Electronic Supplementary Information

Cryopreservation of human erythrocytes through high intracellular trehalose with membrane stabilization of maltotriose-grafted ε-poly(L-lysine)

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Materials

ε-Poly(L-lysine) (ε-PL, average molecular weight ~4000) was purchased from Nanjing Shineking Biotech Co., Ltd., China. Benzyl alcohol (BA, 99.9%) was purchased from Anhui Senrise Technology Co., Ltd., China. α,α-Trehalose (99% anhydrous) was provided by Beijing J&K Scientific Co., Ltd., China. 16-Doxyl-stearic acid (16-DSA) was obtained from Beijing Innochem Technology Co., Ltd., China. Pyrene (98%) was purchased from Tianjin Heowns Biochemical Technology Co., Ltd., China. Maltotriose (96%) and 6-dodecanoyl-*N*,*N*-dimethyl-2-naphthylamine (Laurdan, ≥97% HPLC) were provided by Shanghai Macklin Biochemical Co., Ltd., China. Ethyl acetate (EtOAc, AR) was obtained from Tianjin Kermel Chemical Reagent Co., Ltd., China. All chemicals were used as received without further purification.

Enhanced adenosine triphosphate (ATP) Assay Kit and Detergent Compatible Bradford Protein Assay Kit were supplied by Beyotime Biotechnology, China. Human 2,3-diphosphoglycerate (2,3-DPG) ELISA Kit was purchased from Nanjing Herb-Source Bio-Technology, China. Methemoglobin (MetHb) Assay Kit was supplied by Nanjing Jiancheng Bioengineering Institute, China. FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, USA) was used for phosphatidylserine (PS) exposure determination.

Synthesis

The chemical structure of PL-*g*-M was verified by a ^{1}H NMR spectrometer (AVANCE III TM HD 400 MHz NanoBAY, Bruker, Germany) and a FTIR spectrometer (IRtracer100, Shimadzu, Japan) using KBr pellet technique ranging from 4000 cm^{-1} to 400 cm^{-1}. The corresponding chemical shift (δ, ppm) of protons in the ^{1}H NMR spectra as well as the wavenumber of the vibration absorption bands and α-glycosidic bonds in the FTIR spectra on the glycopeptide PL-*g*-M were displayed in Fig. S1 and Fig. S2, respectively, which had been detailedly analyzed in our previous publication.[1] Its molecular weight was assessed in an aqueous gel permeation chromatography (GPC, Viscotek, UK) system with HAc/NaAc (0.5M, pH 4.5) buffer as the eluent, and calibrated with the poly(ethylene glycol) standard.

