XII JORNADA DE JÓVENES INVESTIGADORES/AS DEL I3A

# Development of cutting-edge DNA nanocarriers with cardioregenerative capacity using the therapeutic potential of microRNAs

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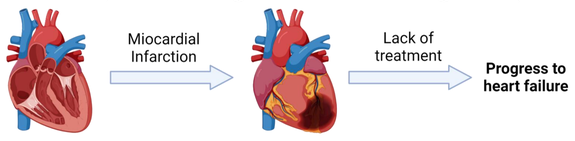
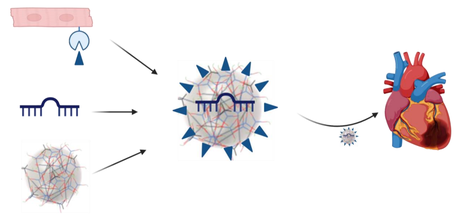
## Introduction

Cardiovascular diseases, including myocardial infarction, are leading causes of death globally



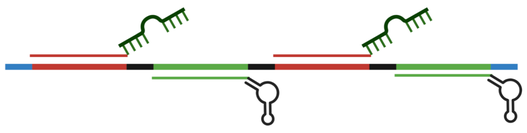

## Purpose

To create a **novel nanotherapy** capable of delivering **cardioregenerative miR-199a-3p** specifically to the heart using **cardiospecific targeting ligands** across the **vascular barrier.**



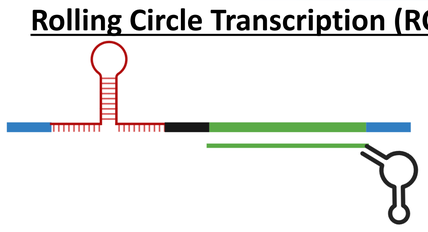
## Approach

### Rolling Circle Amplification (RCA)



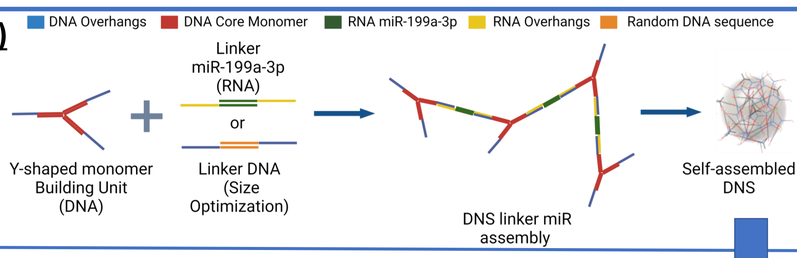
Binding sequence to miR-199a: Random spacer sequence:
Binding sequence to targeting aptamer: Circularization sequence:

### Rolling Circle Transcription (RCT)

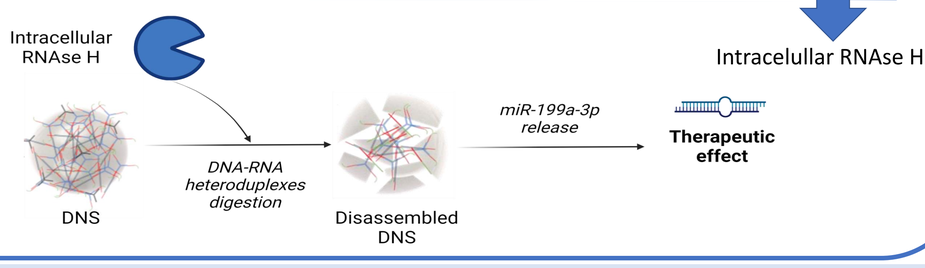


Pre-miR-199 sequence: Random spacer sequence:
Binding sequence to targeting aptamer: Circularization sequence:

### Nanohydrogel (DNS)




### miR release




## *Results*

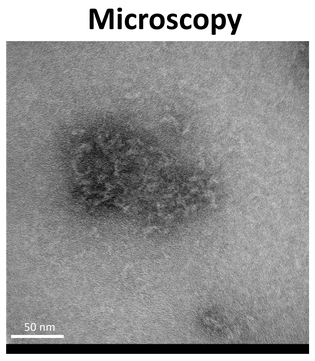
### Characterization of the Nanoparticles

