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Layer-by-layer assembly of graphene oxide on thermosensitive liposomes for photo-chemotherapy

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ABSTRACT

Stimuli responsive polyelectrolyte nanoparticles have been developed for chemo-photothermal destruction of breast cancer cells. This novel system, called layer by layer Lipo-graph (LBL Lipo-graph), is composed of alternate layers of graphene oxide (GO) and graphene oxide conjugated poly (L-lysine) (GO-PLL) deposited on cationic liposomes encapsulating doxorubicin. Various concentrations of GO and GO-PLL were examined and the optimal LBL Lipo-graph was found to have a particle size of 267.9 ± 13 nm, zeta potential of +43.9 ± 6.9 mV and encapsulation efficiency of 86.4 ± 4.7%. The morphology of LBL Lipo-graph was examined by cryogenic-transmission electron microscopy (Cryo-TEM), atomic force microcopy (AFM) and scanning electron microscopy (SEM). The buildup of LBL Lipo-graph was confirmed via ultraviolet-visible (UV–Vis) spectrophotometry, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis. Infra-red (IR) response suggests that four layers are sufficient to induce a gel-to-liquid phase transition in response to near infra-red (NIR) laser irradiation. Light-matter interaction of LBL Lipo-graph was studied by calculating the absorption cross section in the frequency domain by utilizing Fourier analysis. Drug release assay indicates that the LBL Lipo-graph releases much faster in an acidic environment than a liposome control. A cytotoxicity assay was conducted to prove the efficacy of LBL Lipo-graph to destroy MD-MB-231 cells in response to NIR laser emission. Also, image stream flow cytometry and two photon microcopy provide supportive data for the potential application of LBL Lipo-graph for photothermal therapy. Study results suggest the novel dual-sensitive nanoparticles allow intracellular doxorubin delivery and respond to either acidic environments or NIR excitation.

Statement of Significance

Stimuli sensitive hybrid nanoparticles have been synthesized using a layer-by-layer technique and demonstrated for dual chemo-photothermal destruction of breast cancer cells. The hybrid nanoparticles are composed of alternating layers of graphene oxide and graphene oxide conjugated poly-L-lysine coating the surface of a thermosensitive cationic liposome containing doxorubicin as a core. Data suggests that the hybrid nanoparticles may offer many advantages for chemo-photothermal therapy. Advantages include a decrease of the initial burst release which may result in the reduction in systemic toxicity, increase in pH responsivity around the tumor environment and improved NIR light absorption.

1. Introduction

Liposomes have been one of the most investigated drug delivery systems, especially in clinically relevant cancer models [1,2]. In

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recent years, important advances to improve the therapeutic effect and targeting of administered drugs to cancer patients have been made [3–6]. In particular, efficacy of treatment is highly dependent on the presence of sufficient drug in the tumorigenic region [1,7]. This, in turn, is dependent on effective delivery via a high payload capacity, high cellular uptake, and also programmed drug release [8]. Recently, a layer-by-layer deposition method has been shown to be an effective process to improve therapeutic efficacy of administrated liposomes [9–11].

Layer-by-layer liposomal nanoparticles fabricated by deposition of alternate layers of charged polyelectrolytes onto the liposomes is intriguing due to the high loading capacity, multi-functionality, tailored drug release and stimuli-responsiveness that can be achieved using this design [12–14]. Although some studies have focused on the application of LBL assemblies in drug delivery, the main emphasis of these studies is to fabricate barrier layers to control cargo release encapsulated in the core while fewer attempts have been made to fabricate an LBL engineered system to target tumor and facilitate site-specific triggered cargo release [14–16].

A variety of stimuli sensitive LBL constructions have been reported to modify liposomes by using polyethylene glycol-reprivatized nanotubes [17], nanogolds [18], magnetic nanoparticles [19]. Among other stimuli sensitive delivery vehicles, near infrared (NIR) light triggered drug release has some advantages as light at this wavelength has its maximum penetration depth through the tissue, while the main interaction of tissue and light is scattering [20–22]. Thus, in recent years different types of photo-absorbers with NIR absorption have been investigated including gold nanorods [23–27], gold nanoshells [28,29], carbon nanotubes [30–33] and graphene oxide [34,35].

Graphene oxide (GO) is a single layer of graphite with unique thermal, electrical and optical properties. Graphene oxide is able to absorb NIR light due to the delocalization of the electron states, and light absorption rapidly transformed into thermal energy [34,36–38]. Hence, GO with a large number of surface functional groups on the surface, low production cost and acceptable thermal conductivity, can be considered as a versatile stimuli-triggered candidate for photothermal therapy [36,39–43]. However, only a few studies have been reported on the application of GO for photothermal therapy. Yang et al., studied the influence of size and surface chemistry of GO coated PEG on photothermal destruction of cancer cells [39]. Cheng et al., examined chemo-photothermal properties of PEGylated GO containing doxorubicin on cancer cells [44].

