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APPLICATION OF GRAPHENE IN EXPERIMENTAL PHYSIOLOGY RESEARCH

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Abstract. Graphene is a novel advanced material with numerous potential applications in fundamental and clinical medicine. Graphene compounds can be successfully used as an integral part of drug delivery systems for treatment of various diseases. Graphene-based controlled drug release in target tissues may particularly be useful in designing new cancer therapy strategies. This material might also be used for gene transfection and delivery, as a basis for future gene therapy. Finally, some graphene compounds can be used for design of probes for imaging and detection of physiological and pathological changes on a cellular level. Before any of these approaches and strategies can be successfully applied in clinical medicine, potential cytotoxicity and genotoxicity of graphene needs to be disproven. This concise review is focused on recent research on graphene in experimental physiology, pharmacology and related fields.

1. INTRODUCTION

Graphene is a novel, advanced, carbon-based material in which carbon atoms form a two-dimensional structure resembling a honey-comb lattice. Although the chemical and physical properties of other carbon allotropes, such as graphite are known, graphene has some unique qualities that make it suitable for use in various industrial and scientific disciplines. For example, the strength of graphene may be many times greater than many commonly used strong materials in industry (i.e. steel). Also, graphene is an extraordinary conductor of electricity and heat, which makes it a good candidate for electrical engineering innovations and patents. Furthermore, this material is relatively affordable to manufacture and store. All these facts have contributed to the growing interest in graphene applications in natural sciences [1-5].

Potential value of graphene in fundamental and clinical medicine has particularly been under investigation in recent years. It was suggested that derivatives of this material might be incorporated into contrast agents for cellular imaging. Graphene oxide (GO) may also be of great importance in gene transfection. Interactions between graphene and nucleic acids are unique, and are potentially valuable in designing novel gene therapies. Graphene

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nanoparticles could also act as a potential part of drug delivery system [6-10], especially during cancer therapy. Before any of these approaches and strategies can be applied in clinical medicine, potential cytotoxicity and genotoxicity of graphene needs to be disproven. This concise review is focused on recent research on graphene in experimental physiology, pharmacology and related fields.

2. GRAPHENE AS POTENTIAL MEDIUM FOR DRUG AND PROTEIN DELIVERY

So far, there have been numerous attempts to synthetize a graphene – based nanosystem capable of efficient delivery and release of medications to target cells. Many of these systems in in vitro conditions appear effective, however in the future, it remains to be seen whether they behave similarly in a living organism (laboratory animals).

Nanoscale graphene oxide system functionalized with polyethylene glycol, was associated with camptothecin analog, SN38 and tested by Liu et al. (2008). Camptothecin generally is a highly potent anticancer substance with strong inhibiting effects on a DNA enzyme which participates in cell proliferation. One of the main disadvantages of camptothecin is its low solubility. By associating it with a graphene-based nanocomposite, its water solubility substantially improved, and was higher when compared with other FDA-approved soluble versions of SN38 currently present in the market [6].

In 2011, Depan and colleagues synthesized a graphene mediated drug delivery system for controlled release of another anticancer medication, doxorubicin. Graphene oxide system was encapsulated by folic acid conjugated chitosan. Doxorubicin attachment/detachment was dependent on pH, and acidic environment increased the probability of release. This is one of first studies to investigate graphene-chitosan interactions, and provides a basis for further research on applicability of these compounds in oncology [7].

5-fluorouracil as an anticancer medicament, was also successfully associated with graphene system. Fan et al. (2013) described a simple and relatively affordable procedure of manufacturing a graphene nanosheet-carbon nanotube-iron oxide nanoparticle hybrid. This hybrid was designated as a potentially useful platform for delivery of 5-fluorouracil with good loading capacity and pH-activated release. The platform was tested on Hep G2 a human liver cancer cell line and showed considerable cytotoxicity [8].

