Gene delivery

https://doi.org/10.1038/s44286-024-00118-1

Fine-tuning extracellular fluid viscosity enhances gene delivery

Ajay S. Thatte, Dongyoon Kim & Michael J. Mitchell

Successful gene delivery is predicated on the effective cellular uptake of encapsulated nucleic acid cargo. Now, a study identifies extracellular fluid viscosity as a key factor that governs gene delivery via non-viral and viral vectors across a range of cell types.

In the era of genomic medicine, delivery of transgenes has become an integral component of precision cell therapies for genetic disorders and cancer[1]. For example, chimeric antigen receptor (CAR) T cells, an adoptive cell therapy for the treatment of B cell malignancies, are manufactured ex vivo (outside the body) using both viral and non-viral delivery platforms to introduce the CAR genetic construct into T cells[2]. Inside the cell, the CAR gene is translated into CAR protein, arming the T cells with the capability to target and eliminate cancer cells once transfused back into the body. Effective manufacturing of CAR T cells and similar cell therapies hinges on the performance of delivery platforms in overcoming biological barriers to cellular delivery[3].

Delivery platforms and their encapsulated cargo are taken up by cells via endocytosis[4]. These pathways have been shown to be sensitive to biophysical cues such as shear stress, extracellular matrix stiffness and hydraulic pressure from the physiological environment[5–7]. Now, reporting in *Nature Chemical Engineering*, Ma, Zhu and co-workers identify extracellular fluid viscosity as a parameter that mediates the cellular uptake and delivery efficacy of both non-viral and viral gene delivery vectors[8].

To demonstrate the impact of extracellular fluid viscosity on gene delivery, Ma, Zhu and co-workers utilized methylcellulose — a high-viscosity thickening agent — to adjust in vitro culture medium viscosities from 0.77 cP (normal medium) up to 15 cP. For proof of concept, the authors formulated lipid nanoparticles (LNPs) encapsulating reporter messenger ribonucleic acid (mRNA) and treated 21 different cell lines cultured in media of varying viscosity. In all cell types tested, the authors observed that increasing the media viscosity to 1–3 cP, similar to the viscosity of extracellular fluid found in the body, resulted in enhanced mRNA delivery. In concordance with these results, the authors demonstrated that both the uptake of LNPs encapsulating fluorescently labeled mRNA and endosomal escape of mRNA into the cytosol were optimal at a media viscosity of 2–3 cP. Taken together, these data support the critical role of extracellular fluid viscosity in mRNA–LNP uptake, endosomal escape and transfection across cell types (Fig. 1).

To probe the mechanism behind this phenomenon, the authors interrogated the specific endocytic pathways used by mRNA–LNPs at varying extracellular fluid viscosities. At normal cell culture media viscosity, mRNA–LNP endocytosis was governed by clathrin- and/or caveolin-mediated, clathrin- and/or caveolin-independent, macropinocytotic and phagocytotic pathways. By contrast, at a media viscosity optimal for gene delivery (2 cP), mRNA–LNP endocytosis was facilitated by clathrin-mediated and macropinocytotic pathways, suggesting

