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Hybrid polymer-lipid nanoparticles as innovative transfection vectors for microRNA delivery

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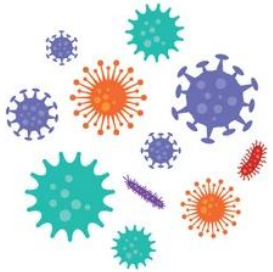
CRS 2025 ANNUAL MEETING & EXPOSITION

PHILADELPHIA, PA
JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Traditional transfection agents

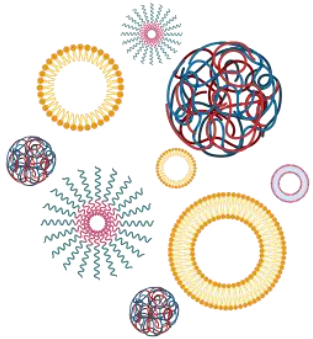
VIRAL VECTORS



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

STATE OF THE ART TRANSFECTION REAGENTS

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



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

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Can we do better?

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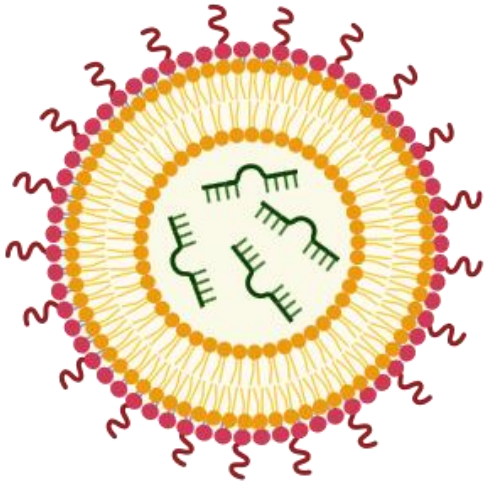
Novel hybrid polymer-lipid nanoparticles



Hybrid nanoparticles (NPs)

- **microRNA (miRNA)**
- **DE-DOPE liposome (lipidic core)**

  Efficient encapsulation efficiency
- **PLGA- based polymeric shell**

  Improves stability

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

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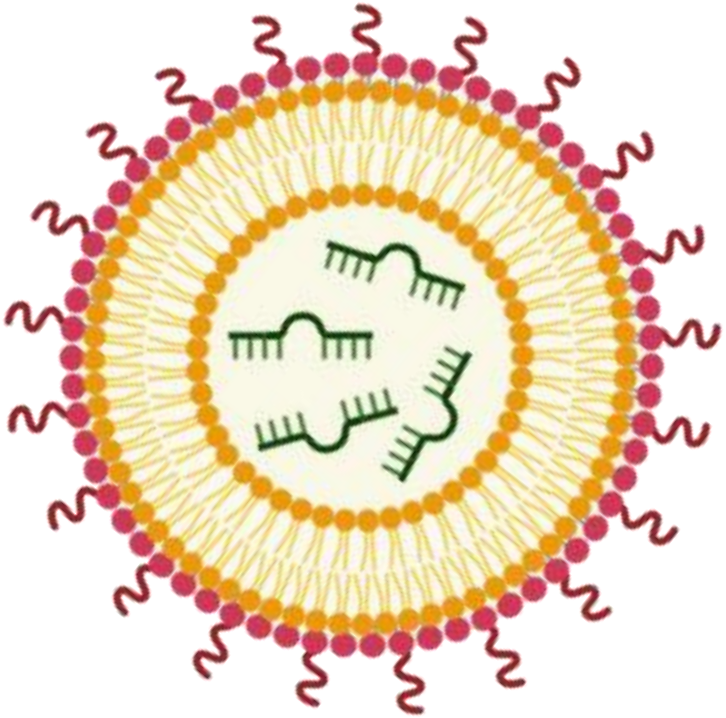
One nanoparticle many possibilities



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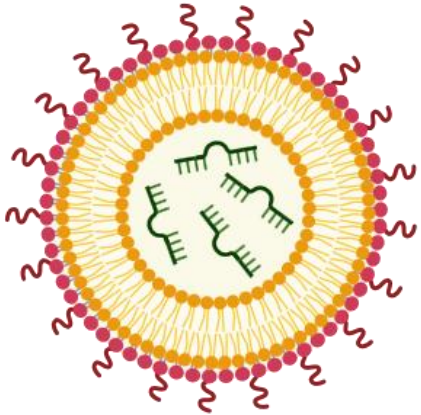
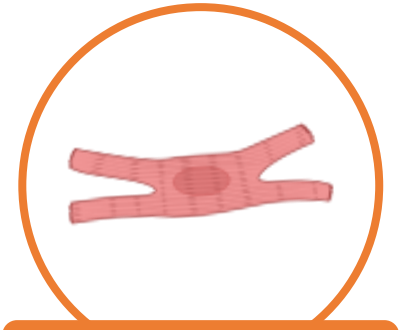
One nanoparticle many possibilities

Versatile and efficient
***in vitro* transfection kit**



Fibroblasts



Cardiomyocytes

Muscle cells

Tumor cells

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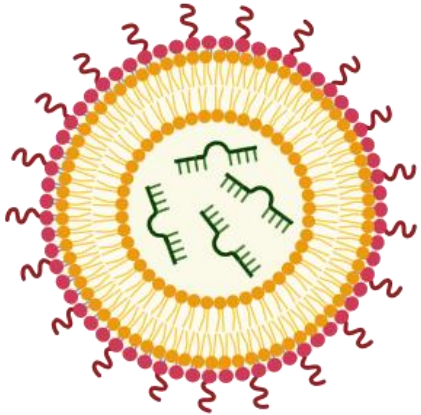
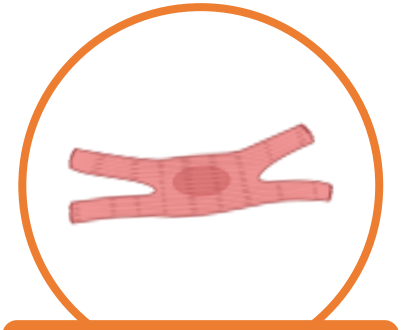
One nanoparticle many possibilities

