LNP-mediated *in situ* gene editing as novel therapy for genodermatoses

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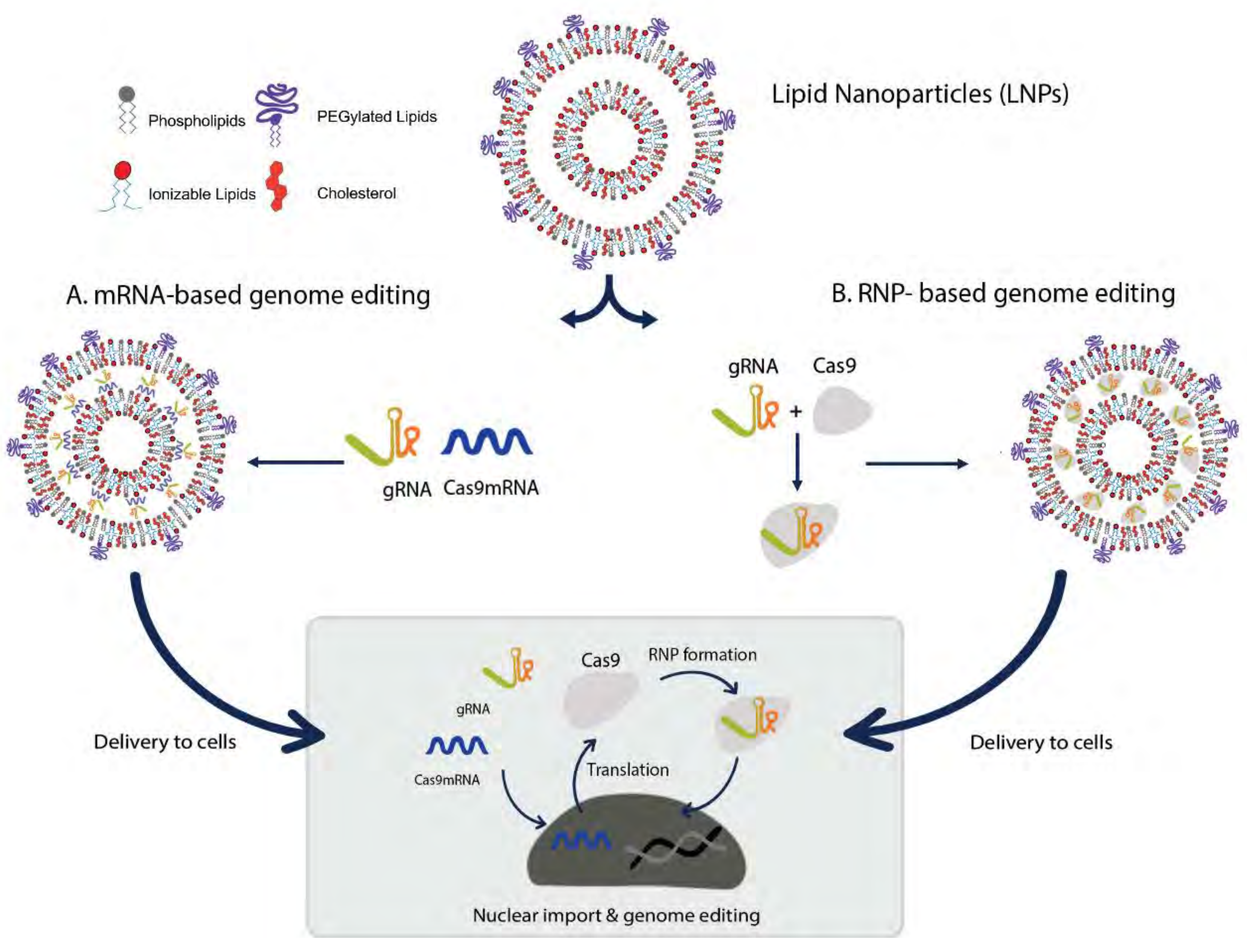
Introduction

The treatment of genetic skin disorders is impaired by the skin's outer most layer, the stratum corneum, that restricts the passage only to molecules with specific physicochemical parameters. Overcoming this layer and reaching the mutation site is one of the biggest challenges for topical drug delivery.

Due to this difficulty, genodermatoses remain with little or no treatment options. The RNA-guided CRISPR-Cas9 nuclease system allows editing of specific DNA sequences in target cells and therefore provides a potential cure to genetic diseases. To reach the target cells, lipid nanoparticles (LNP) stand out as a vector for their high bioavailability and nucleic acid complexation.

We aim to explore the feasibility of *in situ* gene editing in skin through physical modulation of the skin barrier to facilitate intradermal absorption of LNPs.



Results

MTT assay showed 73.8% cell viability for unloaded LNPs (Figure 1).

The editing efficacy in primary keratinocytes for RNP loading ranged from 5.7% to 15.6% (Figure 2.a), and from 8.2% to 16.6% for LNP/mRNA (Figure 2.b). We selected LNP C at ratios 500 and 6.

