Radio-graphene in Theranostic Perspectives

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Received: 26 November 2015 / Revised: 22 January 2016 / Accepted: 3 March 2016 / Published online: 18 March 2016
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Abstract Owing to its unique physicochemical properties such as high surface area, notable biocompatibility, robust mechanical strength, high thermal conductivity, and ease of functionalization, 2D-layered graphene has received tremendous attention as a futuristic nanomaterial and its-associated research has been rapidly evolving in a variety of fields. With the remarkable advances of graphene especially in the biomedical realm, in vivo evaluation techniques to examine in vivo behavior of graphene are largely demanded under the hope of clinical translation. Many different types of drugs such as the antisense oligomer and chemotherapeutics require optimal delivery conveyor and graphene is now recognized as a suitable candidate due to its simple and high drug loading property. Termed as ‘radio-graphene’, radioisotope-labeled graphene approach was recently harnessed in the realm of biomedicine including cancer diagnosis and therapy, contributing to the acquisition of in vivo information for targeted drug delivery. In this review, we highlight current examples for bioapplication of radiolabeled graphene with brief perspectives on future strategies in its extensive bio- or clinical applications.

Keywords In vivo cancer targeting · PET imaging · Radio-graphene · Theranostics

Since two eminent scientists, Andre Geim and Konstantin Novoselov, were given the 2010 Nobel Prize in Physics for 2-dimensional sheet-like material graphene, the research in this field has exponentially expanded its territory beyond electronics and chemistry with developing an ultrafast conductor toward biological and biomedical field [1–7]. Known for its remarkable structural and physicochemical properties, graphene has attracted a substantial amount of interest in numerous fields in science including/especially in nanoscience/medicine/technology. The sp^2 -hybridized carbon atoms in a honeycombed network provide graphene with a large surface area ($2630 \text{ m}^2\text{g}^{-1}$), outstanding thermal conductivity ($\sim 5000 \text{ Wm K}^{-1}$), high mechanical strength ($\sim 1.1 \text{ TPa}$), and elasticity [8]. These properties of graphene have provided a stepping-stone for the development of nanocarriers for drug and gene delivery, biosensors, cell imaging, and phototherapy of cancer [9–20]. Doxorubicin loaded onto PEG-NGO conjugate is a primary example that utilizes the delocalized surface π -electrons in graphene to enhance the solubility of poorly soluble drugs [6]. The cationic polyethylenimine (PEI) polymer that has a strong electrostatic interaction with the negatively charged phosphate of RNA or DNA can be easily decorated onto the surface of graphene in gene therapy, which enables it to treat genetic disorders like cystic fibrosis, Parkinson’s disease, and cancer whilst significantly lowering the cytotoxicity of PEI [6]. Furthermore, the ability to adsorb single-stranded DNA onto graphene sheets, the capacity to quench electron donors, and the function of transportation in living cells and in vivo systems has made graphene even more promising [21]. However, in order to apply these outstanding capabilities of graphene to the biomedical field, numerous

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unsolved issues such as non-homogenous size, undesirable toxicity, and uncertainty of distribution data exists and hampers the clinical application. Especially, understanding in vivo whereabouts of graphene to find out where the injected graphene is localized and how much graphene is successfully targeted (disease targeting efficacy) and how long the graphene is retained in the target site is crucial for the drug development. To this point, the concept for radio-graphene approach is significant for tracing the drug-loaded graphene nanocarrier in vivo.

Radio-graphene has been designed in various synthetic methods [22–27]. Functional moiety exposed at the edge of graphene oxide (GO) can be modified for radionuclide labeling. Carboxylate group on GO surface can be used to synthesize bifunctional chelating agents such as *1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid* (DOTA) or *1,4,7-triazacyclononane-*N,N',N''*-triacetic acid* (NOTA). These have high affinity with various radionuclides such as ^{68}Ga or ^{64}Cu and can also chelate with other radionuclides, ^{177}Lu (or ^{89}Zr using DFO as its chelator) that is available for cancer theranostic approach owing to their dual emitting-radiation capacity. In this case, even though chelator is simply conjugated with GO via amide bond-based synthesis, *1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide* (EDC)-based chemical conjugation has a tendency to cause undesirable particle aggregation. Recently, click chemistry strategy for radiolabeling has been recognized as a potential conjugation method owing to the simple, rapid, stable, and specific labeling reaction in mild condition [28, 29]. It is reported that the reaction between clickable moieties of azide group and its corresponding azadibenzocyclooctyne (ADIBO) occurs quickly within 10 min at room temperature [24]. The well-defined and robust surface modification of GO with a simple reaction could become a driving force toward exponential development of a radio-graphene capable of decorating multifunctional theranostic biomolecules (Fig. 1).

