Revolutionizing Cardiac Therapy: 3FEEP - A Nanofunctionalized Scaffold for Post-Myocardial Infarction Tissue Protection and Regeneration

D. Rossin[1], R. Vanni[1], S. Perveen[1], N. Barbani[2], R.A. Pires[3,4], R.L. Reis[3,4], R. Rastaldo[1], C. Cristallini[2], C. Giachino[1]

[1] *Department of Clinical & Biological Sciences, University of Turin, Italy*
[2] *CNR-IPCF, Institute for Chemical and Physical Processes, Pisa, Italy*
[3] *3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, 4805-017 Barco, Portugal*
[4] *ICVS/3B's–PT Government Associate Laboratory, Braga/Guimarães, Portugal*

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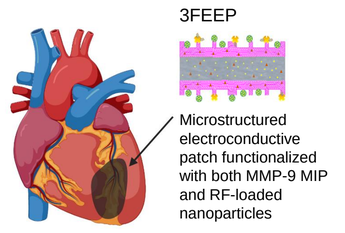
Introduction

Background - Cardiac tissue engineering is a cutting-edge approach with high potential for treating cardiovascular diseases, the leading global cause of death. Advanced nanofunctionalized cardiac scaffolds hold promise for repairing and protecting damaged heart tissue, primarily from ischemic injuries.
In this context, our patented 3D nanoFunctionalized and Engineered Electroconductive Patch (3FEEP) has been developed as a result of the European research project INCIPIT. Thank to the functionalizations with nanoparticles (NPs) loaded with recruitment factor (RF) and with hMMP-9 molecularly imprinted nanoparticles, this innovative scaffold aims to promote tissue regeneration after myocardial infarction (MI) by attracting stem cells, regulating ventricular remodeling and facilitating cardiac cell electrical connectivity.

Aims – Validate the biocompatibility of 3FEEP and evaluate its efficacy in restoring cardiac functionality following myocardial infarction (MI). Given the limited regenerative capacity of myocardial tissue, central goal is to attract cells capable of regenerating the affected cardiac region. To address this objective, migration assays were conducted to demonstrate 3FEEP's capacity to attract mesenchymal stem cells (MSCs) and non-myocyte cardiac cells (NMCs). Additionally, gene expression analysis was employed to elucidate the cardioinductive effects of 3FEEP on cardiac stem cells. Furthermore, in vivo studies were conducted to confirm 3FEEP's ability to recruit cells to the damaged cardiac tissue and promote the formation of new blood vessels.

Materials and Methods

NMCs were cultured in α-MEM supplemented with 10% or 0.5% FBS. H9C2 cell line and hMSC primary cells were cultured in low glucose DMEM supplemented with 10% FBS. Cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) were cultured in RPMI with specific supplementation. Fibronectin coating was used to favour iPSC-CMs adhesion. Citocompatibility of the scaffolds was verified with Propidium Iodide Flow Cytometry assays. NMCs and hMSCs migration ability was assessed through transwell migration assays. Morphology of iPSC-CMs cultured on 3FEEP was observed using Calcein-AM live staining. Expression levels of target genes were analyzed with Real-Time PCR to test scaffold cardioinductive potential on iPSC-CMs. Statistical analyses were performed using the paI/Red Student's t-test and differences with $p < 0.05$ were considered as statistically significant.
An in vivo study was conducted in four groups of rats: SHAM, SHAM + 3FEEP, Ischemia/reperfusion (I/R), I/R + 3FEEP. Animals were sacrified after 4 weeks from patch implantation. Explanted hearts were cut in several 5 μm sections. For immunofluorescence analyses, tissue sections were incubated incubated O/N at 4°C with primary antibodies, 2 hours at RT with secondary antibodies and 30 minutes at RT with nuclear dye. Samples were stored at 4°C in the dark and imaged using a laser scanning confocal microscope.



