Neuropeptide receptor Y as potential target for specific cancer treatment

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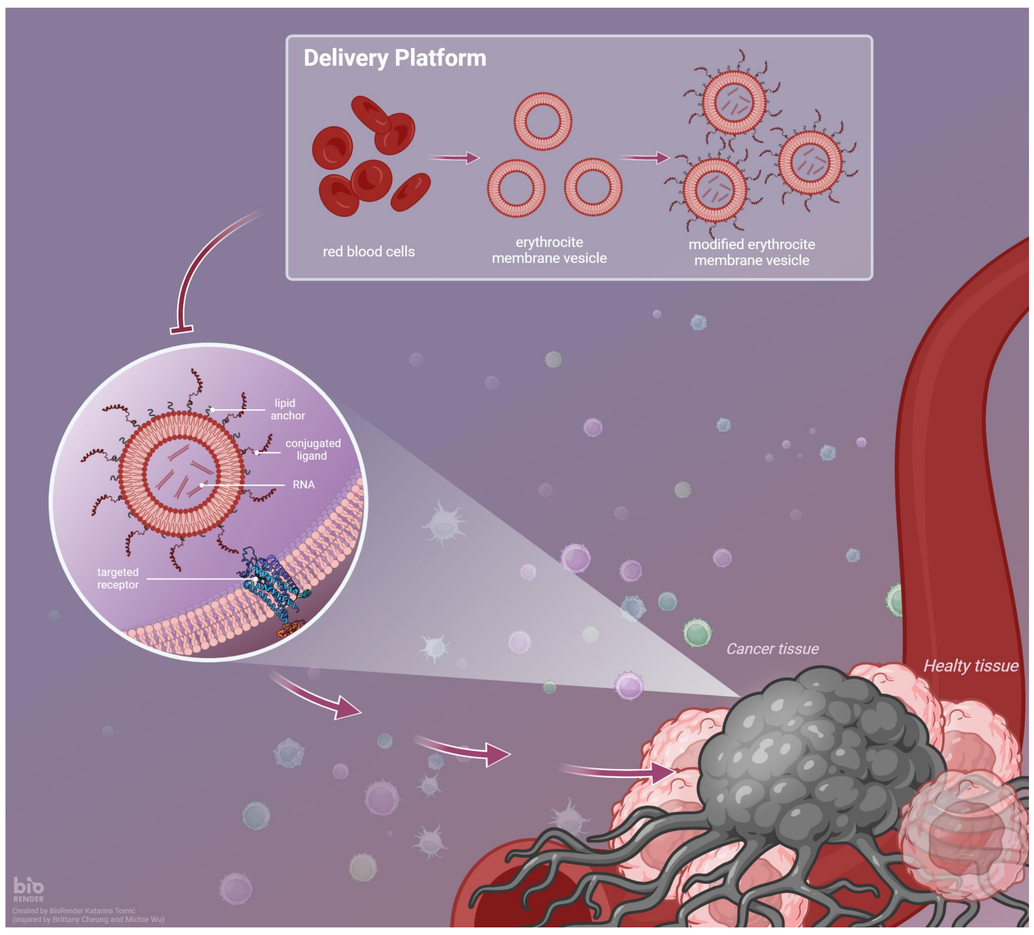
MEDNARODNA PODIPLOMSKA ŠOLA JOŽEFA STEFANA

