INFLUENCE OF 3D CULTURE MICROENVIRONMENT ON MICRORNA-MEDIATED DIRECT REPROGRAMMING OF HUMAN CARDIAC FIBROBLASTS INTO CARDIOMYOCYTES

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Introduction & Aim of the work

Myocardial infarction (MI) remains one of the leading causes of death worldwide. MI results in the loss of up to 1 billion of cardiomyocytes (CMs) and the formation of a fibrotic scar, mainly populated by cardiac fibroblasts, which impair normal cardiac function [1]. Direct reprogramming of human cardiac fibroblasts into CMs may hold a great potential for this purpose. Recently, direct reprogramming of murine fibroblasts into induced cardiomyocytes (iCMs) using the combination of four different microRNAs (miR-1, 133, 208 and 499), named "miRcombo", has been demonstrated in 2D monolayer culture. [2]. However, aiming at mimicking the physiological microenvironment, a more complex culture method is required in order to increase the reprogramming efficiency and maturation of iCMs *in vitro.* For this reason, 3D hydrogel culture have been shown to better mimic the native cardiac tissue environment compared to classic tissue culture plate (TCP)[3].

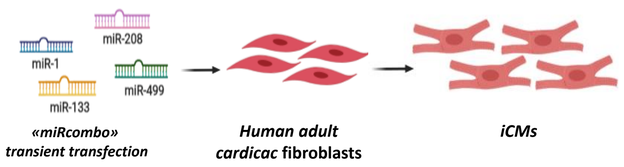
In this work, we investigated:

- ✓ miRcombo-mediated reprogramming oh human cardiac fibroblasts into iCMs in classic monolayer culture
- ✓ A 2D and 3D fibrin-based hydrogel role in enhancing the reprogramming efficiency of fibroblasts into iCMs

Material and Methods

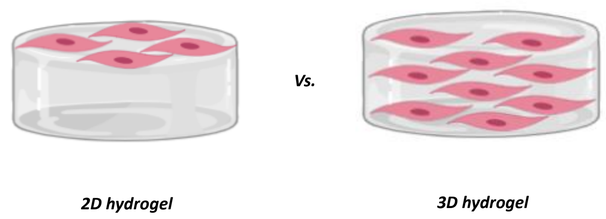
Cell culture and transfection

AHCFs (Lonza, CC-2903) were transfected with miR-1, 133, 208 and 499 (mirVana) using DharmaFECT1 (Dharmacon). NegmiR (mirVana) transfected AHCFs were used as control. After 24 hours, medium was changed to complete medium.



Fibroblast culture in fibrin-based hydrogels

After transfection, cells were detached by trypsin and cultured on the surface (2D) or embedded into 3D fibrin based hydrogels composed of 5 mg/mL fibrinogen (Merck) and 50 u/mL trombin (Merck).



Digital Droplet PCR (ddPCR)

Digital Droplet PCR was performed according to manufacturer's instructions (BIO-RAD). Results are reported as number of cDNA copies/µL of the gene of interest normalized on GAPDH number of cDNA copies/µL.

Calcium Analysis

Cells were loaded with in 5 µM Fluo-4 AM (Invitrogen, codice) at 37 °C for 15 and 30 minutes in modified Tyrode solution (140 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2 and 10mM glucose at pH 7.4) and analysed using Spinning disk microscope (Nikon)

Flow Cytometry

Cell were trypsinized and incubated with Cardiac Troponin T (Abcam) and Alexa-488 (Abcam) antibodies.

Results and Future Work

AHCF reprogramming into iCMs on monolayer culture

We first evaluated if the combination of four microRNAs was able to induce the expression of cardiomyocyte markers in AHCFs cultured in TCP. miRcombo-transfected AHCFs showed increased expression of cardiac transcription factors (TFs) after 7 days **(Fig. 1A)** and higher TnnT2 expression after 15 days of culture compared to negmiR controls **(Fig.2A)**.

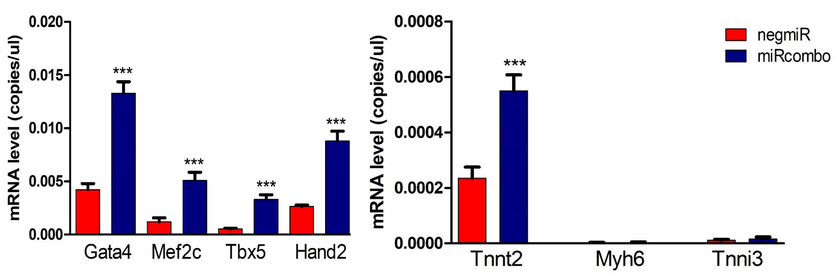


Fig.1A

Fig.2A

Moreover, we evaluated reprograming efficiency using flow cytometry. Interestingly, after 15 days of culture, miRcombo-transfected cells exhibited nearly 11% of cTnT positive cells, whereas no positive cells were found in negmiR controls **(Fig. 3A)**. After 4 weeks of culture, miRcombo transfected cells also showed calcium transient activity, while no calcium activity was found in negmiR controls **(Fig.4A)**

