pulses induced by a self- powered triboelectric nanogenerator. Loh et al. studied the culture of bone marrow-derived MSCs on graphene and GO substrates [47]. They found that the strong noncovalent binding abilities of graphene arising from p–p stacking, electrostatic, and hydrogen bonding allowed it to act as a pre concentration platform for osteogenic inducers, which accelerated the growth of MSCs toward the osteogenic lineage. In addition to their mechanical

and electrical properties, the surface functionalization of graphene-based materials is also useful for tissue-engineering applications.

Gu et al. reported that amine- functionalized GO showed superior cell viability and proliferation with excellent anticoagulation effects [48]. Estrada and co-workers reported the elongated structure of multinucleated myoblasts on laminin-coated graphene, indicating their differentiation into myotube cells [49]. GO has also been shown to help cells differentiate into skeletal muscle, likely because of their roughness and serum protein absorption [50].It is also believed that absorbing fibronectin on a graphene-based material surface would promote articular cartilage regeneration [51]. GO has been found to help adipose tissue generation, the mechanism of which was attributed to absorption of insulin [47]. Additionally, Zeng et al. reported gelatin-functionalized GO (GO-Gel) for the biomimetic mineralization of HA [53]. They demonstrated that the GO-Gel promoted cell adhesion, proliferation, and osteogenic differentiation of MC3T3-E1 cells, indicating that GO-Gel could be used as osteogenesis-promoting scaffold for bone tissue engineering. Zeng and co-workers also reported similar results using GO functionalized with the polysaccharide carrageenan [54]. In addition, the chemical inertness and impermeability of graphene enable it to be used as a biocompatible anticorrosion coating for metallic biomedical devices. Rao et al. reported that graphene coating enhanced both the bio- and hemocompatibility of implant materials [55, 56]. Yang and co-workers demonstrated the use of graphene as a protection film in biological environments [57]. They conducted both in vitro cellular experiments and in vivo animal experiments to demonstrate the effective protection of metal by graphene under biological conditions (Figure 1).

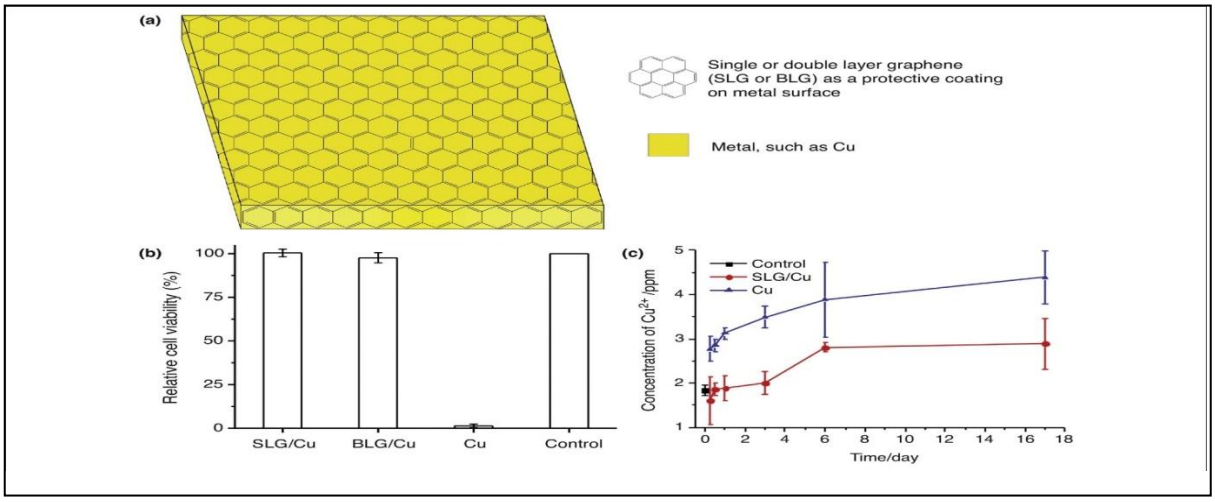

Figure 1: Courtesy - Zhang, W. et al. (2014) Use of graphene as protection film in biological environments. Sci. Rep. 4, 4097

Their results highlight the potential of using graphene coatings to protect metal surfaces in biomedical applications, such as metal implants. Wei et al. reported that a GO/HA-coated Ti substrate also exhibited high corrosion resistance, with enhanced coating adhesion strength and superior cell viability [57].

