J. Cell. Mol. Med. Vol 16, No 7, 2012 pp. 1640-1647

Repetitive transplantation of different cell types sequentially improves heart function after infarction

Schuh Alexander [a, c], Alexander Sasse [a], Simone Konschalla [b], Andreas Kroh [b], Marc W. Merx [a, d], Christian Weber [b, e, #], Elisa A. Liehn [a, b, c, #, *]

[a] *Department of Cardiology, Pulmonology, Angiology and Internal Medicine Intensive Care, Medical Faculty, RWTH Aachen University, Aachen, Germany*
[b] *Institute for Molecular Cardiovascular Research (IMCAR), RWTH Aachen University, Aachen, Germany*
[c] *IZKF-Biomat, RWTH Aachen University, Aachen, Germany*
[d] *Department of Medicine, Division of Cardiology, Pneumology and Angiology, Heinrich-Heine-University Hospital Düsseldorf, Germany*
[e] *Institute for Cardiovascular Prevention, Ludwig-Maximilians-University (LMU) Munich, Germany*

Received: November 13, 2009; Accepted: October 1, 2011

Abstract

Cell-based therapy is considered a novel and potentially new strategy in regenerative medicine. But the efficacy of cell-based therapy has been limited by the poor survival of the transplanted cells in an ischaemic environment. The goal of the present study is to present a possibility to increase survival of the transplanted cardiomyocytes, by increasing the vascularization of the infarcted area. First, we injected endothelial progenitor cells (EPCs) to augment the vascular density in infarcted areas and to improve the benefit of a subsequent Tx of foetal cardiomyocytes. Serial echocardiography indeed showed significant improvement of the left ventricular function after application of EPC and a significant additive improvement after Tx of foetal cardiomyocytes. In contrast, repetitive EPC transplantation as a control group did not show an additional improvement after the second transplantation. Histologically, cells could be readily detected after Tx by BrdU-staining for EPC and by carboxy-fluorescein diacetate succinimidyl ester (CFSE)-staining for foetal cardiomyocytes. Staining for CD31 revealed a significant increase in vessel density in the infarction area compared with medium controls, possibly contributing to the benefit of transplanted foetal cardiomyocytes. Notably, a significant increase in the number of apoptotic cells was observed in cell-transplanted hearts accompanied by an increase in proliferation, collagen content and neutrophil infiltration, suggesting an active remodelling concomitant with sustained inflammatory processes. In conclusion, repetitive Tx of different cell types after myocardial infarction in rat hearts significantly improved left ventricular function and could represent a feasible option to enhance the benefit of cell therapy.

Keywords: cell-based therapy • myocardial infarction • repetitive cell transplantation

Introduction

Recent years have seen a considerable rise in studies employing transplantation of various cell types in different models of myocardial injury [1]. The ultimate goal of cell-based cardiac repair is regeneration of healthy, functionally integrated, myocardial tissue. However, it remains unclear which cell type is most effective, which cell preparation and transfer technique is optimally suited and, last but not least, which mechanisms are primarily responsible for improving heart function. Several studies have postulated that neovascularization is the main reason for improved heart function [2–5]. We could previously demonstrate that transplantation of endothelial progenitor cells (EPCs) [6] or endothelial cells [7] improved heart function after transplantation in border zones of myocardial infarction (MI). Evidence for a significantly higher amount of vessels in infarcted areas pointed towards neovascularization to be responsible for a better contractile function. More recently, we could show that an inflammatory reaction to

[#]These authors equally contributed to this paper.
*Correspondence to: Dr. Elisa LIEHN,
Department of Cardiology, Pulmonology,
Angiology and Internal Medicine Intensive Care,
Institut für Molekulare Herz-Kreislaufforschung,
Universitätsklinikum der RWTH Aachen,
Pauwelsstrasse 30, 52057 Aachen, Germany.
Tel.: +49-241/80 35983
Fax: +49-241/80 82545
E-mail: eliehn@ukaachen.de

doi:10.1111/j.1582-4934.2011.01477.x

transplanted cells seemed to further support the scar remodelling and paracrine regeneration of damaged myocardium [8]. But also the transplantation of foetal cardiomyocytes improved myocardial function in a rat model [5]. This effect seemed to be due to intercellular connection of transplanted cells to host cells and an additive contractile effect, which could in part account for preserved myocardial function. But independent of the cell-type, the efficacy of cell-based therapy has been limited by the poor survival of the transplanted cells in an ischaemic environment.

The goal of the present study is to present a possibility to increase survival of the transplanted cells, in our case cardiomyocytes, by increasing the vascularization of the infarcted area. Premaratne *et al.* could demonstrate that a repetitive transplantation of skeletal myoblasts is possible and can lead to a significant improvement of LV function, which can secure a sufficient graft cell mass without tumour-like overgrowth [9]. The EPC transplantation should improve neovascularization and reconstitute vascular structures in damaged myocardial areas. That should increase the survival chances and the benefit of transplanted foetal myocardial cells in damaged myocardium.

This is the first study that attempts to elucidate for the first time whether repetitive cell transplantation of two different cell types augments myocardial function in a synergic way and can represent a promising and accessible strategy to optimize cardiac repair in the future.