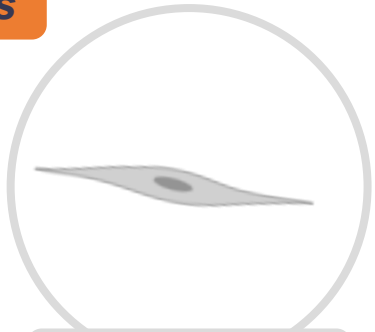
Versatile and efficient
***in vitro* transfection kit**



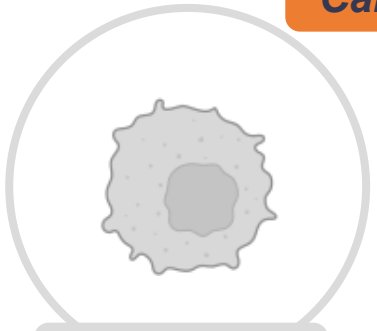
Fibroblasts



Cardiomyocytes



Muscle cells



Tumor cells

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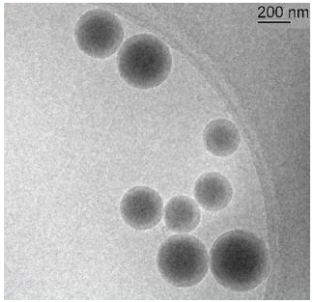
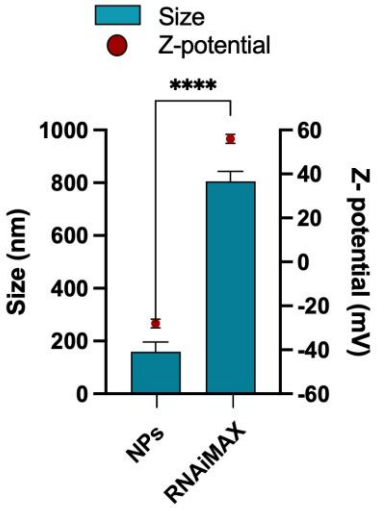
Physicochemical characterization

TEM

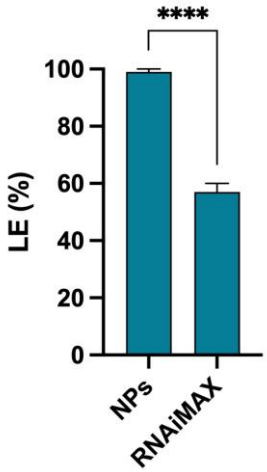


Lipofectamine RNAiMAX used as a control.

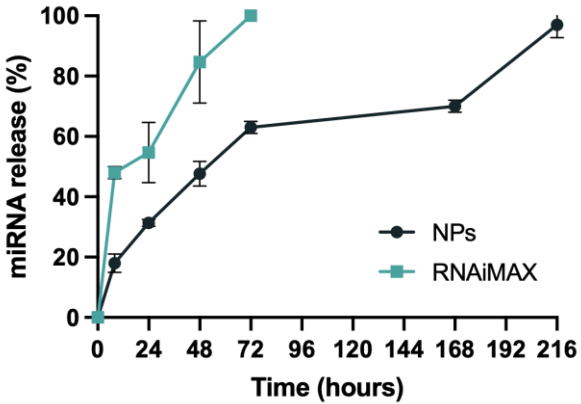
DLS analysis



Loading efficiency (LE)



miRNA release in PBS



- Nanometric size and negative zeta potential; smaller than RNAiMAX
- **Higher loading efficiency** than RNAiMAX
- **Controlled release** in PBS at 37 °C (9 days), more gradual than RNAiMAX (72 h)

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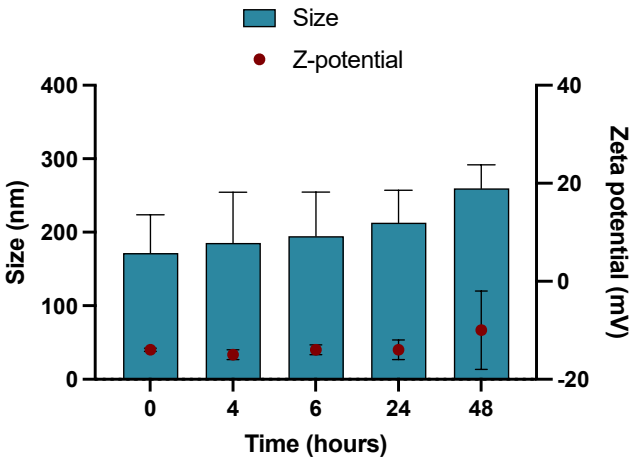
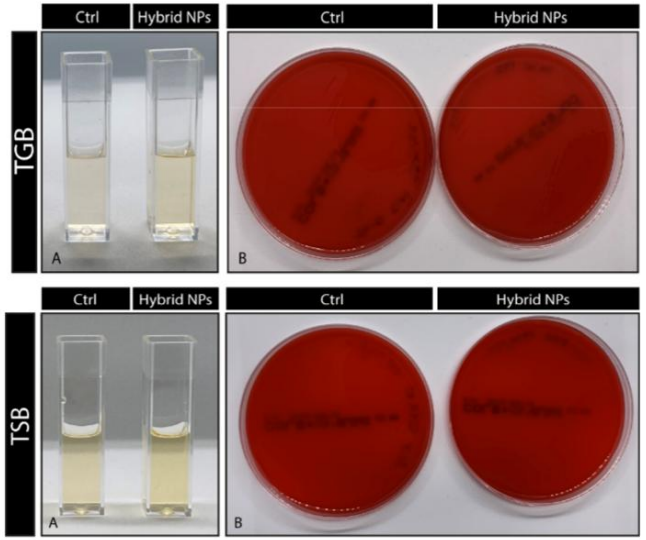
Physicochemical characterization

Stability at 37 °C in DMEM + FBS



Stable in physiological conditions for up to 48 h.

