Tracking Endosomal Escape of Tunable Polymer Nanoparticles for Inhibition of Inflammatory Pathways

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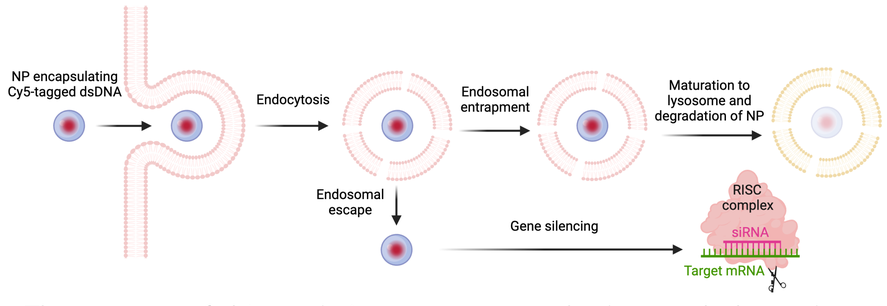
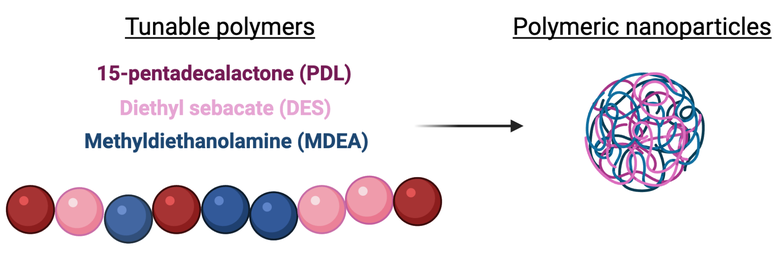
Motivation

- Endothelial cells are a promising target for immunomodulation as they are key regulators in the inflammatory response.[1]
- Non-viral delivery vehicles are a promising method to transport cargo, such as nucleic acids, into cells and can influence the success of delivery.[2]
- Endosomal escape is a critical part of the delivery process and therefore, accurately quantifying endosomal escape could inform decisions about delivery vehicles.
- Delivery of silencing nucleic acids can be used to knockdown inflammatory genes in endothelial cells to prevent harmful or chronic inflammation.

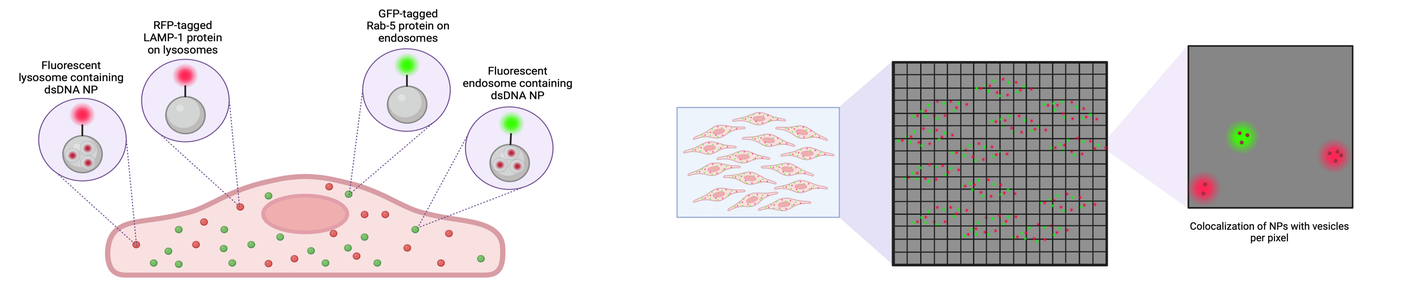


- The purpose of this study is to create a method to track the endosomal escape of a library of different poly(amine-co-ester) (PACE) nanoparticles (NPs) with various surface and chemical properties in human umbilical vein endothelial cells (HUVECS) and validate it with subsequent gene silencing.

