Materials Science & Engineering C 82 (2018) 19–24

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Nanographene oxide as a switch for CW/pulsed NIR laser triggered drug release from liposomes

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ARTICLE INFO

Keywords:
Graphene oxide
Liposome
NIR light
Pulsed laser
Triggered release

ABSTRACT

The application of pulsed and continuous wave (CW) lasers in nanomedicine has increased significantly over the last decade. Near infrared (NIR) lasers can be used for the precise control of drug release at the target site in a non-invasive manner. In this study, we have prepared nanographene oxide (nGO, size ~40 nm) integrated liposomes (size ~900 nm). The nGOs were not simply adsorbed onto the liposome surface but was embedded inside the liposomes as characterized by cryo-TEM, selected area electron diffraction (SAED), and fluorescence quenching studies. The embedded nGOs could act as a molecular switch for NIR light controlled drug release from the liposomes. Calcein was encapsulated into the liposome as a model drug to evaluate the efficiency of light controlled release. An on-demand pulsatile drug release was achieved by irradiation of CW/pulsed NIR lasers into the nGO-liposome suspension. Triggering with a pulsed laser resulted in larger release of calcein with a minimal temperature increase (~2 °C) of the liposome solution, compared to lower release rate and a significant temperature increase (~8 °C) by a CW laser with the same light energy, suggesting two separate mechanisms and different potential applications depending on the laser type.

1. Introduction

Development of novel drug and gene delivery systems is one of the major research fields in biomaterial science [1–3]. The objective of these delivery systems is to increase the efficiency of the therapeutic molecules and improve the patient compliance. Various different delivery systems based on micro/nanoparticles, micelles, supramolecular structures, and hydrogels have been developed for this purpose [4–8]. However, delivery of a therapeutic molecule to the target site with an optimal dose remains a significant clinical challenge [9–10]. Providing a proper dosage of drugs at the site of action is important for efficient therapeutic responses. Lower or higher concentration of drug might lead to unwanted side effects such as drug resistance or toxicity. Therefore, a drug delivery system that would allow the on-demand release of therapeutic molecules at the target site is highly desirable [9,11,12]. Delivery systems responsive to external stimuli, such as light, ultrasound, and magnetic fields, have been developed to provide the controlled on-demand drug release [13–16]. The light-triggered release is particularly attractive because it can be applied noninvasively with precise spatial and temporal control [9,10,13]. Among different sources of light, near infrared (NIR) light has gained increasing interest because of its relatively high tissue penetration and non-toxicity [17].

Liposomes have a long history as drug delivery vehicles and some liposomal formulations have been approved for clinical applications or currently are undergoing clinical trials [18,19]. Thermosensitive liposomes which are prepared with lipids having a higher phase transition temperature (transition from ordered gel phase to liquid-crystalline state) than physiological temperature (37 °C) can release their cargo in response to a mild hyperthermia. However, modulation of local temperature at the diseased site in the real clinical application is difficult to achieve. NIR light, which can penetrate into the tissue over several cms, has been actively pursued as a remote trigger for controlled drug release from liposomes [9,10,13]. In the past, gold nanomaterials such as nanoparticles, nanorods, nanoshells, and nanocages, which are known for their NIR light absorption properties, were used as an optically active switch for the controlled on-demand drug release from liposomes [20–23].

Among different types of lasers, pulsed and continuous wave (CW) lasers have been increasingly used for biomedical applications [17]. Pulsed lasers are predominantly used for the surgical applications [24].

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http://dx.doi.org/10.1016/j.msec.2017.08.057
Received 1 June 2017; Received in revised form 12 July 2017; Accepted 10 August 2017
Available online 12 August 2017
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Nanosecond or femtosecond pulsed lasers can be also used for the delivery of biomolecules such as DNA, protein, *etc.* across the cell membrane as well as through the skin by a process called optoporation [25–27]. Furthermore, nanosecond pulsed laser-induced photomechanical waves were applied for transvascular drug delivery [28]. Near IR CW laser is mainly used for photothermal therapy of tumor tissues, but can also be used for on-demand drug release [29,30].

Graphene oxide (GO) is a unique two-dimensional (2D) nanomaterial with interesting physiochemical properties. The application of GO in nanomedicine and drug delivery systems have increased immensely in recent years [31]. GO also possesses a high photothermal efficiency in response to NIR light, useful for photothermal ablation of tumor tissues [29,31,32]. A core-shell nanocapsule prepared using a protein (lactoferrin) and GO showed photoresponsive drug release behavior [33]. Therefore, GO can be an effective candidate for NIR light-driven release from liposomes. Previous studies of GO and liposomes have mainly focused on the adsorption of small liposomes (< 200 nm) on large (> 1 μm) GO sheets and their interaction [34–36]. In contrast, no study has ever reported the integration of nanosized (< 100 nm) GO into the liposome. In this study, our objective was to apply nanosized GO (nGO) sheets as an NIR light controlled switch for the controlled on-demand release from liposomes. To demonstrate that, we have integrated small size nGO sheets (~40 nm) into larger size (> 800 nm) liposomes and compared the *in vitro* release by NIR light. Furthermore, we employed two different light sources, CW and femtosecond pulsed NIR laser, as an efficient trigger for encapsulated molecules from nGO-incorporated liposome, and analyzed the effect of them on the release profile as well as temperature change.

