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Supplementary Information

Photosensitizer-Induced Self-Assembly of Antigens as Nanovaccines for Cancer Immunotherapy

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Materials and methods

2.1. Materials and reagents

Ovalbumin (OVA) were supplied by Sigma-Aldrich (St. Louis, MO, USA). ICG-sulfo-OSu, a derivative ICG, was obtained from AAT Bioquest, Inc. (Sunnyval, CA, USA). LysoTracker probes were supplied by Life Technologies (Grand Island, NY, USA). 2', 7'-dichlorodihydrofluorescein diacetate acetyl easter (H_2DCFDA) was supplied by Life Technology (USA). All other chemicals used were of the highest quality commercially available.

2.2. Synthesis and characterization of the ICG-OVA nanovaccines

OVA was dissolved in 0.5 mL of phosphate buffer (pH 8.0) and reacted with ICG in 0.5 mL of DMSO at room temperature for 4 h. The reaction mixture were added 10 mL of distilled water before ultrafiltration by a centrifugal filter device (molecular weight cutoff (MWCO) = 10 kDa) to remove free ICG and DMSO. The morphology of ICG-OVA nanovaccines was visualized by using a Dimension™ 3100 AFM (Veeco Instruments, Santa Barbara, CA, USA). The average particle size was measured by a 90Plus particle size analyzer (Brookhaven Instruments Corporation, Holtsville, NY, USA). The circular dichroism (CD) spectroscopy was used to study OVA conformations in solution or conjugated in ICG-OVA nanovaccine. CD spectra analysis was carried out by JASCO J-815 Spectropolarimeter (Jasco, Japan) at 25 °C over the range 200-250 nm under a nitrogen atmosphere. The absorption spectra of ICG and ICG-OVA nanovaccines were analyzed by UV spectroscopy from 400 nm to 900 nm. The amount of ICG in the ICG-OVA nanovaccines was determined by fluorescent spectrometer (excitation at 780 nm and emission at 815 nm). The amount of ICG in the ICG-OVA nanovaccines was determined by fluorescent spectrometer (excitation at 780 nm and emission at 815 nm). ICG-OVA nanovaccines were dissolved in 5 wt% sodium dodecyl sulfate (SDS) to destroying the structure of ICG-OVA nanovaccines and thus releasing the encapsulated ICG adequately. Calibration curve was obtained with ICG/5 wt% SDS solutions with different ICG concentrations.

2.3. Preparation and viability of bone marrow-derived dendritic cells (BMDCs)

DCs were generated from bone marrow cells of C57BL/6 mouse femur and tibia as previously described. A CCK-8 assay was used to assess the cell viability of ICG-OVA nanovaccines. DCs were seeded into 96-well culture plates (1×10^4 cells/well) in growth medium. Then, the cells were incubated with ICG-OVA nanovaccines (equivalent ICG concentration of 10 μg/mL). After 6 h, PBS washed the cells and fresh medium were replaced. The cells were irradiated with a 35 mW/cm^2 808-nm laser for different time. After further incubated for 24 h, the medium was removed and the wells were washed three times with PBS. Finally, cells were incubated with CCK-8 solution for 4 h and OD450 was measured using a Thermo Varioskan Flash Multifunction Microplate Reader.

2.4. Intracelluar distribution of ICG-OVA nanovaccines in the dark and under NIR light exposure

To evaluate intracelluar distribution of ICG-OVA nanovaccines in BMDCs, the cells were incubated with ICG-OVA nanovaccines containing FITC-labeled OVA (equivalent OVA concentration of 20 μg/mL and ICG concentration of 10 μg/mL) for 6 h. Then the cells were washed 3 times with PBS and irradiated with/without a 35 mW/cm^2 808-nm laser for 1.5 min.

After further incubated for 1.5 h, the cells were labelled with Lyso Tracker-Red DND-99 and DAPI to identify lysosomes and nuclei, respectively. Finally, CLSM (CLSM 410; Zeiss, Jena, Germany) was used to observe the cells using Fluoview FV500 imaging software.

