Versatile RBC-derived vesicles as nanoparticle vector of photosensitizers for photodynamic therapy

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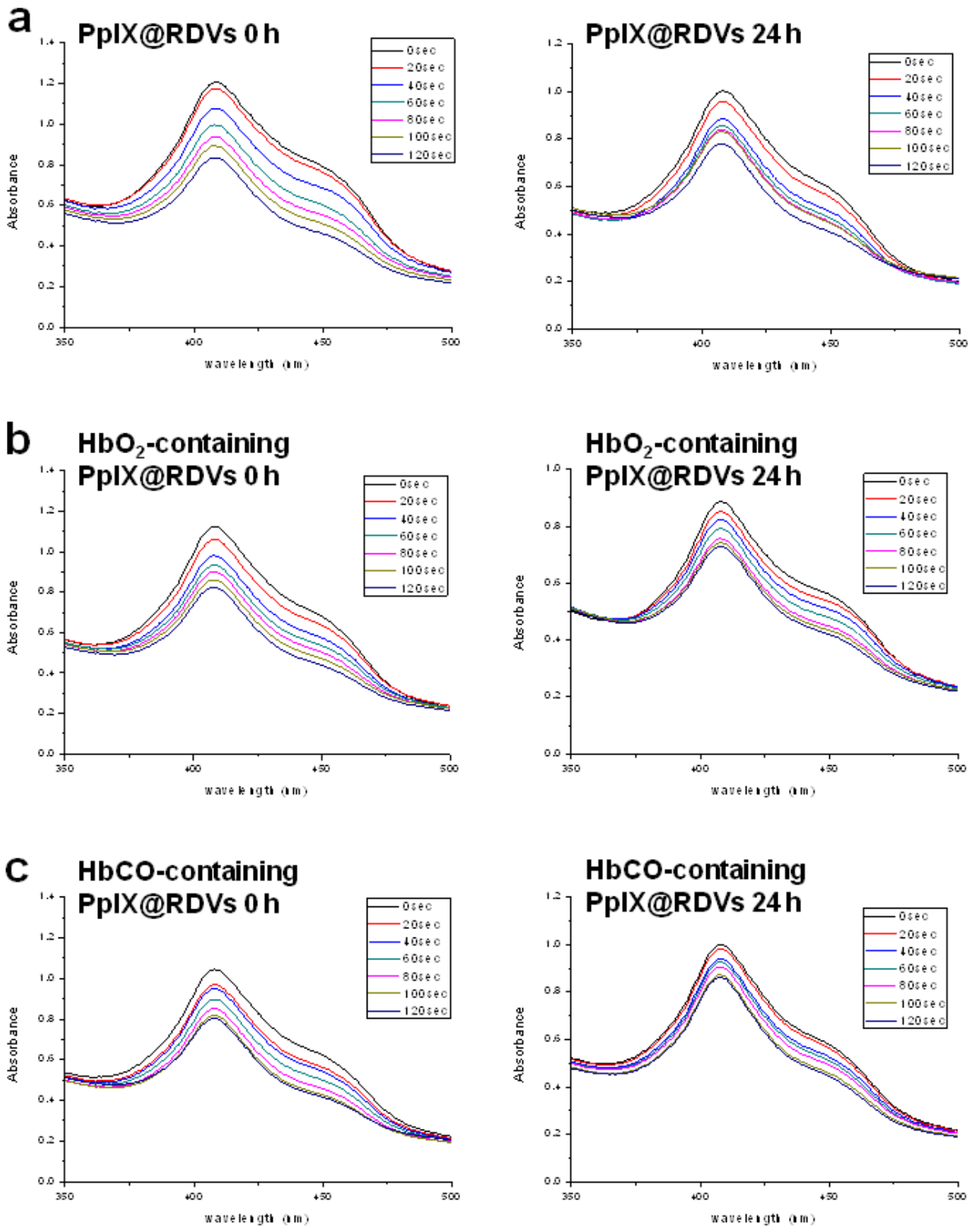
Detection of singlet oxygen

