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Neutron-activatable radionuclide cancer therapy using graphene oxide nanoplatelets

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ABSTRACT

Neutron-activation is a promising method of generating radiotherapeutics with minimal handling of radioactive materials. Graphene oxide nanoplatelets (GONs) were examined as a carrier for neutron-activatable holmium with the purpose of exploiting inherent characteristics for theranostic application. GONs were hypothesized to be an ideal candidate for this application owing to their desirable characteristics such as a rigid structure, high metal loading capacity, low density, heat resistance, and the ability to withstand harsh environments associated with the neutron-activation process. Non-covalently PEGylated GONs (GONs-PEG) offered enhanced dispersibility and biocompatibility, and also exhibited increased holmium loading capacity nearly two-fold greater than GONs. Holmium leaching was investigated over a wide pH range, including conditions that mimic the tumor microenvironment, following neutron irradiation. The in vitro cell-based cytotoxicity analysis of GONs-based formulations with non-radioactive holmium confirmed their safety profile within cells. The results demonstrate the potential of GONs as a carrier of neutron-activatable radiotherapeutic agents.

1. Introduction

With the advent of the nano-revolution, carbon-based nanomaterials have gained much attention from various fields, and nanomedicine was not the exception [1]. Specifically, carbon nanotubes and fullerenes have been the initial subject of much attention owing mostly to their unique physical and chemical characteristics [2,3]. Soon after the isolation of graphene by Geim and Novoselov in 2004, this material arguably became the 'hottest' carbon-based nanomaterial. Once called "the thinnest material in the universe and the strongest ever measured" [4], graphene has a myriad of attractive qualities: an extremely large surface area, chemical and mechanical strength, and superior thermal and electrical conductivity [5–7].

In the field of nanomedicine, graphene oxide nanoplatelets (GONs) are a preferred choice over graphene as a drug delivery system, because the hydrophobic nature of graphene makes it difficult to disperse in aqueous solvents [8]. Two specific findings dramatically increased the potential of GONs as a drug delivery carrier: they are biocompatible followed by functionalization (e.g., with polyethylene glycol (PEG)), and they exhibit π–π stacking with aromatic-structured chemotherapeutics (e.g., doxorubicin) which offers exceptionally high loading capacity (400 w/w%) [9,10]. High propensity for metal adsorption is an additional feature that makes GONs attractive as a carrier for therapeutic radionuclides. In one study, graphene oxide was reported to adsorb a 10-fold higher amount of copper ion compared to activated carbon, which has been a heavily investigated material for adsorbents by virtue of large surface area [11]. While there have been a number of published studies assessing the merits of GONs-based delivery of small molecules (e.g., SN38, camptothecin analog, paclitaxel and cisplatin) and large molecules (e.g., vaccines, proteins and genes), publications detailing their utility for delivery of radionuclides are scarce [12–17].

The conventional methods of preparing radiotherapeutics begin with a radioactive isotope which is then conjugated or loaded onto a carrier (e.g. liposomes, polymeric micelles, peptides, or proteins) [18–21]. This method requires the researcher to deal with highly radioactive materials from the very start of the formulation preparation process. Neutron activation – a process by which stable isotopes are converted into radioactive isotopes through the capture of an additional neutron – is a promising approach to minimize the direct handling of radioactivity. Moreover, this strategy is able to offer the additional benefits of minimal use of excipients being able to control the amount of radioactivity produced by varying the neutron flux or irradiation time. In spite of these advantages, the neutron-activation process has not been widely utilized for the production of radiotherapeutics. This is because a significant amount of heat is generated during the neutron-activation process which may result in the degradation of conventional nanocarriers [22]. To overcome this, Di Pasqua et al. [23] previously explored the application of mesoporous silica nanoparticles (MSNs) as

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neutron-activatable radionuclide carriers. These carriers were found to be able to withstand the harsh environment required for neutron-activation. However, the relatively high density of the formulation has limited their utility to intraperitoneal administration.

In the current study, GONs were investigated as a lower density and more durable alternative to MSNs for neutron activation and delivery of radionuclides. Among the possible neutron-activatable radionuclide candidates, holmium (Ho) was selected for the current study. Ho possesses desirable characteristics for the proposed application, including its high natural abundance (^{165}Ho can only be activated to ^{166}Ho) and its large neutron capture cross-section (64 barns, cf. 1.3 barns of ^{89}Yttrium where 1 barn $= 10^{-24}$ cm^2) [24–26]. Once stable Ho is activated, ^{166}Ho decays by emission of high energy β-particles (E_{max} = 1.84 MeV, 8.7 mm maximum tissue penetration) and low energy γ-photons (81 keV, 6.7% photon yield) which allow for its theranostic application via SPECT/CT imaging (Fig. 1) [27]. The relatively short half-life (26.8 h) reduces the concern of radioactive retention in the human body for extended periods of time. Previous work in our lab involved the synthesis of holmium oxide in the pores of mesoporous carbon nanoparticles (MCNs) as a potential neutron-activated radiotherapeutic [28]. However, incorporation of Ho into the MCNs was a multi-step process that required several steps to achieve complete prevention of Ho leaching. To overcome this limitation, GONs were investigated as a carbon-based carrier for radiotherapeutics that could be loaded in a shorter period of time as well as to achieve better tumor penetration. The successful development of GONs as a neutron-activatable Ho matrix is projected to be utilized for radionuclide therapy which can potentially target multiple targets.

