Introduction

Cardiovascular diseases, including myocardial infarction, are leading causes of death globally. The development of cutting-edge DNA nanocarriers with cardioregenerative capacity using the therapeutic potential of microRNAs is a promising area of research.

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

1. **Characterization of the Nanoparticles**
   - Size evaluation
     - Nanoparticles DLS
       - Nanoparticle | Hydrodynamic diameter (nm)
       - RCA | 158 ± 60
       - RCT | 327 ± 51
       - DNS | 70 ± 20
     - Transmission 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.

2. **Functional Characterization**
   - Creation of a reporter system of miR-199a-3p activity
     - Workflow
       1) Hek293 plating & co-transfection
       2) Transfected nanoparticles

3. **Assessment of the reporter system in vitro (Luciferase Reporter Assay)**
   - 1) Transfected nanoparticles
     - Transfect DNA transfection
     - Luciferase assay
   - 2) Non-transfected nanoparticles
     - DNA transfected with lipofectamine is able to release functional miR-199a-3p, causing a decrease in the reporter signal, while non-transfected DNA is not, suggesting stability issue in serum.

4. **DNS aptamer functionalization**
   - Binding strategy
     - Agarose gel
   - The DNA aptamers correctly integrate in the nanoparticle by standard basepairing with the overhangs.

5. **In vitro model of cardiac and endothelial barrier**
   - No Endothelial Barrier
   - High cell density
   - Low cell density

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 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.

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