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Short communication

Phototherapeutic functionality of biocompatible graphene oxide/dendrimer hybrids

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ABSTRACT

Hydroxyl-terminated fourth generation poly(amido amine) dendrimer and folic acid were chemically bound on graphene oxide. The resultant hybrids exhibited one-photon and two-photon fluorescence emission, since the excitation irradiation at 390 and 780 nm on the hybrids brought a fluorescence emission in the visible region around 450 nm. In addition, the photocytotoxicity study revealed that under the two-photon excitation at 780 nm, the hybrids can absorb near-infrared light and generate reactive oxygen species which can oxidize the HeLa cells and cause their death, suggesting the phototherapeutic behavior. Cytotoxicity measurement revealed the high biocompatibility of the hybrids toward HeLa cells. Thus, the present biocompatible hybrids consisting of only dendrimer, folic acid and graphene oxide have potentials as photodynamic therapeutic agents for medical treatment.

1. Introduction

Graphene oxide, which is an oxidized graphene composed of a graphene-like sheet, is chemically functionalized with oxygen-including groups such as hydroxyl, carboxylic acid and epoxide [1]. Hybrid materials of graphene oxide (GO) have been investigated as new promising materials for biomedical applications including cellular imaging [2], drug delivery [2,3], and photodynamic therapy [4]. Poly(amido amine) (PAMAM) dendrimers, highly-branched polymers with a multi-functionalized peripheral surface, have high degree of molecular uniformity, monomolecular weight, and specified size and shape [5–8]. PAMAM dendrimers also possess a strong fluorescence emission [5–8]. It has been confirmed by the visual observation of fluorescent dendrimers that fluorescent dendrimers-bound avidins interact selectively with biotins immobilized on the patterned substrates [9]. The fluorescent PAMAM dendrimers have revealed lower in vitro cytotoxicity than the non-fluorescent ones toward rat C6 glioma cells [10].

In the present work, hydroxyl-terminated PAMAM dendrimer (DEN-OH) (see Fig. S1) was chemically bound on GO as a vehicle, and folic acid as a target reagent to specific cells with folate receptors [3] was further attached on GO. Since it has been reported that neutral PAMAM dendrimers are less toxic than ionic dendrimers [11], DEN-OH should be preferable to utilize for preparing biocompatible novel nanohybrid materials. Biomedical characteristics of the prepared hybrids such as fluorescent property and cell cytotoxicity were examined. Moreover, the ability to generate reactive oxygen species (ROS) in the hybrids under laser irradiation was investigated for interpreting the obtained characteristics of photocytotoxicity toward HeLa cells under two-photon excitation at 780 nm of these hybrids. Altogether, the present work provides new hybrid materials consisting of GO, DEN-OH and folic acid (FA), GO/DEN-OH/FA, that should have a potential to use as a drug in the photodynamic therapy and also a drug carrier for the efficient delivery in target cancer cells, since GO is a new type of drug carrier, dendrimer is necessary as a stabilizing and photosensitizing drug and FA works as a cancer-targeting molecule.

2. Experimental

2.1. Binding of DEN-OH and FA on GO

Esterification reagents (*N*,*N*′-dicyclohexylcarbodiimide and 4-dimethylaminopyridine) were added in a dimethylformamide

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solution of dendrimer and GO under vigorous stirring, and the mixture was further vigorously stirred at room temperature for 3 days. The dispersion was centrifuged, and the centrifugate (GO/DEN-OH) was rinsed with dimethylformamide. *N*-hydroxysuccinimide and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride were added into an aqueous suspension of GO/DEN-OH, and the mixture was ultrasonicated for 2 h. Then, an aqueous $NaHCO_3$ solution (pH 8) of FA was added and the mixture was stirred overnight at room temperature. The purification of the product (GO/DEN-OH/FA) was carried out by the dialysis against a $NaHCO_3$ solution (pH 8.0) and water. The details are given in Supporting information.

2.2. In vitro study of the photocytotoxicity of GO/DEN-OH/FA

An aqueous suspension (100 μl) of GO/DEN-OH/FA (or GO/FA) was incubated with HeLa cells (1×10^6 cells), which were seeded onto coverslips in a 6-wells plate for 2 h. Microscopic cell viability was investigated by using a multiphoton and high-velocity spectral confocal microscope under the laser irradiation at 780 nm (1.07 W) for 15 min at five different positions.

3. Results and discussion

3.1. Hybridization of DEN-OH and FA on GO

DEN-OH and FA were attached to GO using condensing agents for esterification and amidation, respectively (Fig. 1). TEM and AFM images revealed that after DEN-OH and FA were conjugated on GO, the resultant hybrids exhibited an aggregates flat sheet (see Fig. 1 and Fig. S2). The average zeta potential and particle size of GO, and the resultant hybrids were then measured by DLS and zeta potential as shown in Table S1. The increased in the average particle size of GO/DEN-OH/FA might be due to the aggregation of GO hybrid materials. Zeta potential value of the aggregated GO/DEN-OH was 23.7 mV when compared with the well-dispersed GO (−78 mV); while after FA was conjugated on GO/DEN-OH, zeta potential value was −59.7 mV, indicated GO/DEN-OH/FA was formed a stable dispersion in water.

