Photothermally controlled drug delivery using a functionalized graphene oxide

Hyunwoo Kim\textsuperscript{1}, In-Kyu Park\textsuperscript{2}, Mi-Kyeong Jang\textsuperscript{3} Tae-il Kim\textsuperscript{4} and Won Jong Kim\textsuperscript{1}

\textsuperscript{1}Center for Self-assembly and Complexity, Institute for Basic Science, and Department of Chemistry, Pohang University of Science and Technology, San 31, Hyoja-dong, Pohang 790-784, Korea.
\textsuperscript{2}Department of Biomedical Sciences, Chonnam National Univ. Medical School, Gwangju, Korea
\textsuperscript{3}Department of Nano Polymer Science and Engineering, Sunchon National University, Suncheon, Korea
\textsuperscript{4}Department of Biosystems, Biomaterials Science and Engineering, Seoul National University, Korea

wjkim@postech.ac.kr

**ABSTRACT SUMMARY**

In the present work, functionalized reduced graphene oxide (PEG-BPEI-rGO) has been developed as a nanotemplate for photothermally triggered cytosolic drug delivery by inducing endosomal disruption and subsequent drug release. PEG-BPEI-rGO has ability to load more amount of Doxorubicin (DOX) than unreduced PEG-BPEI-GO via π - π and hydrophobic interactions, showing high water stability. Loaded DOX could be efficiently released by glutathione (GSH) and photothermal effect of irradiated near IR (NIR) in test tubes as well as in cells. Finally, it was concluded that more cancer cell death efficacy was observed in PEG-BPEI-rGO/DOX complex-treated cells with NIR irradiation rather than that in no irradiation.

**EXPERIMENTAL METHODS**

The preparation of BPEI-GO and BPEI-rGO nanomaterials were followed by previous report.\textsuperscript{1,2} In brief, BPEI 1.8K was covalently conjugated to carboxylic acid group of GO using EDC/NHS chemistry. The reducing process of BPEI-GO was conducted by treating 0.05 % v/v of hydrazine monohydrate (80 %) followed by heating to 80 °C for 15 min. In order to enhance colloidal stability of BPEI-rGO, MPEG was incorporated by CDI coupling. The cytotoxicity of free DOX and PEG-BPEI-rGO with/without DOX was evaluated by MTT assay.

**RESULTS AND DISCUSSION**

PEG-BPEI-rGO with two aromatic sheets is capable of adsorbing most of aromatic anticancer drugs through its π-π stacking and hydrophobic interactions as illustrated in Figure 1. DOX has been non-covalently loaded onto functionalized rGO could be a nanotemplate as tailor-made anticancer drug delivery carrier by photothermally controlled drug release via endosomal disruption, finally inducing higher cancer cell death.
water-dispersible PEG-BPEI-rGO by simple mixing for 12 h. The amount of DOX loaded onto PEG-BPEI-rGO was estimated by measuring the absorbance at 520 nm for DOX, after subtracting the absorbance from that for the PEG-BPEI-rGO. It was found that the drug loading capacity for PEG-BPEI-rGO was remarkably higher than that of unreduced form, PEG-BPEI-GO (about 100 % for PEG-BPEI-rGO and only about 10 % for PEG-BPEI-GO Figure 1). The difference in the loading capacity of these two carriers could be attributed to the large number of the aromatic ring structure obtained upon reduction, and that results the difference in the π-π stacking and hydrophobic interactions with DOX. Subsequently, the interaction between PEG-BPEI-rGO and DOX was confirmed by measurement of fluorescence change. The loading process results in fluorescence quenching of DOX due to the photo-induced electron-transfer effect. GO-containing nanomaterials can transfer absorbed energy to thermal energy, and thus we investigated the heat generation upon NIR laser irradiation (808 nm, 6 W/cm2) of the various solutions containing PEG-BPEI-rGO/DOX, PEG-BPEI-GO, PEG-BPEI-GO, GO and a control without GO. As expected, control water had no response to NIR irradiation, whereas solutions containing PEG-BPEI-rGO/DOX, PEG-BPEI-rGO, PEG-BPEI-GO, and GO showed different GO-mediated heat generation. Moreover, both reduced PEG-BPEI-rGO and PEG-BPEI-rGO/DOX showed higher temperature increase (~ 40 °C) as compared to unreduced PEG-BPEI-GO and GO possibly because of effective restoration of the π conjugation by chemical reduction.

The evaluation of the therapeutic efficacy of DOX loaded PEG-BPEI-rGO was carried out in vitro by quantifying the cell viability of PC-3 and HeLa cells using the MTT assay. To evaluate in vitro cytotoxicity of the free DOX and PEG-BPEI-rGO/DOX under NIR irradiation, PC-3 and HeLa cells were incubated with different concentration of DOX for 48 h, where the PEG-BPEI-rGO/DOX has an equivalent DOX dosage to free DOX (Figure 2a and b). As expected, cell viability of PEG-BPEI-rGO/DOX-treated cells was about 20 % decreased in NIR irradiation condition as compared with dark condition, which clearly shows the enhanced and controllable release of drug molecules from PEG-BPEI-rGO by photothermal effect. In the case of free DOX, there was no significant differences of cell viability between NIR irradiation and dark condition. The low cytotoxic effect observed in PEG-BPEI-rGO/DOX-treated cells could be attributed to delayed DOX release from PEG-BPEI-rGO and endocytosis-mediated cytosolic delivery which is controlled to some extent by endosomal disruption.

CONCLUSION
Here, we developed a photothermally triggered drug delivery system based on functionalized reduced graphene oxide nanotemplate. Photothermal endosome disruption and resulting NIR responsive release of loaded DOX from PEG-BPEI-rGO in combination with GSH-mediated drug release was confirmed with NIR irradiation.

REFERENCES

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