Toxicity of graphene based nanomaterials

So far, graphene-based materials have exhibited huge potential in numerous biomedical applications. To further expand their use and advance the translation of these applications from the lab to a clinical setting, a deeper understanding of their potential toxicity is required. Intense efforts have been devoted to elucidating the toxic effects of graphene and its derivatives. However, the molecular mechanism of the toxicity of graphene-based materials is still largely unclear. Therefore, it is currently inappropriate to draw any generalized conclusions about the toxic effects of

graphene-based materials, particularly when regarding the variety of different forms of graphene and methods of synthesis Graphene and its related congeners have been reported to cause cell damage via inducing generations of reactive oxidant species [45, 59] and/or mutations during cell replication [60, 61].Their toxicity has been demonstrated to be time [62, 63], dose and/or concentration [64, 180], and characteristic [61] dependent. Cell–graphene interactions in vitro have been shown to depend on multiple parameters, including surface chemistry, graphene size and/or shape, and impurities. The surface chemistry of graphene has an essential role in determining its cytotoxicity. Generally, bare graphene or GO can interfere with the cell membrane via adhesion or bonding with cell receptors to block the supply of nutrients, induce stress, and activate apoptotic mechanisms, resulting in high toxicity even at low concentrations [65]. Functionalization of graphene based materials with biocompatible polymers or molecules will greatly reduce cytotoxicity. Dai et al. showed that PEGylation of GO exhibited no cellular toxicity up to 100 mg/ml [66, 1]. Alexis and co-workers reported that graphene with PEG and PLA modification showed no dose-dependent toxicity up to 250 mg/ml for U138 cells, whereas hydroxylated graphene showed toxicity at 50 mg/ml in the same study [67].

In addition to surface functionalization, the size of graphene nanomaterials also affects their interaction with cells and their cytotoxicity. Wang et al. studied the in vitro toxicity of GO on A549 cells [64]. They reported that graphene oxide of size 160 T 90 nm induced less cell mortality compared with 780 T 410 nm at the same concentration of 200 mg/ml. However, larger size does not always cause less cytotoxicity. Akhavan and collaborators indicated that, in human MSC, rGO nanoplatelets with smaller lateral dimensions (11 T 4 nm) showed higher cytotoxicity than those with larger lateral dimensions (3.8 T 0.4 mm) [61]. These contradictory observations could be explained by different interaction between cells and particles with different sizes, because Yan et al. demonstrated that small nanosheets entered cells mainly via clathrin mediated endocytosis, while the increase in graphene size enhanced the phagocytotic uptake of the nanosheets [68].Impurities from the preparation process of GO could affect its biocompatibility. Kostarelos and co-workers reported that purified GO had negligible negative effects in vitro and in vivo compared with as-synthesized GO [69]. Therefore, it is necessary to conduct careful biological effects studies of these impurities. In addition to in vitro studies, experiments were also performed in vivo to investigate graphene toxicity, which demonstrates more complexity when animal models are used. Typically, after intra- venous injection, graphene materials were found in spleen, liver, and lung. However, Liu et al. reported that intraperitoneal injection of PEGylated graphene resulted in accumulation in spleen and liver, instead of lung [70]. By contrast, when graphene materials are administered via inhalation, they typically accumulate in the lung and often induce inflammation [71]. Intraocular administration of GO was studied in rabbits, and showed little effect on eyeball appearance, intraocular pressure eyesight, or histology sections [72]. Dash and collaborators reported that intravenous administration of GO induced extensive pulmonary thromboembolism in mice [73], whereas fewer effects were observed for rGO in the same study. Contradictory results were also observed in in vivo experiments, because GO was found to induce high hemolytic activity [74], which was inconsistent with the results of Wang et al. who reported that intravenous injection of GO at 0.1 mg and 0.25 mg showed no obvious toxicity [75]. Thus, we are still some way from completely understanding the toxicity of graphene-based materials.

