Minicircle DNA transfection by SM-102 lipid nanoparticles into HEK cells led to prolonged transient expression compared to plasmids or mRNA

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Introduction

Lipid nanoparticles proved their worth as reliable non-viral gene transfer vehicles for transient delivery of mRNA to the cytoplasm. In gene-modified cell therapy, there are instances where a transient transgene expression can be sufficient or even desirable.
In this work, we examined Minicircles as DNA vectors for LNP-based delivery into HEK cells and tested the efficiency and duration of GFP reporter gene expression following successful nuclear entry and in the absence of targeted genomic integration.

Methods

Minicircles were produced in *E. coli* from their corresponding plasmids through *in vivo* intramolecular recombination and purified.

Lipid Nanoparticle Production
To produce the lipid nanoparticles, the Lipid Nanoparticle (LNP-102) Exploration Kit from Cayman Chemical was used. The LNPs were prepared with 100 µg of DNA or 70 µg of RNA. Buffer exchange was performed using an Amicon Ultra 4 centrifugal filter (10 kDa cut-off) against 1x PBS, and the LNPs containing DNA or RNA were stored at 4 °C until use.

Transfection of HEK293 Cells with LNPs
24 h prior to transfection, HEK293 cells were seeded in 6-well or 24-well plates at a cell density of $5.1x10^5$ or $1x10^5$ cells per well, respectively. Shortly before transfection, DMEM was replaced with Opti-MEM to 50 % of the typical culture volume, and the LNP solution was added in the corresponding concentration.
HEK-293 cells were transfected at a concentration of 1 µg DNA per 1 million cells. Fluorescence microscopy and flow cytometry were used to measure transfection efficiency.

Results

In a comparison experiment against plasmids, Minicircles showed peak transfection efficiency at day 3 with a 3.1-fold increase of transfection efficiency over plasmids. The transfection efficiency remained higher for Minicircles over seven days and was still detectable at day seven.
The transient responses between cytoplasmatically delivered mRNA and nuclear active Minicircle DNA has not been studied before. Firstly, LNPs designed for mRNAs could be quickly repurposed for DNA. Minicircle DNA was found to be well-suited for transient applications that would require a longer expression. *In vitro* transcribed RNA resulted in a relatively lower expression profile and the transfection efficiency dropped drastically already after 48 h.

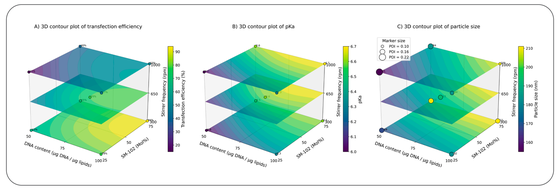

Fig. 1: DoE-based optimization of various parameters for packaging DNA into SM102 lipid nanoparticles.

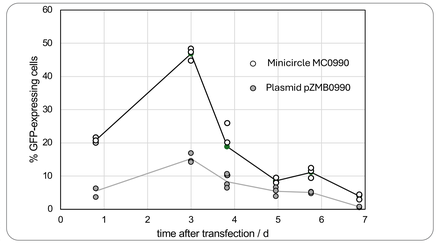

Fig. 2:
Comparison of reporter gene expression following transfection of Minicircle DNA and plasmid DNA

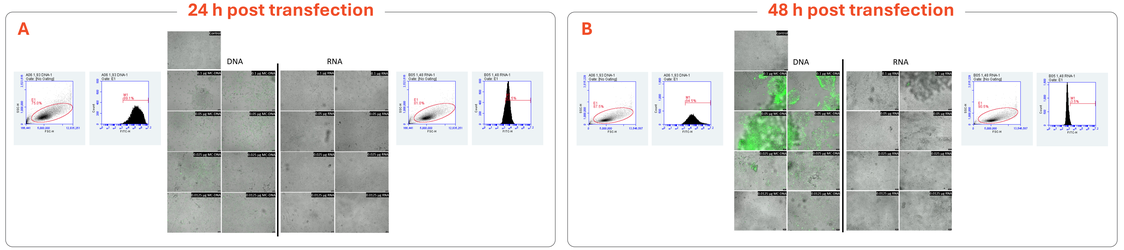

Fig. 3 (A – D):
Fluorescence microscopy and flow cytometry analysis to follow transfection efficiency at various time points following transfection with mRNA or Minicircle DNA using SM102- Lipid Nanoparticles

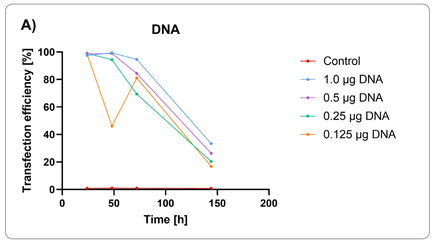

Fig. 4:
Summary of transfection efficiencies for different amounts of minicircle DNA (A) and mRNA (B) over time upto 144 h

✓ Minicircles packaged into LNPs. **✓ Extended transient expression**

Outlook

In gene-modified cell therapy, especially for non-malignant conditions, transient gene expression allows for controlled activity and redosing as required.
In this work, we show that LNP vehicles developed for mRNA delivery could be quickly adapted to deliver a Minicircle DNA cargo. Possibly through repeated transcription, we see an extension of the timeframe of action and thus a more sustained expression while still maintaining a transient character.
Another possible area of application would be in pre-clinical research, where non-viral LNP vehicles could help accelerate the study of *in vivo* gene modification effects.

More about
Reporter constructs and pEPito plasmids

Acknowledgements: We thank Katja Schleef for designing this poster. | Presented at the ASGCT Annual Meeting on 14th of May 2026 in Boston. | © 2026 PlasmidFactory GmbH