Sterility



Absence of microbial contamination after incubation of NPs in Tryptic-Soya Broth (TSB) and Thioglyocolate Broth (TGB) for 14 days.

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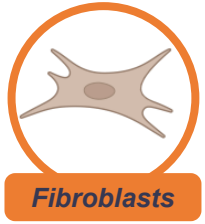
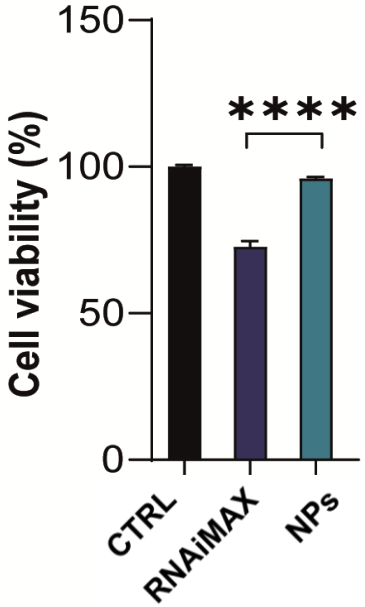
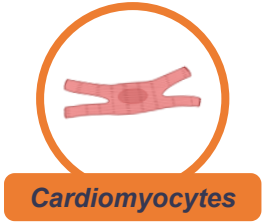
Cytocompatibility

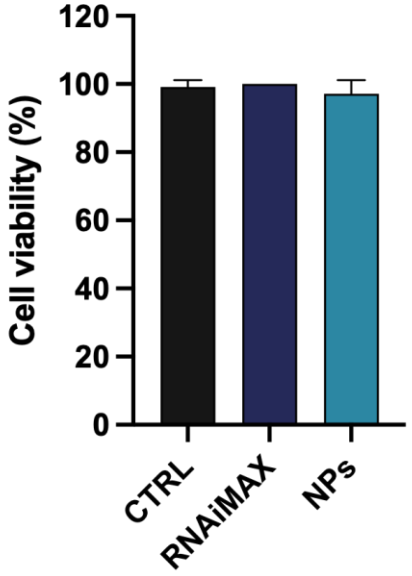


Adult human cardiac fibroblasts (AHCFs)





H9c2 rat cardiac myoblasts cell line



NPs exhibited excellent **cytocompatibility** in different cell types 24 h post-transfection.

NPs 25 nM

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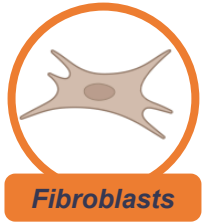
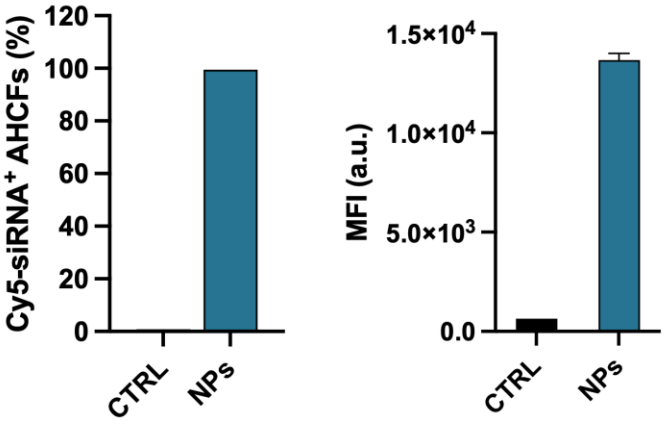
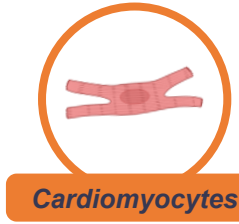
Cellular uptake

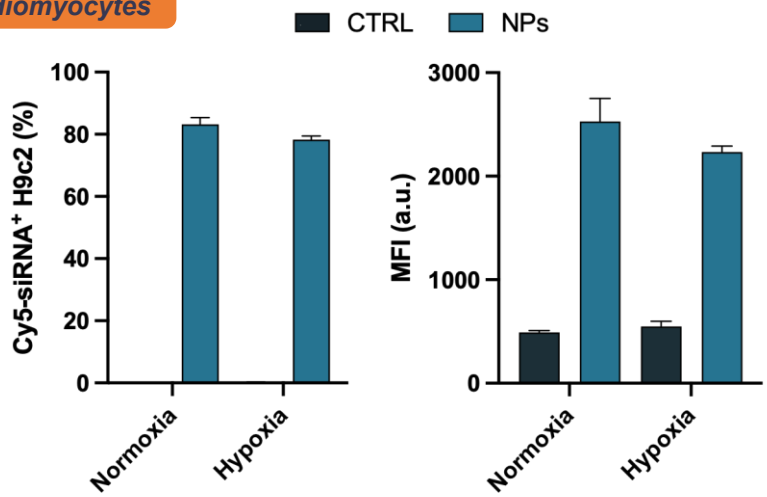


AHCFs





H9c2



NPs showed **efficient uptake** by AHCFs and H9c2 24 h post-transfection.

