Small structural changes in siloxane-based lipidoids improve tissue-specific mRNA delivery

From a single library of siloxane-based lipidoids, siloxane-incorporated lipid nanoparticles (SiLNPs) involving minor alterations in lipid chemistry yield tissue-specific mRNA delivery to the liver, lung, or spleen. Upon enhanced intracellular delivery, these SiLNPs show clinical promise for protein replacement therapies, regenerative medicine, and CRISPR–Cas-based gene editing applications.

This is a summary of:
Xue, L. et al. Combinatorial design of siloxane-incorporated lipid nanoparticles augments intracellular processing for tissue-specific mRNA therapeutic delivery. *Nat. Nanotechnol.* https://doi.org/10.1038/s41565-024-01747-6 (2024).

Published online: 28 October 2024

The mission

Ionizable lipid nanoparticles (LNPs) have emerged as a clinically advanced and modular platform for delivery of nucleic acids such as messenger RNA (mRNA), in particular since the approval by several agencies of mRNA-based COVID-19 vaccines[1,2]. In the LNP formulation, the ionizable lipid (lipidoid) plays a crucial part in targeting tissue or cells and facilitating translation[3]; yet, upon systemic administration, conventional lipidoids facilitate preferential LNP accumulation in the liver. As a result, the field has exploited various targeting approaches to achieve tissue-specific LNP delivery, such as endogenous targeting (in which LNPs bind to specific plasma proteins) and active targeting (in which the LNPs are conjugated with molecules that selectively bind to cell-specific receptors)[4,5]. Nevertheless, the structure–activity relationships that directly link lipidoid structure to tissue-tropic mRNA delivery have not been well-established. We sought to rationally design novel lipid-like materials whose chemical structures can be easily altered to achieve tissue-specific mRNA delivery for gene therapy applications.

The solution

Biomaterials containing siloxanes (organic compounds derived from silicone and oxygen) have stability, biocompatibility, and low chemical reactivity, and therefore they have been used across diverse applications ranging from medical devices to drug delivery[6]. We utilized a combinatorial approach to design a library of 252 siloxane-based lipidoids and used them to formulate siloxane-incorporated LNPs (SiLNPs) for mRNA delivery (Fig. 1a). After identifying lead SiLNP formulations in vitro, we further explored the crucial effect of siloxane moieties on intracellular processing of SiLNPs. For a lead SiLNP and an identical formulation that lacked siloxane moieties, we compared the rate of cellular uptake, membrane permeability (which increases the efficiency of mRNA in disrupting the membrane), endocytosis pathways and the LNPs capacity to exit the endosomes. Finally, from a single synthetic library, we identified lead SiLNPs that enabled potent and selective in vivo mRNA delivery to the liver, lung, or spleen.

We found that small structural alterations in siloxane-based lipidoids resulted in substantial changes in organ tropism; substituting the ester linkers on siloxane lipidoid cores with amine linkers redirected SiLNP targeting from the liver to the lung. Similarly, cyclic siloxane structures attached to negatively charged sulfonic groups promoted spleen-targeted delivery. These lead SiLNPs targeting the liver, lung, or spleen led to robust transfection of various cell types in vivo. In a mouse model of hereditary transthyretin (TTR) amyloidosis, which is caused by the misfolding and aggregation of transthyretin protein that results in the formation of amyloid fibrils, our lead liver-tropic SiLNP facilitated the delivery of CRISPR–Cas9 gene editing cargoes for durable therapeutic *Ttr* knockout, with enhanced delivery efficacy than the gold-standard LNP formulation used in the clinically approved drug (Fig. 1b). Similarly, our lead lung-targeting SiLNP demonstrated robust CRISPR–Cas9 gene editing in the lung of transgenic mice and mice bearing lung tumours (Fig. 1c). Finally, in a mouse model of viral infection-induced lung damage, lung-tropic SiLNPs deliver fibroblast growth factor-2 mRNA to lung endothelial cells, rescuing lung function, alveolar architecture, and leukocyte infiltration.

Future directions

There is a substantial unmet need for the rational design of novel lipid-like materials for the development of safe and effective mRNA LNP therapeutics for a broad range of clinical applications. By analysing the in vitro and in vivo mRNA delivery efficacy of our SiLNP library, we were able to establish the structure–activity relationships that guide tissue-specific SiLNP mRNA delivery. This platform holds remarkable promise for precision medicine and biotechnology.

Nevertheless, we have tested the clearance in vivo of only a representative siloxane-incorporated lipidoid, and the degradability of these kinds of materials still needs to be thoroughly investigated before clinical applications. Furthermore, the weak correlation between the in vitro and in vivo potencies of SiLNPs for mRNA delivery can affect the field's ability to accurately identify potent LNPs from new lipidoid libraries. Thus, future work will not only involve both safety and efficacy evaluations in larger animals and additional models of genetic disorders, but also focus on the development of next-generation technologies to accelerate discovery of ionizable lipids for mRNA delivery.

