Journal of Clinical & Experimental Cardiology

Yu, et al., J Clin Exp Cardiolog 2014, 5:9
DOI: 10.4172/2155-9880.1000333

Research Article Open Access

Ultrasound-mediated Microbubble Destruction Promotes Bone Marrow Mesenchymal Stem Cell Transplantation for Myocardial Infarction Therapy

Fuchao Yu, Jinyu Li, Zhuo Xu, Zhouzhou Lu, Xiaohui Zhang, Dan Li and Jiayi Tong*

Institute of Cardiology, Southeast University, Nanjing, Jiangsu Province, China

***Correspondence author:** Jiayi Tong, Institute of Cardiology, Southeast University, Nanjing 210009, Jiangsu Province, China, Tel: +86-25-83262415; E-mail: 13701464321@163.com

Rec date: Sept 03, 2014, **Acc date:** Sept 08, 2014, **Pub date:** Sept 18, 2014

Abstract

The study was designed to investigate whether ultrasound-mediated microbubble destruction can enhance the transplantation of bone marrow mesenchymal stem cells (BMSCs) for myocardial infarction therapy, twenty myocardial infarction pigs were assigned into the ultrasound microbubble destruction group (n=12, Sonovue were infused into coronary artery with ultrasonic radiation, then BMSCs were injected into infarction region) and the cell control group (n=8, only subjected to BMSCs treatment). The increase of left ventricular ejection fraction was larger in the ultrasound microbubble destruction group (P<0.01), and more Prussian blue-positive cells and higher density of heart muscle capillary in the peripheral infarct regions were found (P<0.01). Prussian blue-positive cells were differentiated into new vascular endothelial cells in two cases, among which the gaps were widened in the microbubble destruction group. Our study first time testifies in pig models that ultrasound-mediated microbubble destruction promotes BMSC transplantation for myocardial infarction therapy.

Keywords: Ultrasound; Microbubble; Bone marrow; Mesenchymal stem cells; Transplantation; Myocardial infarction

Introduction

Myocardial infarction is caused by the loss of myocardial cells and decreased cardiac systolic and diastolic functions, and inevitably leads to heart failure. It has been reported that intramyocardial stem cell transplantation can notably increase the perfusion of blood in ischemic myocardium and improve heart functions [1,2]. The extent of improvement is linearly correlated with the number of colonized stem cells in the heart [3]. Among the various transplantation methods, intramyocardial injection can have the highest transplantation efficiency, on the order of 10%. Intra-coronary artery transplantation achieves the second highest efficiency, usually 1% to 2%, although 10% has also been reported [4]. Since these results are not satisfying, it is of great importance to look for novel and effective approaches to increase the efficiency of stem cell transplantation.

Ultrasound is a type of wave motion that has several biological effects, such as mechanical and sonic cavitation effects. Radiation pressure is generated when ultrasound is transmitted across different tissues, and it can induce the marginal accumulation of blood cells by pushing them to the vascular wall. Ultrasound microbubble contrast agents in liquid can serve as the cavitation nuclei. In response to ultrasound, the microbubbles undergo asymmetric contraction and expansion and are prone to rupture, which markedly widens the gaps between vascular endothelial cells and elevates the membrane permeability. This effect may also give rise to transient and reversible pores in the membrane, increase the permeability of the biological barriers around the tissues where the microbubbles rupture, and ultimately elevate the efficiency of stem cell transplantation.

The present study investigated the influence of the ultrasound-mediated microbubble destruction on the stem cell transplantation therapy against myocardial infarction and provided objective data for clinical therapeutic strategies of stem cell transplantation.

Materials and methods

Experimental design

Randomized controlled animal studies were performed in the Medical College of the Southeast University from October 2006 to May 2008.

Materials

In total, 20 pigs, aged 2 months and weighing 29 ± 1 kg, were provided by the experimental animal center of the Clinical Medical College of the Southeast University. Pigs were randomly assigned into the ultrasound microbubble destruction group (n=12) and the cell control group (n=8). All animals were treated according to the Guidelines for Virtuous Treatment of Experimental Animals that was released by the Science and Technology Ministry in 2006. Reagents and instruments were listed in table 1.

Reagents and equipment's	Resources
Fetal bovine serum	Hangzhou Sijiqing Biological Engineering Materials
DMEM (Low glucose)	Gibco
Ficoll, trypsin	Sigma
Trypan blue	Beyotime Institute of Biotechnology
Prussian blue Kit	Shanghai Chunzhu Biotechnology
Superparamagnetic iron oxide	Research Center for Nanotechnology, Southeast University

Citation: Yu F, Li J, Xu Z, Lu Z, Zhang X, et al. (2014) Ultrasound-mediated Microbubble Destruction Promotes Bone Marrow Mesenchymal Stem Cell Transplantation for Myocardial Infarction Therapy. J Clin Exp Cardiolog 5: 333. doi:10.4172/2155-9880.1000333

SonoVue (License No: J20030117)	Shanghai Bracco Sine Pharmaceutical
64-spiral CT	Simens

Table 1: Reagents and instruments

Methods and observation indexes

In vitro culture of bone marrow mesenchymal stem cells (BMSCs)

The animals were subjected to intramuscular induction of anaesthesia using ketamine in a dose of 10-15 mg/kg behind the pig ear. Once anaesthesia is induced, 0.01 g/kg pentobarbital sodium was intravenously injected to maintain anaesthesia.

Anterior superior iliac spine was chosen as puncture site after conventional sterilization. After puncture, 50 ml syringe containing 1000 Units heparin was used to collect bone marrow, and 5 ml was collected each time, confirming the amount of bone marrow fluid obtained is sufficient. A total of 30 ml bone marrow fluid was transferred into a 50 ml sterile centrifuge tube. MSCs and HBSCs were separated by density centrifugation on Ficoll. The supernatant was diluted with low-glucose DMEM medium containing 10% FCS. Then the MSCs were seeded at a density of 2-3×10^5 in 25 ml culture flasks, and then incubated at 37 in 5% CO_2. The culture solution was changed 48h after incubation, removed the non-adherent cells. After that, the culture fluid was regularly refreshed every 2-3 days. Passage cultured when MSCs reached 80%-90% confluence. The MSCs were purified after multiple passages. Generally, the stem cells for transplantation were harvested after passage 3.

Establishment of the acute myocardial infarction model

The animals were subjected to induction of anaesthesia as described above. Once anaesthesia is induced, the needle was inserted at the maximum pulse point of right lower limb femoral artery. Guide wire and catheter were inserted through sheathing canal in femoral artery. The site of left anterior descending branch was identified using radiography. Then guide wire was placed at left anterior descending branch. OTW Foley's tubes with proper size were chosen by using coronary arteriongraphy, and were placed at the distal one-third site of the left anterior descending branch. Refill through the tubes with the same stress following ischemic preconditioning. The elevation of ST-segment in anterior precordial leads indicated that heart muscle ischemia has been caused by ischemic preconditioning. Filling through the tubes was maintained for 60 min to establish the model of acute anterior myocardial infarction. Subsequently, the models were transferred to animal laboratory [5].

Labeling of BMSCs with super-paramagnetic iron oxide

Thirteen days after model establishment, BMSCs were incubated with super-paramagnetic iron oxide particles that were coated with arginine for 24 h. Cells were then trypsinized, precipitated by centrifugation and re-suspended in fresh medium. Cell survival rates were measured using trypan blue staining [6,7].

Transplantation of BMSCs

Fourteen days after model establishment, animals were subjected to MSCs implantation through coronary artery. The procedures are as follows: The animals underwent coronary arteriongraphy. When TIMI grade III flow was observed, guide wire was inserted through OTW Foley's tubes into the middle segment of the left anterior descending branch. OTW Foley's tubes were dilated afterwards. Totally, 2.4 ml Sono Vue was infused into the pigs via perfusion catheter in ultrasound microbubble destruction group. Using X-ray observation, ultrasonic detector was positioned at the distal one-third site of the left anterior descending branch (myocardial infarction). The necrosis area was treated with ultrasonic radiation at a dosage of 1 MHz and 2 W/cm^2 for 90 seconds. Subsequently, 5×10^6 isolated MSCs labelled by superparamagnetic iron oxide were injected into the pigs. In contrast, the pigs in the cell control group were only subjected to the injection of BMSCs [8].

