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Development and characterization of human-derived nanoerythrosomes for biomimetic drug delivery applications

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INTRODUCTION

Nanoparticles (NPs) represent a promising and innovative approach in the field of drug delivery, offering several key advantages over conventional therapeutic methods, such as targeted and controlled drug release, and reduced systemic toxicity. Among the different types of nanoparticles, nanoerythrosomes (NERs), vesicles derived from Red Blood Cell (RBC) membranes, stand out due to their excellent biocompatibility, low immunogenicity, and minimized off-target effects. These properties make NERs a highly attractive platform for the development of effective biomimetic drug delivery systems [1].

AIM

This study focuses on developing human RBC membrane (RBCm)-derived biomimetic NERs as a novel biomimetic drug delivery system.

MATERIALS AND METHODS

Whole human blood was centrifuged to isolate RBC and separate other cellular components. RBC were lysed with a hypotonic solution to remove their internal contents, including hemoglobin. The empty membranes, called RBC ghosts, were collected for further use [1-2]. NERs were obtained from RBC ghosts using extrusion and purified by dialysis (Figure 1). NERs were characterized for mean size and ζ-Potential using dynamic light scattering (DLS), concentration using nanoparticle tracking analysis (NTA), and morphology through transmission electron microscopy (TEM) and cryo-TEM. Their membrane proteins (MPs) content was evaluated using the microBCA Protein assay. NERs storage stability over time was also studied.

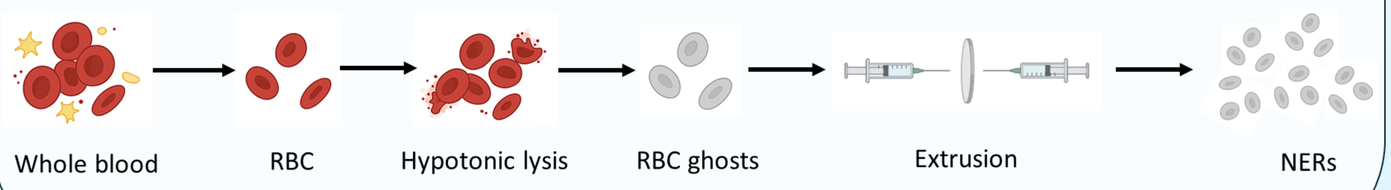

Figure 1. Isolation of human RBC ghosts and fabrication of NERs.

RESULTS AND DISCUSSION

- RBC, treated with a hypotonic solution, were deprived of hemoglobin, turning into RBC ghosts (Figure 2).

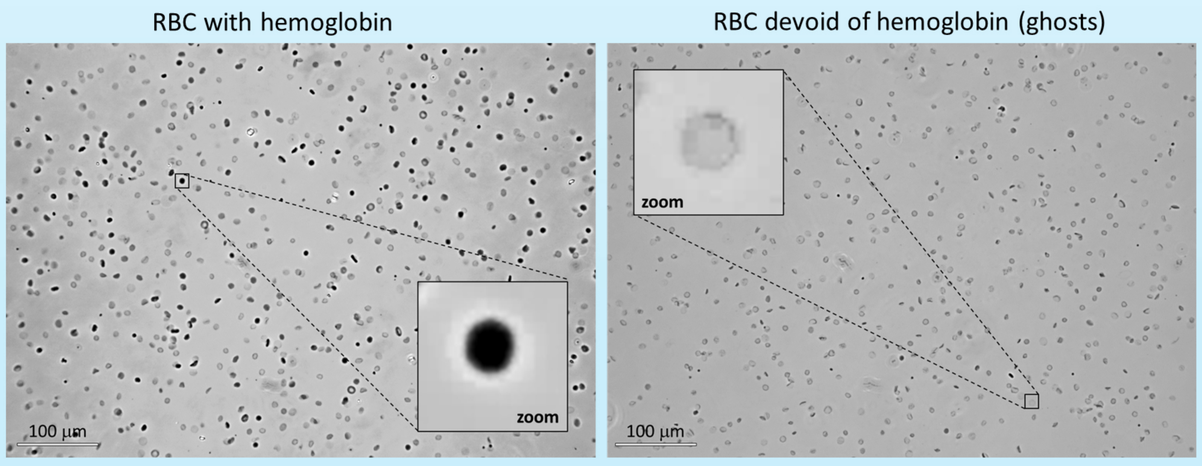

Figure 2: Optical microscope images of RBCs before (left) and after (right) hypotonic lysis. Scale bar: 100 µm.