#### Size evaluation

**Nanoparticles DLS**

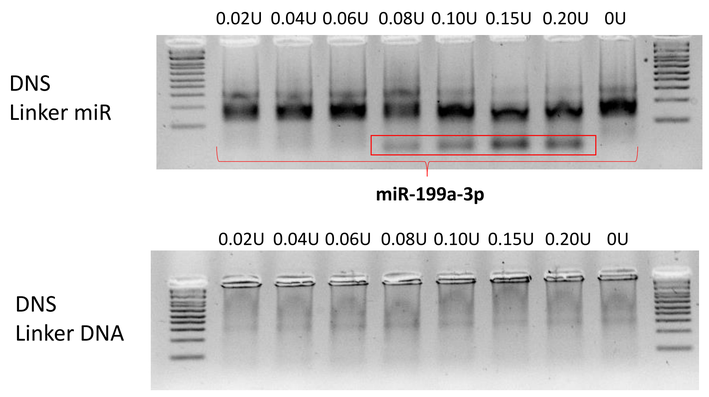
| Nanoparticle | Hydrodynamic diameter (nm) |
|---|---|
| RCA | 158 ± 60 |
| RCT | 327 ± 51 |
| DNS | 70 ± 20 |

**Transmision Electron Microscopy**



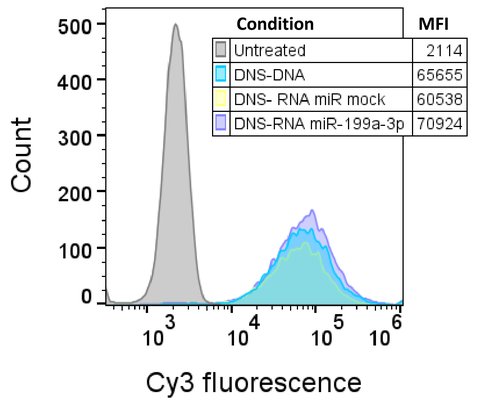

According to the DLS, the three types of nanoparticles assembled in structures of above 70 nm. Furthermore, the **DLS size data agree** with those obtained by **TEM** for the **DNS**.

### RNAse H *in vitro* degradation assay




**RNAse H** can **digest** the **DNA-RNA heteroduplexes** leading to the release of **miR-199a-3p**. No digestion is observed for the fully DNA-based DNS.

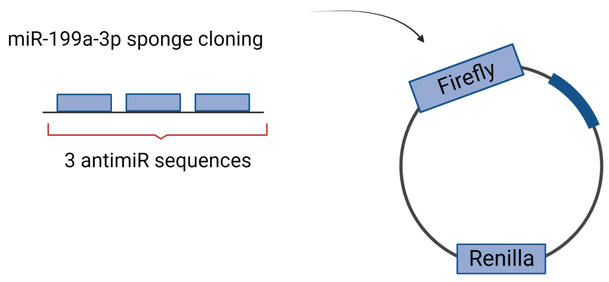
### Internalization



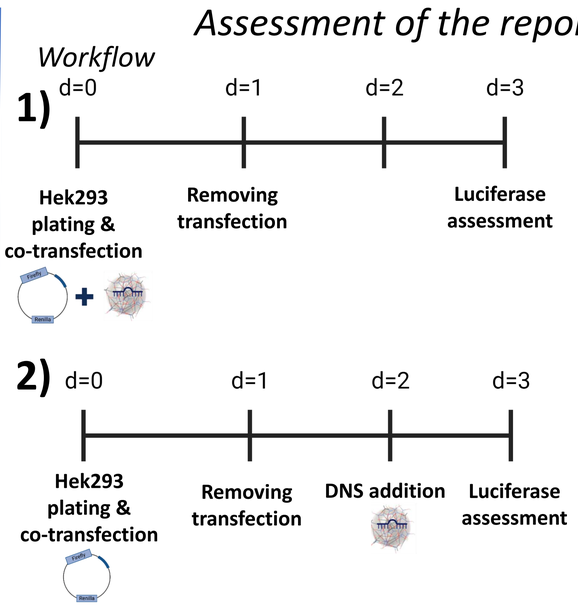

All the tested fluorescently-labelled DNS **internalize efficiently** in Hek293 at 24h.