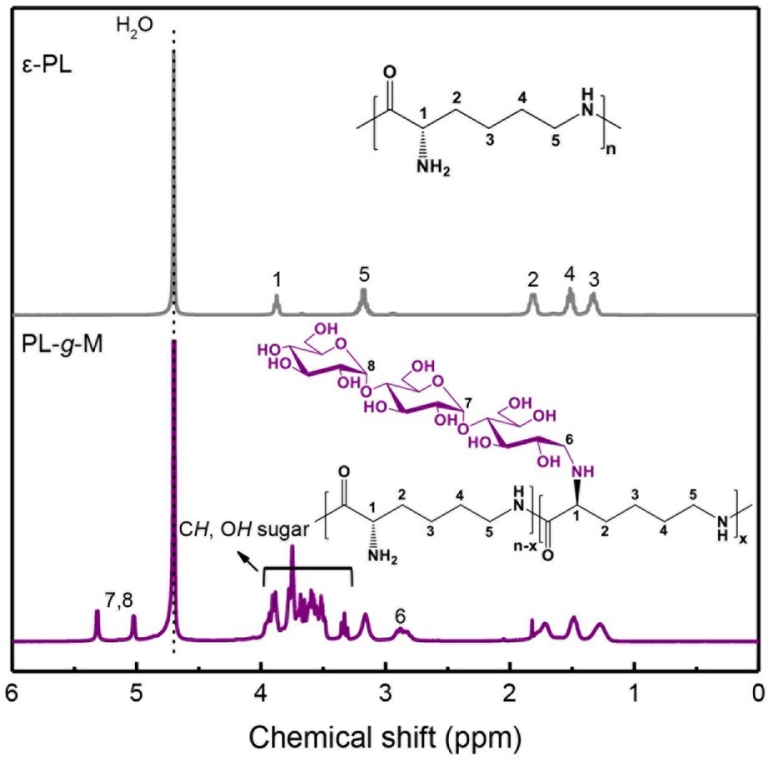

Fig. S1 ^{1}H NMR spectra of ε-PL and PL-*g*-M.

Fig. S2 FTIR spectra of ε-PL and PL-*g*-M.

Benzyl alcohol residue

For the benzyl alcohol residue determination, the supernatant after washing was mixed vigorously with ethyl acetate in equal volume, followed by centrifugation at 1800*g* for 10 min at 4 °C. Absorbance of the supernatant at 258 nm was measured in an UV-vis spectrophotometer (UV3600 PLUS, Shimadzu, Japan) to calculate the residual benzyl alcohol amount.

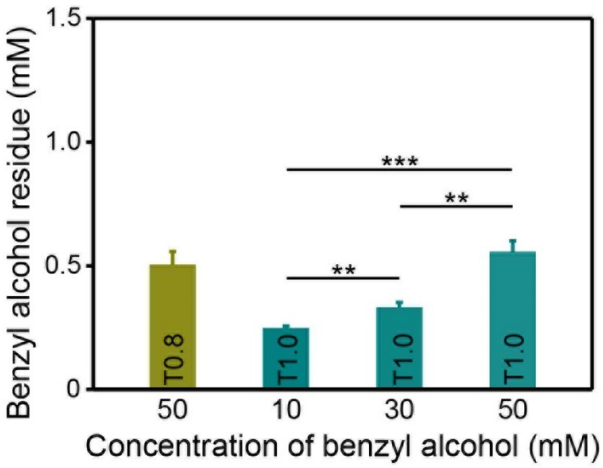

Fig. S3 Benzyl alcohol residue of human erythrocytes after incubation with 0.8 or 1.0 M trehalose containing different concentrations of benzyl alcohol at 4 °C for 22 h.

Hemolysis and intracellular trehalose uptake

Fig. S4 Hemolysis (a) and intracellular trehalose (b) of human erythrocytes as a function of the incubation time after incubation with 50 mM benzyl alcohol and 0.8 or 1.0 M trehalose in PBS (pH 7.4, 100 mOsm/L) at 4 °C.