CONCLUSIONS

The biomedical applications related to the unique physiochemical properties of Graphene based Nanoparticles focus on their thermal, mechanical and electrochemical features. The intrinsic optical properties of Graphene based Nanoparticles -based hybrids in the visible and NIR range along with their small size effects, low toxicity and low production costs make the hybrids attractive for bioimaging in clinical diagnostics and photo-thermal cancer therapy. This targeted therapy aids in their high therapeutic effects and

fewer side effects. The use of Graphene based Nanoparticles in bioimaging and biosensing fields are an emerging biomedical application. The existing literature does not provide detailed information on the various synthesis procedures and characterization techniques before proceeding to the toxicological assays. Currently, most of the Graphene based Nanoparticles are focused on lungs and liver. Studies on other organs including brain/central nervous system are very limited or remain unexplored. To our knowledge, the reports on Graphene based Nanoparticles as endocrine disruptors are very limited. Additional studies in these areas are also necessary. Also, more research is required to optimize the synthesis with proper characterization methods to the Graphene based Nanoparticles with unique properties. Such research would provide a scientific basis to manage their uses and control/prevent their toxic effects.

REFERENCES

[1]. Sun, X. *et al*. Nano-graphene oxide for cellular imaging and drug delivery, *Nano Res.* 1, 2008, 203–212
[2]. Jin, R. *et al.* Self-assembled graphene-dextran nanohybrid for killing drug- resistant cancer cells. *ACS Appl. Mater Interfaces* 5, 2013, 7181–7189
[3]. Dinda, S. *et al.* Grafting of ZnS:Mn-doped nanocrystals and an anticancer drug onto graphene oxide for delivery and cell labeling. *ChemPlusChem* 81, 2016, 100–107
[4]. Chen, H. *et al.* Fe2O3@Au core@shell nanoparticle-graphene nanocomposites as theranostic agents for bioimaging and chemo-photothermal synergistic therapy. *RSC Adv.* 5, 2015, 84980–84987
[5]. Zhou, K. *et al.* One-pot preparation of graphene/Fe3O4 composites by a solvothermal reaction. *New J. Chem.* 34, 2010, 2950
[6]. Chen, W. *et al.* Assembly of Fe3O4 nanoparticles on PEG-functionalized graphene oxide for efficient magnetic imaging and drug delivery. *RSC Adv.* 5, 2015, 69307–69311
[7]. Xu, Z. *et al*. Covalent functionalization of graphene oxide with biocompatible poly (ethylene glycol) for delivery of paclitaxel. *ACS Appl. Mater Interfaces* 6, 2014, 17268–17276
[8]. Zhang, C. *et al.* (2016) Co-delivery of paclitaxel and indocyanine green by PEGylated graphene oxide: a potential integrated nanoplatform for tumor theranostics. *RSC Adv.* 6, 15460–15468
[9]. Angelopoulou, A. *et al.* Graphene oxide stabilized by PLA-PEG copolymers for the controlled delivery of paclitaxel. *Eur. J. Pharm. Biopharm.* 93, 2015, 18–26
[10]. Tian, L. *et al.* Functionalized nanoscale graphene oxide for high efficient drug delivery of cisplatin. *J. Nanopart. Res.* 16, 2014, 2709
[11]. Zhang, L. *et al*. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. *Small* 6, 2010, 537–544
[12]. Pan, Y. *et al.* Water-soluble poly(N-isopropylacrylamide)-graphene sheets synthesized via click chemistry for drug delivery. *Adv. Funct. Mater.* 21, 2011, 2754–2763
[13]. Tian, B. *et al.* Photothermally enhanced photodynamic therapy delivered by nanographene oxide. *ACS Nano* 5, 2011, 7000–7009
[14]. Rana, V.K. *et al.* Synthesis and drug-delivery behavior of chitosan- functionalized graphene oxide hybrid nanosheets. *Macromol. Mater. Eng.* 296, 2011, 131–140
[15]. Fan, X. *et al.* The preparation and drug delivery of a graphene-carbon nanotube-Fe3O4 nanoparticle hybrid. *J. Mater. Chem. B* 1, 2013, 2658–2664
[16]. Zhou, L. *et al.* Graphene oxide noncovalent photosensitizer and its anticancer activity *in vitro*. *Chem. Eur. J.* 17, 2011, 12084–1209
[17]. Goenka, S. *et al.* Graphene-based nanomaterials for drug delivery and tissue engineering. *J. Control. Release* 173, 2014, 75–88
[18]. Yang, Z.R. *et al.* Recent developments in the use of adenoviruses and immunotoxins in cancer gene therapy. *Cancer Gene Ther.* 14, 2007, 599–615
[19]. Hu, H. *et al.* Folate conjugated trimethyl chitosan graphene oxide nanocomplexes as potential carriers for

