Design and validation of a hydrogel-based advanced delivery system for cardiac regeneration after myocardial infarction

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Abstract

Cardiac regeneration after myocardial infarction (MI) remains a global clinical challenge due to heart limited regenerative capacity. Non-viral direct reprogramming of cardiac fibroblasts (CFs) into induced cardiomyocytes (iCMs) triggered by four reprogramming microRNAs (miRcombo) has emerged as a promising strategy. We recently developed novel polymer-lipid hybrid nanoparticles (NPs) with a lipoplex core for efficient miRNA encapsulation and a stabilizing polymeric shell, functionalized with a CF-specific antibody via click chemistry (F-NPs) for targeted delivery. In this work, to improve NPs *in situ* retention, an injectable bioactive hydrogel was developed based on Shiff base reaction between alginate dialdhehyde (ADA) and gelatin modified with carbohydrazide (GEL-C). The approach was validated using a human cardiac scar-on-a-chip model simulating hydrogel–tissue interaction. Co-delivery of human amniotic fluid stem cell-derived extracellular vesicles (hAFS-EVs) is under investigation to enhance reprogramming efficiency.

Hydrogels with ADA/GelC 30/70 w/w exhibited optimal rheological properties (G'~1.5 kPa), self-healing capabilities, and high *in vitro* stability up to 21 days. ADA/GelC with embedded negmiR-loaded NPs showed sustained and controlled miRNA delivery up to 15 days. F-NPs loaded with model miR-1, released from the hydrogel, showed higher transfection efficiency than unfunctionalized NPs, as assessed by TWF-1 downregulation in adult human CFs, both in 2D cell tests and using a human cardiac scar on a chip model (in both static and dynamic conditions). Finally, direct reprogramming efficiency of F-NPs/miRcombo, released from the hydrogel, was demonstrated *in vitro*. Preliminary findings suggest hAFS-EVs have antifibrotic effects and their release from ADA/Gel-C hydrogel is controlled within 7 days. The synergistic delivery of F-NPs/miRcombo and hAFS-EVs from hydrogel is under investigation in 2D and 3D human scar models.

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