Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

Supplementary Information

Hemolysis assay

Blood compatibility was evaluated with hemolysis assay. Fresh rat blood was extracted from hepatic vein and stabilized with Heparin. 2 ml of whole blood sample was added to 4ml Dulbecco's phosphate-buffered saline (D-PBS) and then centrifugated at 10000 g for 5 min to isolate red blood cells (RBCs). The RBCs were further washed five times with 10 ml of D-PBS and finally diluted to 20 ml with D-PBS. 0.2 ml of diluted RBC suspension was exposed to 0.8 ml of the nanoparticle D-PBS suspension at a concentration of 2.5, 12.5, 62.5, 125, 250 or 500 μg/ml to make the final nanoparticle concentration 2, 10, 50, 100, 200 or 400 μg/ml (test group), distilled water (positive group), and D-PBS (negative group). Every group was represented for four tubes. After incubation at room temperature for 4 h and centrifugation for 5 min at 10016g, 100 μl of the supernatant of all samples was transferred to a 96-well plate and the absorbance was measured by a microplate reader (TECAN Znfinite M200, Austria) at 577 nm with the 655 nm as a reference. The hemolytic degree was expressed by the hemolytic ratio as the following formula: hemolysis ratio = $(OD_{(test)} - OD_{(negative\ control)})/(OD_{(positive\ control)} - OD_{(negative\ control)}) \times 100\%$.

Toxicity test of PNIPAM/AA@SiO_2 nanoparticles

Furthermore, incubation of MSN and PNIPAM/AA@SiO_2 nanoparticles with the concentration of 0.8–400 μg/ml showed that PNIPAM/AA@SiO_2 had almost no cytotoxicity to the HEK 293 normal cells, which was lower cytotoxicity than MSN (Fig. S2a). The result of cytotoxicity to normal cells showed that PNiPAM/AA@SiO_2 is a non-toxic material at the low concentration and is suitable as a drug carrier.

To further evaluate the possibility of in vivo application of PNiPAM/AA@SiO_2 particles, the hemolysis tests was used to characterize the blood compatibility of the PNiPAM/AA@SiO_2 and compared with that of MSN. Red blood cells (RBCs) was

treated for 4 h with various concentrations (from 2 to 400 μg/mL^{-1}) of PNiPAM/AA@SiO_2 and MSN samples. It could be seen from Fig. S2b, c and d that the percentage of hemolysis of MSN exhibited does-dependent while the group of PNiPAM/AA@SiO_2 had almost no hemolysis at all the tested concentrations. The result indicated that PNIPAM/AA@SiO_2 was more biocompatible and lower cytotoxicity than MSN. It is well known that the interactions of silica nanoparticles with the membranes of RBCs are mainly dependent on the presence of silanol groups on the surface of the particles.[1] Maybe because steric hindrance from the polymer chains of hybrid silica shells reduces the interactions between silanol groups of the particles and the RBCs, PNIPAM/AA@SiO_2 shows the lower hemolytic activity.