Currently, the use of radio-graphene has been rapidly growing in terms of nanodiagnosis in cancer research and such an example is illustrated by the successfully established ^{125}I -

labeled nanographene sheet (NGS) led by the Zhuang Liu group [25]. Although there is the critical issue of blood stability of ^{125}I -labeled materials, ^{125}I -labeled NGS was quite stable in serum, maintaining more than 95 % in serum condition with a very small portion (less than 5 %) of ^{125}I detached. This result from high serum stability allows for longitudinal tracking study of ^{125}I -labeled NGS in vivo. More interestingly, when they investigated in vivo distribution of ^{125}I -labeled NGS for 60 days, intravenously injected ^{125}I -labeled NGS showed high accumulation in the liver at 1 h and its gradual excretion from the liver tissue, and finally ^{125}I -labeled NGS is eliminated via the feces. This fecal excretion process provides useful information to boost the disease targeting efficiency of NGS with minimizing non-specific uptake of normal tissue. However, further investigation for understanding the exact hepatobiliary excretion of ^{125}I -labeled NGS is necessary to find out whether this is resulting from degradation of NGS or from an unknown cell membrane penetration mechanism for maintaining intact NGS. Nevertheless, this finding will open up new opportunities of advancing GO in drug delivery area.

Researchers have been working to find strategies for the development of in vivo active tumor targeting of graphene through the incorporation of specific ligands [26–29]. Using PET imaging, Hong et al. evaluated the tumor targeting efficacy and pharmacokinetics of ^{68}Ga -labeled GO conjugated with TRC105 antibody that targets CD105, an ideal marker for tumor angiogenesis [26]. The images showed that GO conjugated with TRC105 accumulated very rapidly in the tumor and the uptake remained stable even after 24 h post injection [26]. Targeting CD105 present in tumor neovasculature may be beneficial to tumor detection in terms of the limited extravasation of large nanoparticles. The same group also labeled a radioisotope (^{64}Cu) that has longer physical half-life (12.7 h) and TRC105 antibody onto GO before successfully determining the biodistribution, pharmacokinetics, and tumor targeting efficacy of this compound [27]. Another cancer-targeting approach using graphene was reported by Nahain, who designed a functionalized graphene by conjugating GO with photochromic dye spiropyran and hyaluronic acid (HA) [30]. The spiropyran, fluorescent material, enables finding the location of the graphene in the cells by circumventing the use of toxic effects of other dyes whilst the HA targets the cancer cells by easily binding to the CD44 receptor. Also, Shi et al. has conjugated vascular endothelial growth factor 121 (VEGF121) to GO in order to target the VEGF receptor to enhance the accumulation of GO to the tumor [31].

Meanwhile, evidence suggests that graphene exhibits dose-dependent toxicity (roughly 10–200 $\mu\text{g}/\text{ml}$ in vitro) in cells and in animals [32–34]. Li et al. exhibited that GO sheets were rapidly internalized into cells within 3 h and the results showed widespread distribution in the cytoplasm, mitochondria, endoplasmic reticulum, and even the

Fig. 1 Radio-graphene-based cancer theranostic approach. Surface functionalization of graphene oxide enables labeling with theranostic radionuclides such as ^{177}Lu and the cancer targeting agent such as antibody, peptide. Broad surface area can be used to be loaded with either chemotherapy drug or antisense drug with high affinity