Histological characterization and ultrastructure observation

Samples from the cell control group were collected at 0 h and 6 weeks after transplantation, whereas samples from the ultrasound microbubble group were collected at 0 h, 24 h and 6 weeks after transplantation. Samples from 4 animals were collected at each time point. Normal regions, infarct border regions and infarct regions in the heart were selected for examination. Some of the collected tissues were fixed by formaldehyde and used for preparation of 3 μm thick paraffin sections. Sections were stained with hematoxylin-eosin or Prussian blue. The sections from the 6 week samples were also used for immunohistochemical staining of desmin and capillary counting. Other tissues were fixed with glutaraldehyde and subjected to scanning electron microscopy to examine the ultrastructure of vascular endothelial cells.

Examination of heart functions by 64-spiral computerized tomography (CT)

All animals were examined by 64-spiral CT (scanning slice thickness 0.6 mm) at 1 day before and 6 weeks after transplantation. End-diastolic volume, end-systolic volume and left ventricular ejection fraction (LVEF) were obtained by image processing approaches such as volume rendering, multilayer reconstruction and maximum intensity projection.

Statistical analysis

Data were analysed using the statistic software SPSS (version 11.5) and were expressed as x ± s. Comparisons between groups were carried out by a single factor analysis of variance. Significant differences are defined as $P<0.05$.

Results

Quantitative analysis of experimental animals

The model of myocardial infarction was successfully established in each of the 20 experimental pigs. No animals were lost during the experiment.

Labelling of BMSCs with super-paramagnetic iron oxide and the cell viability test

Before transplantation, BMSCs labelled by super-paramagnetic iron oxide were mounted on slides and stained with Prussian blue. Blue particles in the cytoplasm and red nuclei were observed under a microscope. The labelling rate was nearly 100%. No blue particles could be found in the unlabelled BMSCs. In addition, the survival rate of the labelled and unlabelled BMSCs was about 98% before transplantation, as demonstrated by trypan blue staining.

Changes of heart functions

The LVEFs of the animals in both groups were significantly decreased 1 day before transplantation. No significant difference was observed between the groups (P>0.05), which suggested that myocardial infarction had a similar influence on heart functions of the animals in both groups. Six weeks after transplantation, the LVEFs in both groups were notably elevated compared with the fractions before transplantation, and the increment in the ultrasound microbubble destruction group was significantly higher than that in the cell control group (P<0.01) (Table 2).

Time	Ultrasound microbubble group			Cell control group		
	LEDV (mL)	LESV (mL)	LVEF	LEDV (mL)	LESV (mL)	LVEF
1 day pretransplantation	59.75 ± 0.95	32.25 ± 0.96	0.42 ± 0.02	60.25 ± 0.96	33.25 ± 0.96	0.42 ± 0.02
6 wk aftertransplantation	61.75 ± 0.96^a	27.75 ± 1.71^a	0.55 ± 0.01^a	63.50 ± 1.29	30.25 ± 0.96	0.53 ± 0.01

Table 2: Comparison of cardiac function in both groups before and after transplantation LEDV: Left Ventricular End Diastolic Volume; LESV: Left Ventricular End Systolic Volume; LVEF: Left Ventricular Ejection Fraction; ^{a}P<0.01 vs. the cell control group

Histological characterization of myocardium

Hematoxylin-eosin staining

Infarcted cardiac fibres were dissolved in the infarct regions. Myocardial structure could not be seen and was substituted by infiltrated erythrocytes and neonatal fibroblasts, lymphocytes and capillaries. Dispersed fresh acute infarct regions that had not been repaired were also observed, and they were demarcated by the lack of myocardial structure and cell nuclei. In contrast, it was found that the myocardial structure in the peripheral infarct regions was intact. In these locations, fibroblasts, lymphocyte infiltration and neonatal capillaries were seen.

Prussian blue staining

Prussian blue-positive cells could be seen in the samples of both groups, even at 6 weeks after transplantation; however, more positive cells were found in the ultrasound microbubble destruction group (P<0.01). Prussian blue-positive cells were predominantly distributed in the peripheral infarct regions (Figure 1a). In two cases, Prussian blue-positive cells were differentiated into new vascular endothelial cells (Figure 1b).

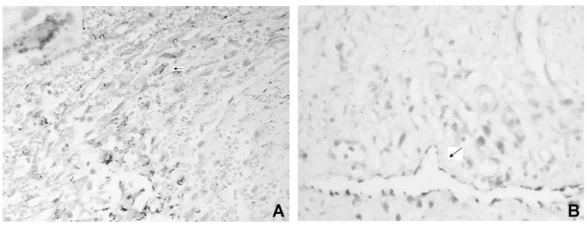

Figure 1: Prussian blue staining of heart pathological sections from the samples in the ultrasound microbubble destruction group (×200). (a) Transplanted BMSCs (indicated by an arrow). (b) Vascular endothelial cells (indicated by an arrow).

Immunohistochemical staining of desmin

Six weeks after transplantation, desmin-positive cells could be seen in samples from both groups (Figure 2).

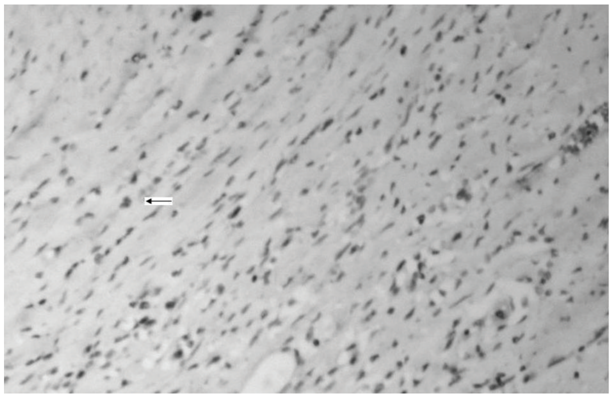

Figure 2: Immunohistochemical staining with anti-desmin of a heart histological section from the ultrasound microbubble destruction group (×200). A desmin-positive cell is indicated by an arrow.

The density of capillaries

The density of capillaries in the peripheral infarct regions was significantly higher in the ultrasound microbubble destruction group than in the cell control group (P<0.05) (Figure 3).

Ultrastructure changes of vascular endothelial cells

Observation of ultrastructure demonstrated that the integrity of vascular endothelial cells with diameters of about 6 μm was disrupted. Karyopyknosis and chromatin margination on the nuclear membrane were also observed. No thrombosis was seen in the vessel lumen (Figure 4). Notably, there was no obvious difference in these ultrastructure changes between the two groups. Moreover, at 0 h after transplantation, a widened gap between two endothelial cells was seen in a blood vessel with a diameter of 11.5 μm in the ultrasound microbubble destruction group, but not in the cell control group (Figure 5).

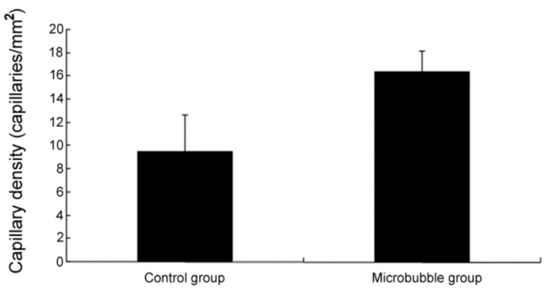

Figure 3: Compare of capillary density between control group and microbubble group

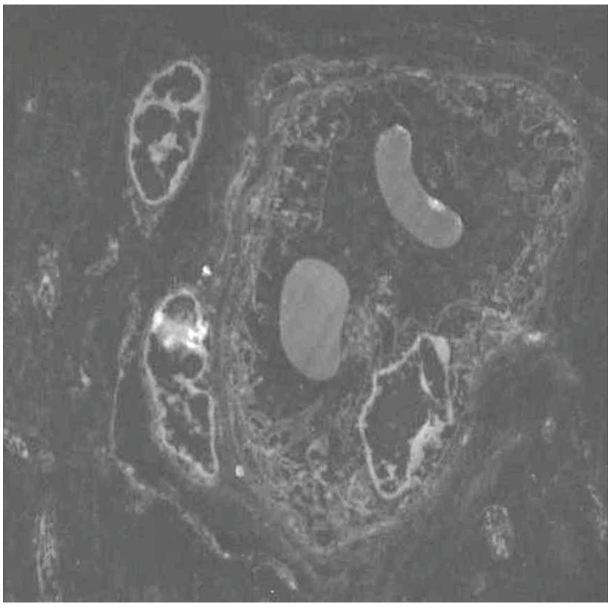

Figure 4: Disrupted integrity of vascular endothelial cells, karyopyknosis and chromatin margination on the nuclear membrane revealed by ultrastructure observation (×4000).

Discussion

Ultrasound microbubble therapy has been widely utilized in clinical diagnosis to enhance the accuracy of diagnosis, besides; it gradually plays an undiscovered role in clinical treatments [9]. It has been reported that ultrasound-targeted microbubble destruction could destroy the skeletal muscle blood capillaries, inducing local infiltration of erythrocytes and promoting cellular penetration of the endothelial physiologic barriers [10]. Therefore, our aim was to clarify whether ultrasound-mediated microbubble destruction could enhance the therapeutic effect of intramyocardial BMSC transplantation on myocardial infarction.

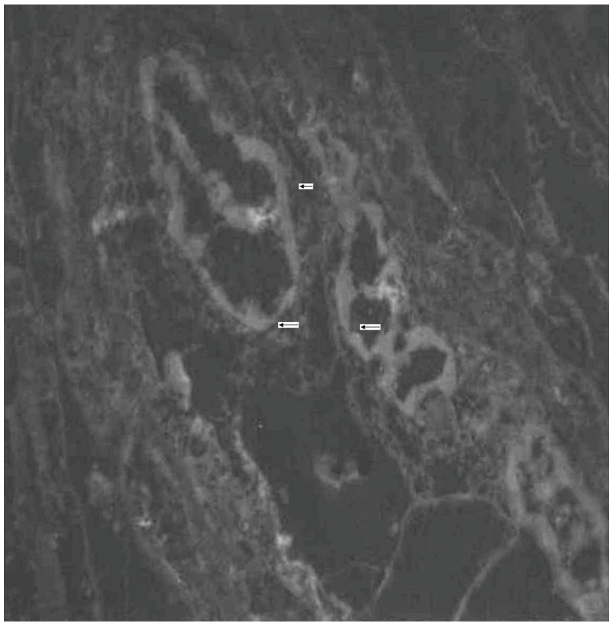

Figure 5: The gaps between vascular endothelial cells are widened. The short arrow indicates a gap between two cells, whereas the long arrows indicate the nuclei of vascular endothelial cells (×4000).

Our data showed that ultrasound-mediated microbubble destruction increased the efficiency of intramyocardial BMSC transplantation, which was demonstrated by greater numbers of Prussian blue-positive cells in the ultrasound microbubble destruction group than in the cell control group. This result is consistent with the finding of Takanobu et al., who reported that low-frequency ultrasound associated with microbubble destruction increased the efficiency of BMSC transplantation into ischemic skeletal muscle [11]. Ghanem et al. recently found that targeted endothelial adhesion and myocardial engraftment after intravascular delivery of MSCs can be enhanced with hf-UMS [12]. These effects may arise from the biological responses to ultrasound-mediated microbubble destruction. These responses include the radiation pressure generated by ultrasound transmission through different tissues, the shear stress generated by microbubble rupture, and the cavitation effect generated by ultrasound-mediated microbubble destruction. Radiation pressure can promote the local accumulation of blood cells by pushing them to the vascular wall, whereas cavitation can induce the contraction of endothelial cells, the widening of gaps between endothelial cells and the subsequent destruction of microvessels [13,14]. Moreover, shear stress can help the transplanted cells in the blood vessel to penetrate the vessel wall and enter tissues. In addition, slight damage-induced inflammatory responses that result from ultrasound-mediated microbubble destruction can also promote the production of inflammatory factors (e.g. interleukin-1) and induce the intramyocardial migration of mesenchymal stem cells. Expression of adhesion molecules is up-regulated in damaged endothelial cells, which allow the circular mesenchymal stem cells to stick to the damaged vascular endothelium via adhesion molecules and avoid blood scouring. All these biological responses facilitate the entrance of transplanted BMSCs into damaged myocardium and increase the efficiency of BMSC transplantation. Indeed, our results demonstrated that ultrasound-mediated microbubble destruction notably elevated transplantation efficiency. The integrity of vascular endothelial cells with diameters of about 6 μm was disrupted. Chromatin margination on the nuclear membrane and widened gaps between endothelial cells were also observed.

Ultrasound-mediated microbubble destruction promoted myocardial neovascularization. Immunohistochemical staining showed that the myocardial capillary density was significantly higher in the ultrasound microbubble destruction group than in the cell control group. Aseptic inflammation induced by microvascular fracture during ultrasound-mediated microbubble destruction can promote myocardial neovascularization, which may increase blood flow in normal and ischemic myocardium [15-18]. It has been reported that transplanted BMSCs are able to secrete vascular endothelial growth factors and basic fibroblast growth factors under certain conditions [19,20]. Moreover, the BMSCs transplanted into myocardium can differentiate into not only myocardial-like cells but also into vascular endothelial-like cells. All these biological effects can induce neovascularization in ischemic myocardium.