2. Materials and methods

2.1. Materials

Single layer graphene oxide (X, Y dimension ~500 nm) was obtained from Angstrom Materials Inc. (Dayton, OH, USA) as a 0.5% (w/v) solution. 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-{12-[(7-nitro-2-1,3-benzoxadiazol-4-yl) amino] dodecanoyl}-*sn*-glycero-3-phosphocholine (NBD-PC) was purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Calcein, chloroacetic acid, Triton X100, and sodium hydroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals were of analytical grade and used without further purification.

2.2. Preparation of nanographene oxide (nGO)

Nanographene oxide (nGO) was prepared by ultrasonication of originally large size (X, Y dimension ~500 nm) single layer graphene oxide (GO) solution as described previously [32].

2.3. Synthesis and characterization of nGO incorporated liposome (nGO-liposome)

Thermosensitive DPPC liposomes were prepared by thin film rehydration method. DPPC (5 mg) dissolved in chloroform (1 ml) was taken in a glass tube. The lipid film was prepared by slowly evaporating the solvent under a steady flow of nitrogen gas and the tube was kept in vacuum for 24 h to remove any residual solvent. Then, the dry lipid film was hydrated with nGO solution (200 μg/ml) at 50 °C with gentle swirling and extruded through an 800 nm polycarbonate membrane using a mini-extruder system (Avanti Polar Lipids, Inc. Alabaster, AL, USA) to obtain nGO containing DPPC liposomes (nGO-liposome). After extrusion, the nGO-liposomes were separated from free nGO by centrifugation at 5000 rpm for 10 min at 4 °C. The precipitated nGO-liposomes were re-dispersed in phosphate buffer (10 mM, pH 7.4) and stored at 4 °C for further analysis. The absorbance of the supernatant containing free nGO was measured to calculate the amount of nGO loaded into the liposomes.

To prepare calcein loaded nGO-liposomes, the dry lipid film was hydrated with nGO solution containing calcein at 45 °C, extruded through the 800 nm polycarbonate membrane, and then purified by centrifugation as described above. The amount of calcein loading was quantified by destroying the liposomes completely and analyzing the released calcein amount. Calcein loaded nGO-liposomes were incubated with 1% Triton X100 at 45 °C for 30 min and analyzed by fluorescence measurement to calculate the loading content of calcein.

The structure of nGO-liposomes (nGO-incorporated liposomes) was observed by cryogenic transmission electron microscopy (Cryo-TEM). 3 μl of nGO-liposome sample was transferred to a lacey supported grid through the plunge/dipping method. To prevent evaporation of water from the sample solutions, the thin aqueous films were prepared at ambient temperature and a humidity of 97–99% within a custom-built environmental chamber. The thin aqueous films were rapidly vitrified through plunging into liquid ethane (cooled by liquid nitrogen) at its freezing point. Finally, the specimen was observed using an HR-TEM (JEOL-JEM-3011, Tokyo, Japan) apparatus operated at 300 kV and the data were analyzed using Gatan Digital Micrograph program.

Fluorescent liposomes were prepared by mixing NBD-PC (50 μg) with DPPC (5 mg). To study the effect of nGO on the fluorescence of the liposomes, several samples of fluorescent liposomes were prepared with increasing concentrations of nGO. After separating the free nGOs by centrifugation, liposomes were collected and their fluorescence was analyzed.

2.4. Near infrared (NIR) laser-mediated in vitro release of calcein from nGO-liposome

Two separate NIR lasers, 808 nm continuous wave laser (Dragon Lasers Co., Changchun, China) and 805 nm femtosecond pulsed laser (Micra-5, Coherent Inc. Santa Clara, CA, USA), were used for the light triggered on demand *in vitro* release studies. Calcein loaded nGO-liposome solutions were incubated at 37 °C and they were irradiated with the NIR lasers (1 W/cm^2) for 10 min at specific time points. At each time points, the amount of released calcein from the liposome was quantified by fluorescence measurement. The temperature increase of the solutions during laser irradiation was measured in real time by an infrared (IR) thermal imaging system (FLIR SC-300, FLIR Systems Inc., Danderyd, Sweden).

3. Results and discussion

Nanographene oxide (nGO) was prepared by ultrasonication of large size GO [29]. The average size of nGO was ~40 nm, as measured by DLS (Fig. 1a). nGO showed a highly negative surface charge (− 47.5 ± 3.5 mV) (Fig. 1c) due to the presence of carboxyl (−COOH) groups. This nGO has a high photothermal conversion efficiency upon NIR light irradiation, as previously reported [29,32]. nGO integrated 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) liposomes (nGO-liposomes) were prepared by the conventional thin film hydration followed by extrusion and non-integrated nGOs were separated from nGO-liposomes by centrifugation. The centrifugation speed was set to be 5000 rpm, which was optimal to precipitate the nGO-liposomes but not enough to precipitate the free nGOs (due to their very small size). After removal of free nGOs in the supernatant, precipitate nGO-liposomes were collected, washed and the amount of nGO integrated into the liposomes was measured by absorbance measurement [29,32]. In nGO-liposome, 4 μg of nGO was integrated into 1 mg of lipid, as calculated from the absorbance measurement. The average size of the blank liposomes and nGO containing liposomes were 890 ± 65 nm and 920 ± 75 nm, respectively (Fig. 1b), which shows that the integration of nGO into liposome did not have a significant effect on their size. The liposomes showed an almost neutral surface charge (− 2.3 ± 1.0 mV), as they were entirely made from DPPC (Fig. 1c). Interestingly, similar to

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