2. Materials and methods

2.1. Chemicals

Graphite flakes, hydrogen peroxide (30%, USP testing grade) and holmium (III) acetate monohydrate (99.99%) were acquired from Sigma-Aldrich (St. Louis, MO). Sulfuric acid (ACS grade), sodium nitrate (analytical grade), sodium hydroxide (ACS grade), nitric acid (trace metal grade) and potassium permanganate (reagent grade) were obtained from Fisher Chemical (Fair Lawn, NJ). 1,2-Distearoyl-*sn*-glycero-3-phospho-ethanolamine-*N*-[methoxy (polyethylene glycol)-3000] (DSPE-PEG) was purchased from Avanti Polar Lipids Inc. (Alabaster, Alabama).

2.2. Synthesis of GONs

GONs were synthesized based on a modification of the graphite flake oxidation and ultra-sonication method established by Marcano et al. [29]. Briefly, graphite flakes were sieved through a U.S. standard testing sieve (300 μm). A 9:1 mixture of H_2SO_4 and H_3PO_4 (400 mL) in addition to 18 g of $KMnO_4$ was added to 3 g of sieved graphite flakes and stirred for 12 h at 50 °C. When the reaction had cooled to room temperature (taking on a purple color), 400 mL of Milli-Q water and 3 mL of 30% H_2O_2 were added. The mixture was sieved again (300 μm) and filtered through the polyester fiber. The resultant yellowish filtrate was centrifuged at 2400*g* for 2 h. The filtrate was washed with Milli-Q water, 30% HCl and ethanol (2 times) to remove unreacted precursors, and the washed product was vacuum-dried overnight at room temperature. Graphene oxide was further exfoliated and reduced to nano-size GONs by 2 h of sonication with probe sonicator (Fisher Scientific Model 505 Sonic Dismembrator; power: 500 W, operating frequency: 20 kHz and amplitude: 25%). For additional purification, GONs were centrifuged at 1800*g* for 5 min and the supernatant was dialyzed for 3 days before their use (MWCO: 12 KDa).

2.3. Non-covalent PEGylation on GONs

In order to enhance colloidal stability and biocompatibility, GONs were PEGylated by a non-covalent method. Since DSPE-PEG was stored in chloroform, the solvent was evaporated and DSPE-PEG was re-suspended in Milli-Q water. Ten mL of GONs (0.5 mg/mL), suspended in Milli-Q water, was mixed with 5 mL of DSPE-PEG (2.5 mg/mL) and

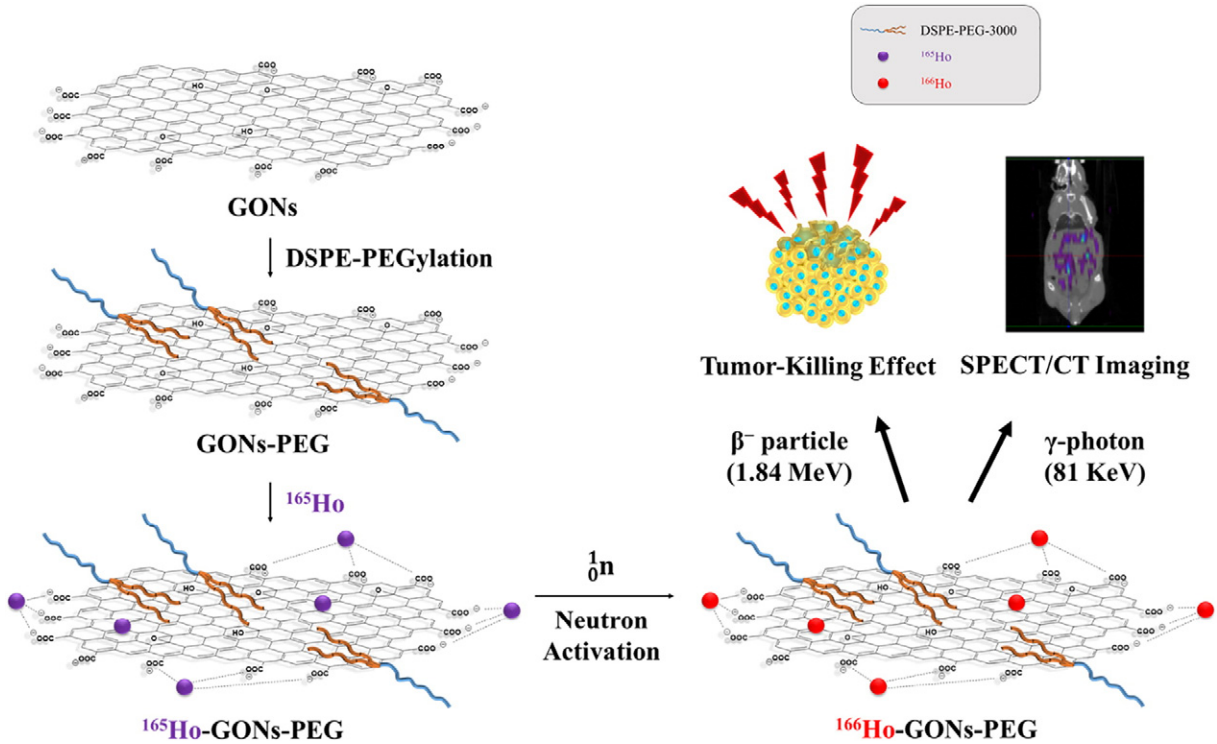

Fig. 1. Schematic representation of neutron-activatable GONs-based therapy.