border area of the nucleus [32]. Dose- and time dependent decrease in cell viability was found to be more than 50 µg/ml. Long term exposure (48 h) of GO induces release of intracellular lactate dehydrogenase and leads to oxidative stress and eventually apoptosis. Interestingly, GO exposure leads to dysregulation of microRNA, which activates apoptosis-associated molecules such as caspase-3. To overcome this toxic issue, the surface modifications can play a pivotal role in the improvement of biocompatibility by reducing the strong hydrophobic interaction of graphene or GO with cells and tissues as well as reducing the production of reactive oxygen species which is associated with apoptosis [35]. Polyethylene glycol (PEG) is an example of functionalized graphene that can facilitate the excretion of the nanoparticle without noticeable toxicity in vivo [30]. One of the most significant merits of using radio-graphene would be the ability to overcome the dose-dependent toxicity issue by using a very small amount of radioactive materials for in vivo radionuclide imaging. In contrast to MR imaging which has low sensitivity, high sensitive radionuclide imaging modality seems to have lower toxicity due to the low dose and low amount of nanoparticle utilized [36]. Furthermore, graphene oxide has the tendency to reside and possibly induce inflammation, oedema, and granuloma formation in the lung tissue [37]. The poor clearance of graphene oxide in kidneys is another limitation for its use [34, 38]. However, a recent interesting study led by Kostas Kostarelos group demonstrated the urinary excretion pattern of GO sheet even though GO size is not permeable to glomerular filtration [39]. Biodistribution study of functionalized graphene oxide with ¹¹¹In conjugated via DOTA chelator showed that GO underwent rapid urinary excretion followed by gradual accumulation in the spleen. The thin sheets used in this study presented that dimensions and surface groups are essential to determine its ability to cross the renal filtration slit (<40 nm). Another report supported the phenomenon of urinary excretion via kidney for Au-labeled graphene oxide [40]. ^{198,199}Au-labeled GO was used to target and to image tumors. The characteristics of Au to interact with the negatively charged cells and sub-cellular membranes allows efficient cancer cell targeting whilst the high loading capacity ensures greater amounts of Au to be grafted onto the nano-conveyor. The low lipophilicity of the particle allows rapid clearing of the particles by the kidneys within 24 h [40].

Graphene has emerged as an impressive material for cancer therapy and/or diagnosis. A literature review shows that 73 % of the research works have been associated to drug and gene delivery applications; 32 % on photothermal therapy, 31 % on imaging, 10 % on photodynamic therapy, and 27 % of the studies on the theranostic applications [16]. Considering that in vivo delivery issue has been

continuously emphasized as a main bottleneck in a drug development area, numerous trials using nanomaterials such as graphene could contribute to overcoming the in vivo targeted delivery issue. The Weibo Cai group revealed that reduced graphene oxide (rGO) labeled with ⁶⁴Cu was conjugated to the anti-CD105 antibody TRC105 to specifically target the tumor vasculature in 4 T1 murine breast tumors in vivo [41]. The success of this experiment demonstrated the potential of reduced graphene oxide as a promising theranostic agent due to its desirable properties for photothermal therapy. Approximately one third of the research using graphene belong to photothermal therapy. Many studies proved graphene's superiority for photothermal effect, showing quite high therapeutic efficacy for ablation of tumor volume [42–44].

Graphene surface functionalization and graphene itself confer us many opportunities for combination cancer therapy. Recently, combination therapy of rGO-gold nanorod using chemical cancer drug and photothermal approach enhanced therapeutic effect with dramatic tumor regression with amplified photothermal effects [45]. Various types of therapeutic materials such as anti-sense oligonucleotide, chemical drug, photothermal/photodynamic agent, and therapeutic radionuclides can be good candidates for combination therapy and can be selected as a proper condition [46–49]. In addition, high cellular penetration capacity of graphene via cell membrane might allow for augmented therapeutic responses to the combined therapeutic drugs targeting DNA (ex. doxorubicin and alpha particle that possesses short range with strong energy (3–8 MeV)) [50, 51].

Unlike optical imaging approach, radionuclide imaging has been recognized to be a more accurate and reliable imaging modality in terms of quantitative analysis, tomographic information, and human application. There is no doubt that these advantages will enhance the acceleration/advancement of radio-graphene approach toward clinical application. Moreover, due to the simple and rapid radiolabeling method on the graphene, application of radioisotope-labeled graphene to targeted cancer theranostics will rapidly progress. As graphene in clinical practice lies in the early stage due to the lack of defined in vivo characteristics, radio-graphene research could provide useful information capable of proving well-defined and reliable in vivo performances of GO-based therapeutic drugs. It also helps to evaluate the quantified in vivo kinetics, decision of optimal dose, and prediction of therapeutic efficacy with a simple and rapid experimental procedure. This far-reaching application of radio-graphene multi-functionality in biomedical imaging perspectives will pave the way to build the new platform for in vivo drug delivery and therapy.

Acknowledgments This research was supported by a grant of the Korea Health Technology R&D Project funded by the Ministry of Health & Welfare, Republic of Korea (HI14C3344), and funded by the Ministry of Health & Welfare, Republic of Korea (HI14C1277), and the Technology Innovation Program (10052749) funded by the Ministry of Trade, Industry and Energy (MOTIE) of Korea.

Compliance with Ethical Standards

Conflict of Interest Do Won Hwang declares that he has no conflict of interest.

Ethical Statement This article does not contain any studies with animals performed by any of the authors. The manuscript has not been published before or is not under consideration for publication anywhere else.

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