Direct reprogramming of human fibroblasts as a novel tool in cardiac tissue engineering

Direct reprogramming of human cardiac fibroblasts to cardiomyocytes using microRNA mimics

C. Paoletti[1], C. Divieto[2], F. Di Meglio[3], D. Nurzynska[3], V. Chiono[,1]
Presenting Author: Camilla Paoletti, camilla.paoletti@polito.it
[1]Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy [2]INRIM, Metrology for Life Quality, Turin, Italy [3]Department of Public Health, University of Naples "Federico II", Naples, Italy

INTRODUCTION: The combination of four different microRNAs (miR-1, 133, 208 and 499), named "miRcombo", has been used for the direct reprogramming of murine fibroblasts into cardiomyocytes (CMs) for myocardial infarction (MI) treatments.[1,2] Here, we evaluated miRcombo mediated reprogramming of human adult cardiac fibroblasts (AHCFs) into CMs in 2D and 3D culture.

METHODS: For digital droplet PCR (ddPCR) analysis, $3x10^5$ AHCFs were plated in 6-well plates, for Immunocytochemistry (ICC) $5x10^4$ cells were plated in 24-well plates. AHCFs were transfected with miRcombo (mirVana) using DharmaFECT1 (Dharmacon). Untrasfected and NegmiR (mirVana) transfected AHCFs were used as controls. After 24 hours, medium was changed to medium with 1 μM Jak1 Inhibitor for 4 days for 2D experiments; for 3D experiments, cells were cultured in fibrin-based hydrogels.

RESULTS & DISCUSSION: ddPCR analysis showed significant increase expression of early cardiac transcription factors (TFs) Hand2 and Mef2c ($p < 0.005$) slight increase expression of Tbx5 and Nkx2.5, although non-significant ($p > 0.05$), and reduced Vimentin expression ($p < 0.05$) in miRcombo-transfected AHCFs compared to controls after 4 days in 2D culture. ICC analysis showed increased expression of late cardiac markers α-sarcomeric actinin and cTnT in miRcombo-transfected AHCFs after 10 and 20 days of culture in 2D. However, ddPCR showed no significant differences of late cardiac markers Myh6 and cTnI expression between the groups after 15 days in 2D culture. On the other hand, cells cultured in 3D fibrin-based hydrogels showed enhanced cardiac TFs expression compared to 2D. However, miRcombo transfection did not significantly enhance cardiac gene expression in AHCFs cultured in 3D hydrogels respect to controls after 4 days. After 15 days, AHCFs cultured in 3D hydrogels showed a strongly enhanced expression of cardiac genes such as cTnI and Myh6 compared to 2D.

CONCLUSIONS: Together, these results showed that a 3D environment was found to play a key role in enhancing direct reprogramming of AHCFs into CMs.

ACKNOWLEDGEMENTS: This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 772168; BIORECAR project).

REFERENCES

[1] Jayawardena TM et al. Circ Res. **2013**, 110, 1465–1473
[2] Li Y et al. Sci. Rep. **2016**, 6, 1–11