Embryonic cardiomyocytes improve contractility and viability of ischemic myocardium

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Introduction: Chronic cardiac ischemia induces extensive necrosis of non-proliferative cardiomyocytes, which is accompanied by an irreversible loss of rhythmic contractile abilities. Intramyocardial transplantation of embryonic cardiomyocytes (eCMs) has led to electrical coupling of transplanted cells to native myocardium, which enabled eCMs to respond to cardiac action potentials [1]. Therefore, eCMs might actively contribute to cardiac contraction, which is not expected from cell types as skeletal myoblasts (SMs) and mesenchymal stem cells (MSCs). SMs and MSCs lack the intrinsic ability of electrical coupling and also did not differentiate into cardiomyocytes in previous studies [1,2].
The **aim** of this work was to establish with *in vivo* cardiac MRI whether eCM transplantation can improve global cardiac performance, reduce infarct size and also recover local contractility in infarcted myocardium.

Methods: Myocardial infarction (MI) was induced in CD-1 mice (n=28) by permanent ligation of the left coronary artery, followed by injection of medium or $2{\cdot}10^5$ GFP-positive eCMs, SMs or MSCs in the anterior-lateral center of the infarction [1,2]. Healthy CD-1 mice served as controls (n=6). Bioluminescence imaging of luciferase expression by eCMs performed one week after transplantation was used to assess cell viability.
After 14 days, long- and short-axis ECG- and respiratory-triggered CINE FLASH images (TE/TR/α/NEX/FOV/matrix=1.8ms/7ms/15°/6/30^{2}mm^2/192x192) were acquired at 9.4T to determine global and regional cardiac functional parameters. Infarct size was assessed approximately 5min after administration of 0.3mmol Gd-DTPA/kg on T_1w short-axis multislice FLASH images (α/number of slices/slice thickness=60°/9/1mm). Data analysis was performed with CAAS-MRV FARM software (Pie Medical Imaging) and PASW Statistics software (SPSS). Stem cell engraftment was confirmed in all mice with *ex vivo* fluorescence microscopy of GFP expression. Histological assessment of infarct composition was performed using picrosirius red staining.

Results: *In vivo* MRI indicated that left ventricular (LV) dilation after myocardial infarction was reduced by eCM, SM and MSC transplantation (Fig 1, row 1). Quantitative analysis of LV dimensions showed that LV end-diastolic and -systolic volumes were significantly decreased at 14 days after myocardial infarction by transplantation of all three cell types ($p<0.05$ vs. MI). Importantly, improvements in global cardiac function, as described by the ejection fraction (EF) and cardiac output (CO), were only significant in case of eCM transplantation ($p<0.05$ vs. MI, Figs 2a,b). Additionally, only the transplantation of eCMs led to a reduction in the relative volume of infarcted myocardium on T_1w MR-images ($p<0.05$ vs. MI, Fig 1 row 2, Fig 2c). *Ex vivo* histology confirmed the changes in LV dimensions and infarct morphology by stem cell therapies that were observed with *in vivo* MRI (Fig 1, row 3).
To study whether eCMs contributed to regional contractility, wall thickening (WT) was evaluated after segmentation of the LV according to the AHA-model (Fig 1, row 4). No significant improvements in WT were observed in the anterior-lateral center of myocardial infarction by stem cell transplantation (Fig 2d). However, at the mid-LV level, WT was locally enhanced by eCMs in all but the anterior-lateral segment ($p<0.05$ vs. MI, Fig 1). Also in the apex, a positive contribution of eCMs to inferior WT was detected ($p<0.05$ vs. MI).