drug and gene delivery. *Mater. Lett.* 125, 2014, 82– 85
[20]. Liu, X. *et al.* Polyamidoamine dendrimer and oleic acid-functionalized graphene as biocompatible and efficient gene delivery vectors. *ACS Appl. Mater Interfaces* 6, 2014, 8173–8183
[21]. Hu, H. *et al.* Folate conjugated trimethyl chitosan graphene oxide nanocomplexes as potential carriers for drug and gene delivery. *Mater. Lett.* 125, 2014, 82– 85
[22]. Tian, B. *et al.* Photothermally enhanced photodynamic therapy delivered by nanographene oxide. *ACS Nano* 5, 2011, 7000–7009
[23]. Feng, L. *et al.* Graphene based gene transfection. *Nanoscale* 3, 2011, 1252–1257
[24]. Cao, X. *et al.* Functionalized graphene oxide with hepatocyte targeting as anti-tumor drug and gene intracellular transporters. *J. Nanosci. Nanotechnol.* 15, 2015, 2052–2059
[25]. Hu, H. *et al.* Folate conjugated trimethyl chitosan graphene oxide nanocomplexes as potential carriers for drug and gene delivery. *Mater. Lett.* 125, 2014, 82– 85
[26]. Liu, X. *et al.* Polyamidoamine dendrimer and oleic acid-functionalized graphene as biocompatible and efficient gene delivery vectors. *ACS Appl. Mater Interfaces* 6, 2014, 8173–8183
[27]. Yang, H.-W. *et al.* Gadolinium-functionalized nanographene oxide for delivery combined drug and microRNA and magnetic resonance imaging. *Nano Res.* 12274, 2014.
[28]. Sun, Y. *et al.* Fabrication of mPEGylated graphene oxide/poly (2- dimethyl amino ethyl methacrylate) nanohybrids 2016.
[29]. Teimouri, M. *et al.* Graphene oxide-cationic polymer conjugates synthesis and application as gene delivery vectors. *Plasmid* 2016, 84–85, 51–60
[30]. Choi, H.Y. *et al.* Efficient mRNA delivery with graphene oxide- poly ethylenimine for generation of footprint-free human induced pluripotent stem cells. *J. Control. Release* 235, 2016, 222–235
[31]. Shen, H. *et al.* PEGylated graphene oxide-mediated protein delivery for cell function regulation. *ACS Appl. Mater Interfaces* 4, 2012, 6317–6323
[32]. Choi, M. *et al.* Multilayered graphene nanofilm for controlled protein delivery by desired electro-stimuli. *Sci. Rep.* 5, 2015, 17631
[33]. La, W.-G. *et al.* Delivery of a therapeutic protein for bone regeneration from a substrate coated with graphene oxide. *Small* 9, 2013, 4051–4060
[34]. Li, H. *et al.* Spontaneous protein adsorption on graphene oxide nanosheets allowing efficient intracellular vaccine protein delivery. *ACS Appl. Mater Interfaces* 8, 2016, 1147–1155
[35]. Langer, R. and Vacanti, J. Tissue engineering. *Science* 260, 1993, 920–926
[36]. Shin, S.R. *et al.* Cell-laden micro engineered and mechanically tunable hybrid hydrogels of gelatin and graphene oxide. *Adv. Mater.* 25, 2013, 6385–6391
[37]. Cha, C. *et al.* Controlling mechanical properties of cell-laden hydrogels by covalent incorporation of graphene oxide. *Small* 10, 2014, 514–523
[38]. Xie, X. *et al.* Graphene and hydroxyapatite self-assemble into homogeneous, free standing nanocomposite hydrogels for bone tissue engineering. *Nanoscale* 7, 2015, 7992–8002.
[39]. Faghihi, S. *et al.* Graphene oxide/poly (acrylic acid)/gelatin nanocomposite hydrogel: experimental and numerical validation of hyper elastic model. *Mater. Sci. Eng. C* 38, 2014, 299–305.
[40]. Depan, D. *et al.* Structure-process-property relationship of the polar graphene oxide-mediated cellular response and stimulated growth of osteoblasts on hybrid chitosan network structure nanocomposite scaffolds. *Acta Biomater.* 7, 2011, 3432–344.
[41]. Park, S. *et al.* Biocompatible, robust free-standing paper composed of a TWEEN/graphene composite. *Adv. Mater.* 22, 2010, 1736–1740
[42]. Lu, B. *et al.* Graphene-based composite materials beneficial to wound healing. *Nanoscale* 4, 2012, 2978–2982.
[43]. Park, S.Y. *et al.* Enhanced differentiation of human neural stem cells into neurons on graphene. *Adv. Mater.* 23(36), 2011, H263–H267.
[44]. Li, N. *et al.* The promotion of neurite sprouting and outgrowth of mouse hippocampal cells in culture by