Another important work on 5-fluorouracil-graphene interactions is the one of Rana et al. (2011) in which the authors manufactured chitosan-functionalized graphene oxides. The overall controlled release behavior of these compounds was shown to be promising in terms of potential future applicability in biomedicine. Apart from 5-fluorouracil, chitosan-functionalized graphene oxides were also loaded with ibuprofen with interesting results [9].

A water-insoluble anticancer medicament, camptothecin was also successfully associated with graphene. In 2011, Pan et al. described poly (N-isopropylacrylamide)–graphene sheets created using click chemistry methods. The in vitro drug loading and release profiles were determined to be excellent, and cytotoxic effects on cancer cells were potent [10].

Apart from medications, it was hypothesized that graphene-based systems can be used for delivery and release of different proteins, mediators and growth factors. For example, PEGylated graphene oxide can be used as nanovector for transport of proteins to cytoplasm. Because of the unique chemical and physical properties of this compound, the proteins are protected from enzymatic hydrolysis. Ribonuclease A and protein kinase A are some of the proteins that can be delivered this way, with the resulting cell death or growth, respectively [11].

Titanium (Ti) substrates coated with graphene oxide may also serve as mediums for delivery of therapeutic proteins. La et al. (2013) demonstrated that these compounds may be loaded with bone morphogenetic protein-2 (BMP-2), which plays a significant role in differentiation (osteogenic) of human bone marrow-derived mesenchymal stem cells. On an animal experimental model of calvarial defects, it was shown that this system may be important part of titanium implants for therapeutic purposes [12].

3. GRAPHENE-NUCLEIC ACID INTERACTIONS

Physical and chemical interactions between graphene and ribonucleic acids are particularly interesting. Recent research has demonstrated that graphene compounds, such as graphene oxide, have different affinity to single stranded DNA, compared to double stranded DNA (Goenka et al. 2014; He et al. 2014). These differences underline potential importance of graphene-DNA conjugation in designing novel methods in molecular genetics [13,14].

Lu et al (2010) suggested that graphene may have certain protective effect, when associated with DNA molecule. Functionalized nanoscale graphene oxide may form strong and stable connections with oligonucleotides and protect them from enzymatic cleavage. Also, this compound facilitates transport of oligonucleotides to intracellular space which may be valuable for future strategies in genetic engineering [15].

Zhang et al. (2011) used polyethylenimine (PEI)-functionalized graphene oxide for sequential delivery of Bcl-2-targeted siRNA. Bcl-2 gene and its protein is considered to be an important regulator of programmed cell death. Known anti-apoptotic effects of Bcl-2 makes it a potentially valuable target for anticancer therapy. Transfection of Bcl-2-targeted siRNA using graphene-based compounds is a novel approach in cancer molecular biology with promising applications [16].

Polyethylenimine functionalized graphene oxide is also a potentially potent vector for gene delivery. A study by Chen et al. (2011) suggested that this system might have relatively good transfection efficiency [17]. Delivery of plasmid DNA to the nucleus of the cells is this way safe and less toxic. Apart from these researchers, other authors also described and evaluated graphene –associated gene transfection as a candidate for novel non-viral based gene therapies [18,19].

Finally, it should be mentioned that graphene oxide can be functionalized with chitosan biopolymer to become a nanocarrier for gene delivery (Bao et al. 2011). Chitosan – grafted graphene oxide are soluble in water and may condense plasmid DNA into a system which has relatively good stability in in vitro conditions. It was shown that this platform is capable of effective gene transfection to cancer HeLa cells [20].

4. GRAPHENE AND CELLULAR IMAGING

Contemporary imaging methods for detection of physiological and pathological changes on a cellular level rely on application of effective contrast agents. In fluorescence microscopy, an ideal contrast must be specific for its target, nontoxic, and its effects should be measurable. Furthermore, it must not interact or modify the reaction/physiological process it is trying to mark and quantify. Graphene and its compounds are potentially good candidates to become an addition, or even replacement of dyes used for fluorescence and photoluminescence techniques.

