Supplementary Information

An Optical/Magnetic Imaging Nanoplatform-Mediated Convection-Enhanced Delivery of Oxaliplatin for Glioma Treatment

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Materials and methods

Materials

Poly (ethylene glycol) methyl ether (average Mw 5000, mPEG113), and 2-bromoisobutyryl bromide were purchased from Adamas-beta (Shanghai, China). 2-(diisopropylamino)-ethyl methacrylate (iDPA, 97%) and tris[(2-pyridyl) methyl] amine (TPMA), and 2-aminoethyl methacrylate (AMA) were from Aladdin (Shanghai, China). Dipropylamine, bromoethanol, 2-bromoisobutyryl bromide, $CuBr_2$, and potassium carbonate were from Energy Chemical. $GdCl_3 \cdot 6H_2O$ were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Cyanine 5 N-hydroxysuccinimide ester (Cy5-NHS) was purchased from Shanghai Acmec Biochemical Technology Co., Ltd. (Shanghai, China). Cell-Counting-Kit-8 (CCK-8) was purchased from New Cell &Molecular Biochemical Technology Co., Ltd. (Suzhou, China). Cell culture dish and centrifuge tubes were obtained from NEST Biotechnology Co. Ltd. (Wuxi, China). Solvents were purchased from Sinopharm Chemical Reagent Co., Ltd and used as received unless otherwise specified.

Characterization and preparation of Gd/Cy5-Oxa@NPs

Preparation of PiDPA

MeO-PEG_{113}-Br was synthesized and purified according to the literature[1]. PiDPA copolymers were synthesized using the atom transfer radical polymerization (ATRP) method. iDPA (426.63 mg, 2.00 mmol), AMA (19.88 mg ,0.12 mmol), TPMA (5.81 mg, 0.02 mmol), $CuBr_2$ (4.46 mg, 0.02 mmol), and MeO-PEG_{113}-Br (100 mg, 0.02 mmol) were charged into a polymerization tube. To dissolve the monomer and initiator, a 2 ml mixture of 2-propanol and 2 ml of dimethylformamide was added. After argon gas was injected into the system for 30 min to remove oxygen, the mixture was stirred at 70 °C for 2 d. After completion of the reaction, the mixture was diluted with 10 ml of tetrahydrofuran (THF) and passed through an Al_2O_3 column to remove the catalyst. The solvent was removed using rotary evaporation, and the resulting residue was dialyzed in distilled water before being lyophilized to yield a

white powder. The nuclear magnetic resonance hydrogen spectrum (^{1}H-NMR) spectra of PiDPA were recorded on a 400 MHz NMR spectrometer (Zhongke-Niujin, China).

Preparation of PiDPA-Cy5

First, 5 mg of PiDPA was dissolved in 0.5 ml of dichloromethane. Next, an equivalent molar amount of Cy5-NHS, based on the primary amino group content, was introduced to the solution. The reaction was performed at room temperature for 24 h in the dark and was distilled under reduced pressure to remove the solvent and obtain the product.

Preparation of Gd/Cy5-Oxa@NPs

To prepare Gd/Cy5-Oxa@NPs, Oxa and PiDPA-Cy5 were dissolved in 1ml of mixed solvent of tetrahydrofuran and dimethyl sulfoxide (*v/v,* 1:10), and then 9 ml of water solution of $GdCl_3$ was dropped slowly under vigorous stirring. The solution was stirred overnight, then transferred to a dialysis tube. It was subsequently washed with distilled water to eliminate any unbound Gd, Oxa, and organic solvents. After dialysis, the final solution was collected and adjusted to a volume of 1 ml. The loading content of Oxa and Gd was characterized by inductively coupled plasma mass spectrometry (ICP-MS, Thermo Fisher, America).

Characterization of Gd/Cy5-Oxa@NPs

Transmission electron microscope (TEM, JEOL, JPN) were used to observe the topography and elemental analysis of Gd/Cy5-Oxa@NPs at pH 7.4 and 6.0. The morphology and elemental analysis of the nanoplatform was characterized using TEM operated at 100 kV. The hydrodynamic diameter, polydispersity index, and zeta potential of nanoplatform were measured by Dynamic light scattering (DLS, Bettersize, China).

pH-responsive property of Gd/Cy5-Oxa@NPs in vitro

MRI and fluorescence imaging of Gd/Cy5-Oxa@NPs in vitro

To examine the MRI related to acid-triggered nanoplatform dissociation, phosphate-buffered saline (PBS) of different pH (6.0, 6.2, 6.4, 6.6, 6.8, 7.0) was used to dilute the Gd/Cy5-Oxa@NPs (with Gd concentration set at 0.25 mM) for MRI. To

investigate fluorescence imaging of nanoplatform in the acidic environment, the Gd/Cy5-Oxa@NPs was diluted to 0.1 mg/ml with PBS at different pH (6.0, 6.2, 6.4, 6.6, 6.8, 7.0). Fluorescence images were recorded using the in vivo imaging system (IVIS, PerkinElmer, America) at an excitation wavelength of 630 nm.