Materials and methods

Generation of myocardial infarctions

Forty-eight female adult Sprague-Dawley rats (same age and same weight 200–250 g) were intubated under general anaesthesia (1 ml/kg ketamine and 10 mg/kg xylasine, intraperitoneal) and positive pressure ventilation was maintained (room air supplemented with oxygen) using a rodent respirator. Hearts were exposed through a 2-cm left thoracotomy and MI was induced by suture occlusion of the left anterior descending artery between the left atrium and the right pulmonary outflow tract (7/0 polyprolene; Ethicons, Norderstedt, Germany). The muscle layer and skin incision were closed with a silk suture. Animal experiments were approved by local authorities and complied with German animal protection law.

Cells

EPCs were isolated from 20 ml of citrate/dextran anticoagulated peripheral blood according to previously published protocols [6]. At day 7, adherent cells were detached with trypsin, counted, resuspended at 10^6 cells/100 μl PBS and transplanted. On the day before transplantation, cells were incubated with BrdU (Zymed, Vienna, Austria) as described by manufacturer. Foetal cardiomyocytes used for transplantation were isolated from donor hearts by enzymatic digestion as previously described [5]. Before transplantation, cells were labelled with CFSE (CellTrace™ Cell Proliferation Kit; Invitrogen, Carlsbad, CA, USA) for cell detection after transplantation as described by the manufacturer. We characterized in detail these cells in our previous works [5, 6, 8], so we do not provide in this study additional data about the markers analysis for the isolated EPCs and foetal cardiomyocytes.

Cell transplantation

Transplantation was performed 4 weeks and 12 weeks after MI. The rats were anaesthetized and the hearts were exposed through thoracotomy as described above. The areas for injection were chosen by visual identification based on surface scarring and wall motion akinesis. Cells were transplanted into marginal zones of the MI by syringe injection (for 1-min injection time) at three distinct but adjacent sites. Animals were divided into three groups, 10–19 animals each: the control group received only culture medium (RPMI), the second group received EPCs and foetal myocardial cells (1×10^6), the third group received twice EPC (1×10^6). We chose these cell types and time points of transplantation based on our recently published studies [5, 6]. After injection, the puncture holes were closed by suture, which served as a marker for the area of transplantation at follow-up thoracotomy. As immunosuppressive treatment, we administrated Cyclosporin A orally in both groups, starting 1 week prior to the first transplantation at a dose of 50 mg/kg [5].

Echocardiography

Four weeks after MI (*i.e.* prior to transplantation), 2 months after first transplantation and 1 month after second transplantation, rats were anaesthetized and two-dimensional and M-mode measurements were performed with a SONOS 7500 (Philips GmbH, Hamburg, Germany) with a 15-MHz linear phased-array probe. The animals were placed in the supine or lateral position and excessive pressure on the thorax was avoided. Parasternal long-axis and short-axis views were obtained, ensuring that the mitral and aortic valves and apex were well visualized. Area fraction and wall area were determined by planimetry of end-diastolic and systolic volumes in parasternal short axis. Measurements of LV end-diastolic and end-systolic dimensions were obtained in M-mode from more than three beats and fractional shortening (FS) was calculated as FS (%) = (LVIDd – LVIDs)/LVIDd) × 100, where LVID is LV internal dimension, s is systole and d is diastole.

Identification of the cells and analysis of the infarction area

For cell identification, slides (5 μm) were stained with anti-BrdU kit (Zymed). CSFE-stained cardiomyocytes appear in red after staining with an anti-FITC (Sigma-Aldrich, St. Louis, MO, USA) antibody and alkaline phosphatase substrate (Vector Laboratories, Burlingame, CA, USA). Universal quick kit and alkaline phosphatase substrate kit (Vector Laboratories) were used with anti-CD31 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for vessel identification. Only the ring-like structure positive for CD-31 in infarcted areas was counted in six different fields in three different sections from the same heart only and express as vessels/mm^2. Accustain trichrome stain (Masson; Sigma-Aldrich) was used to determine collagen content of infarcted regions. The stained areas were measured by computer-assisted planimetry (Diskus software, Hilgers, Königswinter, Germany). Macrophages and Neutrophiles were stained with alpha-naphthyl acetate esterase (Sigma-Aldrich) and naphthol as-d chloroacetate (Sigma-Aldrich), respectively. The nuclei undergoing apoptosis were stained with MEBSTAIN apoptosis kit II (MBL, Woburn, MA, USA). To evidence proliferation, the sections were