IR spectrum of GO/DEN-OH exhibited the absorbance of the C=O band at 1735 cm^{-1} of GO [12] which was significantly decreased after the immobilization of DEN-OH (Fig. S3 and Table S2). Meanwhile, GO/DEN-OH showed absorption bands of amide I and amide II vibration modes at 1629 and 1555 cm^{-1}, respectively, of DEN-OH [13]. Moreover, the bands at 1694 and 1284 cm^{-1}, respectively, can be attributed to C=O and C—O stretching modes of ester bond.

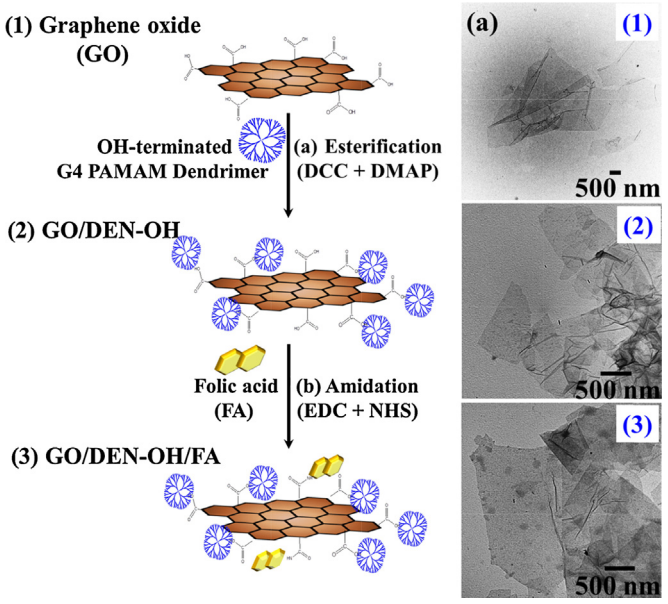

Fig. 1. Schematic illustration of immobilization of OH-terminated PAMAM dendrimer (DEN-OH) and folic acid (FA) on graphene oxide (GO) and TEM images of (1) GO, (2) GO/DEN-OH and (3) GO/DEN-OH/FA.

Thus the covalent immobilization of DEN-OH on GO through the ester linkage was proved. When FA was bound on GO/DEN-OH, GO/DEN-OH/FA showed a band shift of amide I from 1629 cm^{-1} of GO/DEN-OH to 1643 cm^{-1}. Contrary, the NH_2 bending band of FA at 1605 cm^{-1} disappeared. These results mean the successful formation of amide linkage between GO/DEN-OH and FA.

Fig. S4 shows Raman spectra of GO and the resultant hybrids at an excitation wavelength of 633 nm. It was observed that both GO/DEN-OH and GO/DEN-OH/FA exhibited Raman D and G bands in common at 1334 and 1590 cm^{-1}, respectively, similar to GO. The I_D/I_G values of GO/DEN-OH (1.03) is almost comparable to I_D/I_G values of GO (1.06); however, I_D/I_G values of GO/DEN-OH/FA was increased up to 1.29 (Table S3). Since I_D/I_G values provide information of structural defect of carbon materials, it can be indicated the covalent functionalization of DEN-OH and FA on GO might take place at the carboxylic group at the edge of GO sheet.

GO revealed a main absorption band at 232 nm and a shoulder around 300 nm, which correspond to $\pi \rightarrow \pi^*$ transition of aromatic CC bonds and $n \rightarrow \pi^*$ transition of C=O bonds, respectively,

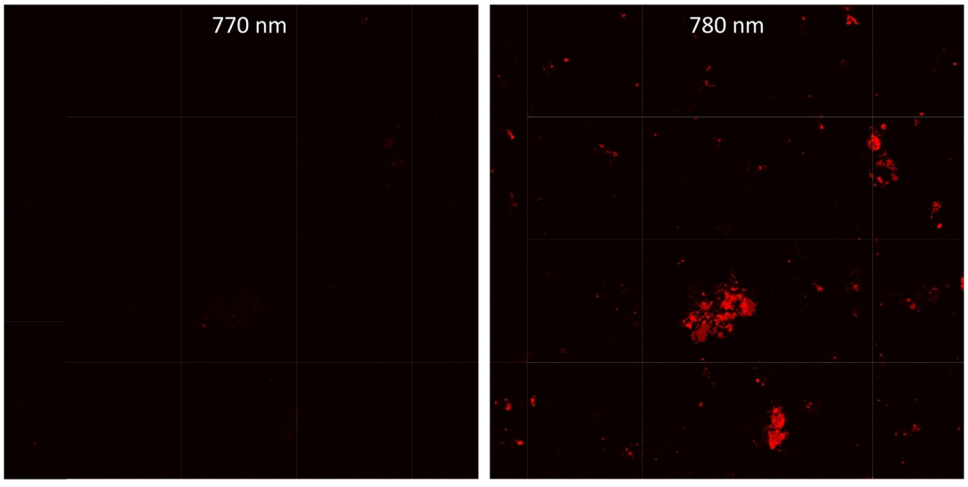

Fig. 2. Two-photon fluorescence images of GO/DEN-OH/FA (DEN-OH = 54 μM) at excitation wavelengths of 770 and 780 nm.