- NERs produced by extrusion exhibited a mean size of 164.96 ± 6.34 nm (PDI: 0.10 ± 0.04), a ζ-Potential of -23.07 ± 1.68 mV, and a concentration of 4.5 × 10^{11} ± 1.98 × 10^{11} particles/mL. Characterization of NERs after purification by dialysis showed comparable results (Figure 3).

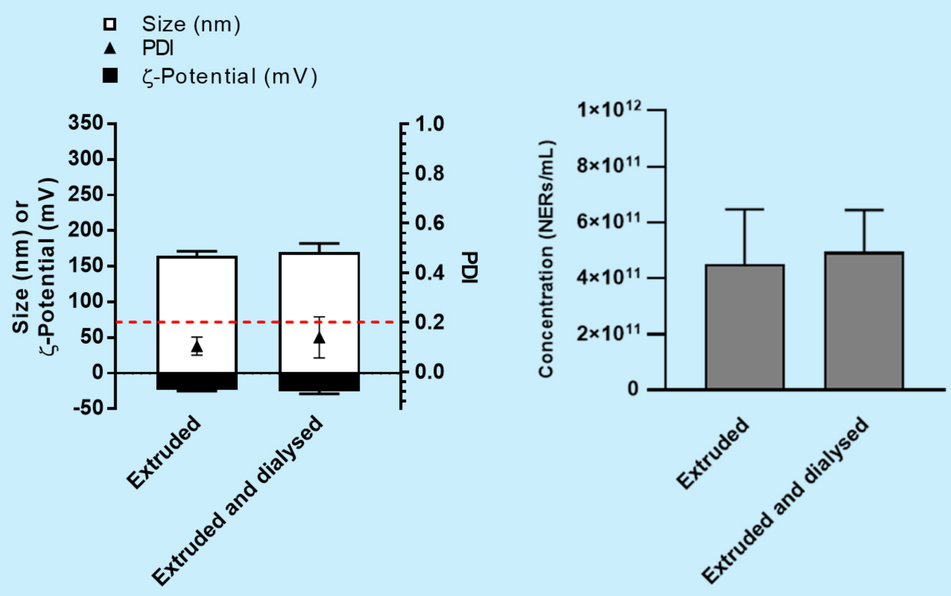

Figure 3: NERs characterization by DLS and NTA before and after purification. Data are presented as mean ± SD (n=3).

- TEM analysis confirmed that extrusion process produced uniform NERs (Figure 4A-B). Furthermore, cryo-TEM imaging revealed that NERs were unilamellar in structure (Figure 4C).

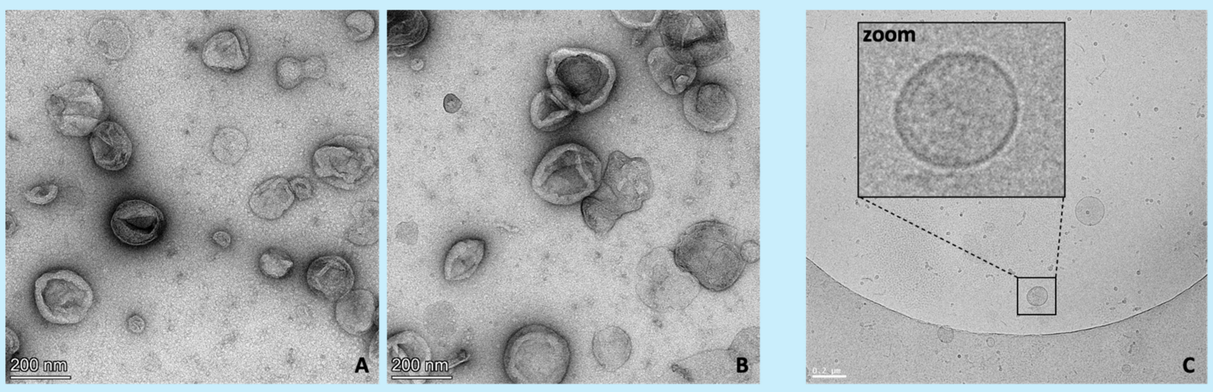

Figure 4: TEM (A: before purification; B: after purification) and cryo-TEM (C) images of NERs. Scale bar: 200 nm.

- About 60% of the RBC ghosts MPs were incorporated into NERs. No significant differences were observed after the purification step, suggesting that the protein content remained stable and the purification process preserved the structural integrity of the NERs.