In vitro examination of Oxa and Gd release

To detect the release of Oxa and Gd in vitro, Gd/Cy5-Oxa@NPs was put into a dialysis bag and placed in a buffer of pH 6.0 or pH 7.4, and the dialysate was collected at 1, 2, 3, 6, 12 and 24 hours. The concentrations of Gd and Pt in dialysate were detected by ICP-MS, and the cumulative release rate was calculated to evaluate the pH-responsive drug release of Gd/Cy5-Oxa@NPs in vitro.

In vitro cellular uptake assay and cytotoxicity assay of Gd/Cy5-Oxa@NPs

In vitro cellular uptake assay

GL261 cells were plated in 12-well plates at a density of 2×10^5 cells per well. Following an overnight incubation, the cells were exposed to various concentrations (50, 25, 12.5, and 6.25 μg/ml) of Gd/Cy5-Oxa@NPs for different durations (1, 2, and 4 h). After washing the cells with PBS, the cells were collected and mixed in PBS to evaluate the cell uptake by flow cytometry.

GL261 cells were plated into 12-well plates, each containing a round cover glass, at a density of 2×10^5 cells per well. After incubating overnight, the medium was replaced with fresh culture medium containing Gd/Cy5-Oxa@NPs. The cells were then incubated for 4 h, washed with PBS, and fixed with 4% paraformaldehyde. The cell nuclei were subsequently stained with 4′,6-Diamidino-2-phenylindol (DAPI). Finally, images were captured using a confocal laser scanning microscope (CLSM, Leica, Germany).

In vitro cytotoxicity assay

Cytotoxicity was evaluated using the CCK-8 assay. GL261 cells were initially seeded into 96-well plates at a density of 8×10^3 cells per well. After 24 h of incubation, the culture medium was replaced with various concentrations of NPs, Oxa, or Gd/Cy5-Oxa@NPs, adjusted to the specified pH conditions (pH 6.0 or pH 7.4).

The medium was substituted with a CCK-8 reagent solution and incubated for an additional 2 h. The absorbance of each well was measured at 450 nm using a microplate reader (Molecular Devices, America).

In vivo cancer therapy and biosafety evaluation

Animals and tumor model

All experiments involving animals were performed in accordance with the Animal Management Rules of the Ministry of Health of the People's Republic of China and were approved by the Institutional Animal Care and Use Committee (IACUC) of Peking University Health Science Center. Male C57BL/6 mice (6-8 weeks old, 20-25 g) were purchased from the Peking University Health Science Center and all mice were kept in a pathogen-free animal house specific to the Department of Laboratory Animal Sciences. C57BL/6 mice were anaesthetized with tribromoethanol and then secured in a stereotaxic frame for sterile survival surgery and craniotomy. A surgical hole was carefully drilled in the left caudate nucleus (2.0 mm lateral, 1.0 mm anterior to the bregma, and 3.0 mm deep) and 2×10^5 GL261 cells were injected into the caudate nucleus at 0.5 μl/min. Tumors grew for 5 d before receiving different treatments.

In vivo antitumor efficiency evaluation

Mice were divided into 4 groups randomly and administered the following treatments: 1) normal saline, 2) NPs, 3) Oxa, 4) Gd/Cy5-Oxa@NPs (n=3, NPs: 15 μg/mouse, Oxa: 0.66 μg/mouse). The volume of different nanoplatform was consistent at 3 μl, and the administration was performed via CED at a rate of 0.5 μl/min every 5 d. Body weights and tumor volume were measured every 5 d. On the 5th day following the inoculation of GL261 cells into mice, T2-weighted imaging (T2WI) MRI scans were conducted on mice's brains. The tumor region appeared as a mass of abnormal high signal intensity on the T2WI images. Tumor volume was then calculated based on the imaging data. Using the software (RadiAnt DICOM Viewer), tumor boundaries were outlined on each slice to determine the tumor area. The tumor area on the n-th image was represented as S1, S2, S3, ..., Sn, with the slice thickness

denoted as ST (2 mm). Tumor volume (V) was subsequently calculated using the following formula: $V = (S1 + S2 + S3 + ... + Sn) \times ST$.