2.5. Cross presentation in vitro

In vitro cross-presentation of OVA by BMDCs was evaluated by a lacZ antigen presentation assay. BMDCs were incubated with free OVA or ICG-OVA nanovaccines (equivalent OVA concentration of 20 μg/mL and ICG concentration of 10 μg/mL) at 37 °C for 6 h. Then the cells were washed 3 times with PBS and irradiated with/without a 35 mW/cm^2 808-nm laser for 1.5 min. B3Z cells (2 × 10^5 cells/well) were incubated with BMDCs for 24 h. Then cells were washed with PBS, and 100 μL of X-Gal buffer was added for up to 24 h at 37 °C. Absorbance was measured at 405 nm using a microplate reader.

2.6. ROS detection in vitro

BMDCs were treated with H_2DCFDA probe to detect intracellular ROS level. In brief, DCs were seeded into 96-well culture plates (1×10^4 cells/well) in growth medium. BMDCs were incubated with medium or ICG-OVA nanovaccines (equivalent OVA concentration of 20 μg/mL and ICG concentration of 10 μg/mL) at 37 °C for 6 h. Following the ICG-OVA nanovaccines treatment, media was removed and cells were loaded with 10μM H_2DCFDA diluted in clear media at 37 °C for 30 min. Subsequently, the cells were washed with PBS and irradiated with or without an 808 nm laser (35 mW/cm^2) for 1.5 min. ROS were immediately measured by In Cell Analyzer 1000 (GE Healthcare, England) using FITC filter set. Morphology of cells was photographed using CLSM (CLSM 410; Zeiss, Jena, Germany).

2.7. Proteasome activity

To determine the proteasome activity, BMDCs were incubated with medium or ICG-OVA nanovaccines (equivalent OVA concentration of 20 μg/mL and ICG concentration of 10 μg/mL) with or without the pretreatment of vitamin C (VC) at 37 °C for 6 h. Subsequently, the cells were washed with PBS and irradiated with or without an 808 nm laser (35 mW/cm^2) for 1.5 min. The proteasome activity was assessed using a fluorometric assay kit (AAT Bioquest, CA, USA) according to manufactory's instruction.

2.8. In vivo tracking of ICG-OVA nanovaccines

To visualize the lymphatic tracking of the ICG-OVA nanovaccines, the female C57BL/6 mice were subcutaneously injected on their tail-base site with ICG-OVA nanovaccines (equivalent OVA concentration of 40 μg/mL and ICG concentration of 20 μg/mL in 100 μL saline per mouse). An 808-nm laser at 50 mW/cm^2 was used to irradiate the immunization site (30 min after immunization) for 3 min. The Maestro imaging system (CRI inc. MA, USA) was used to obtain ICG fluorescent signals (704 nm excitation and 735 nm filter) at different time intervals.

2.9. Tumor implantation and immunization of mice

All the animal experiments were performed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals, Peking Union Medical College, China. E.G7-OVA cells 6, the derivative ovalbumin (OVA)-expressing E.G7 lymphoma cells, were inoculated

subcutaneously into the right flank of female C57BL/6 mice (6-8 weeks old) (Beijing Huafukang Laboratory Animal Technology Co., Ltd, China). On the day 7, mice (n = 5 mice/group) were subcutaneously injected on their tail-base site with saline, ICG-OVA nanovaccines (equivalent 20 μg ICG and 40 μg OVA in 100 μL saline per mouse), free ICG (equivalent 20 μg ICG in 100 μL saline per mouse), free OVA (equivalent 40 μg OVA in 100 μL saline per mouse) or a mixture of ICG and OVA (equivalent 20 μg ICG and 40 μg OVA in 100 μL saline per mouse). An 808-nm laser at 50 mW/cm^2 was used to irradiate the immunization site for 3 min.

Tumor volume were measured every two days with callipers and volume was calculated as $V=(ab^2)/2$, where a and represent length and width (mm). After 3 days post-last immunization, spleens and tumors were both harvested from mice in different groups and prepared into a single cell suspension. Cells were labelled with fluorochrome-labelled antibodies against CD3 and CD8 antibodies and analysed using flow cytometry. For in vitro T lymphocytes proliferation assay, splenocytes T cells from each experimental group were labelled with 5 μmol/L 5,6-carboxyfluorescein acetate N-succinimidyl ester (CSFE) according to the manufacture's instructions. The CSFE-labeled T cells were then restimulated with OVA (10 μg/mL) for 72 h at 37 °C. The proliferation of CD8$^+$ T lymphocytes was determined by measuring live cells with decreased CFSE intensity (CFSElow cells) using flow cytometry.