NPs 25 nM

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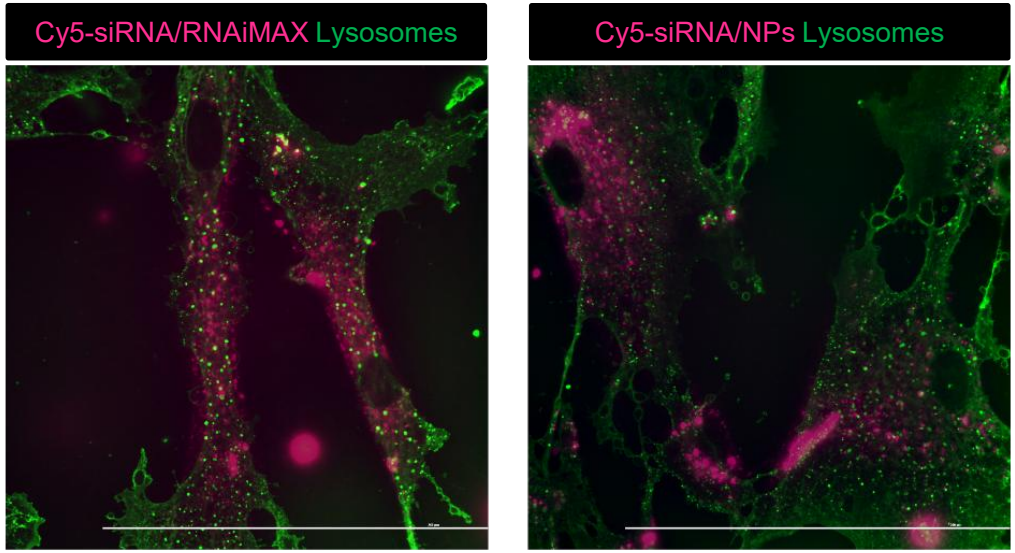
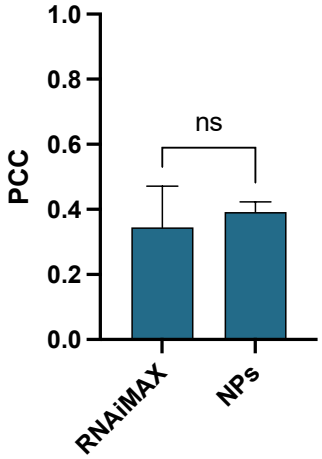
Endosomal escape



Scale bar = 250 μM

NPs 25 nM



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

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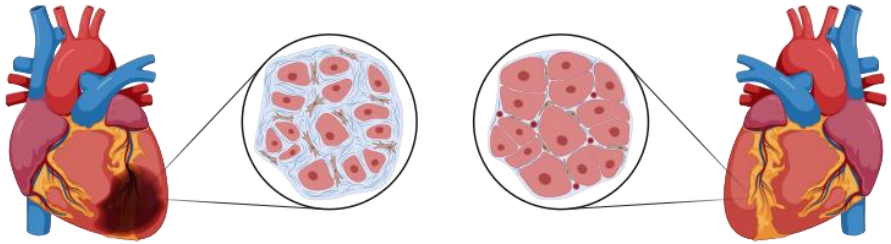
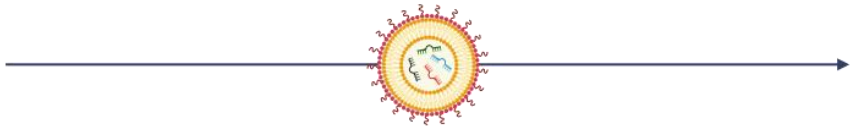
Direct reprogramming for cardiac regeneration

Myocardial infarction



Regenerated heart

DIRECT REPROGRAMMING



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

Fibroblasts  Induced cardiomyocytes

Jayawardena *et al.*, *Circulation research, 2012* – Paoletti *et al.*, *Frontiers in Bioengineering and Biotechnology*, 2020

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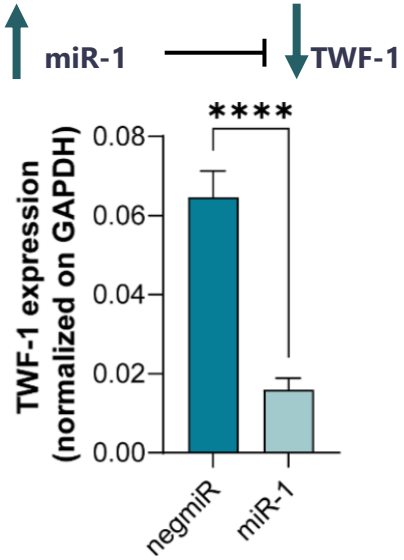
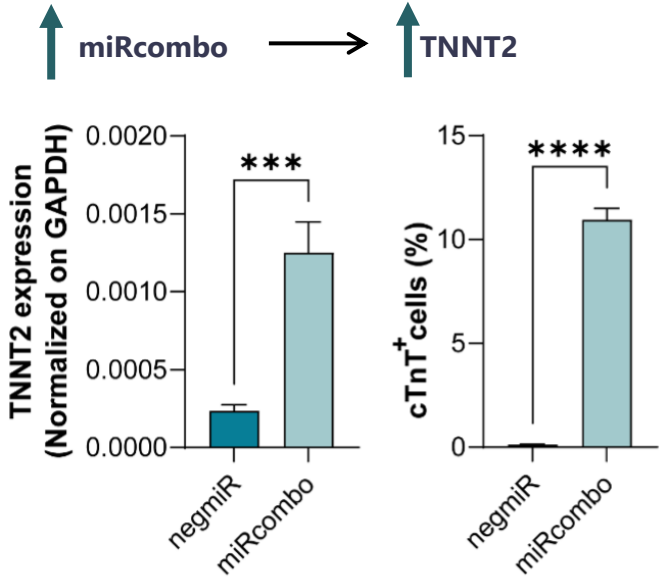
Efficacy tests

Fibroblasts

Transfection efficiency



Direct reprogramming



- 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** (CMs marker) at gene and protein levels after 15 days.