Methods

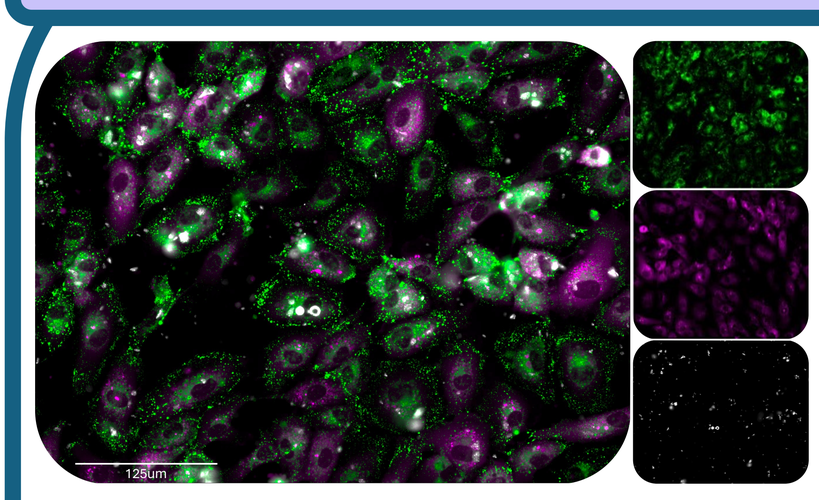


- Five PACE NPs with different end-groups were formulated containing either fluorescent dsDNA for the imaging and isolation assays or TNFRSF1A siRNA for the qPCR and flow cytometry assays.
- The colocalization of the fluorescent dsDNA with fluorescent endosomes and lysosomes was quantitatively measured using fluorescence microscopy (EVOS) over time within a single cell population.



- To confirm the imaging analysis could accurately track the endocytic pathway, endosomes were isolated via probe sonication and evaluated by flow cytometry.
- To further validate these methods and link NP location with therapeutic activity, the relative expression of the TNFRSF1A gene and the reduction of TNFα induced E-selectin expression were measured using qPCR and flow cytometry, respectively, after delivery of the PACE NPs containing TNFRSF1A siRNA.

Results

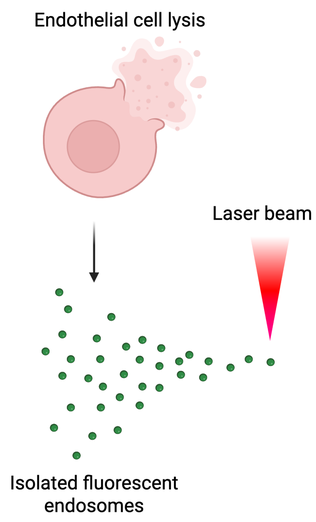


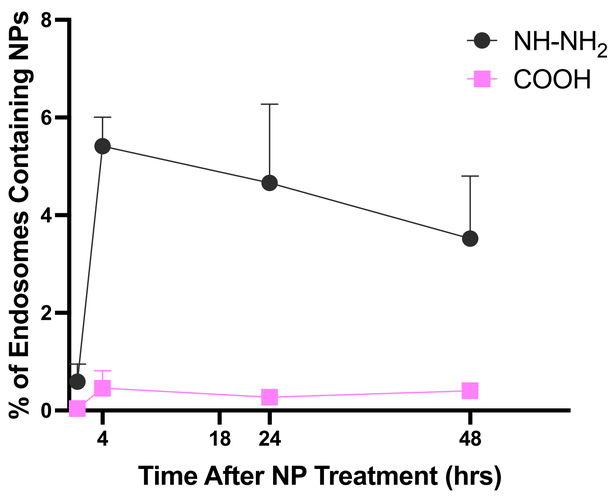
HUVECS with stably transfected fluorescent early endosomes (GFP-RabA5) and lysosomes (RFP-LAMP1) were developed for this work.