graphene substrates. *Biomaterials* 32, 2011, 9374– 9382.
[45]. Yang, K. *et al.* Behavior and toxicity of graphene and its functionalized derivatives in biological systems. *Small* 9, 2013, 14.
[46]. Guo, W. *et al.* Self-powered electrical stimulation for enhancing neural differentiation of mesenchymal stem cells on graphene-poly (3,4- ethylene dioxy thiophene) hybrid microfibers. *ACS Nano* 10, 2016, 5086–5095
[47]. Lee, W.C. *et al.* Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. *ACS Nano* 5, 2011, 7334–7341
[48]. Guo, M. *et al.* N-containing functional groups induced superior cytocompatible and hemocompatible graphene by NH2 ion implantation. *J. Mater. Sci. Mater. Med.* 24, 2013, 2741–2748
[49]. Krueger, E. *et al.* Graphene foam as a three-dimensional platform for myotube growth. *ACS Biomater. Sci. Eng.* 2, 2016, 1234–1241
[50]. Ku, S.H. and Park, C.B. Myoblast differentiation on graphene oxide. *Biomaterials* 34, 2017–2023
[51]. Yoon, H.H. *et al.* Dual roles of graphene oxide in chondrogenic differentiation of adult stem cells: cell-adhesion substrate and growth factor- delivery carrier. *Adv. Funct. Mater.* 24, 2014, 6455–6464
[52]. Lee, W.C. *et al.* Origin of enhanced stem cell growth and differentiation on graphene and graphene oxide. *ACS Nano* 5, 2011, 7334–7341
[53]. Liu, H. *et al.* Gelatin functionalized graphene oxide for mineralization of hydroxyapatite: biomimetic and in vitro evaluation. *Nanoscale* 6, 2014, 5315–5322.
[54]. Liu, H. *et al.* Biomimetic and cell-mediated mineralization of hydroxyapatite by carrageenan functionalized graphene oxide. *ACS Appl. Mater Interfaces* 6, 2014, 3132–3140
[55]. Podila, R. *et al.* Graphene coatings for enhanced hemo compatibility of nitinol stents. *RSC Adv.* 3, 2013, 1660–1665
[56]. Podila, R. *et al.* Graphene coatings for biomedical implants. *Journal Title* XX, e50276.
[57]. Zhang, W. *et al.* Use of graphene as protection film in biological environments. *Sci. Rep.* 4, 2014, 4097
[58]. Li, M. *et al.* Graphene oxide/hydroxyapatite composite coatings fabricated by electrophoretic nanotechnology for biological applications. *Carbon* 67, 2014, 185– 197
