Electronic Supplementary information

Erythrocyte membrane based cationic polymer-mcDNA complexes as an efficient gene delivery system

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Part of experimental details:

The optimization for hypotonic solution formula

Five kinds of hypotonic solution as the following formulas:

Solution 1 (Sol.1): 2.5 mM Tris, 0.025 mM EDTA, 0.25 mM $CaCl_2$.

Solution 2 (Sol.2): 5 mM Tris, 0.05 mM EDTA, 0.5 mM $CaCl_2$.

Solution 3 (Sol.3): 10 mM Tris, 0.1 mM EDTA, 1 mM $CaCl_2$.

Solution 4 (Sol.4): 20 mM Tris, 0.2 mM EDTA, 2 mM $CaCl_2$.

Solution 5 (Sol.5): 40 mM Tris, 0.4 mM EDTA, 4 mM $CaCl_2$.

Erythrocyte membrane was prepared with the same processes described in the manuscript. Intact membrane system structure is crucial for cargoes delivery. In order to evaluate the integrity property of the prepared membrane system, all the samples were stained by trypan blue and measured via flow cytometry (BD FACS CantoTM II, USA).

Herein, the integrity ratio was calculated according to the following formula:

$$\text{Integrity ratio (\%)} = (\text{uncoloured cells} / \text{total cells}) \times 100\%$$

As shown in Fig. S1, the FSC/SSC signal from erythrocyte membrane which prepared in Sol.1 and Sol.2 solutions were different with the natural erythrocyte. It indicated that the low osmotic pressure caused erythrocyte membrane disruption. By contrast, there were more than 90% samples maintained membrane integrality in Sol.3, Sol.4 and Sol.5 solutions. Meanwhile, these samples exhibited the similar SSC and FSC signal intensity. However, there were still several erythrocytes through the preparation via Sol.4 and Sol.5 solutions which were observed under an optical microscope (not shown here). Therefore, the hemoglobin could be nearly totally eliminated and the high membrane integrity could be prepared within Sol.3 hypotonic solution. Finally, the Sol.3 was selected as the optimized hypotonic solution formula for the following experiments in this paper.

The transfection efficiency of EM-XtremeGENE-mcDNA prepared with different hypotonic solution formula

We prepared four kinds of hypotonic solution as the following formulas.

Formulation 1 (EM1): 10 mM Tris, 0.1 mM EDTA, 1 mM $CaCl_2$.

Formulation2 (EM2): 5mM NaH_2PO_4, 5mM $NaH_2PO_4 \cdot H_2O$, 5mM, $Na_2HPO_4 \cdot 7H_2O$, 5mM $MgCl_2$.

Formulation 3 (EM3): 1mM KH_2PO_4, 5mM Na_2HPO_4, 27.4mM NaCl, 0.54mM KCl.

Formulation 4 (EM4): 0.2mM KH_2PO_4, 1mM Na_2HPO_4, 5.5mM NaCl, 0.1mM KCl.

Erythrocyte membrane was prepared with the same processes described in the manuscript. The transfection efficiency of EM-XtremeGENE-mcDNA was evaluated. 293T cell was selected as the targeted cell and the XtremeGENE HP DNA Transfection Reagent was applied to form cationic complexes. Cells were seeded at the density of 1.0×10^4 cells in 96-well plates with complete FBS containing media and were incubated at 37 ^oC for 24 h. Then, the media was removed and 100 μL FBS free DMEM solution contained EM-cationic polymer-mcDNA system (0.5 μg mcDNA per well) was added. The cells were incubated for 4 h and the samples containing media was replaced with fresh FBS containing media. After 24, 48, 72, 96 and 168 h, the EGFP expression was observed using an inverted fluorescence microscope. Then flow cytometry (BD FACS CantoTM II, USA) was performed to measure the gene transfection efficiency. In all experiments, XtremeGENE/mcDNA complexes were used as controls. Free erythrocyte membrane was used as the negative control.

The transfection results (Fig.S2) show that the EM-XtremeGENE-mcDNA (EM1) resulted the highest transfection efficiency. Thus the formulation 1 (10 mM Tris, 0.1 mM EDTA, 1 mM $CaCl_2$) was selected for the subsequent experiment.