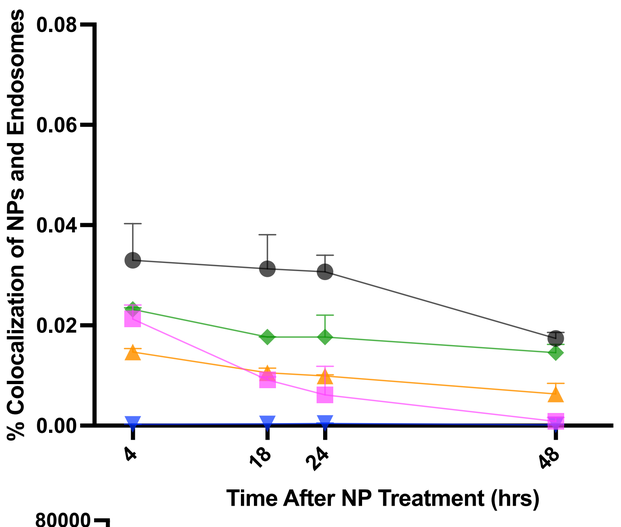
Endosomes

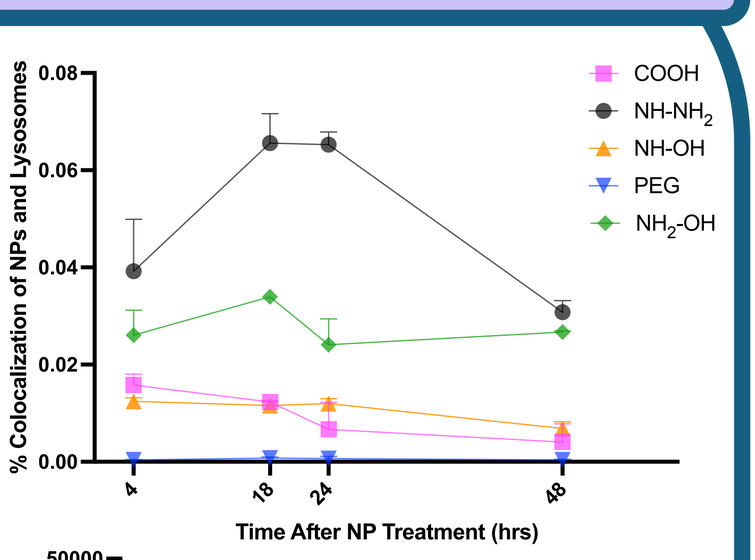
Lysosomes

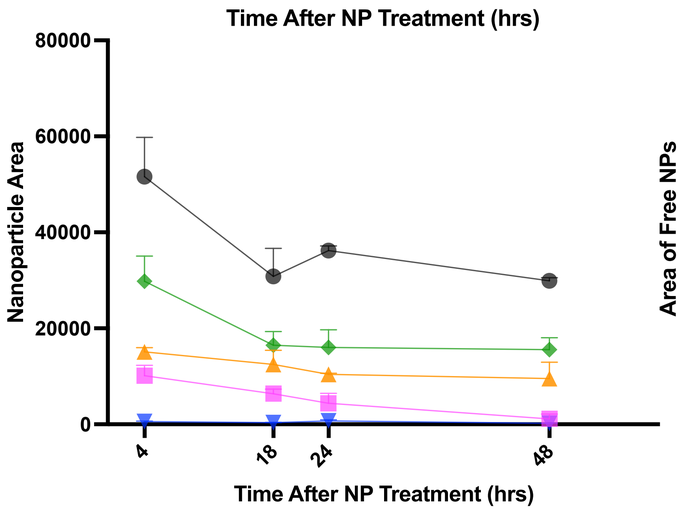
Nanoparticles with dsDNA

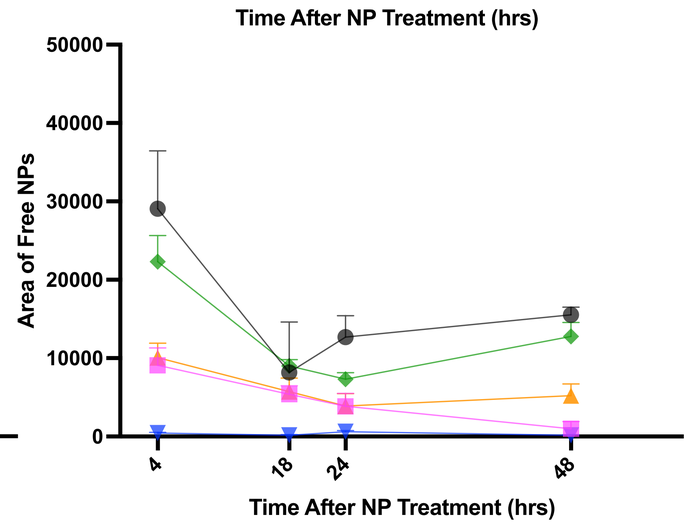






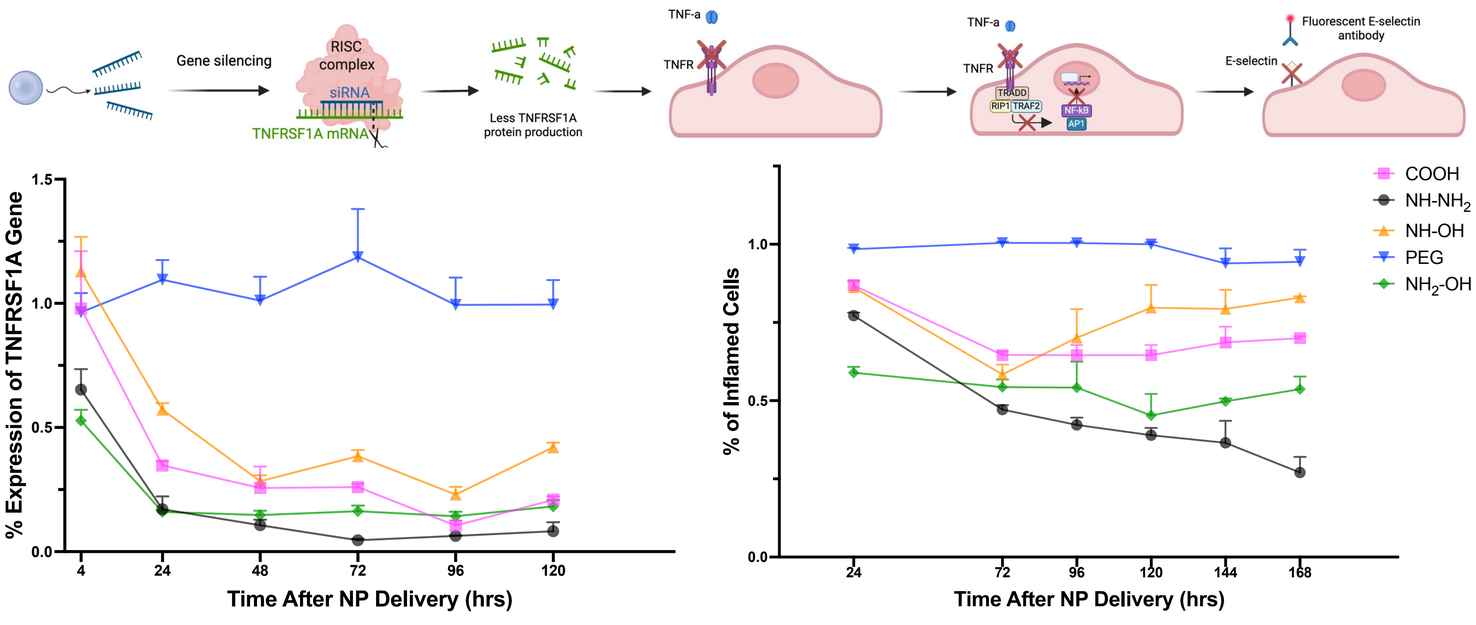






- NH_2-OH and NH-NH_2 end-groups exhibit the greatest cellular uptake and sustained release compared to NH-OH and COOH end-groups by having the largest total and free NP area as well as the greatest and most sustained colocalization of NPs with endosomes.
- There are distinct differences between PACE NP type, suggesting a higher charge and more specifically, more amine groups included, resulted in the greatest cellular uptake and most sustained release.
- These results suggesting greater uptake and more sustained release with the NH-NH_2 end-group compared to the COOH end-group were confirmed by analyzing isolated endosomes over time after treatment with each NP.

Application



- The amount of endosomal escape is associated with subsequent gene silencing.
- The NP tracking methods were verified by the correlating TNFRSF1A gene knockdown with NP trafficking.
- The overall dynamics of NPs correlated location and concentration in the cells with knockdown activity.
- The most robust and sustained knockdown seemed to be influenced the most by the highest NP area in the cell, increases in free NP area at 48 hours, as well as higher and more sustained colocalization of NPs with endosomes.
- The significance of the knockdown in inflammatory gene expression can be seen in the prevention of the inflammatory cascade that would normally occur in ECs after stimulation.