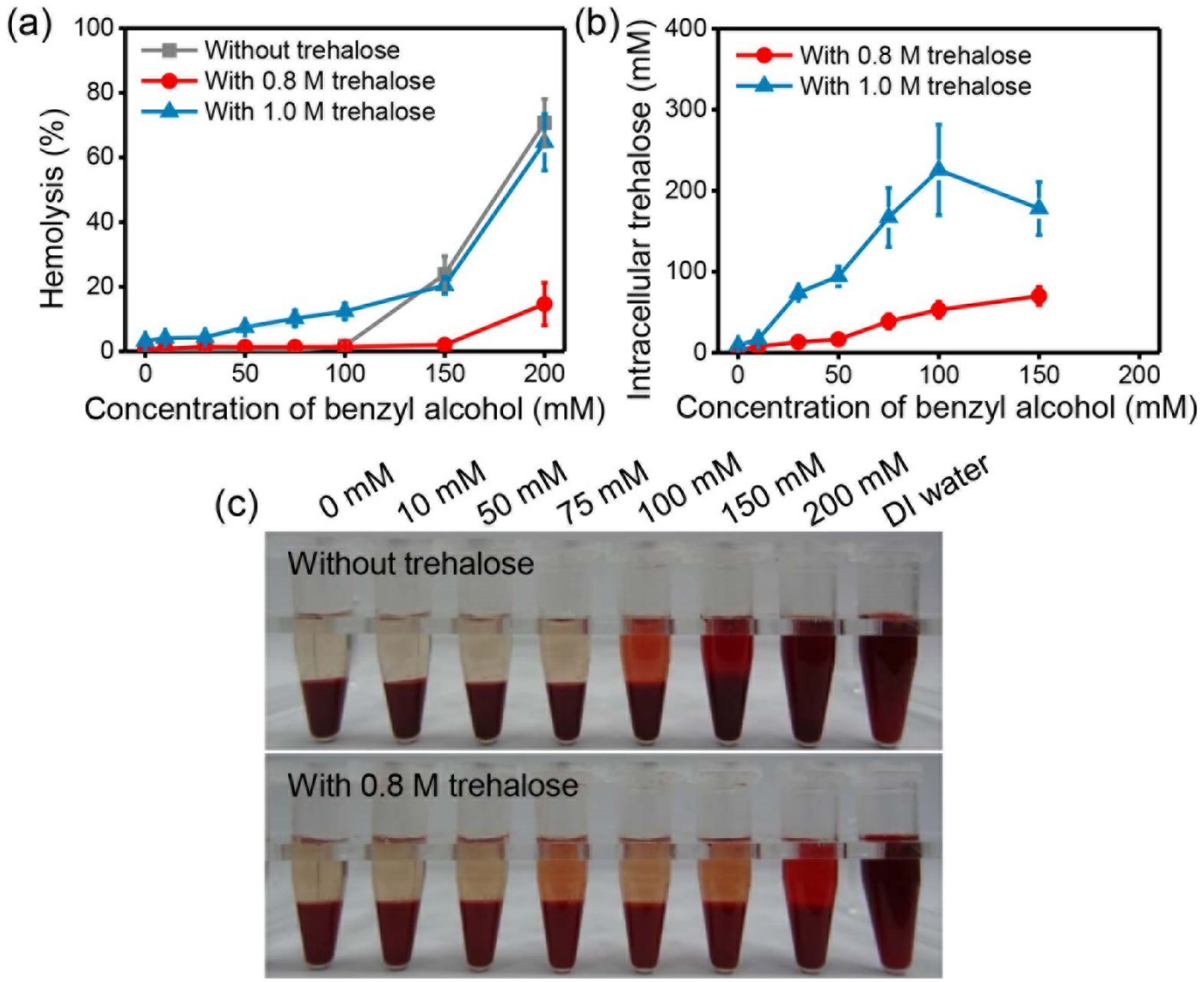

Fig. S5 Hemolysis (a), intracellular trehalose (b) and corresponding photographs (c) of human erythrocytes as a function of the concentration of benzyl alcohol after incubation with 0.8 or 1.0 M trehalose in PBS (pH 7.4, 100 mOsm/L) at 4 °C for 22 h.

Morphology

Erythrocytes after incubation were fixed by 2.5% glutaraldehyde and observed under a scanning electron microscope (SEM, S-4800, Hitachi, Japan) in light of the previous methods.[1,2]