In vivo biocompatibility evaluation

After 2 d of treatment, serum samples were obtained by centrifugation whole blood (4000 rpm, 10 min) to analyze the concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, and uric acid (UA). Mice from various groups were humanely euthanized, and their major organs, including the heart, liver, spleen, lungs, kidneys, and brains, were harvested. The tissues were fixed in 4% paraformaldehyde, and 5 μm thick paraffin sections were prepared. These sections were subsequently stained with hematoxylin and eosin (H&E) and examined under an optical microscope (3DHISTECH, HU).

In vivo imaging and biodistribution

In vivo MRI and fluorescence imaging

For in vivo imaging of MRI, 3 μl Gd/Cy5-Oxa@NPs was injected into the GL261-tumor-bearing mice via CED. After the injection, T1-weighted images were obtained at 1, 2, and 3 h by a 3T MRI scanner (GE750, TR = 300 ms, slice thickness = 2.0 mm). For in vivo fluorescence imaging, GL261 tumor-bearing mice were administered Gd/Cy5-Oxa@NPs (3 μl) via CED. Fluorescence signals were recorded using the IVIS at 1, 2, and 3 h after the injection.

In vivo biological distribution

Mice were euthanized and their major organs (liver, liver, spleen, lungs, and kidneys) and brains were collected 2 d after CED of Gd/Cy5-Oxa@NPs. Fluorescence imaging of the major organ was performed using the IVIS. In addition, the concentrations of Pt and Gd in major organs and the brain were detected by ICP-MS for analyzing Gd/Cy5-Oxa@NPs metabolic pathways.

Statistical analysis

All results are expressed as mean ± standard deviation (SD). All statistical data were processed in Origin 2022 by Student’s t-test or one-way ANOVA. ns indicates

no significance, * indicates significance at $p < 0.05$, ** indicates significance at $p < 0.01$, *** indicates significance at $p < 0.001$, **** indicates significance at $p < 0.0001$.

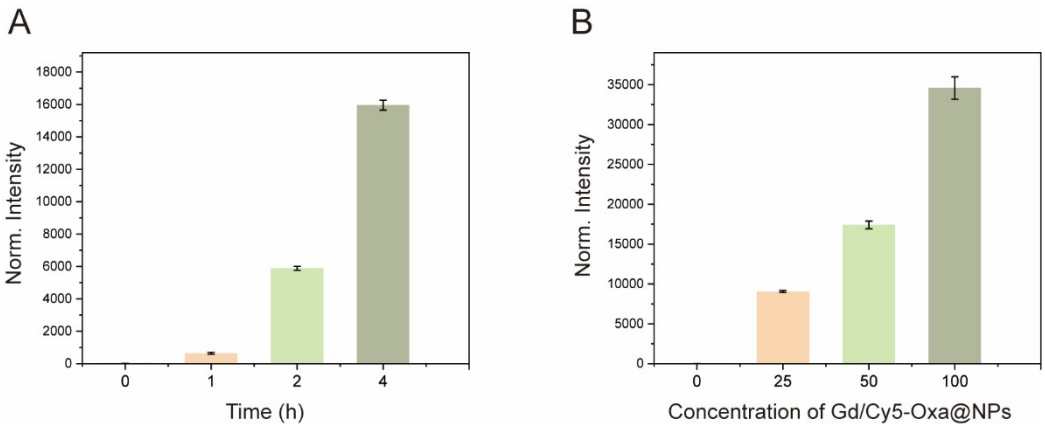

Fig. S1 (A) Quantitative analysis of time-dependent cellular uptake of Gd/Cy5-Oxa@NPs in GL261 cells by flow cytometry (n=3, mean ± SD). (B) Quantitative analysis of concentration-dependent cellular uptake of Gd/Cy5-Oxa@NPs in GL261 cells by flow cytometry (n=3, mean ± SD).

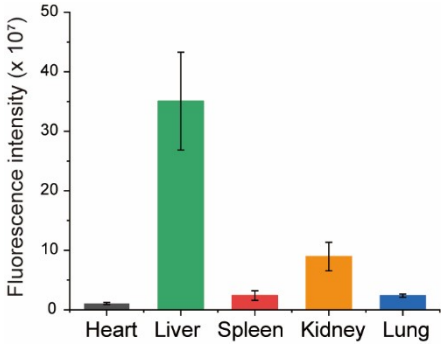

Fig. S2 Quantitative fluorescence intensity analysis of major organs after a single-dose administration of Gd/Cy5-Oxa@NPs via CED (n=3, mean ± SD).

Reference

1. B. Dong, S. Du, C. Wang, H. Fu, Q. Li, N. Xiao, J. Yang, X. Xue, W. Cai and D. Liu, *ACS nano*, 2019, **13**, 1421-1432.