Results

This study unmasked the potential of scaffold to serve as a strategy to promote tissue regeneration in the ischemic damaged heart.
Cytocompatibility evaluation of scaffolds with H9C2 cells and MSCs did not point out any significant toxicity compared to Control or 3FEEP's previous version, an electroconductive patch not functionalized with NPs (**Fig. 1**). Transwell migration analyses highlighted the significant capability of 3FEEPs functionalized with NPs to attract both NMCs and MSCs (**Fig. 2**). Morphological analyses carried out on 3FEEP showed the ability of the patch to promote adhesion and alignment of iPSC-CMs (**Fig.3**). Cardioinductivity evaluation indicated the ability of the scaffolds to direct iPSC-CMs towards a cardiomyocytic lineage by activating specific cardiac genes (**Fig. 4**). Lastly, the *in vivo* study on rats and immunofluorescences analyses on the explanted hearts confirmed the patch ability to partially restore the myocardial tissue after MI (**Fig. 5 and 6**).

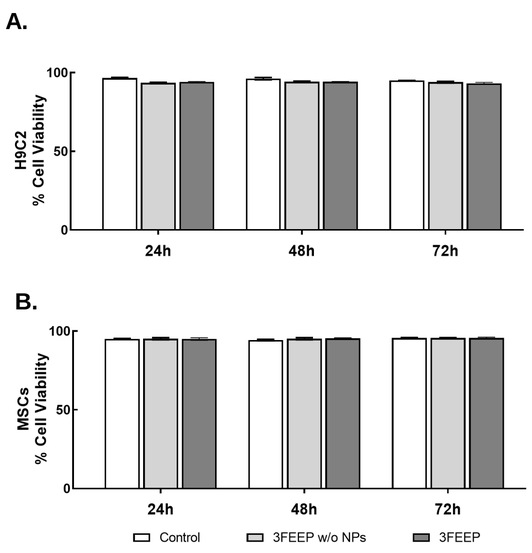
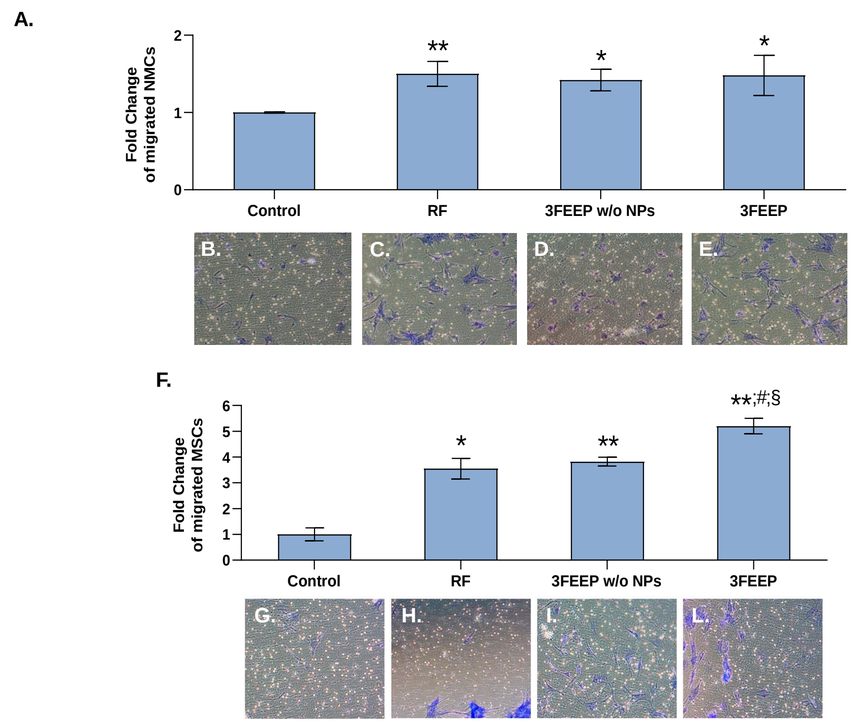


Fig. 1: Biocompatibility analyses
Propidium iodide flow cytometry analyses to evaluate cytocompatibility of the 3FEEP w/o Nanoparticles (NPs) and 3FEEP with H9C2 (**A**) and MSCs (**B**).



