Toward clinically translatable nanotherapeutics: stability of engineered red blood cell-derived vesicles

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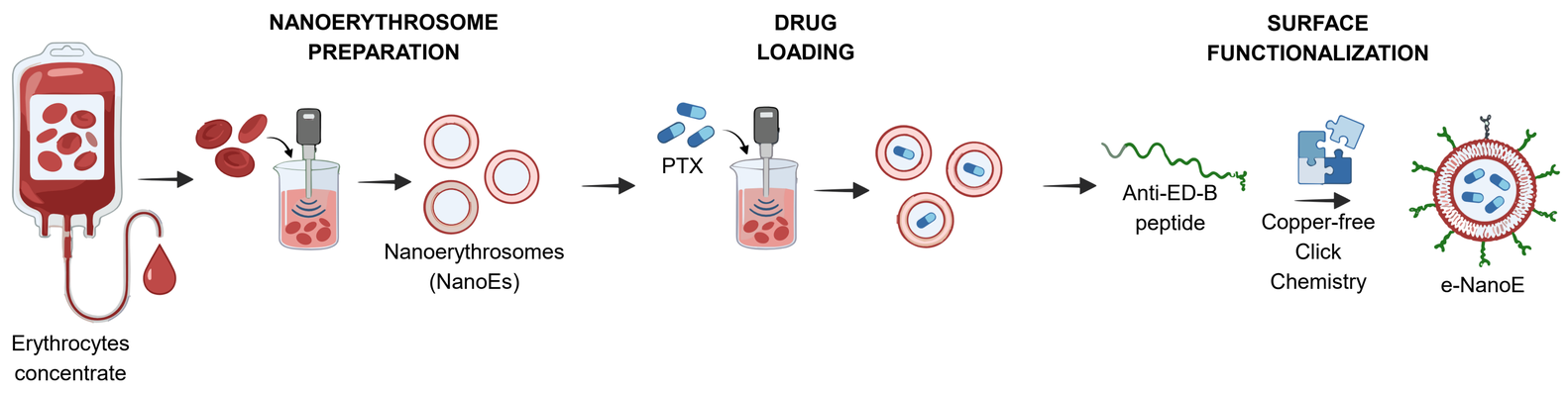
AIM OF THE PROJECT