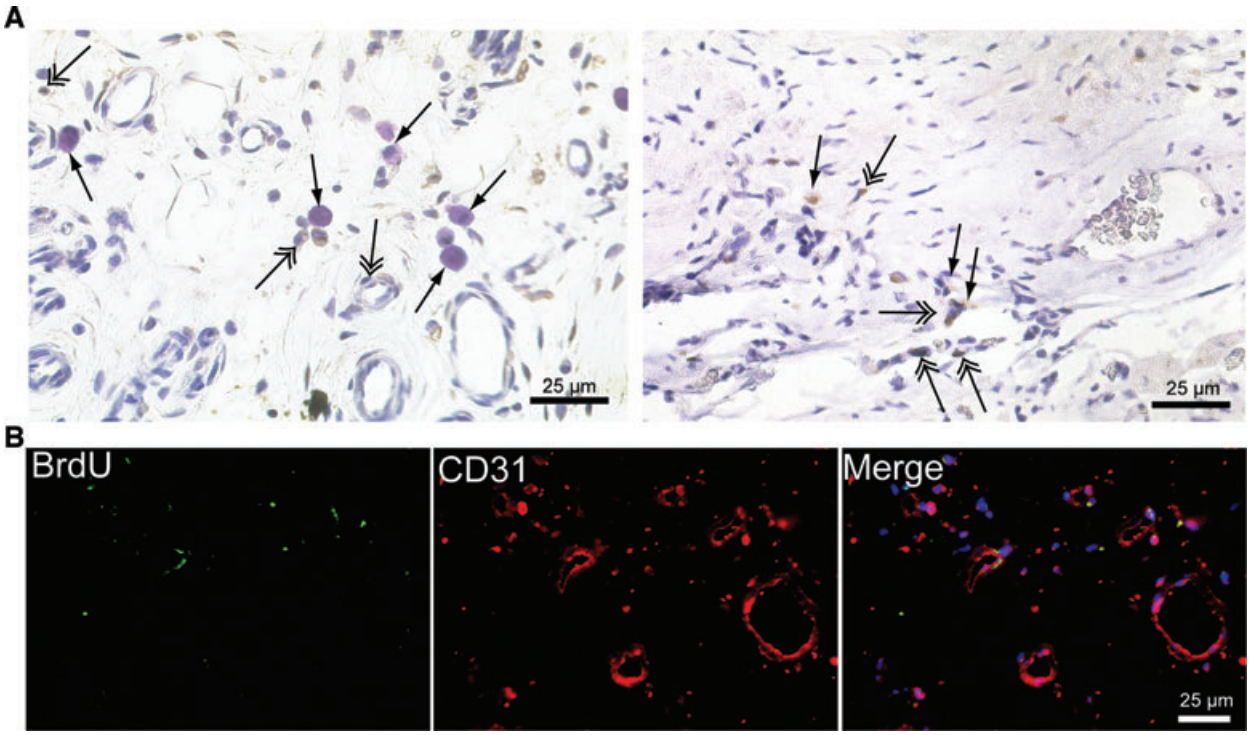

Fig. 1 Detection of transplanted cells in infarcted areas. Immunohistochemical BrdU staining of infarcted areas after sequential cell transplantation shows positive signals for BrDU-labelled EPC-derived cells (see double arrows), whereas transplanted foetal cardiomyocytes (right) and twice transplanted EPC (left) were labelled and detected using carboxy-fluorescein diacetate succinimidyl ester (CFSE, arrows) (**A**). Double fluorescent staining of BrdU and CD31 showed the localization of the transplanted EPCs in vessel structures (**B**). Objective 40×; scale bars: 25 μm.

stained for Ki-67 (DAKO, Hamburg, Germany), followed by an anti-rat-FITC antibody [10]. The stained cells were counted only in infarcted areas and express as cells/mm^2. Double staining were performed for BrdU and CD31 to assess the incorporation of the transplanted cells in the newly formed vessels, as well as for Ki-67 and CD31 or ED-1 (Macrophages marker; Serotec, Oxford, UK) to prove the cells type with proliferative activity.

Data analysis

Data represent mean ± SD. Statistical analysis was performed with Prism 4 software (Graph Pad) using unpaired Student's *t*-test or one-way ANOVA followed by Newman-Keuls test. Differences with $P < 0.05$ were considered significant.

Results

Tracking of transplanted cells

Endothelial progenitor cells were transplanted four weeks after ligation of the left coronary artery. After additional 8 weeks foetal cardiomyocytes (n = 19) or a repetitive EPC transplantation (n = 10) as well as medium (n = 19) were injected into infarcted myocardium and adjacent border zones. Transplanted cells were labelled using BrdU (EPCs) and CFSE (foetal cardiomyocytes or repetitive transplantated EPC) to allow for further tracking. We were able to detect BrdU-labelled EPCs, of which some are incorporated in capillary-like structures (Fig. 1A), as shown also by double staining with CD31 (Fig. 1B), as well as CFSE-labelled foetal myocardial cells surrounded by fibrous tissue in infracted areas of host myocardium after two transplantations (Fig. 1A).

Remodelling and inflammation in infarcted areas

TUNEL staining revealed significantly more apoptotic cells in infarcted areas after cell transplantation in cardiomyocyte-transplanted group (661.5 ± 39.0 TUNEL$^+$ cells/mm^2 *versus* control 441.9 ± 113.5 TUNEL$^+$ cells/mm^2; $P < 0.05$, Fig. 2A). This result differs from our previous findings showing less apoptosis after single transplantation of EPCs [6], microspheres or fibroblasts [8], indicating a more marked remodelling in the present model. This notion is further supported by increased proliferation in the infarcted area of the cardiomyocyte-transplanted group (113.1 ± 20.98 Ki-67$^+$ cells/mm^2 in cell transplanted group *versus* control 41.7 ± 11.4 Ki-67$^+$ cells/mm^2, $P < 0.05$, Fig. 2B). Contrary, the EPC-transplantated group showed no differences in TUNEL$^+$ cells (430.0 ± 41.1 TUNEL$^+$ cells/mm^2, Fig. 2A), as well as in proliferation (42.3 ± 5.9 Ki-67$^+$ cells/mm^2, Fig. 2B)

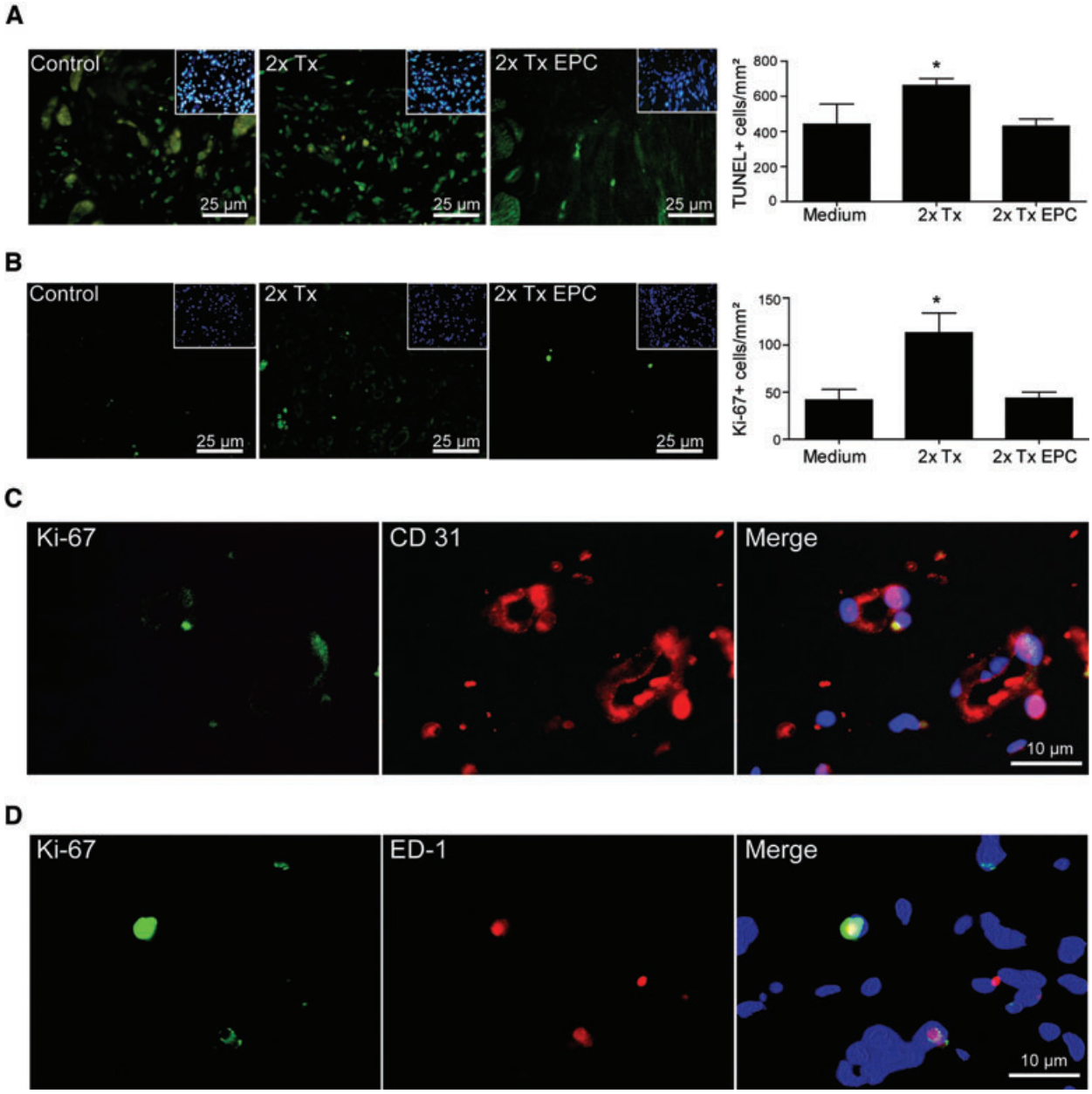

Fig. 2 Apoptosis and proliferation in infarcted areas. TUNEL staining was used to identify increased numbers of apoptotic cells (**A**) in cell-treated hearts (green: TUNEL-positive nuclei; objective 40×; scale bar: 25 μm; *$P < 0.05$ *versus* control; insets = blue DAPI staining for all nuclei). To assess proliferation (**B**), the sections were stained for Ki-67 (DAKO), followed by an anti-rat-Cy3 antibody (green; *$P < 0.05$ *versus* control; objective 40×; scale bar: 25 μm). Double staining of Ki-67 and CD31 (**C**) and ED-1 for macrophages (**D**) showed that proliferating cells were mostly endothelial cells but there are also some macrophages which showed proliferation activity (objective 40×; scale bar: 25 μm).

compared with control. We found the presence of Ki-67 mostly in endothelial cells (Fig. 2C), but we found also some macrophages with proliferating activity (Fig. 2D).

