# Safety and Efficacy of Intramyocardial Implantation of Peripheral Blood Stem Cell for Cardiomyopathy

Ruengsakulrach P, MD, PhD
email : permyos@bangkokheart.com

Permyos Ruengsakulrach, MD, PhD, FRCST, FCCP[1]
Kittipan Visudharom, MD, PhD[1]
Lertlak Chaothawee, MD[2]
Michael Belkin, MD[3]

[1] Division of Cardiothoracic and Vascular Surgery, Bangkok Heart Hospital, Bangkok Hospital Group, Bangkok , Thailand.
[2] Cardiac Imaging Center, Bangkok Heart Hospital, Bangkok Hospital Group, Bangkok, Thailand.
[3] Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel.

**Keywords:**
Stem cells, Cell transplantation, Cardiomyopathy, Heart failure, Coronary artery bypass grafting

**Presentation at the Society of Thoracic Surgeons (STS) 47th Annual Meeting, January 31 - February 2, 2011, San Diego, California, USA*

**OBJECTIVE**. To determine the safety and efficacy of intramyocardial autologous blood stem cell injection for cardiomyopathy.

**MATERIALS AND METHODS**. Between May 2005 and February 2010, 126 consecutive patients underwent intramyocardial cell injection. Fifty two were dilated cardiomyopathy (DCM) and 74 were ischemic cardiomyopathy (ICM). Mean age was 59.2 ± 12.4 years. The stem cells are isolated from the patient's own blood and cultured. The final product is called angiogenic cell precursors (ACPs). The number of cells prior to injection was 46.1 ± 36.5 million cells. ACPs were injected into all areas of the left ventricle in DCM patients, and into the non-viable myocardium and hypokinetic segments in ICM patients. Combined coronary artery surgery and cell injection were performed in 33.8% of ICM cases.

**RESULTS**. There was no new ventricular arrhythmia. The 30-day mortality rate was 3.8% (2/52) and 4.1% (3/74) in DCM and ICM, respectively. New York Heart Association (NYHA) class improved from 3.0 ± 0.6 to 2.0 ± 0.9 at 485.8 ± 370.3 days ($p < 0.001$) in DCM and improved from 2.7 ± 0.6 to 1.9 ± 0.8 at 419.6 ± 345.5 days ($p < 0.001$) in ICM. Left ventricular ejection fraction (LVEF) increased from 23.3 ± 7.0% to 27.7 ± 11.3% at 409.7 ± 352.4 days ($p = 0.03$) in DCM and increased from 23.6 ± 7.7% to 31.5 ± 10.0% at 400.6 ± 350.1 days ($p < 0.001$) in ICM. Quality of life evaluated at 3 months has significantly improved for physical function, role-physical, general health and vitality domains in DCM. For ICM, physical function, role-physical, general health and social function domains were also improved.

**CONCLUSION**. Intramyocardial ACPs injection is feasible and safe in both DCM and ICM. NYHA, quality of life and LVEF had significantly improved in both DCM and ICM.

Cardiomyopathies represent a heterogeneous group of diseases that often lead to progressive heart failure with significant morbidity and mortality. Cardiomyopathies may be primary (i.e., genetic, mixed, or acquired) or secondary (e.g., infiltrative, toxic, inflammatory).[1] Classification was revised several times since the first use of the term *"cardiomyopathy"* in 1957, due to a rapid evolution of molecular genetics in cardiology and the emergence of ion channelopathies.[2] The American Heart Association Scientific Statement divided cardiomyopathies into 2 major groups: (1) primary cardiomyopathies and (2) secondary cardiomyopathies. This statement defines Primary cardiomyopathies (genetic, nongenetic or acquired) as those solely or predominantly confined to the heart muscle. Genetically caused primary cardiomyopathies include hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy/dysplasia, left ventricular noncompaction, conduction system disease and ion channelopathies.