Nanostructured materials

Introduction

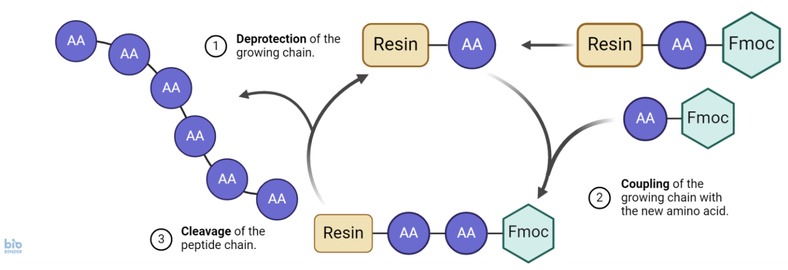
Targeted gene silencing using small interfering RNA (siRNA) has enormous potential in treating various pathologies. However, the efficient and **specific delivery of siRNA** remains a significant challenge due to its vulnerability to enzymatic degradation, limited cellular uptake, and lack of targeted delivery. To address this issue, a novel strategy has been proposed in this study that involves using biocompatible **erythrocyte membrane vesicles** (EMVs) as carriers for siRNA. The idea is to engineer EMVs for active targeting of cancer cells by incorporating neuropeptide Y (NPY) analogs onto their surface. **NPY analogs** act as ligands for G-protein coupled receptors (GPCRs) that are often overexpressed in cancer cells. Therefore, it can potentially serve as a homing beacon for targeted siRNA delivery.

We hypothesize that by incorporating NPY into EMVs, we can achieve significantly enhanced and specific delivery of siRNA to cancer cells compared to untargeted EMVs. This targeted delivery will result in a superior therapeutic effect by silencing the desired gene within cancer cells while minimizing off-target effects on healthy tissues expressing lower levels of the targeted GPCRs.

