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

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Abstract Body

Introduction: MicroRNAs (miRNAs) regulate various physiological and pathological processes [1,2]. Thus, several therapeutic strategies based on viral vectors and non-viral transfection agents for miRNAs delivery have been developed. However, viral vectors face limitations due to safety issues and high costs, while commercial lipidic and polymeric transfection agents suffer from poor stability, incomplete loading efficiency, and uncontrolled release kinetics [3]. To address these challenges, novel polymer-lipid nanoparticles (H-NPs) were designed as innovative transfection agent for miRNA delivery.

Methods: H-NPs were prepared using a patented nanoprecipitation method with a lipoplex core capable of encapsulating miRNAs and a polymeric shell to enhance stability. Physicochemical properties of H-NPs were characterized. As a proof of concept for H-NPs, two complementary strategies were explored to promote cardiac regeneration following myocardial infarction: direct reprogramming of adult human cardiac fibroblasts (AHCFs) into induced cardiomyocytes (iCMs) using miRcombo (miR-1, 133, 208, 499) [4], and promoting cardiomyocytes (CMs) proliferation in H9c2 heart myoblasts with miR-199 [2]. H-NPs cytocompatibility, miRNA uptake, and transfection efficiency were compared with the commercial transfection agent Lipofectamine RNAiMAX (RNAiMAX) in AHCFs and H9c2.

Results: H-NPs showed nanometric size, negative Z-potential, excellent miRNA encapsulation efficiency (99 %), good colloidal stability and scalability. H-NPs demonstrated controlled miRNA release over 9 days, in contrast to RNAiMAX, which completely released within 72 h. AHCFs transfected with H-NPs had superior cell viability (100 % vs. 70 %) compared to RNAiMAX, while in H9c2 viability was comparable (100 %). AHCFs and H9c2 showed efficient uptake of H-NPs and RNAiMAX. AHCFs treated with miR-1 loaded H-NPs displayed significant downregulation of TWF-1 48 h post-transfection. Similarly, H-NPs loaded with miRcombo demonstrated an increased expression of cardiac troponin T, in AHCFs, at both gene and protein levels, 15 days post-transfection, suggesting successful direct reprogramming of fibroblasts into iCMs. In H9c2 cells, miR-199 loaded H-NPs induced the downregulation of HOMER-1 and HOPX 48 h post-transfection. Additionally, H-NPs were successfully freeze-dried with trehalose and stored for 14 days with no alterations in their physicochemical and biological properties.

Conclusion: H-NPs showcased an efficient transfection vector for *in vitro* miRNA delivery. Combining high miRNA loading efficiency of the lipidic core with controlled release and stability of the polymeric shell, H-NPs overcome key limitations of commercial agent. The obtained results highlight H-NPs versatility and potential for broader therapeutic and industrial applications.

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References:

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Presenter biography: Martina Coletto is a PhD student in Bioengineering and Medical-Surgical Science at Politecnico di Torino. She obtained a master's degree in Biomedical Engineering from the same institution. Her research focuses on cardiac tissue engineering, nanomedicine, and RNA-based therapy.

Learning Objectives

Understand the design and advantages of H-NPs as innovative transfection vectors for efficient miRNA delivery.

Explore the potential of H-NPs in promoting cardiac regeneration through fibroblast direct reprogramming and cardiomyocyte proliferation *in vitro*.