Additional evidence for increased remodelling could be provided, namely collagen content, angiogenesis and inflammatory cell content. Analysis of collagen content by specific staining revealed a significant increase in the cardiomyocyte-transplanted hearts (41.8 ± 2.3% of infarcted area), as well as in the EPC-transplanted group (43.7 ± 1.9% of infracted area *versus* 35.1 ± 1.6% of infarcted area in controls, $P < 0.05$, Fig. 3A). The infarct

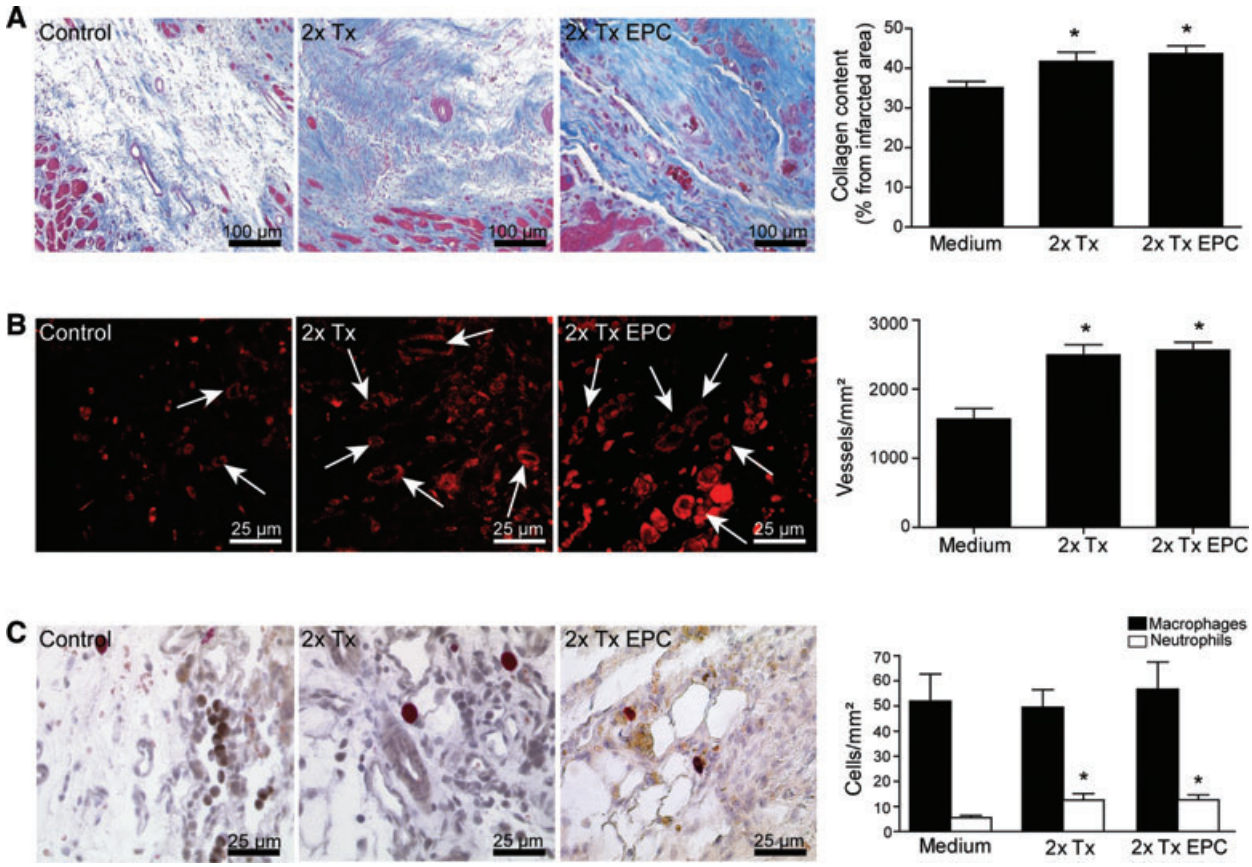

Fig. 3 Vessel density and inflammation in infarcted areas. (**A**) Collagen content (blue; scale bar: 100 μm; $*P < 0.05$ *versus* control) and (**B**) vessel density per mm^2 (red: CD 31 positive vessels; objective 20×; scale bar: 50 μm; $*P < 0.01$ *versus* control) were significantly increased in cell-treated hearts compared with controls. (**C**) Neutrophils were also increased in cell-treated hearts (red; objective 40×; scale bar: 25 μm; $*P < 0.05$ *versus* control), whereas no significant differences were found in the numbers of monocytes/macrophages (yellow-brown) in infarcted areas.

size before injection did not differ between the groups (data not shown). To investigate effects on angiogenesis, vascular density was assessed by staining and quantification of CD31-positive vessels in the area of infarction. Both groups with cell injection resulted in a significantly increased number of vessels after Tx as compared to medium treated control hearts (2495 ± 150 vessels/mm^2 in cardiomyocyte-transplanted group, 2563 ± 116.5 vessels/mm^2 in EPC-transplanted group *versus* 1567±151 vessels/mm^2 in controls, $P = 0.006$, Fig. 3B). Notably, the number of vessels determined after two transplantations was considerably higher than after a single transplantation, as described previously [6, 7]. Furthermore, as an indicator of inflammation, we assessed monocyte/neutrophil cell infiltration in all groups by staining for specific/unspecific esterase. Infiltration of neutrophils in the infracted areas was significantly more marked in cardiomyocytes-transplanted hearts (12.5 ± 2.6 cells/mm^2) and EPC-transplanted hearts (13.83 ± 2.2 cells/mm^2 *versus* 5.6 ± 0.8 cells/mm^2 in medium controls, $P < 0.05$; Fig. 3C), while no difference was found in the numbers of monocytes/macrophages.