### Functional Characterization

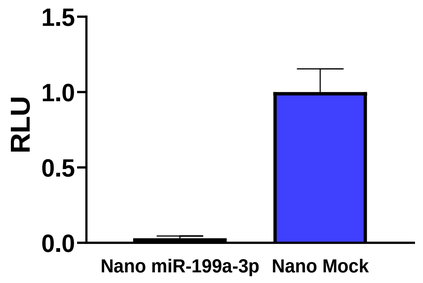
*Creation of a reporter system of miR-199a-3p activity*



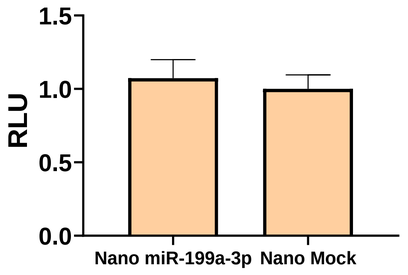

*Assessment of the reporter system in vitro (Luciferase Reporter Assay)*




**1)** Transfected nanoparticles



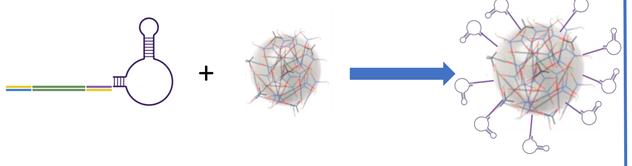

**2)** Non-transfected nanoparticles



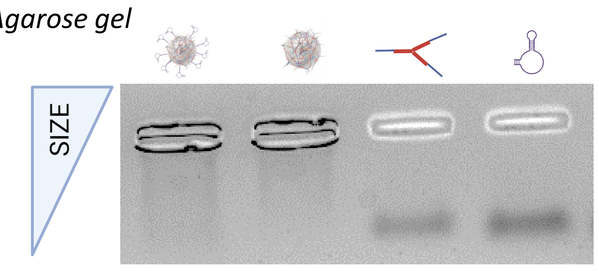

**DNS transfected** with lipofectamine are able to release **functional miR-199a-3p**, causing a decrease in the reporter signal, while **non-transfected DNS are not**, suggesting **stability issue** in serum.

### DNS aptamer functionalization

*Binding strategy*

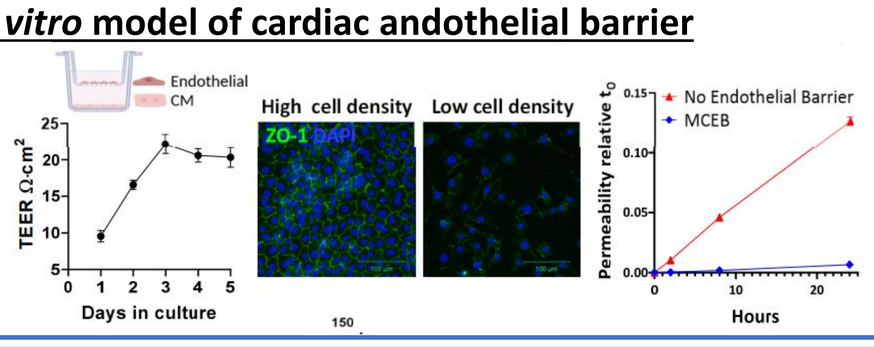


*Agarose gel*




The **DNA aptamers** correctly **integrate in the nanoparticle** by standard basepairing with the overhangs.

### *In vitro* model of cardiac andothelial barrier




## *Conclusions*

- **Three** types of **nanoparticles** capable of **loading miR-199a-3p** have been successfully developed and the *in vitro* **functionality of DNS** has been **verified** in a routine cellular model.
- A strategy for **attaching the aptamer** to the **nanoparticles** has been achieved, in addition to developing a **cardiac endothelial barrier model** that will allow **transcytosis studies** to be carried out in the future.

## *Future Work*

- Carry out **nanoparticle targeting** studies using **aptamers.**
- Assess nanoparticles activity in a model of **human heart cells.**
- **Stabilize the RNA component of the nanoparticles**

## *Acknowledgments*

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