Combined PDT and PTT by Methylene Blue Loaded Graphene Oxide
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Statement of Purpose: We have prepared methylene blue, a hydrophilic photosensitizer, loaded nanoGO for combined photodynamic and photothermal treatment for cancer therapy in vivo.

2. Materials & methods

Methods: Carboxylated nanosize GO sheet (nanoGO) (~40 nm) was prepared first by reaction with chloroacetic acid in alkaline condition then by ultrasonication of the large size GO (~ 450 nm), and it was coated with Pluronic block-copolymer to increase its stability in biological environment. Then, photosensitizer (MB) was loaded onto Pluronic coated nanoGO by simple mixing. As in vitro characterization, release of MB from nanocomplex, singlet oxygen generation by laser, photothermal activity of nanoGO, and cellular uptake as well as cytotoxicity assay were done. In vivo tumor accumulation of nanoGO after i.v. injection was optically monitored, and photodynamic and photothermal effect was compared with xenograft tumor mouse model.

Results: NanoGO itself was unstable in buffer solution or serum-containing media. But, physical coating with Pluronic F 127 completely eliminated the stability issue in all media (Fig.1(A)). MB, a positively charged hydrophilic photosensitizer, could be loaded into Pluronic-coated nanoGO with a negative charge via electrostatic interaction. By simple addition over 20 % loading content and almost complete loading was achieved while maintaining its size. The release of MB from Pluronic coated nanoGO showed a pH-dependent manner: faster release at pH5.0 and slower release at pH 7.4, thus, advantageous for tumor targeted delivery (Fig.1(B)). This nanocomplex maintained its ability to generate ROS (Fig.1(C)) from loaded MB and heat (Fig.1(D)) from photothermal properties of GO by light. The nanocomplex also showed enhanced uptake by cancer cells than normal cells and in the absence of light it showed no major toxicity towards cells, but significant cell death was observed upon irradiation with selective NIR laser. i.v. injection of the complex into tumor bearing mice showed high tumor accumulation, and with the irradiation of NIR light to tumor, a significantly enhanced tumor suppression was observed by the combined action of photodynamic and photothermal effects compared to PDT or PTT alone.

Conclusions: The combination of NIR light induced photodynamic therapy and subsequent photothermal therapy by using MB-loaded Pluronic coated nanoGO showed complete ablation of tumor, indicating synergistic effect of dual phototherapy.

Figure 1. (A) Stability of bare nanoGO and Pluronic coated nanoGO in DIW, PBS, and serum containing DMEM. (B) pH-dependent release of MB from Pluronic coated nanoGO. (C) RNO consumption (ROS generation) by free MB, nanoGO, and nanoGO-MB complex after laser irradiation (650 nm, 150 mW/cm2). (D) Photothermal effect of nanoGO upon NIR laser (808 nm, 2 W/cm2) irradiation.

Figure 2. In-vivo cancer therapy in HeLa tumor bearing mice. (A) Changes in tumor volume (n = 3) after different phototherapies. (B) Complete ablation of tumor tissue and no regrowth in 15 days by combined therapy.

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References: