3D Cardiac Tissue Engineering for Embryonic Stem Cells Therapy after Myocardial Infarction: Preliminary Results.

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Introduction To study using MRI the feasibility of 3D-cardiac tissue engineering for the cell therapy of heart failure in rats.

Material and Methods 3D-cardiac tissue [1] consisted in fibrin matrix incorporating 300000 iron oxide particles magnetofected Embryonic Stem Cells -derived cardiomyocytes (FeESC). 16 rats weighted 200-300g, have been included in this study. Under general anesthesia, chest of the animal was opened: myocardial infarct was induced by left coronary occlusion and then 3D-cardiac tissue was engrafted with fibrin glue, on the heart in place of the suspected infarcted area. 4 conditions (N = 4 rats / conditions) were studied: 1) Normal heart with fibrin matrix containing only iron oxide particles (NFe), 2) Normal heart with 3D-cardiac tissue (NFeESC), 3) Infarcted heart with fibrin matrix containing only iron oxide particles (MIFe), and 4) Infarcted heart with 3D-cardiac tissue (MIFeESC). Each rats were imaged under 1.5T MR magnet (Intera, Philips) 6hours after surgical intervention, 3 days (D3), 7 days (D7) and 45 days (D45) later. The 2hours/animal MR protocol consisted in 12slices FFE sequence (TE/TR/FA= 7ms /350ms /50°, acquired pixel size= 0.2 x 0.3 mm^2, slice thickness= 2mm), SE sequence (TE/TR/FA= 22ms / 375ms/ 90°, acquired pixel size= 0.2 x 0.3 mm^2, slice thickness= 2mm) for iron oxide particles repairing; a C-SPAMM TAG preparation segmented cine FFE sequence (interTAG spacing= 1.25mm, acquired pixel size= 0.6x1.8 mm^2, slice thickness= 3mm) was also used for quantitative regional function study. After 0.6mL injection of contrast agent (Dotarem, Guerbet), a 7 slices FFE cine [2] sequence (acquired pixel size= 0.4 x 0.4 mm^2, slice thickness= 2mm) and with same geometry a delayed enhancement inversion recovery T1 weighted FFE sequence (TE/TR/TI/FA=7.6ms /12ms /300ms /45°, acquired pixel size= 0.3 x 0.3 mm^2, slice thickness= 2mm) were acquired for Left ventricular Ejection Fraction (EF), End-systolic volume (ESV) and End-diastolic volume (EDV) and Infarct zone evaluation. 50 days after engraftment, animals were sacrificed and heart taken out for histological process (24h 10% formalin fixation, embedment in paraffin wax, 3 □m slices). All slices were stained with Anti MEF2 (Santa Cruz, SC-313) for assessment of cardiomyocites presence in areas presenting iron particles (Prussian blue staining). Statistical relationship between animals were performed over time and conditions for EF, ESV, EDV and Infarct zone with a Bonferroni and Dunnett ANOVA test (SPSS software). Measures of wall thickening (WT) and circumferential constrains were performed in 6 sectors and compared for the infarct area for MIFe and MIFeESC.

Results At D3, no significant difference of the infarct size was observed between non treated (MIFe) and ESC treated (MIFeESC) groups. Infarct size remained stable along time as assessed by delayed enhancement MRI from 3 to 45 days after engraftment for these both groups. 3D-cardiac tissue clearly visible as an hypointense signal due to iron particles: for MIFeESC group, the hypointensity decreased in size but was still visible at 45 days after engraftment (Figure 1). EF, ESV and EDV of MIFe and MIFeESC groups presented same time evolution with decrease in EF, increase of ESV and increase EDV. Immuno-histology confirmed the migration of ESC out of the 3D fibrin matrix and the presence of the Prussian blue stained cardiomyocytes in the infarct zone suggesting an efficient homing of the stem cells (Figure3). Significant conservation of the cardiac function was observed in the MIFeESC group by comparison to the non treated group MIFe (p=0.02, Figure2).

Conclusion In this study, we demonstrated the feasibility and the potential benefice of the 3D-cardiac tissue engineering for the cell therapy using embryonic stem cells after myocardial infarct. Further experiments are underway to confirm the embryonic origin of the iron loaded cardiomyocytes in the infarct area..

References [1] Zammaretti P., Jaconi M. Curr Opin Biotechnol. 2004 Oct;15(5):430-4. [2] Montet-Abou K. et al. MAGMA. 2006 Aug;19(3):144-51.

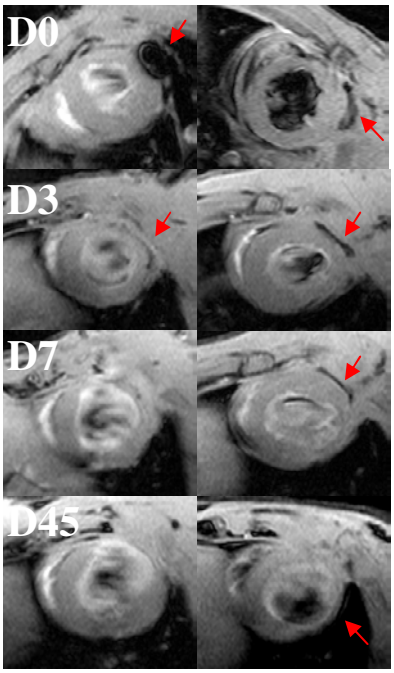

Figure 1: Typical anatomical MR images obtained for 2 animals respectively MIFe (Left) and MIFeESC (Right) for iron oxide particles repairing. Iron loaded fibrin matrix appeared as a hyposignal (Red arrows). For MIFeESC, hyposignal of 3D cardiac patch persisted 45 days after its engraftment, indicating ESC integration.

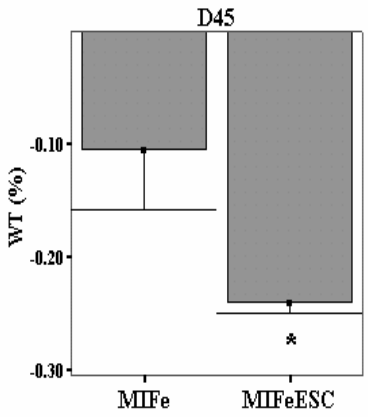

Figure 2: Wall thickening (in %) measured in sector with infarcted area for the non treated (MIFe) and the FeESC treated groups at D45. (* stands for significative difference: p=0.02)

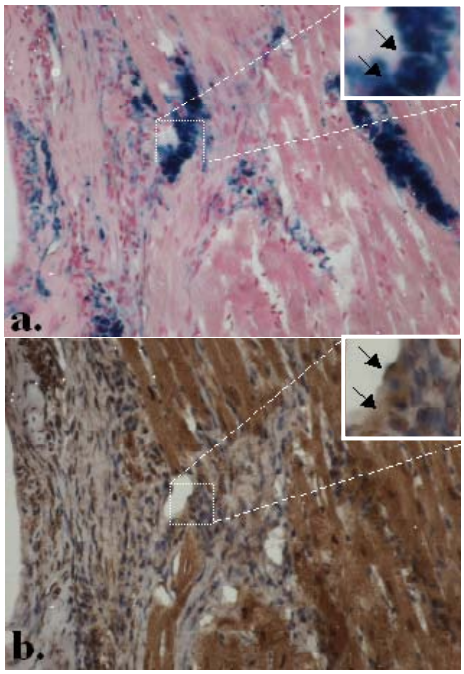

Figure 3: Histological view of myocardial muscle treated with 3D-cardiac tissue. **a.** Presence of iron particles is stained by Prussian Blue coloration. Nucleus appeared in pink (Red nuclear staining). **b.** Anti MEF 2 staining (brown coloration) assessed presence of cardiomyocites in the iron particle stained area.