[59]. Bianco, A. Graphene: safe or toxic? The two faces of the medal. *Angew. Chem. Int. Ed.* 52, 2013, 4986–4997
[60]. Akhavan, O. *et al.* Genotoxicity of graphene nanoribbons in human mesenchymal stem cells. *Carbon* 54, 2013, 419–431
[61]. Akhavan, O. *et al.* Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. *Biomaterials* 33, 2012, 8017–8025
[62]. Akhavan, O. *et al.* Size-dependent genotoxicity of graphene nanoplatelets in human stem cells. *Biomaterials* 33, 2012, 8017–8025
[63]. Liu, S. *et al.* Lateral dimension-dependent antibacterial activity of graphene 2012.
[64]. Chang, Y. *et al. In vitro* toxicity evaluation of graphene oxide on A549 cells. *Toxicol. Lett.* 200, 2011, 201–210
[65]. Jaworski, S. *et al.* In vitro evaluation of the effects of graphene platelets on glioblastoma multiforme cells. *Int. J. Nanomed.* 8, 2013, 413–420
[66]. Liu, Z. *et al.* PEGylated nanographene oxide for delivery of water-insoluble cancer drugs. *J. Am. Chem. Soc.* 130, 2008, 10876–10877.
[67]. Moore, T.L. *et al.* Systemic administration of polymer-coated nanographene to deliver drugs to glioblastoma. *Part. Part. Syst. Charact.* 31, 2014, 886–894
[68]. Mu, Q. *et al.* Size-dependent cell uptake of protein-coated graphene oxide nanosheets. *ACS Appl. Mater Interfaces* 4, 2012, 2259–2266
[69]. Yang, K. *et al.* In vivo pharmacokinetics, long-term bio distribution, and toxicology of PEGylated graphene in mice. *ACS Nano* 5, 2011, 516–522
[70]. Ducssh, M.C. *et al.* Minimizing oxidation and stable nanoscale dispersion improves the biocompatibility

of graphene in the lung. *Nano Lett.* 11, 2011, 5201–5207
[71]. Yan, L. *et al*. Can graphene oxide cause damage to eyesight? *Chem. Res. Toxicol*. 25, 2012, 1265–1270
[72]. Singh, S.K. *et al*. Thrombus inducing property of atomically thin graphene oxide sheets. *ACS Nano* 5, 2011, 4987–4996
[73]. Singh, S.K. *et al*. Thrombus inducing property of atomically thin graphene oxide sheets. *ACS Nano* 5, 2011, 4987–4996
[74]. Liao, K.-H. *et al*. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. *ACS Appl. Mater Interfaces* 3, 2011, 2607–2615.
[75]. Wang, K. *et al*. Biocompatibility of graphene oxide. *Nanoscale Res. Lett.* 6, 2011, 8.