*p<0.05, **p<0.01 vs Control; # p<0.05 vs 3FEEP w/o NPs ; § p<0.05 vs RF

Fig. 2: Transwell migration assay
The graph shows the fold change of NMCs migration (**A**). Images show the migration of NMCs, stained with 0.2% Cristal Violet. Negative control condition (**B**), RF (**C**), 3FEEP w/o nanoparticles (NPs) (**D**), and 3FEEP (**E**). Graph **F** shows fold change of MSC migration. Images show MSC migration. Negative control (**G**), RF (**H**), the 3FEEP w/o NPs (**I**), 3FEEP (**L**).

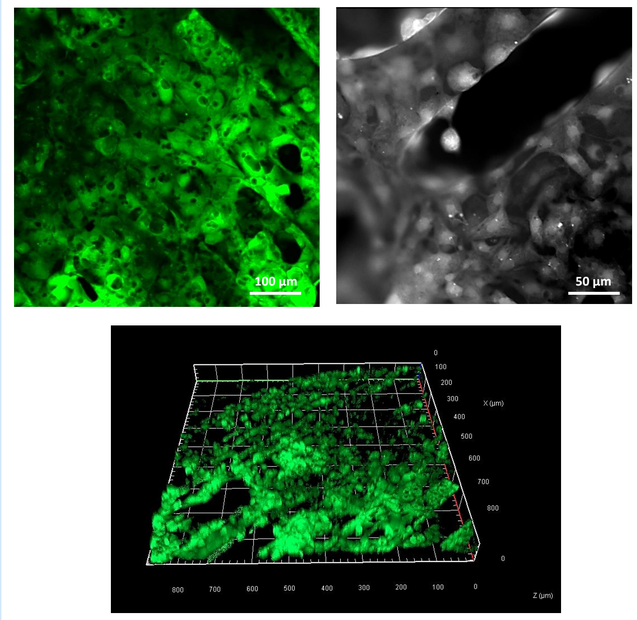
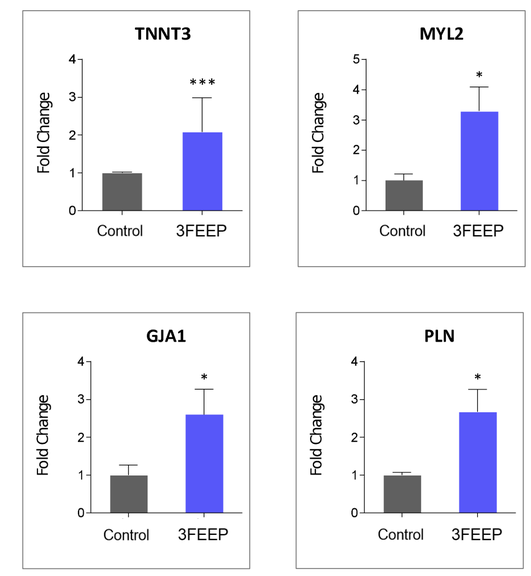


Fig. 3: : **Cell growth and alignement**
Morphology of IPSC-CMs cultured on 3FEEP. Calcein-AM live staining of cells cultured on Fibronectin-coated 3FEEP for 24 hours.



*p < 0.05; ***p < 0.001

Fig. 4: Cardioinductivity evaluation
Evaluation of gene expression levels of cardiac differentiation markers in human iPSC-CMs cultured on 3FEEP for 7 days. The expression of the same genes in 2-D control cultures was used for normalization. The graphs show fold changes related to the RNA expression levels calculated using the $2^{-\Delta\Delta Cq}$ method.