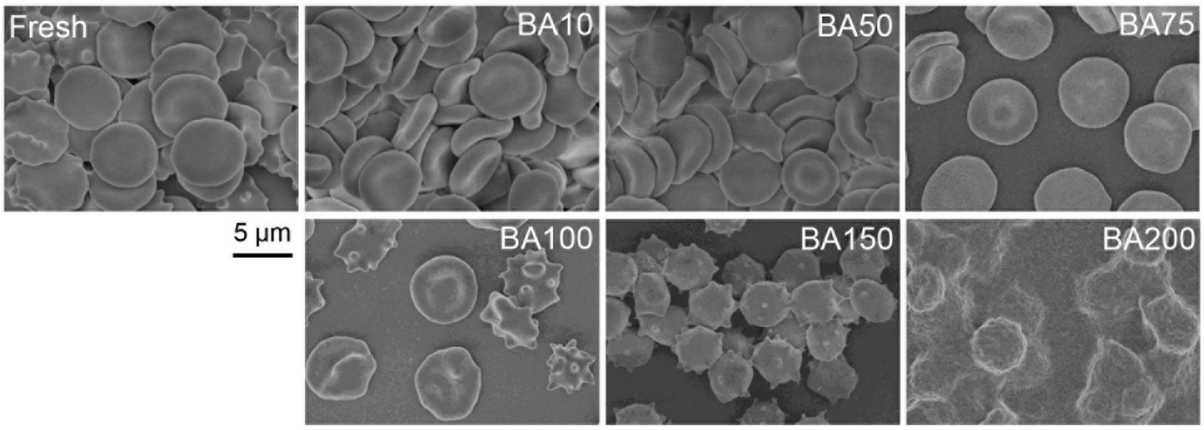

Fig. S6 SEM images of human erythrocytes after incubation with 0, 10, 50, 75, 100, 150 or 200 mM benzyl alcohol in PBS (pH 7.4, 300 mOsm/L) at 4 °C for 22 h.

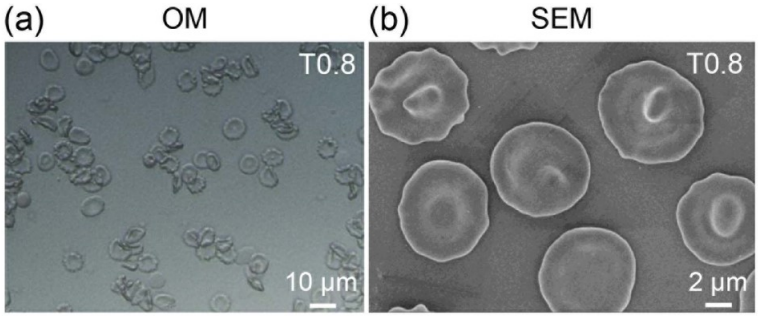

Fig. S7 Optical microscope photograph (a) and SEM images (b) of human erythrocytes after incubation with 0.8 M trehalose in PBS PBS (pH 7.4, 100 mOsm/L) (T0.8) at 4 °C for 22 h.

Cryopreservation

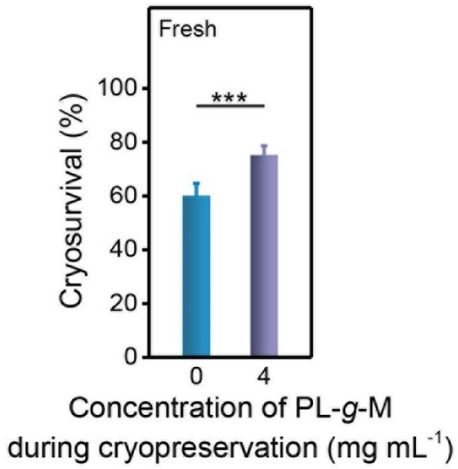

Fig. S8 Cryosurvival of human erythrocytes by direct cryopreservation in liquid nitrogen after mixing with 0.36 M trehalose only or 0.36 M trehalose containing 4 mg/mL PL-*g*-M in PBS (pH 7.4, 100 mOsm/L).

Notes and references

1 S. Gao, K. Zhu, Q. Zhang, Q. Niu, J. Chong, L. Ren and X. Yuan, *Biomacromolecules*, 2022, **23**, 530-542.

2 X. Zhang, X. Xu, Y. Li, C. Hu, Z. Zhang and Z. Gu, *Adv. Mater.*, 2018, **30**, e1707240.