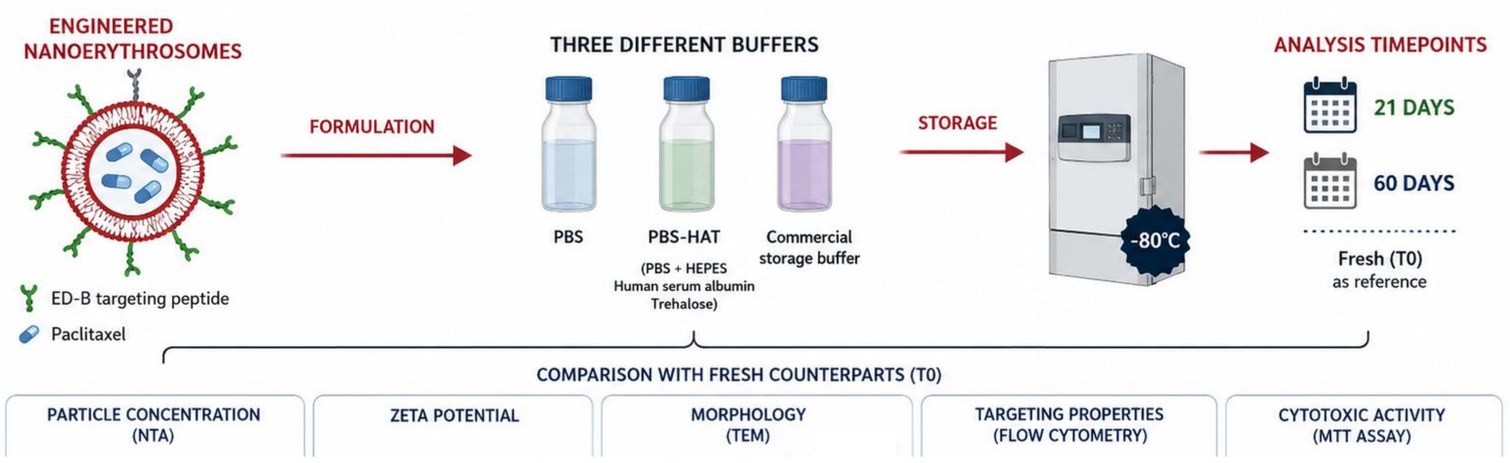
GENERATION AND ENGINEERING

Engineered red blood cell-derived particles, also termed nanoerythrosomes (NanoEs), are promising nanotherapeutic platforms for the targeted delivery of chemotherapeutic agents. Here, NanoEs were engineered to deliver paclitaxel (PTX) to the tumor site through functionalization with an ED-B-targeting peptide, recognizing the Extra Domain-B of oncofetal fibronectin, which is virtually absent in adult healthy tissues, but overexpressed in the tumor microenvironment.



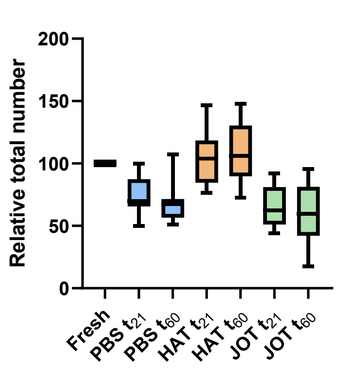
STABILITY ASSESSMENT

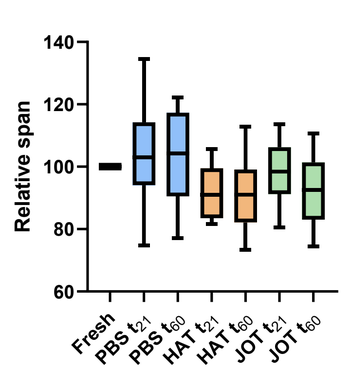
Optimizing cryostorage conditions is essential for e-NanoE clinical translation.
This project therefore aims to assess the stability of PTX-loaded, ED-B-targeted e-NanoEs during cryogenic storage and identify optimal conditions for preserving their physicochemical and biological properties.