We used 1,3-diphenylisobenzofuran (DPBF, Aldrich) to determine the release of singlet oxygen into solution[1] to indirectly estimate the oxygen inside RDVs. The reaction was monitored by recording the decrease in absorption at 400 nm via UV-Vis spectroscopy (DU800, Beckman). In our experiment, 1.25 μL of stock solution of DPBF (8 mM) was added into 200 μL of 0.5 μg μL^{-1} PpIX@RDVs (Fig. S1a), HbO_2-containing PpIX@RDVs (Fig. S1b) or HbCO-containing PpIX@RDVs (Fig. S1c) at 0 or 24 h after the preparation of various RDVs in D_2O. Solutions were then illuminated with a 635 laser and the optical densities at 408 nm recorded every 20 s for total 120 s. The steep decrease of DPBF absorption with time in the PpIX@RDVs solution or HbO_2-containing PpIX@RDVs solution but not in the HbCO-containing PpIX@RDVs solution indicates the efficient generation of $^1O_2$ by PpIX@RDVs and HbO_2-containing PpIX@RDVs but not by HbCO-containing PpIX@RDVs (Fig. S1d). Although the efficiency of $^1O_2$ generation in the PpIX@RDVs or HbO_2-containing PpIX@RDVs solution was less than that at 0 h, PpIX@RDVs and HbO_2-containing PpIX@RDVs still showed good performance of $^1O_2$ generation at 24 h after their preparation. These results show that the oxygen molecules could be maintained inside RDVs for a long period of time.