Some recent research has suggested that graphene oxide can be converted to magnetic graphene which can then make a polyacrylic acid bridge for linking the fluorescein o-methacrylate, a compound used in fluorescence imaging [21]. This novel marker did not exhibit significant toxicity eeither in HeLa cell culture, or in animal experimental models such as zebrafish. It seems that multifunctional graphene is localized specifically in cell cytoplasm.

Graphene quantum dots (GQDs) as a part of imaging bioassays are also under investigation in some laboratories. For example, this new type of quantum dots (obtained from graphene oxide sheets) can be associated with anti-human IgG antibody which has previously been conjugated with Fe3O4 nanoparticles. These complex probes could be used for urine analysis as a part of diagnostic procedures for renal diseases [22].

Graphene quantum dots are also potentially useful in detection of various types of ions. Zhang et al. (2016) reported that these nanoparticles can act as highly sensitive fluorescent probes for identification of iron Fe(3+) ions with wide linear response concentration range. In this work, this platform was manufactured using one-step microwave assisted pyrolysis of aspartic acid and NH4HCO3 [23].

There are many different chemical methods for GQDs synthesis and transformation into imaging probes. Luminescent GQDs can be manufactured during ameliorative photo-Fenton reaction from graphene oxide sheets with hydrothermal processing as reported by Jiang et al. (2015). Strong green luminescence that these GQDs emit in water environment enables them to be used not only for conventional cell imaging but also for biolabelling of different structures. Estimated toxicity in cell cultures and animal models, similarly as in other studies, is relatively low [24].

5. TOXICITY CONCERNS

With the rapid development of nanobiology and nanomedicine, safety of nanomaterials became one of the priorities in research. Many nanooarticle system may in some circumstances have potentially detrimental effects on human health. Also, many nanomaterials in some concentrations, may exhibit cytotoxic, genotoxic, or proapoptotic effects in cell cultures [25-28].

At present, graphene quantum dots as potential probes for imaging are considered relatively safe. Data obtained by Chong et al. (2014) shows that graphene quantum dots, when prepared using fac-

ile oxidation approach, have low cytotoxicity in in vitro conditions. In vertebrates (mice), biodistribution of GQDs indicates that there is neither accumulation in tissues nor damage to main organs [29]. Other authors also reported no significant acute toxicity of graphene or carbon quantum dots [30,31].

However, graphene oxide functionalized with polyethylene glycol, may accumulate in various organs. Organs of reticuloendothelial (RES) system show significant morphological changes due to the GO aggregation. It is thought that this accumulation in liver and spleen may in some cases be so significant that it causes death of the treated animal [32]. In cell cultures, high concentrations of GO can cause oxidative stress and decrease cell viability [32].

Long-term toxicity of graphene is poorly understood, and many questions remain unanswered. For example, there is some limited evidence of teratogenic effects of reduced graphene oxide nanosheets [33]. Although this material does not influence levels of sex hormones, high doses may cause abortions in pregnant mice when treated at a late gestational stage [33].

Potential cancerogenicity of graphene and its derivatives is also unknown. Graphene interacts with DNA molecule in a unique way, but it is unclear if it can cause deletions, translocations or other mutations in a genetic material. Also, immunotoxicity of graphene is unclear and needs to be tested in future studies. It is unclear if long-term administration of graphene compounds has any immunostimulatory of immunosuppressive effects in living organisms.

6. CONCLUDING REMARKS

Graphene is a novel advanced material with numerous potential applications in fundamental and clinical medicine. Graphene compounds can be successfully used as an integral part of drug delivery systems for treatment of various diseases. Graphene-based controlled drug release in target tissues may particularly be useful in designing new cancer therapy strategies. This material can also be used for gene transfection and delivery as a basis for gene therapy. Finally, some graphene compounds can be used for design of probes for imaging and detection of physiological and pathological changes on a cellular level. Before graphene can be applied in clinical medicine, some important issues need to be addressed. Both acute and chronic toxicity of this material is poorly understood. Also it is unknown it this material exhibits cancerogenic or immunotoxic effects.

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