2.11. Statistical analysis

Data were expressed as the mean ± standard deviation (SD). Difference analysis between two groups was made using an unpaired, two-side Student's t-test. *P* values of 0.05 or less were considered to be statistically significant.

Results

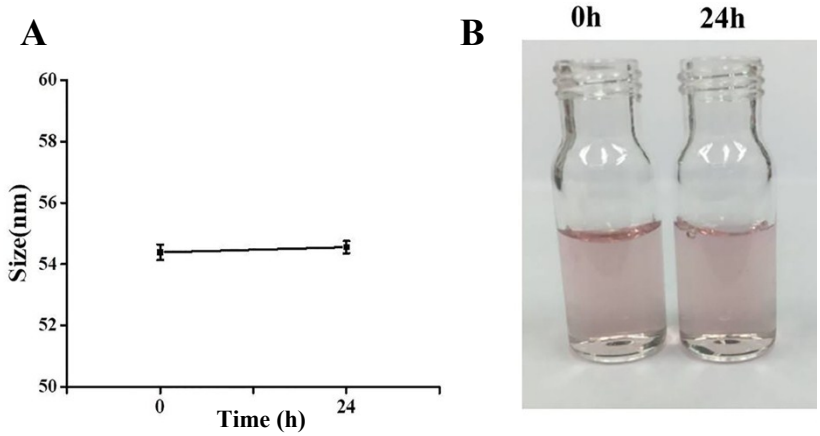

Figure S1. The stability of ICG-OVA nanovaccines over time in serum containing RPMI cell culture medium. (A) Average hydrodynamic diameter and (B) Digital images of ICG-OVA nanovaccines in serum containing RPMI cell culture medium.

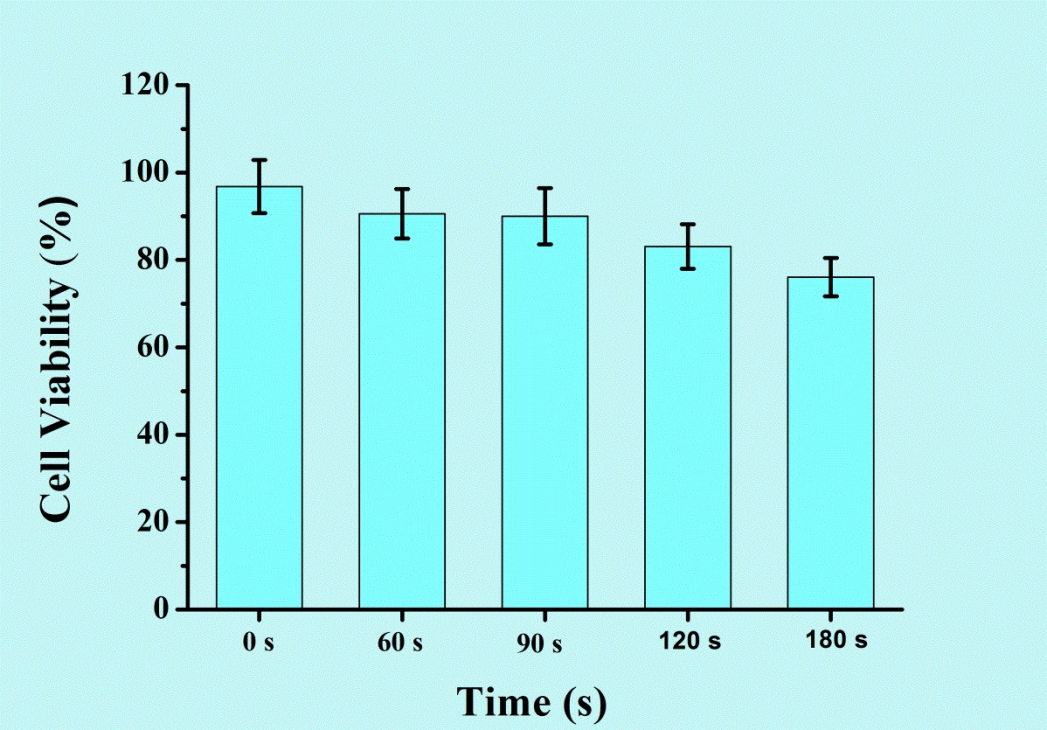

Figure S2. BMDCs viability after incubation with ICG-OVA nanovaccines. BMDCs were irradiated with a 35 mW/cm^2 808-nm laser for different time. The data are presented as the mean ± SD (n = 6).