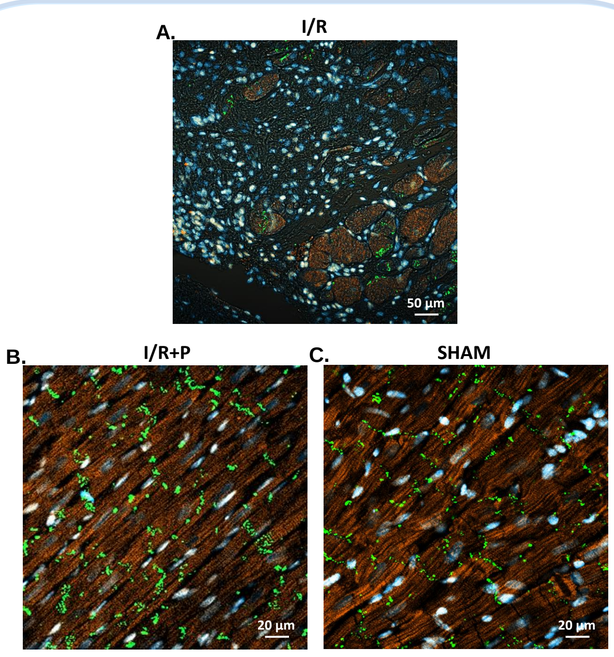


Fig. 5: *In vivo* study in rats: myocardial tissue regeneration
In vivo immunofluorescence images of Cx43 (green), TnC (orange), and nuclei (light blue) of cardiac tissue sections of rat undergone to ischemia-reperfusion (I/R) (**A**), 3FEEP application on the infarcted region for one month (**B**) and control group (sham group) (**C**).

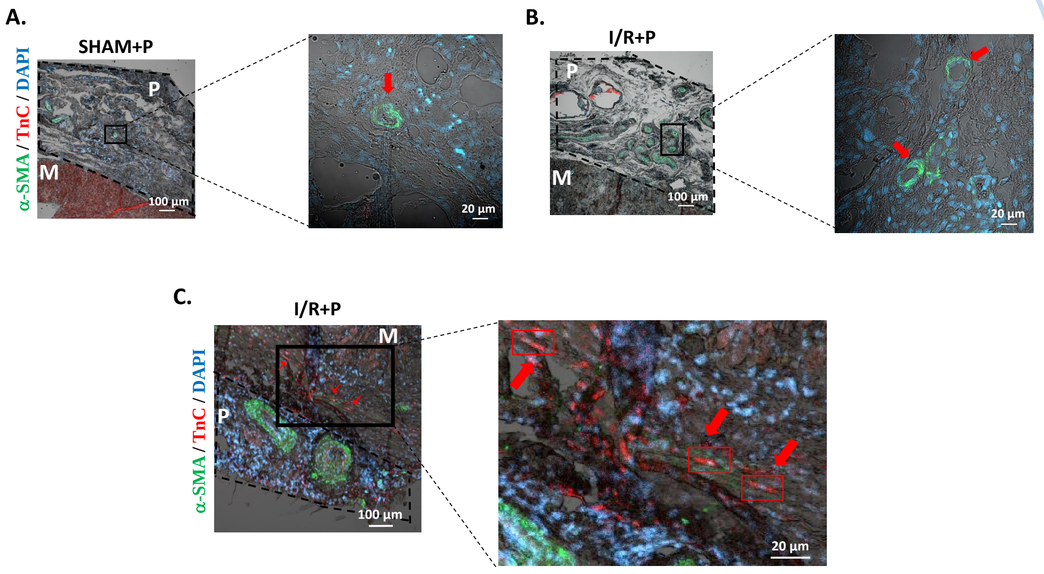


Fig. 6: *In vivo* study in rats: neovessel formation and cell recruitment
In vivo immunofluorescence images of α-SMA (green), TnC (red), and nuclei (light blue) of rat cardiac tissue sections. The 3FEEP application promoted the neovessel formation within the patch in both Sham+P (**A**) and I/R+P groups (**B**). A recruitment of TnC positive cells (indicated by red arrows) was also observed in myocardial region under the patch (**C**).

Conclusions

Overall, these results reinforce the hypothesis of 3FEEP patches as a promising approach to promote myocardial regeneration after MI, providing knowledge to support and encourage future studies in large *in vivo* animal models and possible subsequent translation to clinical trials. Currently more experiments are ongoing to reinforce the already obtained data.