NPs 25 nM

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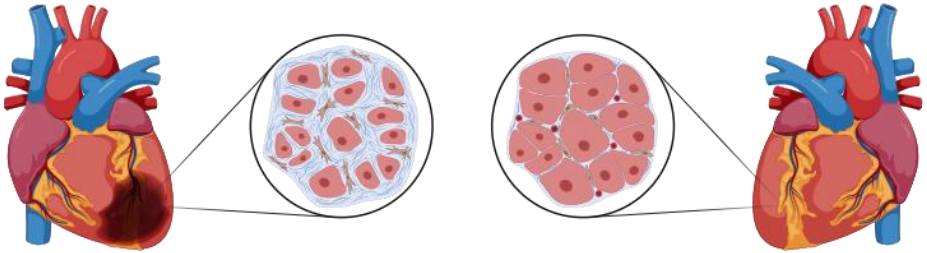
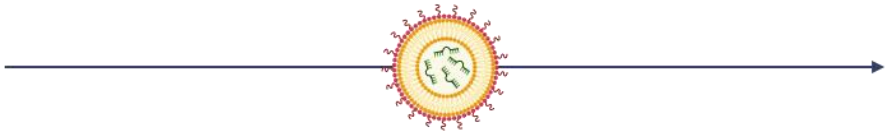
Resident cardiomyocytes proliferation

Myocardial infarction



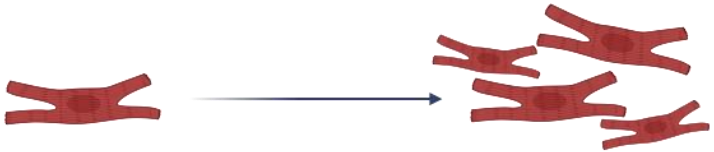
Regenerated heart

CARDIOMYOCYTES PROLIFERATION



miR-199a-3p

Cardiomyocytes



Proliferating cardiomyocytes

Gabisonia *et al*., *Nature*, 2019

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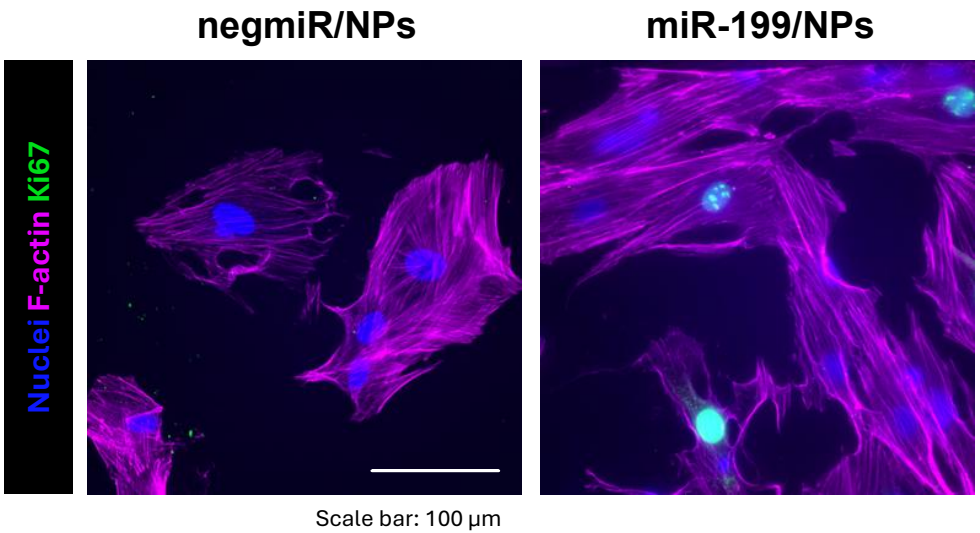
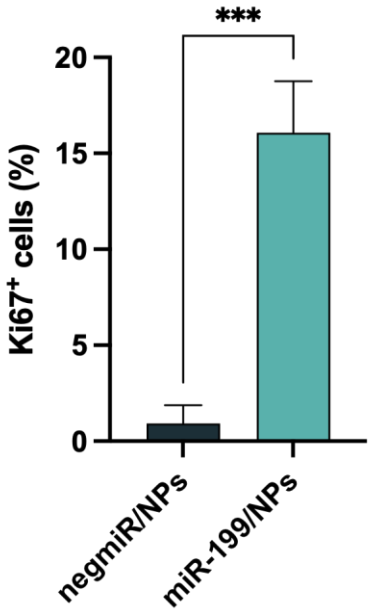
Re-entry into the cell cycle

Proliferation assay





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

NPs 25 nM

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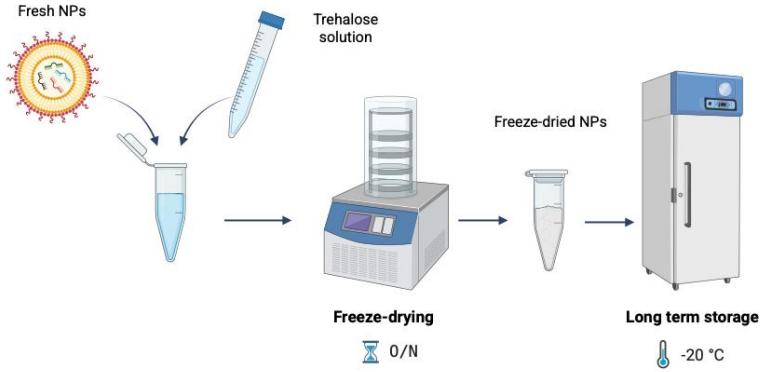
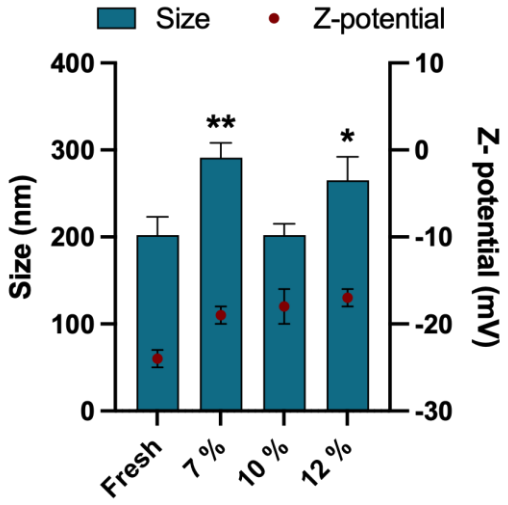
Long-term storage