Cardiac function

To assess cardiac function, we performed 2D echocardiography before and after MI, the first and second transplantation, and at the end of the experiment. Four weeks after MI, reduced FS was observed in all groups. Remarkably, after transplantation of both cell types, echocardiographic analysis revealed a significant improvement of FS after transplantation of EPCs and an additional significant improvement after the second transplantation of foetal cardiomyocytes (FS: after MI 30.3 ± 7.1% *versus* control 30.4 ± 9.1%; after first EPC-Tx 36.8 ± 6.2% *versus* control 30.6 ± 7%, $P = 0.007$; after second foetal cardiomyocyte-Tx 41.2 ± 5.2% *versus* control 31.1 ± 10.4%, $P = 0.008$; Fig. 4). In the EPC-transplanted group, a significant improvement in heart function was observed after the first transplantation, the second transplantation failed to add a sequential benefit, compared with cardiomyocyte-transplanted group (FS: after MI 28.1 ± 6.5% *versus* control 30.4 ± 9.1%; after first EPC-Tx 37.8 ± 7.2% *versus* control 30.6 ± 7%, $P = 0.007$; after second EPC-Tx 35.6 ± 7.8% *versus* control 31.1 ± 10.4%, $P = 0.008$; Fig. 4).

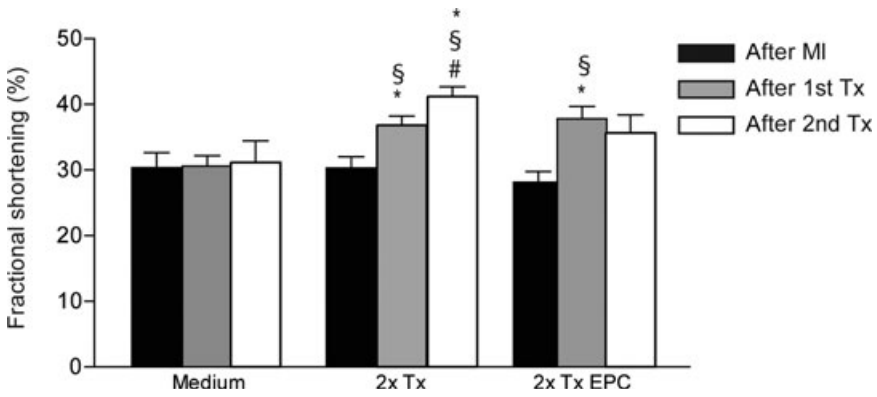

Fig. 4 Echocardiographic results. Fractional shortening in percentage after transplantation revealed significant differences towards improved left ventricular function after transplantation of EPCs and an additional significant improvement after transplantation of foetal cardiomyocytes, which is not present in EPC-twice treated group. $^{*}P < 0.05$ *versus* medium, $^{\S}P < 0.05$ *versus* after MI in the same group, $^{\#}P < 0.05$ *versus* after 1st Tx in the same group.

Discussion

Various studies have made an effort to elucidate and scrutinize the mechanisms, which were responsible for beneficial effects in infarcted myocardium following cell-based therapy [4, 7, 8, 11, 12]. Repairing vascular integrity, stimulating neovascularization [6], attenuated loss of myocardial function [5], modulation of inflammatory processes [8] or paracrine effects [11, 13–16] might be in part responsible for cardiac protection. In the same way, genetically altered cells or cells preconditioned with growth factors have shown an additional impact on improvement of myocardial function [17–19].

The results of different studies lead to the conclusion that attenuation of myocardial dysfunction after cell transplantation seems to be a combination of different mechanisms. Different cell types were suggested to exert different mechanisms in improving myocardial function. Whereas the main mechanism of transplanted endothelial cells or EPCs may be improved neovascularization, through a better nutrition of infarcted regions and a partially reconstituted vascular network, the main beneficial effect of transplanted myocardial cells seems to be an additional reservoir of contractile cells. However, paracrine effects of both cell types have also been suggested as important mechanisms. The goal of the present study was to combine these different mechanisms by sequentially transplanting EPCs and foetal cardiomyocytes.