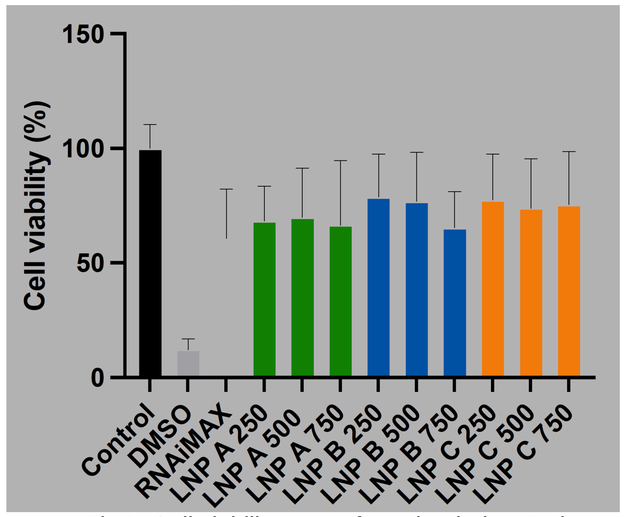


Fig 1. Cell viability assay for unloaded LNPs in primary keratinocytes.

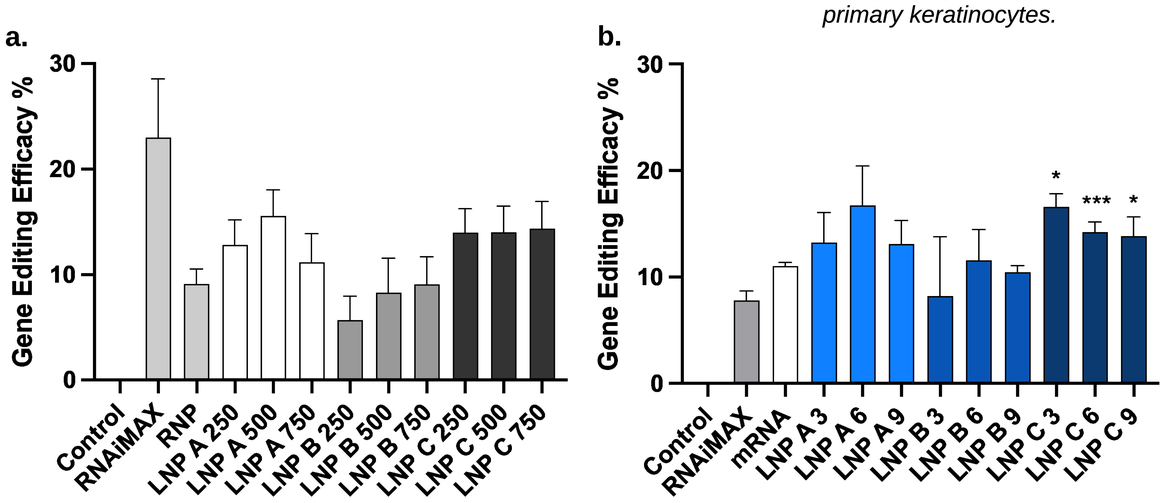


Fig 2. Gene editing efficacy for a. LNP/RNP, and b. LNP/mRNA. (*) represents $p < 0.5$ and (***) $p < 0.001$ vs. RNAiMAX

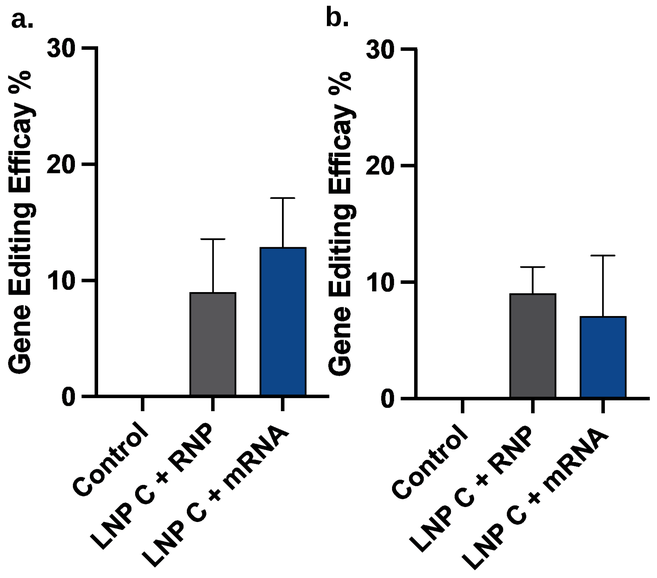


Fig 3. Gene editing efficacy for LNP/RNP and LNP/mRNA in a. skin models and b. excised human skin.

For gene editing efficacy on skin, solid microneedles were used to facilitate permeation of the LNPs, yielding 9.0% editing for RNP-loaded and 12.8% editing for mRNA-loaded LNPs on skin models; and 9.0% editing for RNP-loaded and 7.1% editing for mRNA-loaded LNPs.

Conclusions

Results showed promising outcomes for gene editing on primary skin cells and ex vivo human skin. In vivo assays had good cell viability and gene editing efficacy; and excised human skin, positive editing efficacy. These data suggests in situ gene editing can be a viable therapy option for genodermatoses.

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