Innovative microRNA transfection kit based on hybrid polymer-lipid nanoparticles

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INTRODUCTION

VIRAL VECTORS



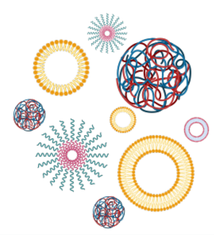
- ✕ Poor control on delivery
- ✕ Immune response and safety issues
- ✕ Uncontrolled expression of miRNAs
- ✕ High costs

Traditional transfection agents come with several limitations.

Can we do better?

TRANSFECTION REAGENTS

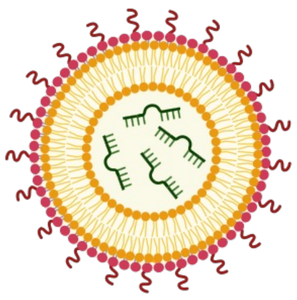
- Poor stability ✕
- Incomplete loading efficiency ✕
- Uncontrolled release kinetics ✕



Lee at al., *Journal of Controlled Release*, 2019 | Gabisonia *et al.*, *Nature*, 2019

OUR SOLUTION

We develop **hybrid polymer–lipid nanoparticles** (NPs) for efficient **microRNA** delivery and ***in vitro*** transfection.



microRNA (miRNA)

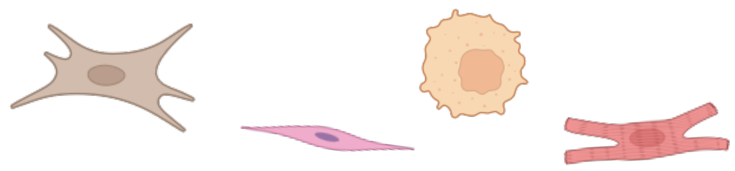
DE-DOPE liposome (lipidic core)

PLGA-based polymeric shell

Versatile *in vitro* transfection kit

- ✓ Good encapsulation efficiency
- ✓ Improved stability

NPs can efficiently transfect fibroblasts (FBs), cardiomyocytes (CMs), muscle cells, tumor cells, and potentially many others.



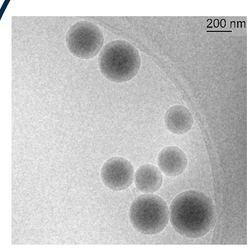
Lipofectamine RNAiMAX used as a control.

Nicoletti *et al.*, *Nanomedicine: Nanotechnology, Biology and Medicine*, 2022 | Nicoletti, Paoletti *et al.*, *Advanced Healthcare Materials*, 2025

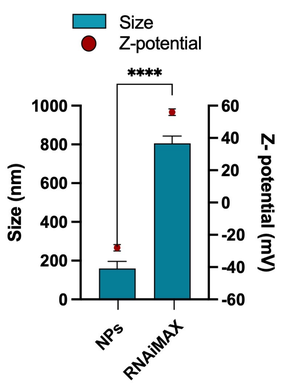
RESULTS

Physico-chemical characterization

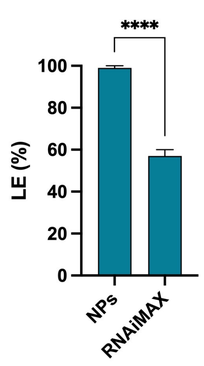
TEM



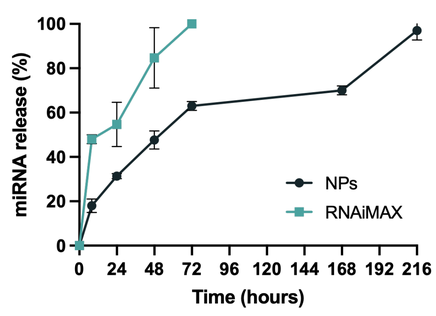
DLS analysis



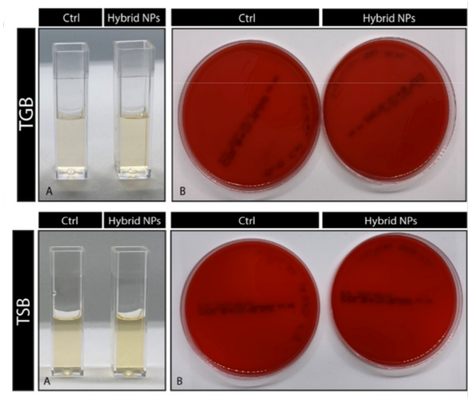
Loading efficiency



miRNA release in PBS



Sterility

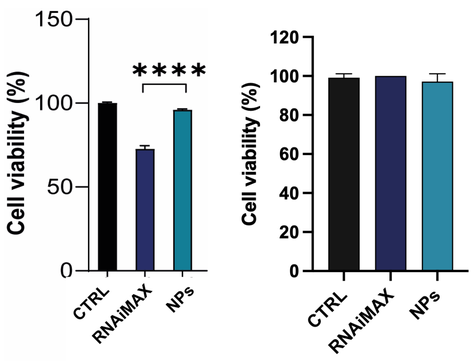


- Core-shell structure
- Nanometric size and negative Z-potential
- **High encapsulation efficiency**
- **Controlled miRNA release**
- **Stable** in DMEM + FBS up to 48 h
- **Absence of micorbial contamination** after 14 days in Tryptic-Soya Broth (TSB) and Thioglycolate Broth (TGB)