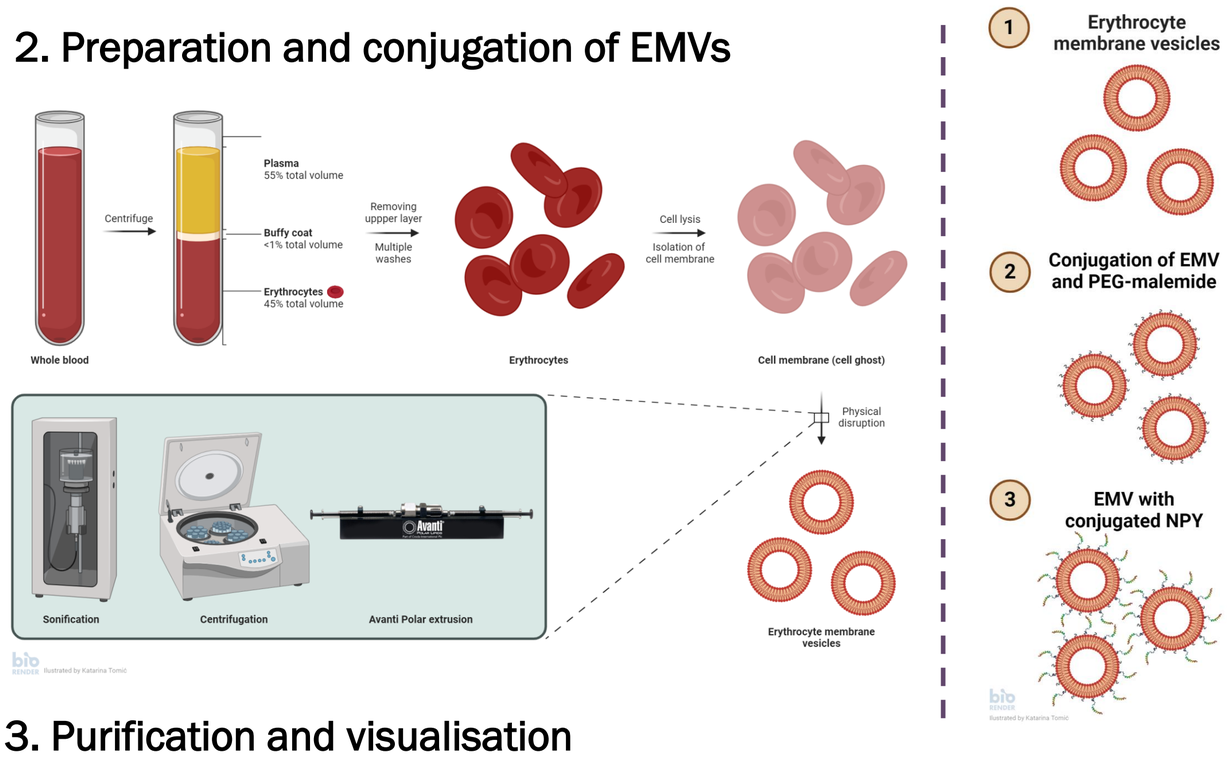


Methods

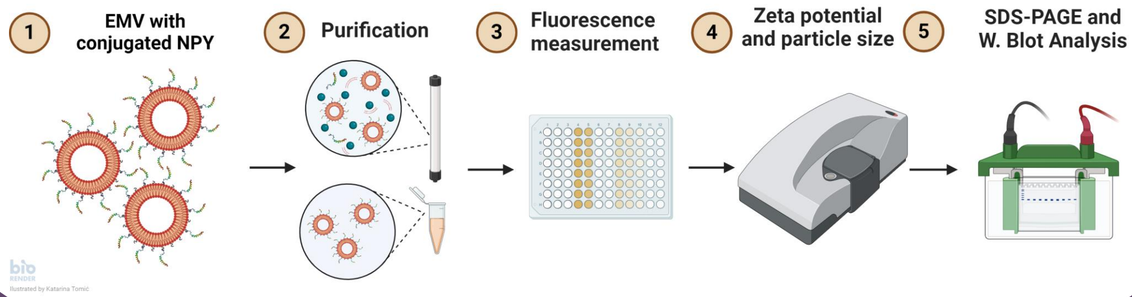
1. Peptide Synthesis



2. Preparation and conjugation of EMVs



3. Purification and visualisation



Conclusions

- We prepared EMVs and fully characterized their size and morphology using conventional TEM, cryo-TEM, freeze-fracture EM, DLS, and zeta potential measurements.
- We demonstrated that SDS-PAGE is an appropriate method for detecting small peptides (1-2 kDa).
- We proposed a suitable conjugation protocol for attaching peptides to the EMVs.
- The characterization of the conjugates is ongoing with the newly synthesized sequences, which were selected based on in silico models to have high affinity and selectivity towards cancer cells.

Results

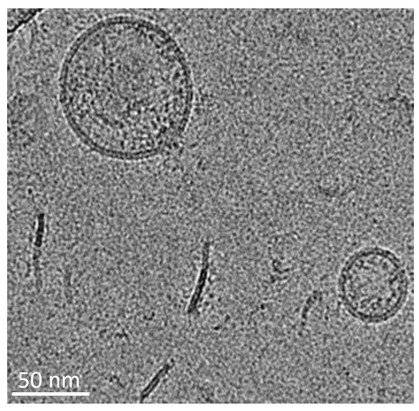


Figure 3: Cryo TEM image of EMVs (Matic Kisovec, National Institute of Chemistry, Ljubljana)

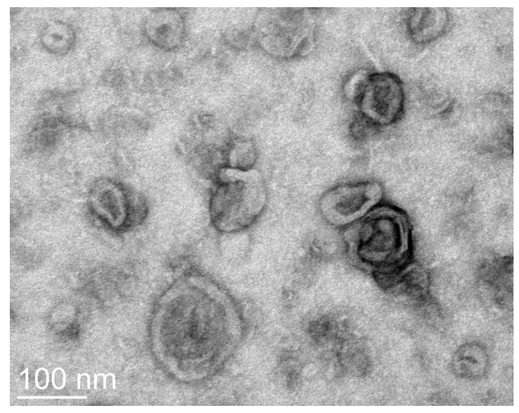


Figure 4: TEM image of EMVs, negatively stained (Nina Kostevšek, Institute Jožef Stefan, Ljubljana)