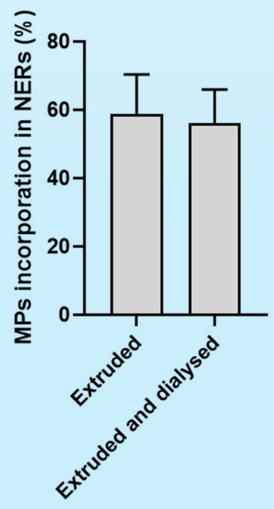

Figure 5: MPs quantification in NERs before and after the purification process. Data are presented as mean ± SD and expressed as a percentage relative to RBC ghosts (set as 100%) (n=3).

- NERs were stable up to 4 weeks when stored at 4 °C (Figure 6).

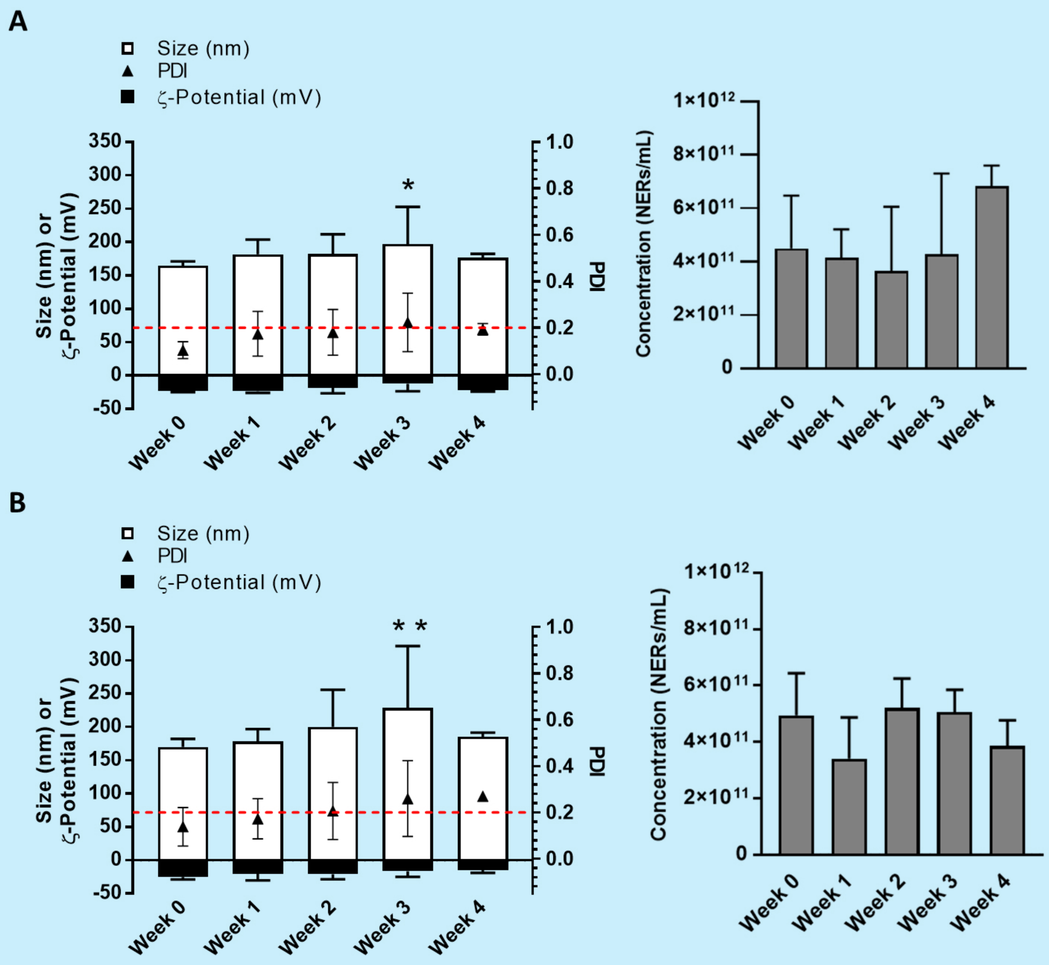

Figure 6: Storage stability of NERs before (A) and after (B) purification, monitored by DLS and NTA analysis. Data are presented as mean ± SD (n=3); * p ≤ 0.05, ** p ≤ 0.01.

CONCLUSIONS AND FUTURE PERSPECTIVES

Extrusion generated uniform NERs. Purification by dialysis enhanced their purification without compromising structural integrity. Further investigations are required to assess NERs biochemical composition, cellular uptake and cytotoxicity. This study lays the groundwork for advancing injectable, drug-loaded NERs as a promising drug-delivery platform.

REFERENCES

[1] Rahikkala et al, RSC Adv, 2020, 10, 35198-35205, 10.1039/d0ra05900e.

[2] Long et al, Nat Commun, 2025, 16, 1909, 10.1038/s41467-025-57048-6.

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