Fibroblasts

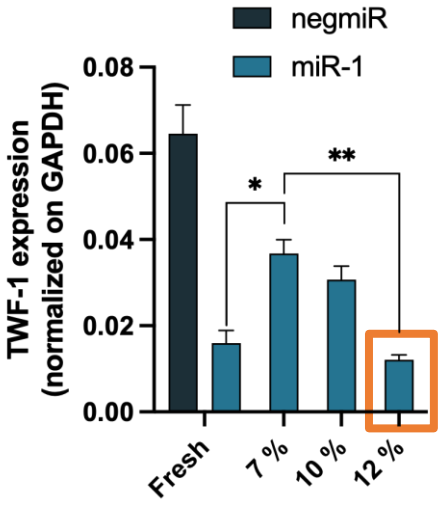


12 % w/v **trehalose** ensures optimal retention of **physicochemical properties** and **transfection efficiency**, which are also maintained after 7 and 14 days of storage.

DLS analysis



Transfection efficiency



NPs 25 nM

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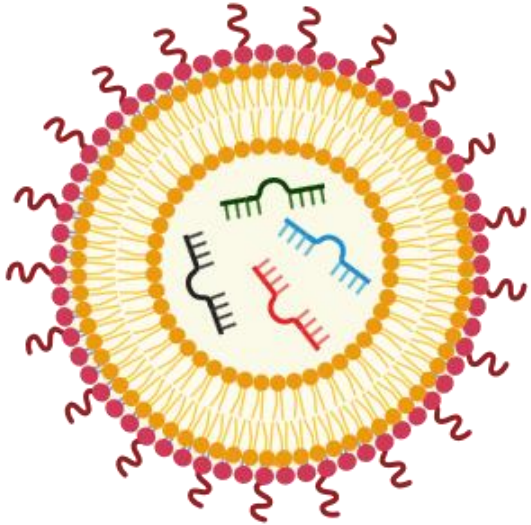
Conclusions



In vitro **transfection kit**

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

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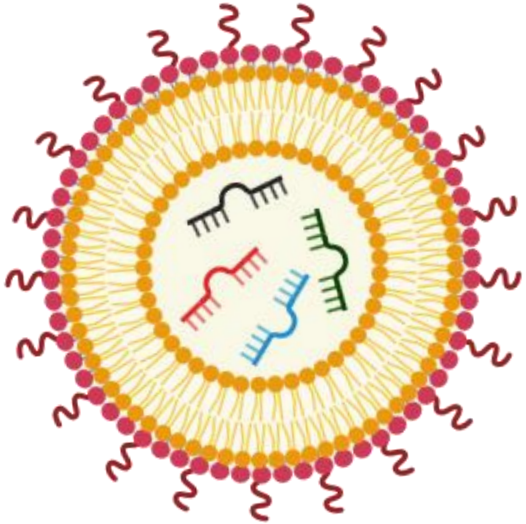
Conclusions



ADVANCED HEALTHCARE MATERIALS

Research Article | Open Access

Design and Validation of Hybrid Polymer-Lipid Nanoparticles as Novel Transfection Vectors for MicroRNA Delivery to Human Cardiac Fibroblasts

Letizia Nicoletti, Camilla Paoletti, Martina Coletto, Elena Marcello, Giovanni Paolo Stola, Francesca Cossetta, Francesco Schiavone, Ilaria Andreana, Barbara Stella, Silvia Arpicco, Clara Mattu, Valeria Chiono ... See fewer authors

First published: 06 June 2025 | https://doi.org/10.1002/adhm.202500971

Read the full paper here!

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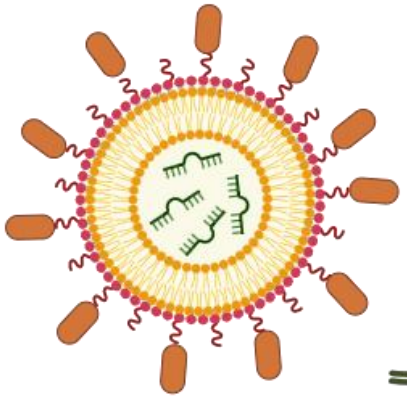
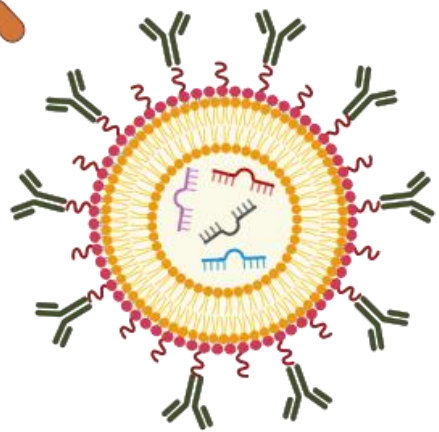
Ongoing work & Future perspectives





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

PoliRNA

Letizia Nicoletti, Giovanni Paolo Stola and Valeria Chiono are co-founders of PoliRNA Srl spinoff company.