- Typically, when TNFα binds to the TNFRSF1A receptor on ECs, it triggers a cascade that causes expression of a molecule, E-selectin, that aids in extravasation of immune cells into the tissue.
- By decreasing the expression of TNFRSF1A, the TNFα molecule is unable to bind, preventing the inflammatory cascade, including the expression of E-selectin.
- These results show a decrease in E-selectin in correlation with the previous data, as the NPs with enhanced endosomal escape and gene knockdown show less staining for CD62E.

Conclusions

- These results suggest that the imaging methods were able to accurately track the endocytic pathway, show large differences between the PACE NP variations, and identify the timeline and relative magnitude of endosomal escape.
- Additionally, knockdown of the inflammatory gene TNFRSF1A was able to prevent the inflammatory cascade in ECs in correlation with the amount of knockdown.
- In future studies, these methods can be used to evaluate future polymer NPs of interest to gain insight on their delivery before use with therapeutics.
- These findings will also be further investigated in vitro and in vivo.

Acknowledgements

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References

1. Young MR. *Endothelial cells in the eyes of an immunologist*. Cancer Immunol Immunother 2013;61(10): 1609–1616.
2. Su, S. and M.K. P, *Recent Advances in Nanocarrier-Assisted Therapeutics Delivery Systems.* Pharmaceutics, 2020. 12(9).