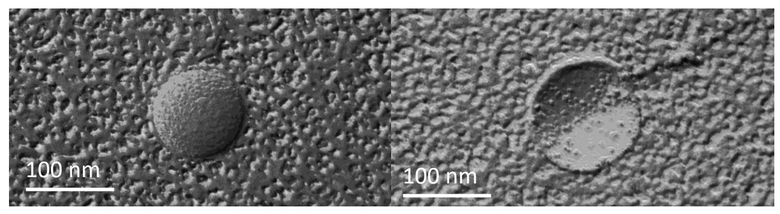


Figure 5. Freeze-fracture electron microscopy images (Samo Hudoklin and Mateja Erdani Kreft, Institute of Cell Biology, Ljubljana)

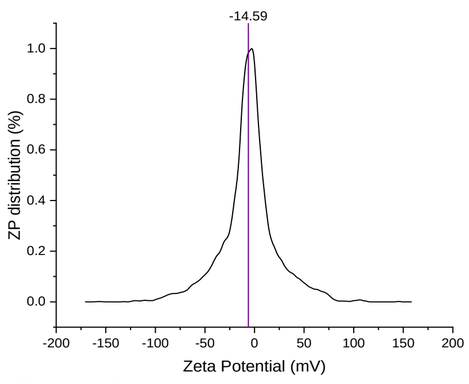


Figure 6. The graph shows the spread of zeta potential values across the EMV population, with a mean value of -14.59 mV

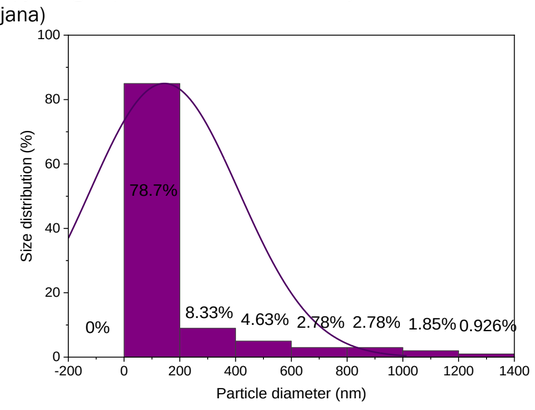


Figure 7: The graph shows the distribution of particle sizes for EMVs. The majority of EMVs (78.7%) fall within the size range of 150 nm to 200 nm.

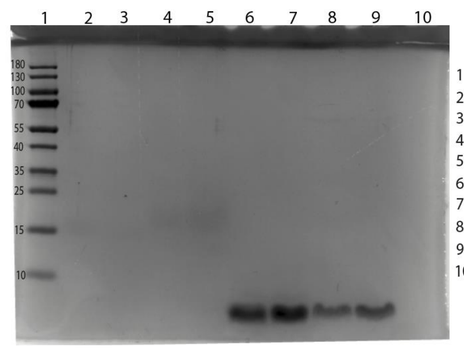


Figure 8: A 10% gel was stained with silver, and, neither CV17 nor CV24 were visible. It is evident that both SL15 and SL23 are around 2 kDa and that they are present in monomeric form

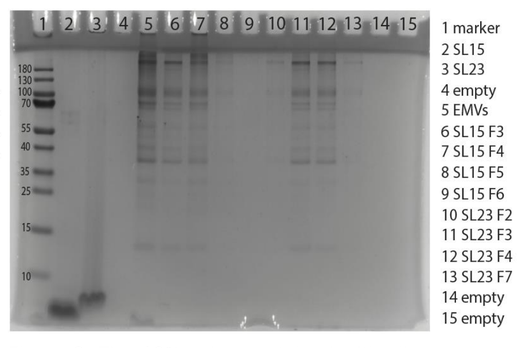


Figure 9: The 10% gel was stained with silver, and SL15 and SL23 are visible. Peptides can be differentiated from the proteins normally present on EMVs. We didn't detect any conjugated peptides, suggesting that the conjugation process needs further optimization

References:

[1] K. Misawa et al., Sci Rep, 2020, vol. 10, no. 1, p. 1007.
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[3] D. Berillo et al., Medicina (B Aires), 2021, vol. 57, no. 11, p. 1209.
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