Supporting Information

Redox-responsive hyperbranched poly(amido amine) and polymer dots as vaccine delivery system for cancer immunotherapy

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1 Gel permeation chromatography (GPC) analysis of the PAA-PEI<sub>600</sub> and PDs

1.1 Experimental

The molecular weight of PAA-PEI<sub>600</sub> was measured by using GPC (Waters Breeze 515, USA) with dextran as a standard. Aqueous sodium nitrate (0.2 mol/L, containing 0.2‰ (w/v) sodium azide) was used as the eluent with a flow rate of 0.6 mL/min.

Redox sensitivity of the PAA-PEI<sub>600</sub> and PDs was studied by measuring their elution times before and after glutathione treatment via the GPC analysis. Briefly, 2 mg/mL of PAA-PEI<sub>600</sub> solution or PDs suspension was added into the same volume of 20 mM aqueous GSH solution. After incubated for 1 h at 37°C, the mixtures were analyzed by GPC, as described above.

1.2 Result

According to the GPC analysis, the molecular weight of PAA-PEI<sub>600</sub> was calculated to be 183.0 kDa. The elution times of the PAA-PEI<sub>600</sub> and PDs before and after glutathione treatment were shown in Figure S1. PAA-PEI<sub>600</sub> and PDs before GSH treatment showed the maximum peaks at 31.06 and 36.55 min, respectively. After the GSH treatment, the maximum peaks of the samples shifted to 41.46 and 41.51 min, respectively. This suggests that the redox-responsive -S-S- broke rapidly by glutathione and the molecular weights of PAA-PEI<sub>600</sub> and PDs decreased along with the breakage of the disulfide bonds. It indicates that the PAA-PEI<sub>600</sub> and PDs had redox-sensitivity and could degrade in the presence of intracellular glutathione.
2 Loading and encapsulation efficiency of OVA and its release kinetics

2.1 Experimental

Aqueous PAA-PEI$_{600}$ solution (20 mg/mL, 250 µL in pure water) or PDs suspension (20 mg/mL, 250 µL in pure water) was mixed with aqueous OVA solution (4 mg/mL, 250 µL in pure water) to form nanoparticles suspension. The suspensions of PAA-PEI$_{600}$/OVA and PDs/OVA nanoparticles were incubated at 37°C for 30 min. Then, the suspensions were centrifuged at $1.5 \times 10^4$ rpm for 5 min. The supernatants were collected. OVA concentration in the supernatants was detected by using Pierce BCA Protein Assay Kit (Thermo Scientific, USA).

Aqueous PAA-PEI$_{600}$ solution (20 mg/mL, 250 µL in pH7.4 PBS) or PDs suspension (20 mg/mL, 250 µL in pH7.4 PBS) was mixed with aqueous OVA solution (4 mg/mL, 250 µL in pH7.4 PBS) to form nanoparticles suspension. The suspensions of PAA-PEI$_{600}$/OVA and PDs/OVA nanoparticles were incubated at 37°C. At 1, 3, 6, 9, 12 and 24 h, the suspension was centrifuged at $1.5 \times 10^4$ rpm for 5 min. The supernatant (100 µL) was collected. OVA concentration in the
supernatant was detected as described above. Meanwhile, 100 µL of the PBS was supplemented into the suspension. The suspension was resuspended and incubated at 37°C for further sampling.

In another release experiment, at 6 h, glutathione was added to the release medium of PAA-PEI\textsubscript{600}/OVA and PDs/OVA nanoparticles to a final concentration of 2 mM, in order to study the release profiles of OVA from the redox-responsive PAA-PEI\textsubscript{600} and PDs in the presence of glutathione. Other procedures were the same as described above.

2.2 Result

The encapsulation efficiency (the mass percentage of loaded OVA in the total OVA input) of PAA-PEI\textsubscript{600}/OVA and PDs/OVA nanoparticles were calculated to be 79.5% and 89.9%, respectively. The loading efficiency (the mass percentage of loaded OVA in the total carrier materials input) of PAA-PEI\textsubscript{600}/OVA and PDs/OVA nanoparticles were calculated to be 15.9% and 18.0%, respectively.