References

1. Y. S. Lin and C. L. Haynes, *Journal of the American Chemical Society*, 2010, **132**, 4834-4842.

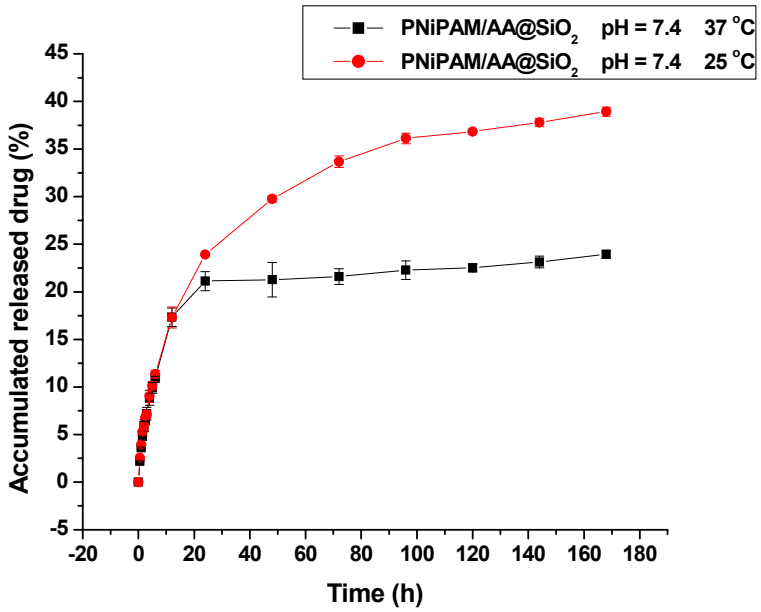

Fig. S1. DOX release profiles from DOX@SiO_2 and DOX@PNiPAM/AA@SiO_2 nanoparticles with decreasing temperature for 7 days.

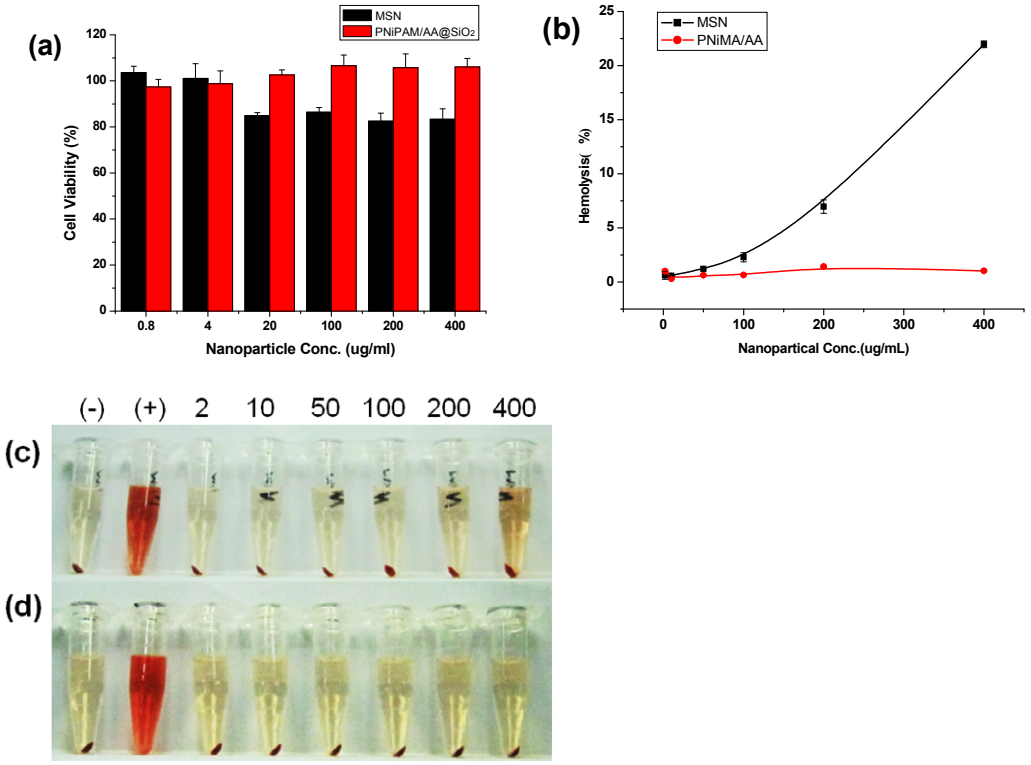

Fig. S2. The comparision of cytotoxicity of PNiPAM/AA@SiO_2 and MSN in vitro. (a) HEK293 cells exposed to PNiPAM/AA@SiO_2 and MSN. (b) Percentage of hemolysis of RBCs incubated with PNiPAM/AA@SiO_2 and MSN at different concentrations ranging from 2 to 400 μg/mL for 4 h. Photographs of hemolysis of RBCs in the presence of MSN (c) and PNiPAM/AA@SiO_2 (d). Water (+) and D-PBS (-) are used as positive and negative control, respectively. From left to right, (-), (+) and 2, 10, 50, 100, 200, 400 μg/ml.