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Acknowledgements

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Valeria Chiono, Professor
Clara Mattu, Professor
Irene Carmagnola, Professor
Alice Zoso, PhD
Elena Marcello, PhD
Camilla Paoletti, PhD
Letizia Nicoletti, PhD
Mattia Spedicati, PhD
Gerardina Ruocco, PhD
Daniele Testore, PhD
Francesca Tivano, PhD student
Giovanni Paolo Stola, PhD student
Francesca Cossetta, PhD student
Francesco Schiavone
Giuseppe Cutietta

UNITO

Barbara Stella, Professor
Silvia Arpicco, Professor
Ilaria Andreana, PhD

This work was supported by the European Research Council (ERC) under the European Union's Horizon research and innovation program (BIORECAR, grant agreement no. 772168 and POLIRNA, grant agreement no. 101113522).

Images created in https://BioRender.com

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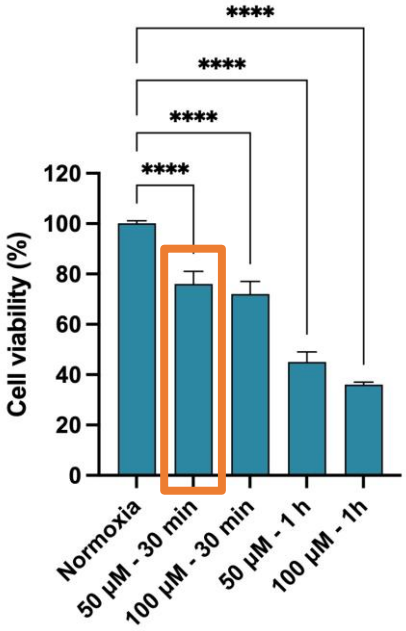
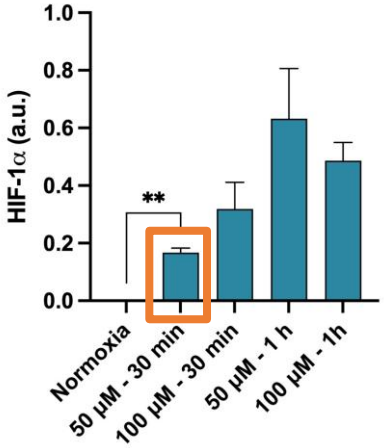
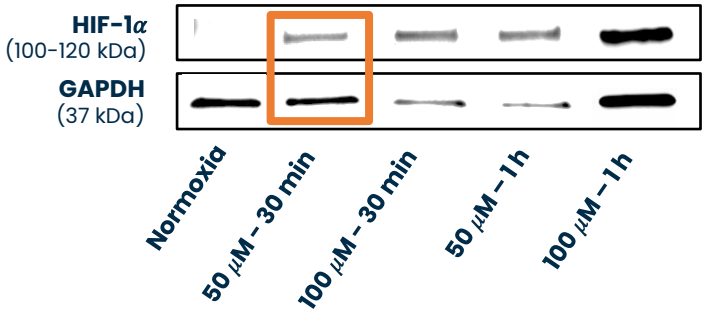
Induction of hypoxia with H_2O_2

Cell viability



Western blot





Hypoxia was induced using 50 µM **H_2O_2** for 30 min, based on cell viability and **HIF-1α** expression.

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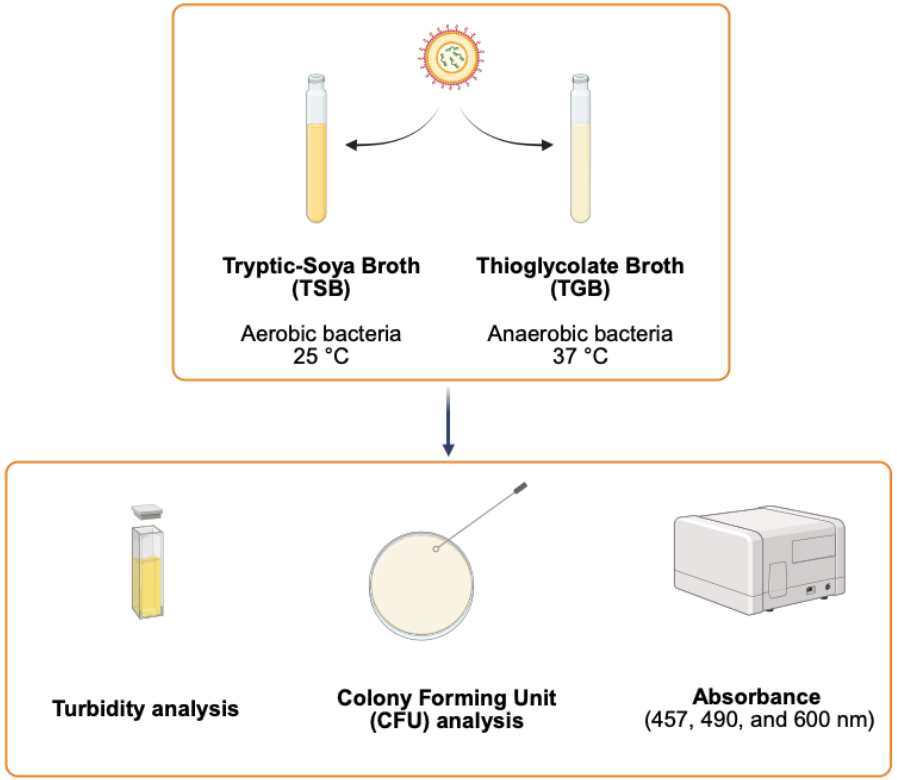
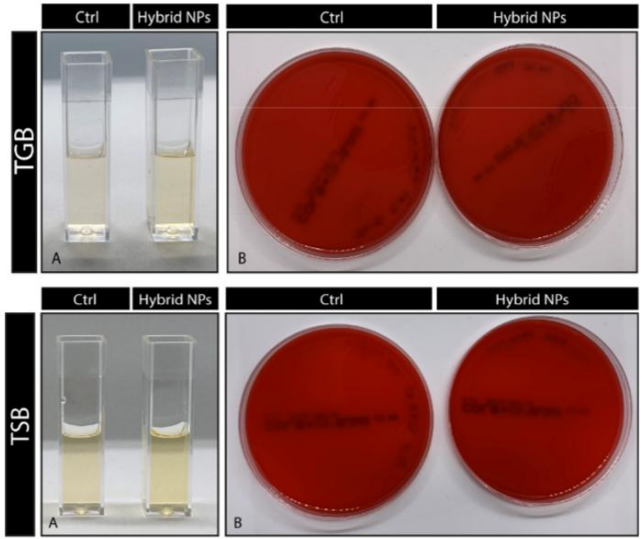
Absence of microbial contamination