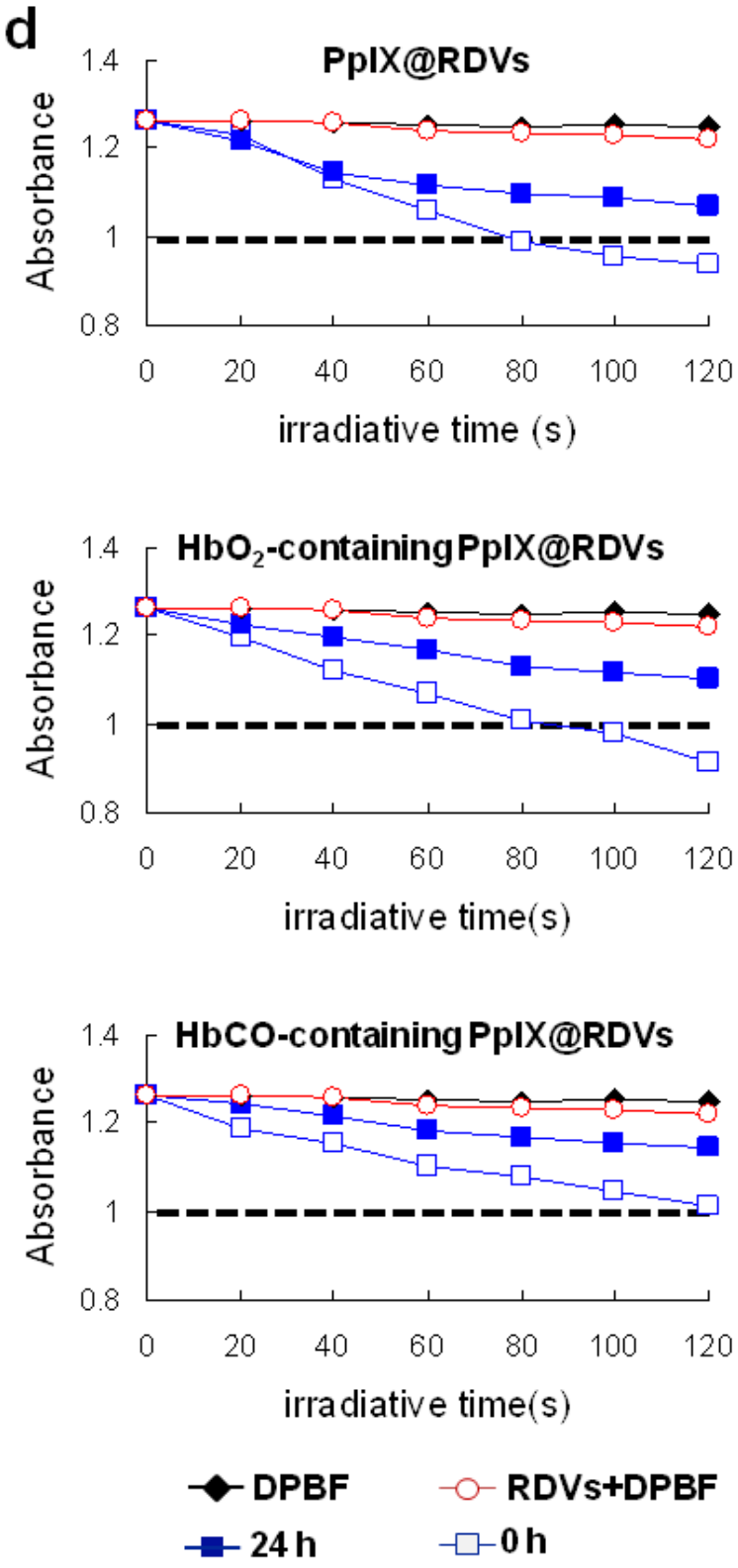
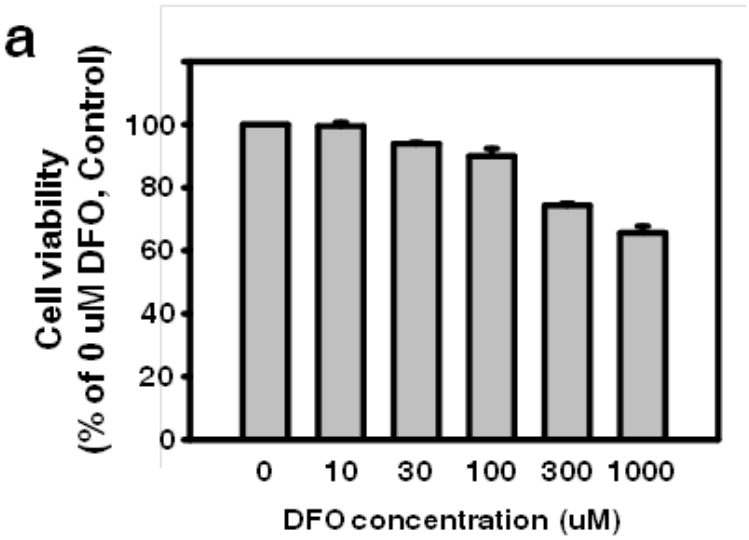


Fig. S1 Photobleaching of DPBF by singlet oxygen generated from PpIX@RDVs (a), HbO_2-containing PpIX@RDVs (b), or HbCO-containing PpIX@RDVs (c) upon photoirradiation at 635 nm. (d) Decay curves of DPBF absorption as function of irradiative time from (a) to (c).

Induction of cellular hypoxia by DFO

Huh7 cells (5×10^3 cells) were seeded in a 96-well plate and allowed to attach for 24 h. These cells were treated with desferrioxamine (DFO) at 0 (Control), 10, 30, 100, 300, and 1000 μM for 24 h and then were processed for MTT reduction assay. We found that DFO would cause a minor cytotoxicity when the treatment concentration was higher than 100 μM (Fig. S2a), and therefore DFO at 30 μM was chosen to induce cellular hypoxia.