The release kinetics of OVA from the nanoparticles is shown in Figure S2. At 24 h, about 76% OVA was released from PAA-PEI\textsubscript{600}/OVA nanoparticles in the absence of glutathione, and about 56% OVA was released from PDs/OVA nanoparticles in the absence of glutathione. The slower OVA release from PDs/OVA nanoparticles could be due to the more positive charge around PDs and the stronger electrostatic attraction between PDs and OVA. In addition, almost 100% of OVA was released from the two nanoparticles after 9 h in the presence of glutathione added at 6 h. It indicates that the presence of glutathione could cause the degradation of the redox-responsive PAA-PEI\textsubscript{600} and PDs, which further leaded to complete OVA release from the nanoparticles.
Figure S2. OVA release from PAA-PEI\textsubscript{600}/OVA and PDs/OVA nanoparticles in the presence or absence of glutathione (GSH).

3 Percentage of OVA-Cy5 positive DC2.4 cells by FACS

3.1 Experimental

The experiment was the same as that in the section 2.4 of the manuscript.

3.2 Result

The percentage of OVA-Cy5 positive DC2.4 cells by FACS was shown in Figure S3.
4 Tracking the antigen protein OVA via fluorescent PDs

4.1 Experimental

The experiment was the same as that in the section 2.4 of the manuscript.

4.2 Result

To track the antigen protein OVA via fluorescent PDs, DC2.4 cells were incubated with PDs/OVA-Cy5 nanoparticles for 2 and 6 h, observed with CLSM, and displayed in Figure S4. Obviously, the blue fluorescence of the PDs and the red fluorescence of OVA-Cy5 in DC2.4 cells could well overlap. This suggests that the location of the fluorescent PDs could well reflect the trace of OVA.
Figure S4. Tracking the antigen protein OVA via fluorescent PDs in DC2.4 cells after 2 and 6 h of incubation.

5 Potential of the PAA-PEI<sub>600</sub> and PDs to enhance cross-presentation

5.1 Experimental

In order to evaluate the level of cross-presentation, the OVA-specific MHC I molecule expression was analyzed. Briefly, C57BL/6 mice (6-8 weeks old) were randomly divided into three groups (n=5) and subcutaneously injected with 100 μL (50 μL/hind leg) of OVA (30 μg) alone, PAA-PEI<sub>600</sub>/OVA (150 μg/30 μg) and PDs/OVA (150 μg/30 μg) nanoparticles, respectively. At 1st, 3rd and 7th d after the immunization, the popliteal lymph nodes were collected and ground to cells. Subsequently, the popliteal lymph node cells were washed with PBS and stained with the following fluorochrome-conjugated anti-mouse antibodies: PE-anti-H-2K<sub>b</sub> bound to SIINFEKL
(eBioscience, CA, USA) and APC-anti-CD11c (1:200 v/v in PBS). Then, the cells were detected by the flow cytometry.

5.2 Result

The potential of the PAA-PEI$_{600}$ and PDs to enhance cross-presentation was evaluated by measuring the OVA-specific MHC I expression on CD11c$^+$ (characteristic marker of DCs) DCs of the popliteal lymph node cells of the immunized mice, as shown in Figure S5. At 1$^{\text{st}}$ d, PDs/OVA nanoparticles induced significantly higher MHC I molecule expression than OVA alone. At 3$^{\text{rd}}$ d, PAA-PEI$_{600}$/OVA and PDs/OVA nanoparticles both induced significantly higher MHC I molecule expression than OVA alone. At 7$^{\text{th}}$ d, PDs/OVA nanoparticles induced significantly higher MHC I molecule expression than OVA alone. The results indicate that PDs/OVA nanoparticles could induce higher expression of MHC I molecule on DCs in the lymph nodes at the initial and later stages after immunization. It seems that the PDs/OVA nanoparticles could induce effective cross-presentation to enhance cellular immune responses.

![Figure S5. MHC I molecule expression on DCs in popliteal lymph nodes of the immunized mice.](image-url)