Biomimetic scaffolds and multifunctional materials in the regeneration of pathological cardiac tissue

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Keywords: biomimetic, cardiac, direct reprogramming*, in vitro* models, 3D scaffolds

Myocardial infarction causes the irreversible loss of billions of cardiomyocytes, followed by myocardial fibrosis and cardiac dysfunction [1]. The complete recovery of heart functionality is currently not possible and heart transplantation remains the only available treatment. For this reason, many advanced therapies are under investigation to regenerate myocardial tissue, including cell and gene therapies and tissue engineering approaches [1]. Among them, direct reprogramming of cardiac fibroblasts into induced cardiomyocytes (iCMs) deserves attention as it could potentially restore cardiac functionality. Previous publications have outlined the possibility to use combinations of transcription factors, small molecules and microRNAs (miRNAs) to the purpose [1]. MiRNAs are particularly advantageous as they offer the possibility to be efficiently administered by non-viral vectors, such as polymer or lipid nanoparticles, increasing patients' safety [2].
The aim of the work was the development of an *in situ* direct reprogramming approach for human adult cardiac fibroblasts using a combination of miRNAs called miRcombo (miR-1, miR-133, miR-209, miR-499) [3,4].
MiRcombo was demonstrated for the first time to drive the transdifferentiation of adult human cardiac fibroblasts (AHCFs) into iCMs [5]. After 7 days from transfection, AHCFs upregulated early cardiac transcription factors such as GATA4, MEF2c, TBX5, HAND2 and NKX2.5, compared to NegmiR transfected cells. After 15 days culture time, around 11% of miRcombo transfected cells expressed cardiac troponin T (cTnT), while immunofluorescence analysis showed the expression of both cTnT and α-sarcomeric actinin only in miRcombo-treated cells. After 30 days culture time, around 38% of reprogrammed cells showed calcium transients while rare or no calcium oscillation was observed in NegmiR transfected cells. AHCF direct reprogramming efficiency was higher in 3D biomimetic microenvironments than in 2D culture plates, suggesting the need for testing in predictive 3D *in vitro* models of human cardiac tissue. Pathological cardiac tissue is characterized by high variability, in terms of size, location and progression degree (maturation) after heart attack. Hence, a versatile scaffold platform for the modelling of pathological cardiac tissues with various characteristics is highly demanded. To the purpose, 2D and 3D "bioartificial" scaffolds were produced by electrospinning and melt-extrusion additive manufacturing, based on polycaprolactone (PCL), a well-known biocompatible synthetic polymer, surface functionalized with gelatin (G). 2D and 3D bioartificial scaffolds supported long-term AHCF culture, while their structure affected cell adhesion, fibrotic marker expression and cardiac protein deposition. As a conclusion miRcombo was demonstrated to directly reprogram AHCFs into iCMs while a versatile platform of scaffolds prepared from commercially available materials was validated for the modelling of human pathological cardiac tissue with different sizes and severity degrees. This work is supported by BIORECAR ERC project (contract number 772168).

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