Cytocompatibility

Fibroblasts

Adult human cardiac fibroblasts (AHCFs)



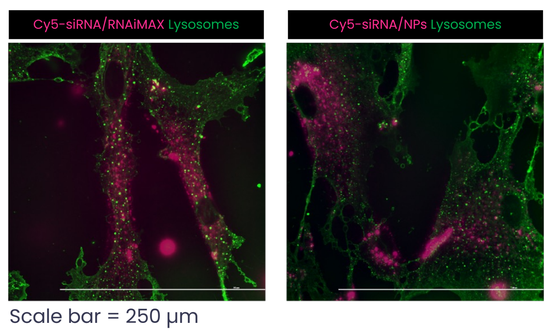
Cardiomyocytes

H9c2 rat cardiac myoblasts cell line

NPs exhibited excellent **cytocompatibility** and **efficient uptake** by AHCFs and H9c2 24 h post-transfection (25 nM).

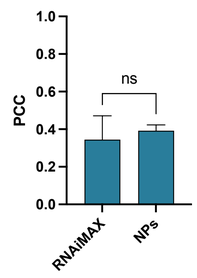
Colocalization analysis using Pearson's correlation coefficient (PCC) 24 h post-transfection suggest efficient **lysosomal escape**.

Endosomal escape



Scale bar = 250 µm

Fibroblasts



Efficacy tests

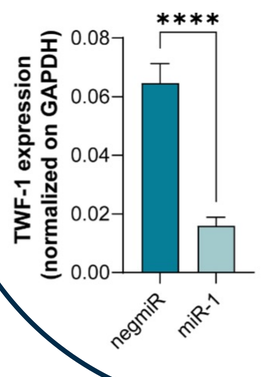
DIRECT REPROGRAMMING

miRcombo
(miR-1,miR-133,miR-208, miR-499)

FBs → Induced CMs

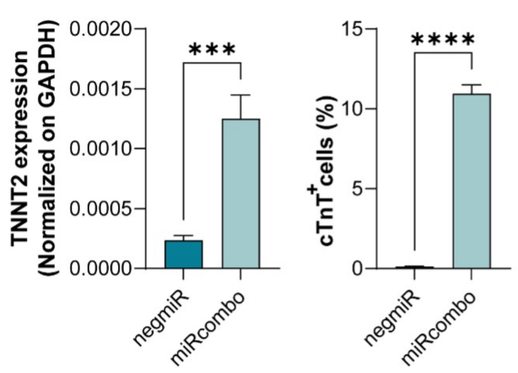
Transfection efficiency

miR-1 ⊣ TWF-1



Direct reprogramming

miRcombo → TNNT2



Myocardial infarction → *Regenerated heart*

CARDIAC REGENERATION

- Transfection efficiency of miR-1-loaded NPs confirmed by **TWF-1 mRNA downregulation** at 48 h.
- miRcombo-loaded NPs induced direct reprogramming, with **increased cTnT expression** at gene and protein levels at 15 days.

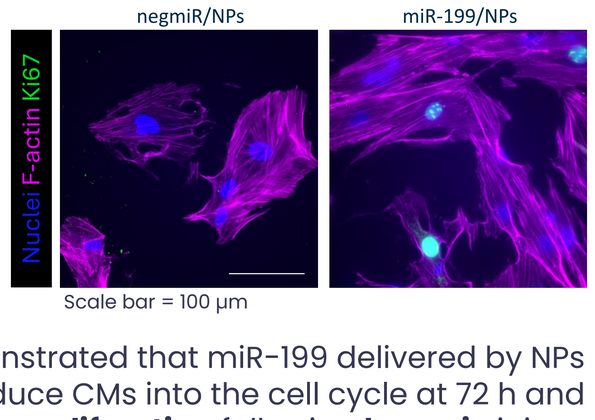
Read the full paper here!

CARDIOMYOCYTES PROLIFERATION

miR-199a-3p

CMs → Proliferating CMs

Proliferation assay



Scale bar = 100 µm

Ki67 proliferation assay demonstrated that miR-199 delivered by NPs can effectively re-introduce CMs into the cell cycle at 72 h and induces their **proliferation** following **hypoxic** injury.

Jayawardena *et al.*, *Circulation research*, 2012 | Paoletti *et al.*, *Frontiers in Bioengineering and Biotechnology*, 2020 | Gabisonia *et al.*, *Nature*, 2019

CONCLUSIONS & FUTURE PERSPECTIVES

- Fast and **scalable production**
- High miRNA **loading efficiency**
- Controlled **miRNA release**
- Excellent colloidal **stability**
- **Cytocompatible**
- **Long-term storage** capability
- Efficient ***in vitro*** **transfection** in various cell types

- Addition of **antifouling** properties
- Surface **functionalization** with ligands for **active targeting**
- Translation to ***in vivo*** applications

CONTACTS & ACKNOWLEDGEMENTS

Let's Connect!

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