e-NanoE STABILITY ASSESSMENT

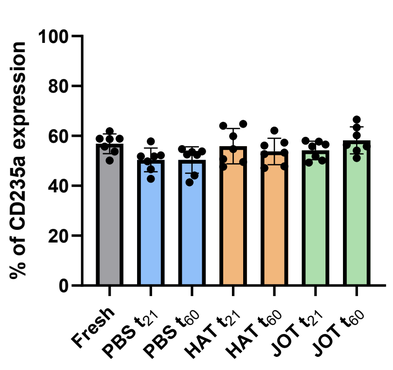
RECOVERY





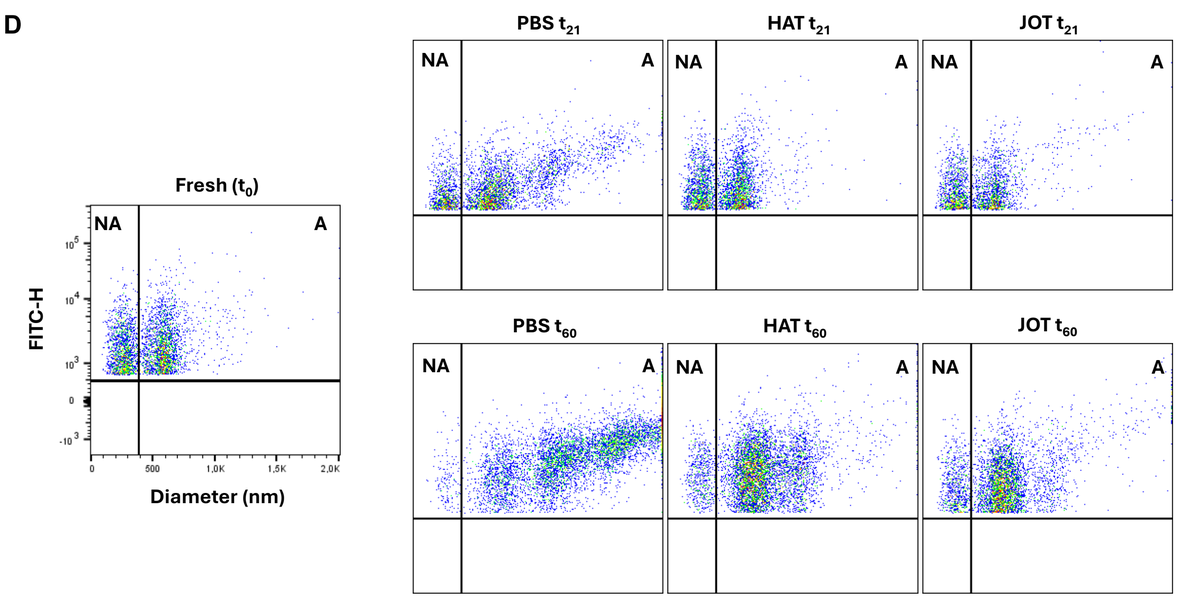
The use of cryoprotectants improves particle recovery and ensures a more homogeneous size distribution.

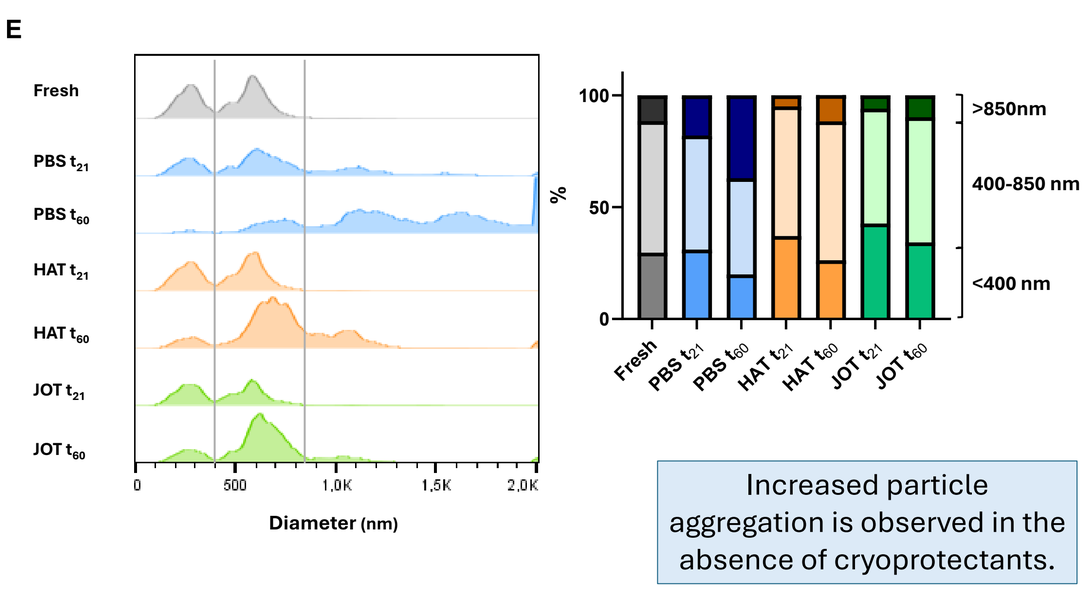
IDENTITY



The e-NanoE identity is preserved under all tested conditions.

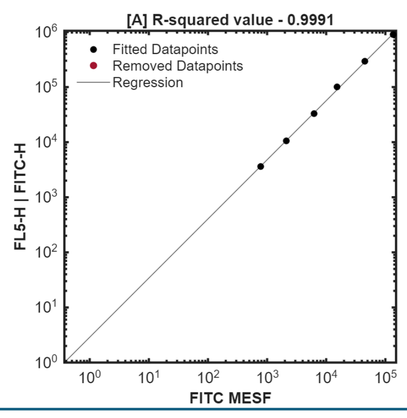
AGGREGATION





Increased particle aggregation is observed in the absence of cryoprotectants.