Huh7 cells were seeded in 35-mm dishes (1×10^5 cells per dish) overnight and then were treated with 30 μM DFO for 24 h. After treatment cells were rinsed with ice-cold 1 × PBS and were lysed by the addition of lysis buffer (25 mM HEPES, pH 7.5, 150 mM NaCl, 1% Igepal CA-630, 10 mM $MgCl_2$, 1 mM EDTA and 2% glycerol, 1μM phenylmethylsulfonyl fluoride, 1 μg mL^{-1} leupeptin, and 10 μg mL^{-1} aprotinin) for 60 min at 4°C. The suspensions were centrifuged at 15700 g for 20 min at 4°C. The protein concentration of the supernatant was assessed by the Bio-Rad protein assay kit. The cellular content of HIF1α was determined by commercially available enzyme-linked immunosorbent assay kit (ELISA; Abcam, ab117996). A dramatic induction of HIF1α content in DFO-treated cells suggests a cellular hypoxia (Fig. S2b).



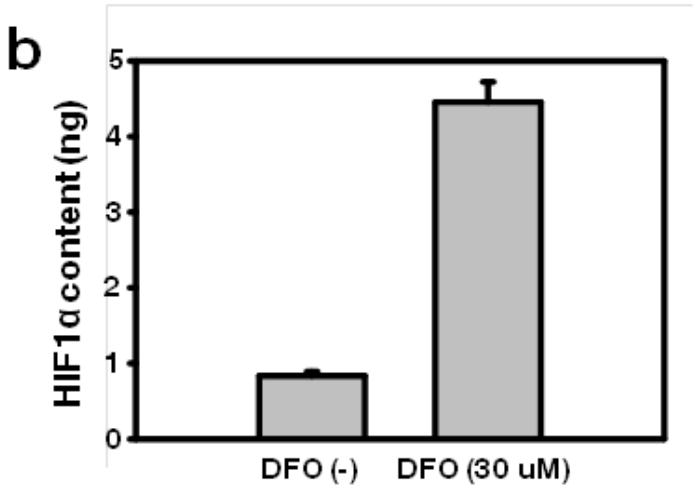


Fig. S2 Induction of hypoxia by DFO in Huh7 cells. (a) The cytotoxic effects of various concentrations of DFO. Data are expressed as mean ± standard error of three determinations (each in quadruplicate). (b) The induction of cellular hypoxia was demonstrated by the increase of HIF1α content. Values represent mean ± standard error of four experiments.

Stability of RDVs

10 μL RDVs (0.1 μg μL^{-1}) were incubated in 990 μL Dulbecco's phosphate buffered saline (DPBS) containing with (blood/DPBS) or without (DPBS) 90 μL whole blood at 4°C for 1, 24 or 72 h. Then the samples were processed for the TEM observation and the particle size and zeta potential measurement. After a long incubation of RDVs, RDVs could be recovered without deformation or aggregation by TEM observation (Fig. S3). The zeta potential assay also suggests the high stability of RDVs (Table S1).

