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Abstract

Cardiovascular diseases is one of the leading causes of death worldwide. Particularly, myocardial infarction (MI) causes the irreversible loss of cardiomyocytes and the formation of dysfunctional fibrotic scar tissue, leading to heart failure [1]. To date, heart transplantation is the only available therapy for end-stage heart failure. Hence, extensive research is in progress to develop novel strategies for post-MI cardiac regeneration. Among them, the administration of microRNAs (miRNAs) has attracted interest as a promising new strategy to modulate gene expression and to potentially induce cardiac regeneration. In our previous research, Paoletti *et al*. demonstrated that transient transfection with four microRNA mimics (termed "miRcombo") using a commercial lipid transfection agent (DF) triggers direct reprogramming of human adult cardiac fibroblasts (AHCF) into induced cardiomyocytes (iCMs) *in vitro* [1]. However, *in vivo* administration of naked miRNAs is hindered by their degradation and poor cell internalization. Hence, safe and efficient nanocarriers for direct reprogramming of AHCFs into iCMs are demanded.

In our work, we demonstrated the key role of safe and efficient delivery systems for miRcombo and biomimetic culture conditions in enhancing direct reprogramming efficiency of AHCFs into iCMs.

New lipoplexes (LP) were designed showing higher miRNA encapsulation efficiency (~99%) and biocompatibility, and similar cell transfection efficiency respect to DF-based lipoplexes. AHCF transfection with LP/miRcombo vs DF/miRcombo increased *in vitro* direct reprogramming efficiency of AHCFs into iCMs. However, the *in vitro* direct reprogramming efficiency of DF/miRcombo-transfected cells strongly increased when they were cultured in 3D biomimetic hydrogels based on fibrin/cardiac extracellular matrix produced *in vitro* by cells [2]. Finally, AHCFs transfection with LP/miRcombo and their culture in a biomimetic hydrogel further enhanced direct reprogramming efficiency of AHCFs.

In parallel, we designed an alginate-based injectable hydrogel with double crosslinked network for controlled *in situ* release of miRcombo-loaded nanocarriers during *in vivo* application. As alginate presents limitations such, as low degradability *in vivo* and limited cell adhesion, it was blended with alginate dialdehyde (ADA) and chemically-modified gelatin. Hydrogel composition was selected to ensure, injectability, cell adhesion, biomimetic stiffness and proper stability in physiological conditions. Modified LP/miRNAs nanocarriers were designed and patented keeping the same miRNA loading efficiency and biocompatibility as LP/miRNA nanocarriers but showing superior stability and easy surface functionalization with ligands for receptor-mediated cell targeted release, as well as the ability to be encapsulated and released from the injectable hydrogel. *In vitro* direct reprogramming experiments from release media and *in vivo* tests in mouse model are in progress

References

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