Analysis after 1,7, and 14 days of incubation



According to European Pharmacopeia (Ph. Eur. 2.6.1, 2008) and ISO 11737-2:2019 Sterilizaton of health care products (Bioburden estimation).

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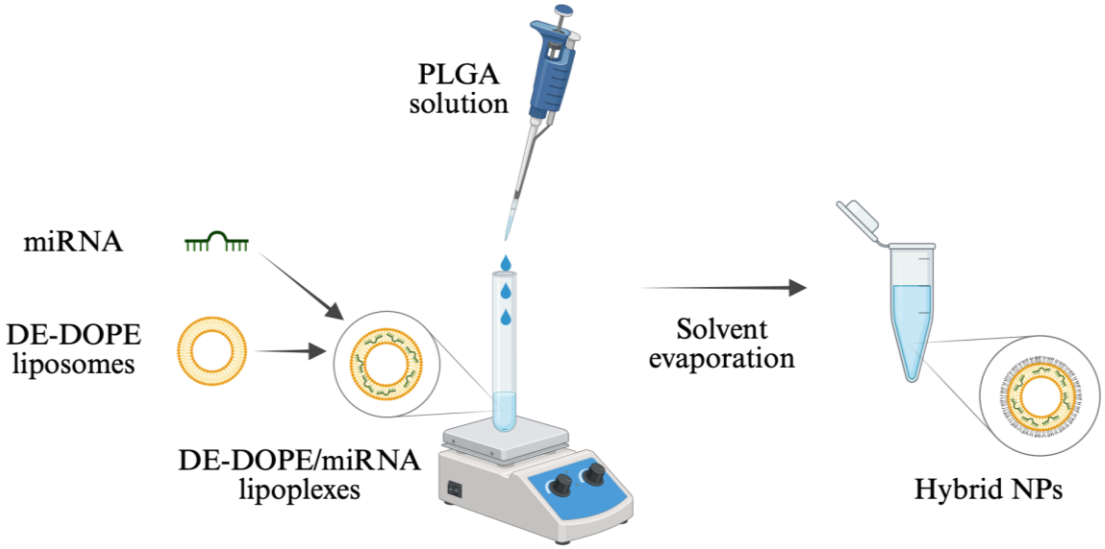
Nanoprecipitation



Nanoprecipitation

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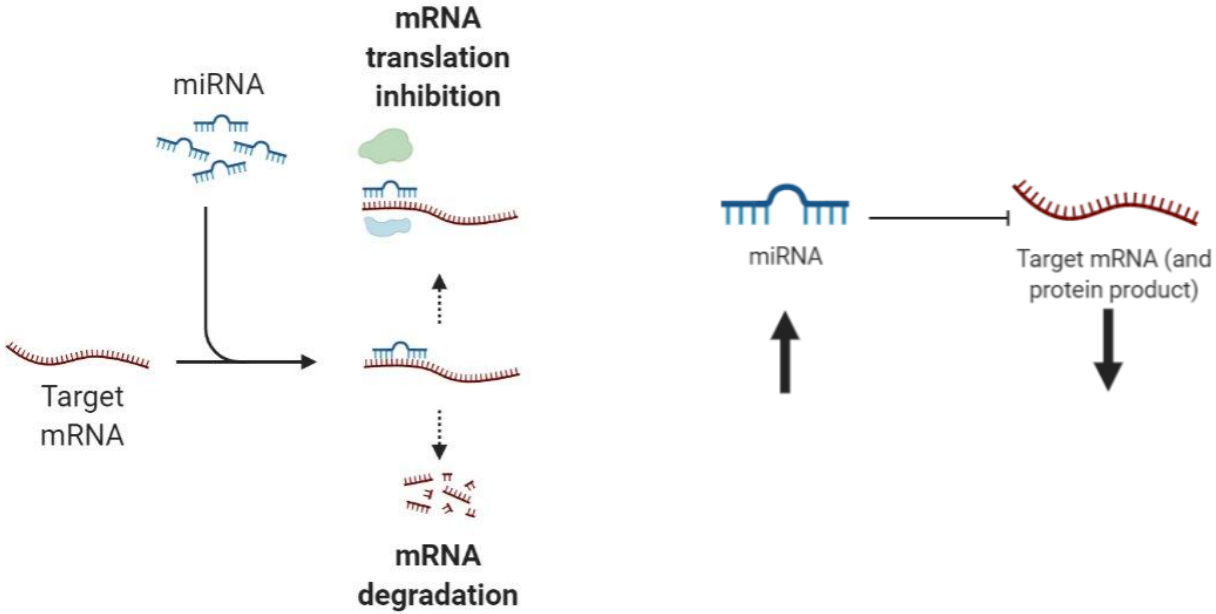
miRNA: regulator of gene expression

miRNAs are defined as small non-coding RNA molecules (about 21 nucleotides) that function as guide molecules in post-transcriptional regulation of gene expression in the RISC complex (RNA induced silencing complex).



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