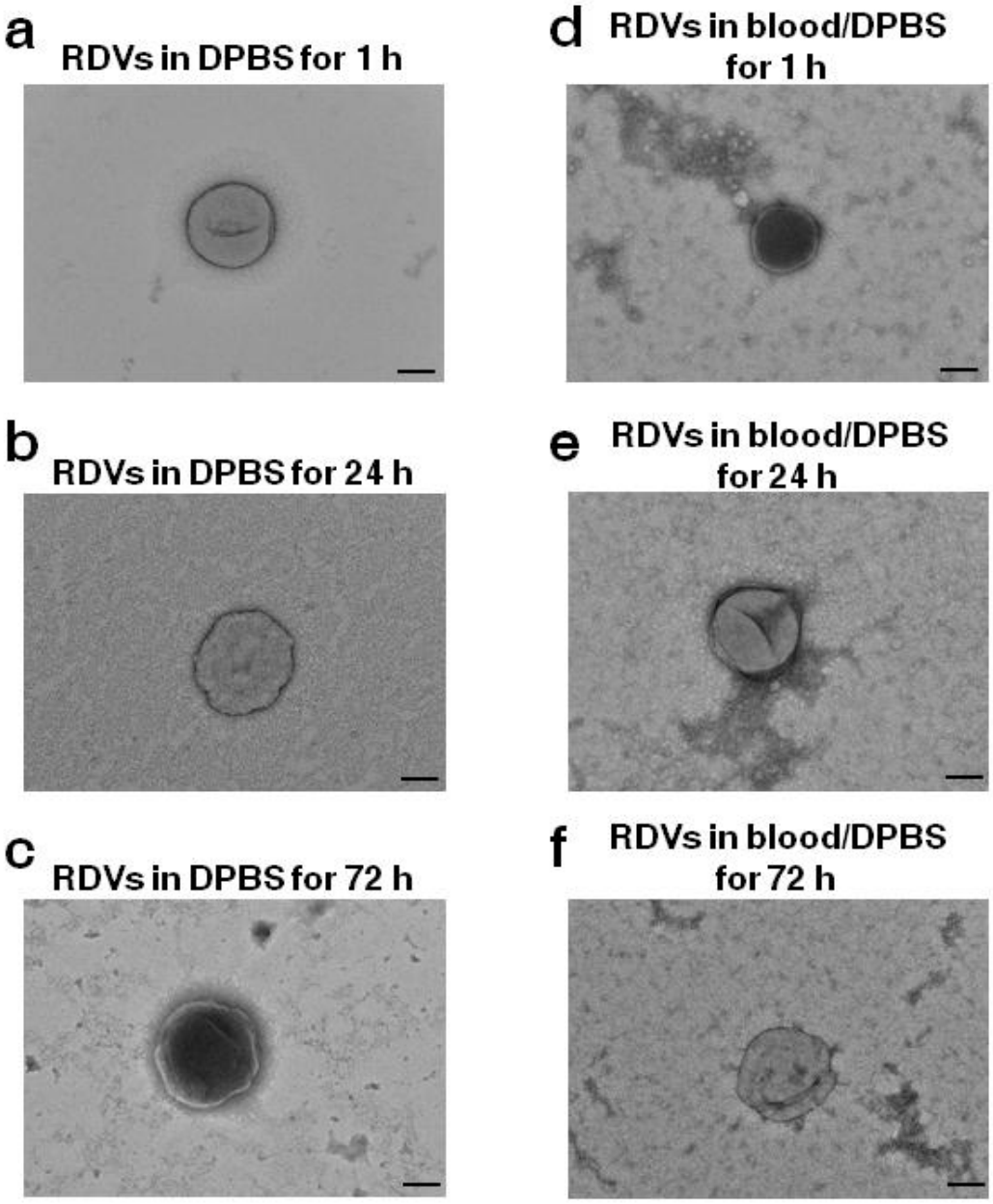


Fig. S3 TEM observation of RDVs after the incubation in DPBS for 1 h (a), 24 h (b), and 72 h (c) or in blood/DPBS for 1 h (d), 24 h (e), and 72 h (f). Scale bar, 100 nm.

Table S1. The particle sizes and zeta potentials of RDVs after their incubation in DPBS or blood/DPBS.

Incubation time╲Buffer	**DPBS**	**Blood/DPBS**
1 h	266.7 ± 5.6 nm -10.52 ± 0.63 mV	260.6 ± 6.2 nm -10.52 ± 1.39 mV
24 h	269.2 ± 4.0 nm -10.33 ± 0.38 mV	255.1 ± 6.7 nm -11.33 ± 0.47 mV
72 h	278.6 ± 7.8 nm -11.13 ± 0.42 mV	271.4 ± 10.2 nm -11.77 ± 0.21 mV

References

(1) D. B. Tada, L. L. Vono, E. L. Duarte, P. K. Itri, M. S. Baptista and L. M. Rossi, *Langmuir*, 2007, **23,** 8194–8199.