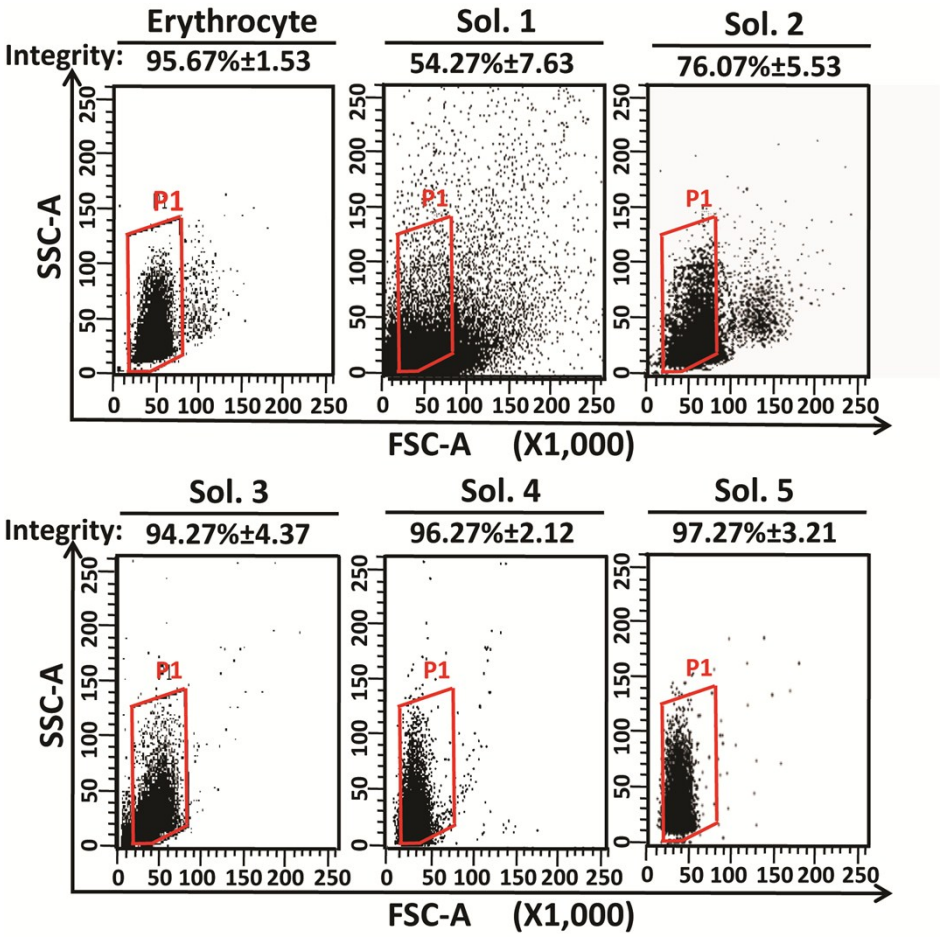

Fig. S1. The calculated integrity ratio and FSC-SSC signals of erythrocyte membrane prepared with different kinds of hypotonic solution.

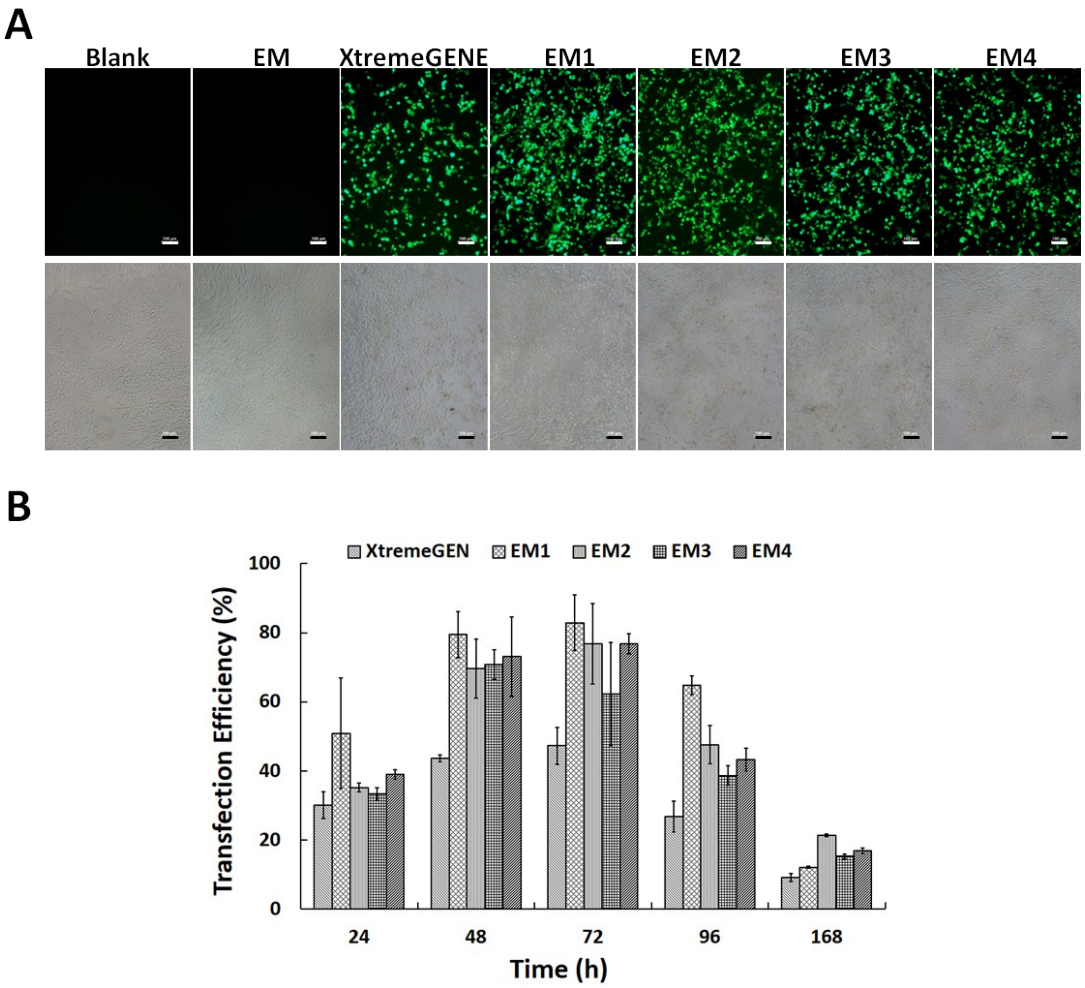

Fig. S2. (A) Fluorescent microscope images of EGFP expression at 48 h, Scale bar 100 μm. (B) Transfection efficiency of different samples.