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EXPERT OPINION

"The authors have designed a new siloxane-containing lipid library and demonstrated that it is capable of organ-specific functional mRNA delivery in vivo. This finding is highly original and significant, and it will provide new chemical space for functional ionizable lipids and important insights into this field of research." **Yusuke Sato, Hokkaido University, Sapporo, Japan**

FIGURE

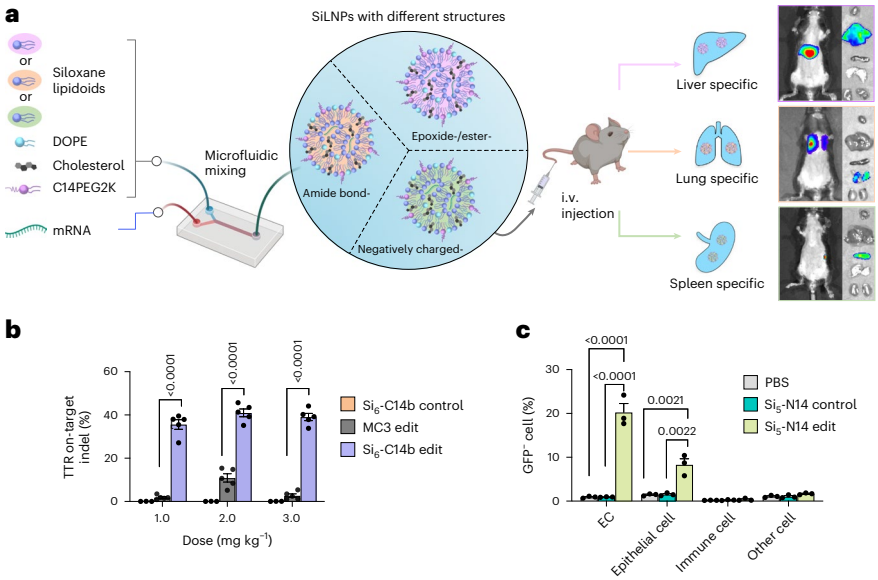

Fig. 1 | Siloxane-incorporated lipid nanoparticles (SiLNPs) mediate tissue-specific mRNA delivery. **a**, Microfluidic-assisted synthesis of SiLNPs with chemically distinct structures for in vivo mRNA delivery to the liver, lung, and spleen. C14PEG2K, lipid-anchored polyethylene glycol (PEG); DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; i.v., intravenous. **b**, Frequency of *Ttr* on-target insertion and deletion (indel) in the liver of mice following administration of our lead liver-targeting SiLNP (called Si_6-C14b) encapsulating Cas9 mRNA and *Ttr* single guide RNA (sgRNA). MC3, DLin-MC3-DMA, the clinically approved LNP formulation. **c**, Quantification of the percentage of green fluorescent protein (GFP)-negative (GFP^-) cells (indicating successful GFP gene editing) in the lung of transgenic GFP mice. Lead lung-targeting SiLNPs (Si_5-N14) encapsulating Cas9 mRNA and GFP sgRNA were systemically administered. Phosphate-buffered saline (PBS) and LNPs co-delivering Cas9 mRNA and scramble sgRNA were used as negative controls.

BEHIND THE PAPER

To date, siloxane-based materials have been applied extensively in materials science and biomedical applications. Looking at the excellent chemical properties of siloxane moieties, we thought that incorporating this kind of material into LNP formulations could have unexpected effects on mRNA delivery. The combinatorial design approach to generate a large library of siloxane-incorporated lipidoids from which we identified quite a few lead candidates was quite exciting. However, the most challenging part of this study was identifying the mechanism by which siloxane moieties accelerated mRNA delivery. Through both in vitro and in vivo screening evaluations, we concluded that small structural alternations of siloxane-incorporated lipidoids enabled potent, tissue-specific mRNA delivery. We really enjoyed exploring the therapeutic efficacy of these lead SiLNPs for various gene therapy applications with our collaborators. **L.X., K.L.S. & M.J.M.**

REFERENCES

1. Sahin, U., Karikó, K. & Türeci, Ö. mRNA-based therapeutics — developing a new class of drugs. *Nat. Rev. Drug Discov.* **13**, 759–780 (2014).
   A comprehensive review article on the development of mRNA drugs.
2. Hou, X., Zaks, T., Langer, R. & Dong, Y. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Mater.* **6**, 1078–1094 (2021).
   A review article that summarizes the design of LNPs for mRNA delivery.
3. Eygeris, Y., Gupta, M., Kim, J. & Sahay, G. Chemistry of lipid nanoparticles for RNA delivery. *Acc. Chem. Res.* **55**, 2–12 (2022).
   A review article that discusses the core features of ionizable lipids for RNA delivery.
4. Cheng, Q. et al. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. *Nat. Nanotechnol.* **15**, 313–320 (2020).
   This paper reports that the addition of a selective organ targeting molecule into LNPs enables mRNA delivery to specific organs.
5. Dilliard, S. A. & Siegwart, D. J. Passive, active and endogenous organ-targeted lipid and polymer nanoparticles for delivery of genetic drugs. *Nat. Rev. Mater.* **8**, 282–300 (2023).
   A review article that highlights emerging materials and biological insights for targeting specific organs and cells.
6. Blanco, I. Polysiloxanes in theragnostic and drug delivery: a review. *Polymers* **10**, 755 (2018).
   A review article that summarizes siloxane-based materials for biomedical applications.

FROM THE EDITOR

"The paper by Xue and colleagues stands out because it offers a strategy to selectively target specific organs for modification of tissue-specific proteins, opening up new therapeutic possibilities in gene therapy." **Editorial Team, *Nature Nanotechnology***