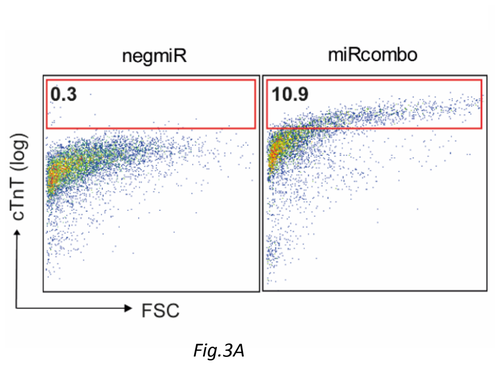


Fig.3A

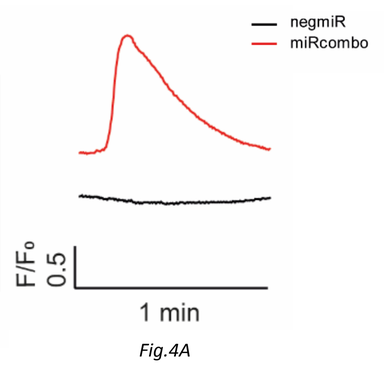


Fig.4A

2D vs. 3D culture systems

Then, we explored the effects of a fibrin 3D culture environment in enhancing miRcombo-mediated reprogramming of AHCFs after 15 days of culture. To elucidate the role of fibrin hydrogel in cell reprogramming, cells were cultured on the surface of fibrin hydrogel (2D) or embedded into 3D fibrin hydrogel (3D). After 2 weeks, 3D culture environment significantly enhanced mRNA levels of Tnnt2 **(Fig.1B)**, Tnni3 **(Fig. 2B)** and Myh6 **(Fig.3B)** cardiac markers in both miR combo and negmiR transfected AHCFs compared to 2D hydrogels.

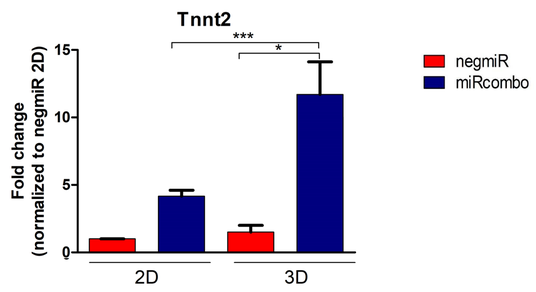


Fig.1B

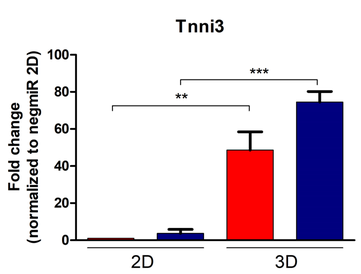


Fig.2B

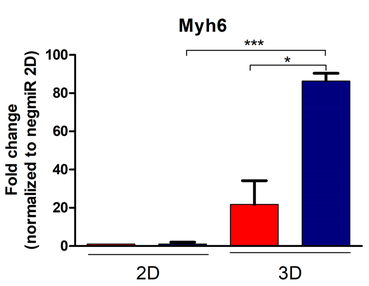


Fig.3B

Future Developments

Future developments will focus on understanding miRcombo-mediated reprogramming in 3D fibrin based hydrogel.

Moreover, in order to recreate a 3D structure that resemble the physiological microenvironment, we will introduce cardiac ECM proteins produced *in vitro* by cardiac fibroblasts.

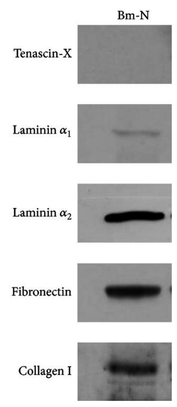
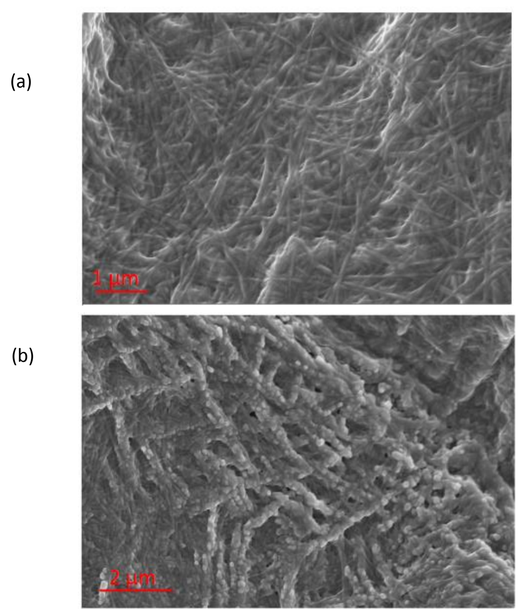


Image adapted from Castaldo et al [4]



SEM images of a 3D fibrin hydrogel (a) and fibrin hydrogel blended with Biomatrix (b)

Conclusions

- ✓ miRcombo transfection triggers the transion of AHCFs towards iCMs
- ✓ a 3D fibrin hydrogel was able to enhance miRcombo-mediated reprogramming of AHCFs into induced CMs. Further investigations are in progress.

References

[1] Paoletti C et al. Cells 2018, 7, 114
[2] Jayawardena TM et al. Circ Res. 2013, 110, 1465
[3] Li Y et al. Sci. Rep. 2016, 6, 1
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Acknowledgements

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