Recently, we demonstrated that transplantation of microspheres (uniform polystyrene, 10 μm diameter) into infarcted myocardial areas was able to improve myocardial function compared to medium injected control hearts [8]. Therefore, it was tempting to speculate that inflammation triggered by transplantation potentially contributes to and modifies remodelling processes. Similar to transplanted microspheres, transplantation of EPCs had a beneficial effect on myocardial function. Mechanisms of improved myocardial function appeared to be attributable to augmented neovascularization, as suggested by several other studies dealing with transplantation of endothelial cells. Indeed, our results showed significantly increased neovascularization, as evident by vessel density, which was even more profound than previously observed with a single transplantation of EPCs alone [6]. On the other hand, two times of EPC transplantation did not augment the vessel density compared to a second transplantation of foetal cardiomyocytes and did not lead to an additional improvement of myocardial function after a second injection. It has been further speculated that transplanted cells were able to acquire or support functional properties of resident cells, such as contractility of myocytes [5]. We could indeed show improved contractile function after transplantation of foetal cardiomyocytes [5]. But to reverse left ventricular remodelling and to improve cardiac function, a large number of cardiomyocytes is needed, which can represent a high risk of tissue overgrowth or massive cell death. Indeed, Reinecke and Murry have demonstrated a risk of tissue overgrowth that sometimes distorts the ventricular contour, when myoblasts are transplanted as a bolus [20]. Based on these findings, our hypothesis was that the EPC transplantation should improve neovascularization and reconstitute vascular structures in damaged myocardial areas. This improved "preconditioning" should increase the survival chances and the benefit of transplanted foetal myocardial cells in damaged myocardium.

Indeed, our results show that repetitive cell transplantation, even after three successive open-chest operations, is feasible and echocardiographic analysis and isolated heart studies according to Langendorff revealed a significant improvement of myocardial function after infarction compared to controls without cell transplantation. FS improved after first cell injection in the transplantation group, whereas no change of myocardial function is detectable in the control group. In contrast, despite the initial significant improvement of heart function after the first transplantation, repetitive EPC-transplantation failed to add a significantly benefit compared with cardiomycyte-transplanted animals. This finding is surprising, since Premaratne *et al.* showed that a repetitive transplantation of a smaller volume of the same cell type can be more effective as a single injection of a larger volume of myoblasts. He showed that a bolus injection of myoblasts may lead to ischaemia in the centre of the area of transplanted cells, which is the case also in cardiomyocyte transplantation. In contrast, repeated transplantation of a smaller volume of cells may have protected the myoblasts from ischaemia-induced cell death [9].

But knowing the effect of the single cell type transplantation, we do not exclude that the mechanisms of the second transplantation are more comprehensive as we analysed and presented. Despite the efficiency of repetitive myoblast transplantation, repetitive EPC transplantation did not present a real benefit, but is able to support and improve the effect of the foetal cardiomyocyte transplantation. Therefore, this strategy should be considered to increase the clinical potential of cell therapy.

It has been suggested in previous studies that beneficial effects of cell transplantation are partially caused by reduced numbers of apoptotic cells in infarcted regions [6, 8]. In the present study we detected significantly more apoptotic cells and increased proliferation after second foetal cardiomyocyte transplantation. Therefore, we assumed that repetitive transplantation of different cell types caused a prolongation of the scar remodelling, explaining the improvement in the heart function. We did not observe this effect after repetitive EPC transplantation. Surprisingly, repetitive EPC transplantation leads to the same increase in collagen content and angiogenesis as the cardiomyocyte group, but no additional improvement in heart function. That could be only explained by different paracrine effects of the EPCs and foetal cardiomyocytes, and this demonstrates the complexity of the processes and necessity for more profound analysis of molecular events preceding cell therapy.

Besides, inflammatory response should play the major role in starting these remodelling processes. As we showed in our previous study [8], the inflammation induced by injection of unlikely candidates such as fibroblasts and even microspheres 1 month after MI contributed to remodelling and improved myocardial function. Furthermore, standard pharmacologic therapy following MI including statin and ACE inhibitor therapy is known to modulate inflammatory processes. Recently, Ciulla *et al.* observed similar mechanisms in improving heart function by standard ACE inhibitor treatment ACE in experimental MI compared with cell-administration [21]. In the present study we observed an increased inflammatory response and we supposed that could be an important mechanism in initiating the remodelling of the infarction scar. As we showed previously, single cell transplantation induces mostly an increase in monocyte/macrophage recruitment, but not neutrophils [6–8]. In this study, the sequential transplantation of EPC and foetal cardiomyocytes as well as the repetitive transplantation of EPC did not affect monocyte/macrophage recruitment, but induced an increased neutrophil infiltration. It seems that despite the effect on inflammation differed between the two different cell types, the sequential transplantation might act synergic and amplified the response.

Beyond the limitations, our study tried to open new sights of the cell-therapy, which have not yet been explored. Our present experimental study has demonstrated that sequential transplantation of different cell types further improves cardiac function concurrent with reverse LV remodelling compared with a single cardiomyocytes transplantation. But of course, a better understanding of these multifaceted inflammatory interactions warrants a complex and comprehensive further investigation. Also further studies in large animal models are required to improve the reproducibility, to monitor long-term effects and to facilitate a clinical approach.

In conclusion, our study demonstrates that repetitive transplantation of different cells is feasible and is associated with a sequential improved myocardial function. The mechanisms of altered myocardial function by cell transplantation are still unclear and need further investigation. At this time point, improvement of myocardial function appears to be a combination of different mechanisms, additional contractility by transplanted contractile cells, augmented neovascularization, paracrine effects and modulation of inflammatory processes. The main impact is likely to depend on the selection of cell types, mode of application, timing of application and combination of these factors.