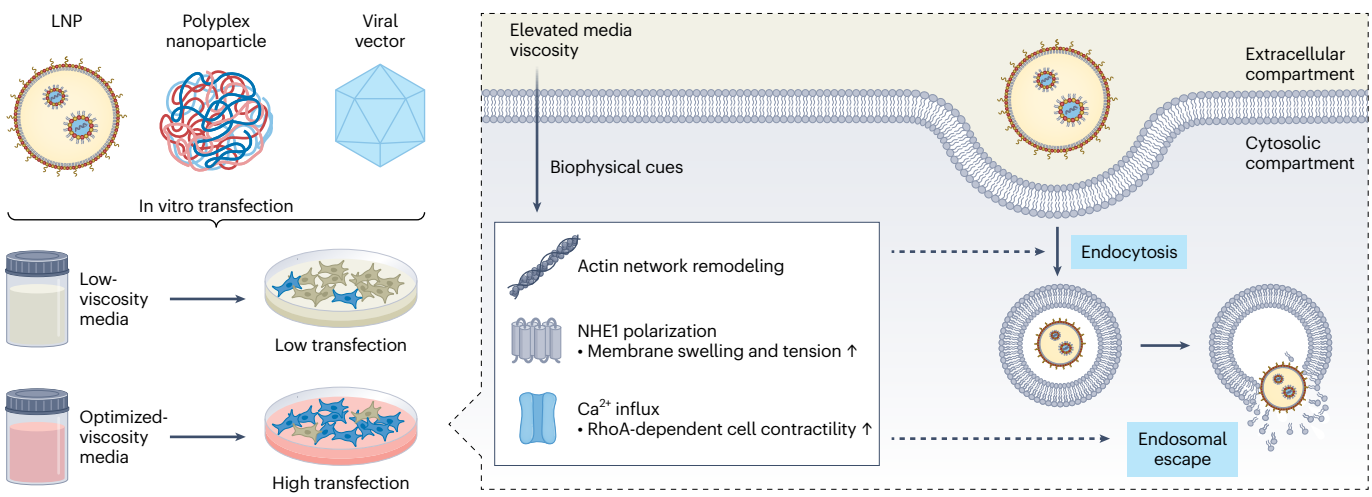

Fig. 1 | Optimizing extracellular fluid viscosity enhances gene delivery. Fine-tuning culture media viscosity using methylcellulose to increase the efficacy of both non-viral and viral gene delivery platforms. Ma, Zhu and co-workers identified optimal fluid viscosities for enhanced gene delivery across a range of cell types. Modulating the extracellular fluid viscosity induced changes in both the cell membrane and the cytoskeleton to improve cell uptake and fundamentally affected the endocytosis and endosomal escape of gene delivery platforms. NHE1, Na^+/H^+ exchanger 1; RhoA, Ras homolog family member A.

that the mechanism of mRNA–LNP uptake is linked to extracellular fluid viscosity. Inhibition of actin polymerization during cell culture further demonstrated the importance of cytoskeletal remodeling in enhanced mRNA–LNP uptake.

Finally, the authors sought to extend their observations to other non-viral and viral delivery platforms. Similar to mRNA–LNPs, LNPs encapsulating plasmid DNA (pDNA), polyplexes encapsulating pDNA and polyplexes encapsulating mRNA exhibited optimal nucleic acid delivery at a media viscosity of 2 cP. By contrast, viral vectors demonstrated a greater tolerance for media viscosity with transduction peaking at higher media viscosities (8–15 cP). Thus, ex vivo gene delivery can be maximized at optimal extracellular fluid viscosity, which in turn varies for non-viral and viral delivery platforms. For products such as CAR T cells, where the manufacturing process contains sequential steps that employ distinct gene delivery platforms, optimization of cell culture viscosity at each stage may improve the efficiency of CAR T cell production[9].

In summary, the authors demonstrate that extracellular fluid viscosity is an important mechanical parameter that governs the efficacy of gene delivery across cell types, irrespective of the class of delivery platform. Although this relationship is well described with reporter nucleic acid cargo in this study, further exploration of optimized extracellular fluid viscosity during the generation of functional cell therapies is necessary to validate the translational potential of these findings. From a biophysical perspective, further investigating the delicate balance of extracellular fluid viscosity with other biophysical parameters and their combined effect on viral and non-viral gene delivery is paramount for advancing the field. Still, in its present form, this study has provided a strong rationale to develop novel viscous biomaterials, potentially conjugated to growth factors, cytokines or agonists, to further improve gene delivery when added to ex vivo culture medium. Optimizing the viscosity of culture media using biomaterials during ex vivo cell therapy manufacturing may improve the yield of these products, motivating the continued study of biophysical factors in ex vivo cell engineering.

Ajay S. Thatte [1], Dongyoon Kim [1] & Michael J. Mitchell [1,2]
[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA. [2]Penn Institute for RNA Innovation, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.
e-mail: mjmitch@seas.upenn.edu

Published online: 11 September 2024

References

1. Wirth, T., Parker, N. & Ylä-Herttuala, S. *Gene* **525**, 162–169 (2013).
2. June, C. H., O'Connor, R. S., Kawalekar, O. U., Ghassemi, S. & Milone, M. C. *Science* **359**, 1361–1365 (2018).
3. Mitchell, M. J. et al. *Nat. Rev. Drug Discov.* **20**, 101–124 (2021).
4. Doherty, G. J. & McMahon, H. T. *Annu. Rev. Biochem.* **78**, 857–902 (2009).
5. Yankaskas, C. L. et al. *Sci. Adv.* **7**, eabh3457 (2021).
6. Discher, D. E., Janmey, P. & Wang, Y. *Science* **310**, 1139–1143 (2005).
7. Zhao, R. et al. *Sci. Adv.* **5**, eaaw7243 (2019).
8. Ma, J. et al. *Nat. Chem. Eng.* https://doi.org/10.1038/s44286-024-00116-3 (2024).
9. Levine, B. L., Miskin, J., Wonnacott, K. & Keir, C. *Mol. Therapy Meth. Clin. Dev.* **4**, 92–101 (2017).

Competing interests

The authors declare no competing interests.