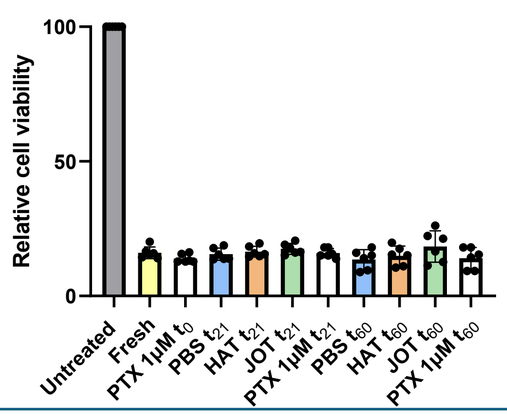
FUNCTIONALIZATION



Sample	Mean MESF FITC-H of NA (mean±SD)
Fresh	442±159
PBS t_{21}	278±50
HAT t_{21}	300±64
JOT t_{21}	277±57
PBS t_{60}	469±256
HAT t_{60}	402±141
JOT t_{60}	372±171

Peptide functionalization is preserved and comparable across all samples in the non-aggregated fractions.

CYTOTOXICITY



e-NanoE cytotoxic activity appears to be preserved under all tested storage conditions based on this preliminary assessment. Further, more sensitive and detailed *in vitro* analyses will be performed to confirm these findings.

Figure 1. Project overview. Nanoerythrosomes (NanoEs) are obtained from erythrocyte concentrates through a sonication-based process. Paclitaxel (PTX) is subsequently loaded into NanoEs by sonication, followed by surface functionalization with an anti-ED-B fibronectin peptide via copper-free click chemistry, yielding engineered NanoEs (e-NanoEs). The physicochemical stability and preservation of biological activity of e-NanoEs are evaluated after 21 and 60 days of storage at −80 °C using three different buffer formulations: phosphate-buffered saline (PBS), PBS supplemented with HEPES, human serum albumin, and trehalose (PBS-HAT), and a commercial storage buffer.

Figure 2. Stability of e-NanoEs upon storage in PBS, PBSHAT (HAT) and a commercial storage buffer (JOT) for 21 and 60 days at -80°C. (A) Box&whiskers plot representing the total particle number of e-NanoEs following storage in the different buffers, normalized to freshly prepared samples (n=14). **(B)** Box&whiskers plot showing the span of particle size distribution under the different storage conditions, normalized to freshly prepared samples (n=14). **(C)** Percentage of CD235a-positive NanoEs following storage, indicating preservation of erythrocyte-associated markers. Results are reported as mean ± SD (n=7). **(D)** Representative dot plot of e-NanoEs, showing non-aggregated (NA, below 400nm of diameter) and aggregated (A, above 400nm diameter) particles. **(E)** Distribution of e-NanoE diameters across the different experimental conditions. The left panel is representative of the size distribution of the differently stored e-NanoE populations, while the right panel summarizes the relative proportion of particles in three size classes: below 400nm, between 400 and 850 nm, and above 850nm of diameter. **(F)** Flow cytometry analysis of e-NanoE functionalization with the FITC-labeled peptide. On the left, representative calibration curve relating FITC fluorescence intensity (FITC-H, y-axis) to FITC molecules of Equivalent Soluble Fluorochrome (MESF, x-axis). On the right, table reporting the mean FITC-H MESF values measured in the different samples referring to the non-aggregated fraction of e-NanoEs; results are reported as mean ± SD (n=12). **(G)** MTT assay showing the percentage of cell viability normalized to untreated samples after treating HT-29 cells with differently stored e-NanoEs and PTX 1 µM, assumed as positive control. Results are reported as mean ± SD (n=6).

CONCLUSIONS

This work highlights the importance of selecting an appropriate storage buffer and storage duration to preserve e-NanoE properties and minimize aggregate formation, thereby supporting the development of a readily available and clinically translatable product.