Acknowledgements

This study was supported by the Interdisciplinary Centre for Clinical Research 'BIOMAT' (VV113-d, K1-2) within the faculty of Medicine at RWTH Aachen University. We thank R. Soltan for excellent technical support.

Conflict of interest

The authors confirm that there are no conflicts of interest.

References

1. **Bonaros N, Rauf R, Schachner T, *et al.*** Enhanced cell therapy for ischemic heart disease. *Transplantation*. 2008; 86: 1151–60.
2. **Marsboom G, Janssens S.** Endothelial progenitor cells: new perspectives and applications in cardiovascular therapies. *Expert Rev Cardiovasc Ther*. 2008; 6: 687–701.
3. **Kawamoto A, Gwon HC, Iwaguro H, *et al.*** Therapeutic potential of *ex vivo* expanded endothelial progenitor cells for myocardial ischemia. *Circulation*. 2001; 103: 634–7.
4. **Hristov M, Weber C.** Endothelial progenitor cells: characterization, pathophysiology, and possible clinical relevance. *J Cell Mol Med*. 2004; 8: 498–508.
5. **Skobel E, Schuh A, Schwarz ER, *et al.*** Transplantation of foetal cardiomyocytes into infarcted rat hearts results in long-term functional improvement. *Tissue Eng*. 2004; 10: 849–64.
6. **Schuh A, Liehn EA, Sasse A, *et al.*** Transplantation of endothelial progenitor cells improves neovascularization and left ventricular function after myocardial infarction in a rat model. *Basic Res Cardiol*. 2008; 103: 69–77.
7. **Merx MW, Zernecke A, Liehn EA, *et al.*** Transplantation of human umbilical vein endothelial cells improves left ventricular function in a rat model of myocardial infarction. *Basic Res Cardiol*. 2005; 100: 208–16.

8. **Schuh A, Liehn EA, Sasse A, *et al.*** Improved left ventricular function after transplantation of microspheres and fibroblasts in a rat model of myocardial infarction. *Basic Res Cardiol.* 2009; 104: 403–11.
9. **Premaratne GU, Tambara K, Fujita M, *et al.*** Repeated implantation is a more effective cell delivery method in skeletal myoblast transplantation for rat myocardial infarction. *Circ J.* 2006; 70: 1184–9.
10. **Liehn EA, Merx MW, Postea O, *et al.*** Ccr1 deficiency reduces inflammatory remodelling and preserves left ventricular function after myocardial infarction. *J Cell Mol Med.* 2008; 12: 496–506.
11. **Du YY, Zhou SH, Zhou T, *et al.*** Immunoinflammatory regulation effect of mesenchymal stem cell transplantation in a rat model of myocardial infarction. *Cytotherapy.* 2008; 10: 469–78.
12. **Schuh A, Breuer S, Al Dashti R, *et al.*** Administration of vascular endothelial growth factor adjunctive to foetal cardiomyocyte transplantation and improvement of cardiac function in the rat model. *J Cardiovasc Pharmacol Ther.* 2005; 10: 55–66.
13. **Bonacchi M, Nistri S, Nanni C, *et al.*** Functional and histopathological improvement of the post-infarcted rat heart upon myoblast cell grafting and relaxin therapy. *J Cell Mol Med.* 2009; 13: 3437–48.
14. **Wragg A, Mellad JA, Beltran LE, *et al.*** VEGFR1/CXCR4-positive progenitor cells modulate local inflammation and augment tissue perfusion by a SDF-1-dependent mechanism. *J Mol Med.* 2008; 86: 1221–32.
15. **Perez-Ilzarbe M, Agbulut O, Pelacho B, *et al.*** Characterization of the paracrine effects of human skeletal myoblasts transplanted in infarcted myocardium. *Eur J Heart Fail.* 2008; 10: 1065–72.
16. **Nakanishi C, Yamagishi M, Yamahara K, *et al.*** Activation of cardiac progenitor cells through paracrine effects of mesenchymal stem cells. *Biochem Biophys Res Commun.* 2008; 374: 11–6.
17. **Liang HL, Yi DH, Zheng QJ, *et al.*** Improvement of heart allograft acceptability associated with recruitment of CD4+CD25+ T cells in peripheral blood by recipient treatment with granulocyte colony-stimulating factor. *Transplant Proc.* 2008; 40: 1604–11.
18. **Yokokura Y, Hayashida N, Okazaki T, *et al.*** Influence of angiogenesis by implantation of bone marrow mononuclear cells in the rat ischemic heart. *Kurume Med J.* 2007; 54: 77–84.
19. **Hahn JY, Cho HJ, Kang HJ, *et al.*** Pre-treatment of mesenchymal stem cells with a combination of growth factors enhances gap junction formation, cytoprotective effect on cardiomyocytes, and therapeutic efficacy for myocardial infarction. *J Am Coll Cardiol.* 2008; 51: 933–43
20. **Reinecke H, Murry CE.** Transmural replacement of myocardium after skeletal myoblast grafting into the heart: too much of a good thing? *Cardiovascr Pathol.* 2000; 9: 337–44.
21. **Ciulla MM, Montelatici E, Ferrero S, *et al.*** Potential advantages of cell administration on the inflammatory response compared to standard ACE inhibitor treatment in experimental myocardial infarction. *J Transl Med.* 2008; 6: 30.