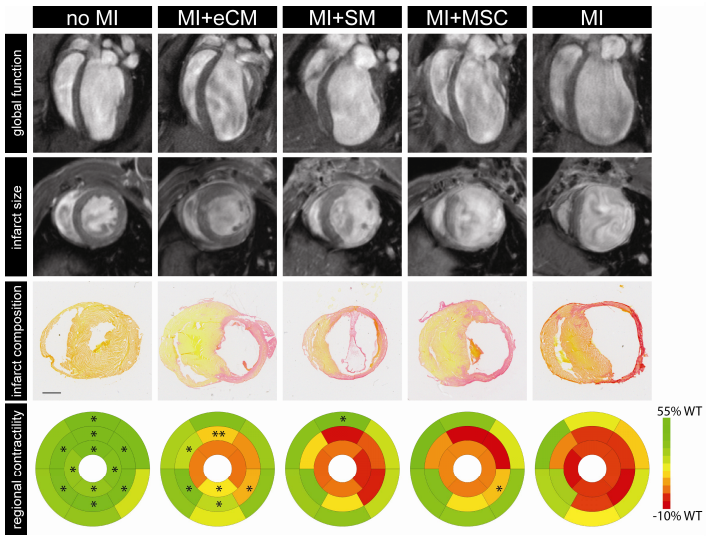

Figure 1: (row 1&2) *In vivo* long- and short-axis MR-images coregistered to (row 3) *ex vivo* picrosirius red staining; (row 4) mean wall thickening per AHA segment. *=p<0.05 vs. MI, **=p<0.1 vs. MI (Dunnet)

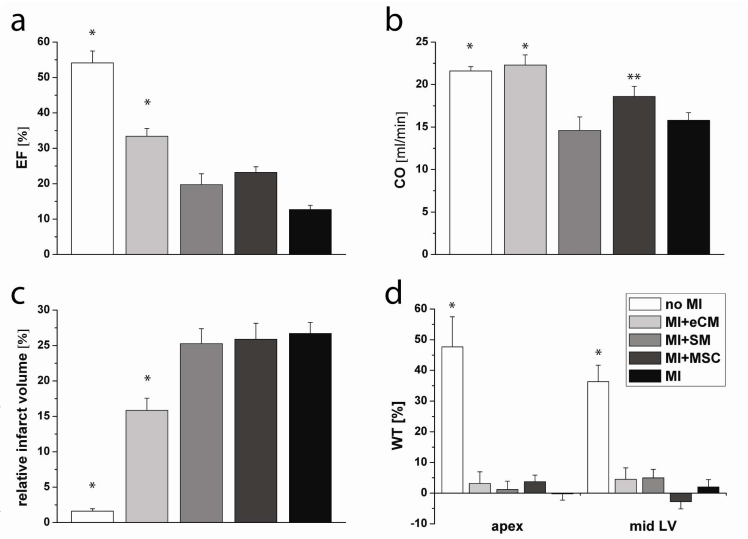

Figure 2: (a) Ejection fraction; (b) cardiac output; (c) infarct size; (d) wall thickening in the infarct center at the apical and mid-LV level. *=p<0.05 vs. MI, **=p<0.1 vs. MI (Dunnet). Bars represent mean±SE

Discussion: Embryonic cardiomyocyte transplantation in areas of infarcted myocardium showed great potential to recover both global and local cardiac contractility. Electrical coupling by gap junction proteins as connexin43 enabled rhythmical contraction of eCMs [1], which improved contractility within infarcted myocardium. Besides the positive effects on cardiac function, eCM therapy also affected the extent of infarct remodeling and expansion, since the infarct volume was smaller after eCM transplantation. By contrast, infarct volume was not reduced by SM and MSC therapy, even though engraftment of these cells was independently confirmed. Therefore, engraftment of stem cells by itself was not sufficient to reduce infarct size. The origin of improved infarct contractility by eCM transplantation could be preservation of local myocardial elasticity or supported survival of native cardiomyocytes, in addition to eCM contraction. Although SMs and MSCs both improved global cardiac morphology, infarct wall thickening was not restored, strongly suggesting that electrical coupling of engrafted eCM stem cells is indeed critical for improved regional contractility.

References: 1. Röll W. et al. *Nature*; 2007:819-826. 2. Breitbach M. et al. *Blood*; 2007:1362-1369.