

Advanced hybrid nanoparticles for targeted microRNA-mediated cardiac reprogramming

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Heart failure remains one of the leading causes of morbidity and mortality worldwide, largely due to the irreversible loss of cardiomyocytes (CMs) and the formation of fibrotic scar tissue following myocardial infarction [1]. In recent years, microRNAs (miRNAs) have emerged as powerful regulators of gene expression with significant potential in cardiac regeneration. Specifically, a combination of miR-1, miR-133, miR-208, and miR-499 (known as miRcombo), has been shown to directly reprogram cardiac fibroblasts (CFs) into induced CM-like cells (iCMs). However, the clinical translation of miRNA-based therapies remains limited by the lack of safe and efficient delivery systems capable of targeting specific cell populations within the injured heart. To address this challenge, we designed novel delivery systems based on patented hybrid nanoparticles (NPs), produced via a nanoprecipitation method. These NPs consist of a lipoplex core for highly efficient miRNA encapsulation and a polymeric shell that enhances colloidal stability [2]. Surface functionalization via copper-catalyzed click chemistry enabled the conjugation of CF-targeting ligands, generating functionalized nanoparticles (F-NPs) for precise delivery. NPs and F-NPs were characterized in terms of physicochemical properties, loading efficiency, cytocompatibility, cellular uptake, and transfection performance in vitro, particularly using adult human cardiac fibroblasts (AHCfs). NPs showed a size of around 150-200 nm, negative Z-potential, excellent miRNA encapsulation efficiency, and sustained miRNA release up to 9 days in vitro. AHCfs transfected with NPs and F-NPs maintained excellent cell viability, with F-NPs showing markedly enhanced uptake compared to non-targeted NPs. The miRcombo-loaded F-NPs significantly increased the expression of cardiogenic markers at gene and protein levels. Early in vivo tests in mouse model confirmed safety and efficacy. Injectable self-healing natural polymer hydrogels have also been developed to further improve local retention of F-NPs and minimize any off-target effects. By integrating the high miRNA loading capacity of the lipid core with the enhanced stability and controlled release of the polymeric shell, and cell-specific targeting of the surface ligand, F-NPs showed promising results in addressing the key limitations of conventional delivery systems.

Keywords: Direct cardiac reprogramming, miRcombo, antibody functionalized nanoparticles, myocardial infarction

References: [1] "MicroRNA-Mediated Direct Reprogramming of Human Adult Fibroblasts Toward Cardiac Phenotype", C., Paoletti et al., *Frontiers in Bioengineering and Biotechnology*. 2020; 8; 529 [2] "Design and Validation of Hybrid Polymer-Lipid Nanoparticles as Novel Transfection Vectors for MicroRNA Delivery to Human Cardiac Fibroblasts", L., Nicoletti, C., Paoletti et al., *Advanced Healthcare Materials*. 2025; 14.18, 2500971

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Matrix Vesicles as a Potent Therapeutic for Hard Tissue Regeneration

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Extracellular vesicles (EVs) are biologically complex nanoparticles with prospective applications in regenerative medicine. EV preparations are often highly heterogeneous with no clear mechanism of action (MoA), limiting their clinical translation. Hard tissues such as bone contain a specialised subpopulation of matrix bound vesicles (MVs) that have well described functions in early tissue mineralisation events. We sought to define the composition and therapeutic potential of MVs for regenerative applications in bone. Primary MSCs were cultured in osteogenic medium. MVs and mEVs were isolated from the ECM and conditioned medium respectively. Vesicle preparations were orthogonally analysed using nanoparticle tracking analysis (NTA), nano-flow cytometry (NanoFCM), western blotting (WB), transmission electron microscopy (TEM) and proteomic analysis. Enzymes and channelling proteins associated with mineralisation were compared and their expression at the single vesicle scale confirmed using dSTORM imaging. Alkaline phosphatase (TNAP) concentration and