BMSC transplantation with ultrasound-targeted microbubble destruction can increase the colonization of stem cells in the infarct regions and the infarct border regions make more stem cells differentiate into myocardial cells, synergistically facilitate angiogenesis, and improve blood supply in both the infarct regions and the peripheral infarct regions. This transplantation strategy may improve cardiac blood perfusion, especially in hibernating and stunned myocardium, and lead to the recovery of systolic function in hibernating myocardium. It also provides the appropriate microenvironment for the survival of transplanted stem cells and their differentiation into myocardial cells. The strategy can also limit ventricular dilatation, especially in the ventricular scar region, inhibit cardiac remodelling, ameliorate cardiac systolic and diastolic functions and ultimately improve cardiac functions impaired by myocardial infarction. In conclusion, the present work suggested a new strategy for the transplantation of intramyocardial BMSCs. This approach may have widespread application in the treatment of myocardial infarction.

Especially, we used spiral CT rather than ultrasonic radiation in the present study due to the fact that the statistical analysis by ultrasonic radiation was more likely to be affected by operator subjectivity compared with by spiral CT. In addition, ultrasonic radiation may potentially produce more errors when measuring the volume of irregular-shaped ventricles, which formed under the symptoms of myocardial infarction and ventricular aneurysm. Second, ultrasonic analysis was one of the affecting factors in our study, which might increase the potential interfering factors during cardiac function analysis. Spiral CT ensures accurate positioning. The device provides accurate data regarding the left ventricular volume, quality and functional evaluation, and it is highly reproducible and less affected by operator subjectivity. Compared with MRI, spiral CT needs less scan time and has lower requirements of animal cooperation. The availability of better cardiac post-processing techniques is also a benefit. It has been reported that the LVEF is better correlated with spiral CT measurement than with MRI measurement [21,22]. Therefore we chose to use spiral CT rather than MRI in the present study.

The present study had several limitations. First, the number of experimental samples was relatively small. Whether the findings in our study could be applied into clinical practice still requires further intense investigations with large sample size. Surprisingly, recent studies proposed the promising prospect of applying ultrasound microbubbles into treatment of myocardial infarction through stem cell transplantation. Song et al suggested that using US-mediated MB destruction prior to BMSCs transplantation into the infarcted myocardium improves the effectiveness of cardiac cell therapy and cardiac function in rabbits [23]. Second, the respective functions of the ultrasound and microbubbles on the transplantation of BMSCs were not analysed in more detail. Third, the physical and molecular mechanisms by which ultrasound-mediated microbubble destruction improves cell homing ability remain unclear [24]. In addition, the exact mechanisms by which stem cells transplantation ameliorated cardiac systolic function and the roles of extracellular matrix during this process also warrant clarification. It is still unknown whether myocardial-like cells differentiated from transplanted BMSCs have systolic and neovascularization functions. Future investigations will focus on these issues.

Acknowledgments

We are grateful to Jiangsu Key Laboratory for Biomaterials and Devices for technical support.

References

1. Wang Y, Johnsen HE, Mortensen S, Bindslev L, Ripa RS, et al. (2006) Changes in circulating mesenchymal stem cells, stem cell homing factor, and vascular growth factors in patients with acute ST elevation myocardial infarction treated with primary percutaneous coronary intervention. Heart 92: 768-774.
2. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, et al. (2006) Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet 367: 113-121.
3. Poh KK, Sperry E, Young RG, Freyman T, Barringhaus KG, et al. (2007) Repeated direct endomyocardial transplantation of allogeneic mesenchymal stem cells: safety of a high dose, "off-the-shelf", cellular cardiomyoplasty strategy. Int J Cardiol 117: 360-364.
4. Meyer GP, Wollert KC, Lotz J, Steffens J, Lippolt P, et al. (2006) Intracoronary bone marrow cell transfer after myocardial infarction: eighteen months' follow-up data from the randomized, controlled BOOST (BOne marrOw transfer to enhance ST-elevation infarct regeneration) trial. Circulation 113: 1287-1294.
5. Krombach GA, Kinzel S, Mahnken AH, Günther RW, Buecker A (2005) Minimally invasive close-chest method for creating reperfused or occlusive myocardial infarction in swine. Invest Radiol 40: 14-18.
6. Kraitchman DL, Heldman AW, Atalar E, Amado LC, Martin BJ, et al. (2003) In vivo magnetic resonance imaging of mesenchymal stem cells in myocardial infarction. Circulation 107: 2290-2293.
7. Amado LC, Saliaris AP, Schuleri KH, St John M, Xie JS, et al. (2005) Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. Proc Natl Acad Sci USA 102: 11474-11479.
8. Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, et al. (2006) Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. N Engl J Med 355: 1210-1221.
9. Ghanem A1, DeMaria AN, Lohmaier S, El-Sayed MA, Strachan M, et al. (2007) Triggered replenishment imaging reduces variability of quantitative myocardial contrast echocardiography and allows assessment of myocardial blood flow reserve. Echocardiography 24:149-158.
10. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, et al. (1998) Basis for detection of stenosis using venous administration of microbubbles during myocardial contrast echocardiography: bolus or continuous infusion? J Am Coll Cardiol 32: 252-260.