The interaction between graphene oxide and liposome was studied previously by Feng et al. [45,46]. They studied the interaction between nondiamond, graphene oxide and carbon nanotube with zwitterionic liposomes. They found that the absorption of carbon based materials to the surface of liposomes neither induces drug leakage nor affect phase transition temperature [45]. In another study by the same group, they demonstrated that the absorption of DOPC and doxorubicin on the surface of graphene oxide was orthogonal because doxorubicin and DOPC didn't displace each other and the adsorption capacity was dependent on the presence of other molecules [46].

In the current studies, we sought to improve the bioavailability and also pharmacokinetic profile of nanoparticles intended for tumorigenic delivery by combining the advantages of liposomes with a layer-by-layer synthesis technique. We chose a combination of GO and GO-PLL as the respective polycation and polyanionic alternating layers and thermosensitive cationic liposome containing doxorubicin as a core to fabricate layer by layer architecture. Positively charged poly-L-lysine (PLL) is known to improve the cellular uptake of the nanoparticles and if PLL is incorporated in the LBL nanoparticles, they have the potential to advance clinical translation of the nanotechnology [47]. NIR light is absorbed by GO and GO-PLL and converted to heat. Photothermal energy is used to activate a liquid phase transition temperature of the phospholipid membrane and lead to the release of the encapsulated toxic cargo. The present study investigates the concentration required for layer-by-layer fabrication of GO and GO-PLL on the liposomal core (LBL Lipo-graph) and their physiochemical characterization. The light matter interaction was studied by calculation the cross section absorption of LBL Lipo-graph at 808 nm pulse laser irradiation in the frequency domain by utilizing the Fourier analysis. Cytotoxicity analysis of LBL Lipo-graph were performed on MD-MB-231 cell lines. The interaction of LBL Lipo-graph with MD-MB-231 cell lines was analyzed by image stream flow cytometry and confocal microcopy.

2. Materials and method

2.1. Materials

Graphene oxide was purchased from Graphene Supermarket (Reading, MA, USA). 1,2-dipalmitoyl-*sn*-glycerol-3-phosphatidylcholine (DPPC), cholesterol (Chol), 1 2-Dioleoyloxy-3-trimethylammonium propane chloride (Dotap) and poly(L-lysine) (PLL, MW: 15,000–30,000) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL). Polyoxyethylene (20) stearyl ether (Brij 78), doxorubicin HCl (DOX), fetal bovine serum (FBS), phosphate buffer saline (PBS), and Dulbecco's Modified Eagle Medium (DMEM) and Cell Proliferation Reagent Kit I (MTT) were purchased from Sigma Aldrich (USA). Fluorescein-5-Isothiocyanate (FITC) and Live/dead cellular viability assay kits were purchased from Thermo-Fisher. PE (Lissamine Rhodamine B) was purchased from Avanti Polar Lipids Inc. Cell tracker green and DAPI (4′,6-diamidino-2-phenylindole) were supplied from Molecular Probe Inc. Mounted media was obtained from Vector Laboratories, CA. All other chemical agents utilized were analytical grade.

2.2. Methods

2.2.1. Preparation of GO and GO-PLL suspension

An aqueous GO suspension was probe sonicated for 3 h (100 W, 5 s on and 2 s off, Misonix, USA) to achieve exfoliated GO nanosheets. Then, GO-PLL was prepared by vigorous stirring of 8 mg of poly-L-lysine, 2 mg of GO, and 10 mg of KOH in 10 ml of distilled water at 70 °C for 24 h following by multiple centrifugation steps with water to remove excess PLL and impurities. The GO-PLL formation was confirmed using FTIR (Perkin Elmer, Waltham, MA, USA).

2.2.2. Fabrication of LBL Lipo-graph

Thermosensitive liposomal nanoparticles (Lipo) were prepared using a thin film hydration and pH gradient loading method. Briefly, 60 μmol of lipid mixture composed of DPPC, Brij 78, Dotap and Chol in a molar ratio of 66/4/20/10 were dissolved in the mixture of chloroform/methanol (3:1) in a round bottom flask. Then the organic solvent was evaporated from the lipid phase under reduced pressure at 37 °C in a rotary evaporator (Buchi Labortechnik, Switzerland) to form a thin lipid film on the inside flask wall. Then the resulting thin lipid film was hydrated using 200 mM phosphate buffer saline at 42 °C (pH = 4). Subsequently, the large multilayered vesicles in a final concentration of 20 $\mu mol \cdot ml^{-1}$ (i.e. 13.72 $mg \cdot ml^{-1}$) were extruded through a 100-nm polycarbonate filter using a mini-extruder (Avanti Polar Lipids, Inc., Alabaster, USA) for 21 cycles. A transmembrane pH gradient across the membrane was induced by exchanging external sulfate buffer with PBS solution using a dialysis bag (MWCO of 10 kDa, BBI, Canada), pH = 8.4. DOX (Lipid·drug^{-1} = 20 (molar ratio)) was loaded into