11. Imada T, Tatsumi T, Mori Y, Nishiue T, Yoshida M, et al. (2005) Targeted delivery of bone marrow mononuclear cells by ultrasound destruction of microbubbles induces both angiogenesis and arteriogenesis response. Arterioscler Thromb Vasc Biol 25: 2128-2134.

12. Ghanem A, Steingen C, Brenig F, Funcke F, Bai ZY, et al. (2009) Focused ultrasound-induced stimulation of microbubbles augments site-targeted engraftment of mesenchymal stem cells after acute myocardial infarction. J Mol Cell Cardiol 47: 411-418.

13. Miller DL, Quddus J (2000) Diagnostic ultrasound activation of contrast agent gas bodies induces capillary rupture in mice. Proc Natl Acad Sci USA 97: 10179-10184.

14. Skyba DM, Price RJ, Linka AZ, Skalak TC, Kaul S (1998) Direct in vivo visualization of intravascular destruction of microbubbles by ultrasound and its local effects on tissue. Circulation 98: 290-293.

15. Zen K, Okigaki M, Hosokawa Y, Adachi Y, Nozawa Y, et al. (2006) Myocardium-targeted delivery of endothelial progenitor cells by ultrasound-mediated microbubble destruction improves cardiac function via an angiogenic response. J Mol Cell Cardiol 40: 799-809.

16. Wang JS, Shum-Tim D, Galipeau J, Chedrawy E, Eliopoulos N, et al. (2000) Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. J Thorac Cardiovasc Surg 120: 999-1005.

17. Liu J, Hu Q, Wang Z, Xu C, Wang X, et al. (2004) Autologous stem cell transplantation for myocardial repair. Am J Physiol Heart Circ Physiol 287: H501-511.

18. Miyahara Y, Nagaya N, Kataoka M, Yanagawa B, Tanaka K, et al. (2006) Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. Nat Med 12: 459-465.

19. Yoshida J, Ohmori K, Takeuchi H, Shinomiya K, Namba T, et al. (2005) Treatment of ischemic limbs based on local recruitment of vascular endothelial growth factor-producing inflammatory cells with ultrasonic microbubble destruction. J Am Coll Cardiol 46: 899-905.

20. Liechty KW, MacKenzie TC, Shaaban AF, Radu A, Moseley AM, et al. (2000) Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep. Nat Med 6: 1282-1286.

21. Boehm T, Alkadhi H, Roffi M, Willmann JK, Desbiolles LM, et al. (2004) Time-effectiveness, observer-dependence, and accuracy of measurements of left ventricular ejection fraction using 4-channel MDCT. Rofo 176: 529-537.

22. Heuschmid M, Rothfuss JK, Schroeder S, Fenchel M, Stauder N, et al. (2006) Assessment of left ventricular myocardial function using 16-slice multidetector-row computed tomography: comparison with magnetic resonance imaging and echocardiography. Eur Radiol 16: 551-559.s

23. Song X, Zhu H, Jin L, Wang J, Yang Q, et al. (2009) Ultrasound-mediated microbubble destruction enhances the efficacy of bone marrow mesenchymal stem cell transplantation and cardiac function. Clin Exp Pharmacol Physiol 36: 267-271.

24. Klibanov AL (2006) Microbubble contrast agents: targeted ultrasound imaging and ultrasound-assisted drug-delivery applications. Invest Radiol 41: 354-362.