Dilated cardiomyopathy (DCM) is classified as a mixed (genetic and nongenetic) primary cardiomyopathy. DCM is characterized by ventricular chamber enlargement and systolic dysfunction with normal left ventricular wall thickness. DCM leads to progressive heart failure and a decline in left ventricular function, ventricular and supraventricular arrhythmias, conduction system abnormalities, thromboembolism, and sudden or heart failure-related death. Indeed, DCM is a common and largely irreversible form of heart muscle disease. It is the most frequent cause of heart transplantation. DCM may manifest clinically at a wide range of ages (most commonly in the third or fourth decade but also in young children) and usually is identified when associated with severe limiting symptoms and disability. Myocarditis (inflammatory cardiomyopathy), stress (*"tako-tsubo"*) cardiomyopathy and peripartum (postpartum) cardiomyopathy are examples of acquired primary cardiomyopathies. Secondary cardiomyopathies show pathological myocardial involvement as part of a large number and variety of generalized systemic (multiorgan) disorders such as amyloidosis, endomyocardial fibrosis and systemic lupus erythematosis. Coronary artery disease is one of the most frequent causes of heart failure (HF).[3] There is no uniform definition for ischemic cardiomyopathy (ICM). Felker GM, et al., in 2002 proposed a new definition of ischemic cardiomyopathy that reclassifies patients with single-vessel disease as nonischemic unless they have left main or proximal left anterior descending disease or a history of revascularization or myocardial infarction.[4] Despite improvements with medical treatment, the prognosis of patients with end-stage HF remains poor.[5] Myocardial viability assessments are useful tools for treatment selection. Patients with viable myocardium have a good prognosis after revascularization and medical treatment carries a higher risk of cardiac event.[6] Patients with severe ischemic cardiomyopathy (ICM) have high rates of adverse events associated with revascularization procedures. Reported perioperative mortality rates from coronary artery bypass grafting (CABG) in this patient subset range from 5% to 30%.[7] Furthermore, revascularization of nonviable myocardium has not proven to be beneficial in terms of either mortality benefit[8] or global left ventricular (LV) functional improvement.[9]

In this study, we only included patients with DCM and ICM. Although cardiomyopathy is asymptomatic in the early stages, symptoms are the same as those characteristically seen in any type of heart failure and may include shortness of breath, fatigue, cough, orthopnea, paroxysmal nocturnal dyspnea, and edema. Diagnostic studies include B-type natriuretic peptide levels, baseline serum chemistries, electrocardiography, and echocardiography.[1]

Heart transplantation is currently the best treatment option for end-stage cardiomyopathy; however it is limited by a shortage of donors.

Stem cell therapy is a rapidly growing new field that promises to improve health and quality of life by repairing or regenerating cells, tissues or organs. The angiogenic cell precursors (ACPs) used in this study were generated from autologous peripheral blood. ACPs represent a heterogenic stem/progenitor cell population of hematopoietic cells that can potentially differentiate in vivo in response to tissue signals at the site of injection and lineage specific angiogenic precursors.[10] Animal experiments demonstrated the efficacy of these cells; there was a significant reduction of myocardial scarring and increased blood vessel density in the direct intramyocardial injected areas.[11] We have previously reported on the feasibility and safety of using these cells to treat cardiomyopathy.[12]

The objectives are to determine the safety and efficacy of intramyocardial angiogenic cell precursors (ACPs) injection for dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM) in a larger group of patients.

## Materials and Methods

### *The Patients*

Between May 2005 and February 2010, 126 consecutive patients underwent intramyocardial autologous peripheral blood stem cell injection. Fifty two were DCM and 74 were ICM. The study was approved by the Ethics Committee and Institutional Review Board (IRB). Informed consent was obtained before the procedure. Screening of severe contagious infections, HIV and hepatitis were done; only patients testing negative were confirmed for inclusion in the study. Malignancy within the preceding 3 years was also an exclusion criterion. All patients had a recent coronary angiogram (within 6 months) confirming the negative coronary artery disease status before the procedure. They all underwent pre-operative workups including routine chest x-ray, electrocardiography, and NT-ProB-type natriuretic peptide (NT-ProBNP). Echocardiography without stressing and/or cardiac MRI (CMR) was performed in all cases. CMR was conducted by using 3.0 Tesla MR Scanner (Achieva 3.0T systems with Philips Quasar Dual gradients, Philips Medical Systems, The Netherlands). The patients were excluded from the CMR study if they had contraindications such as metallic equipment implantation. They all undertook a six-minute walk test, New York Heart Association (NYHA) functional class evaluation and routine laboratory tests required for general anesthesia. Quality of life was evaluated at the preoperative and postoperative periods with Short Form-36 (SF-36, a multi-purpose, short-form health survey).

The SF-36 is a generic instrument consisting of 36 items or questions grouped into eight health-related aspects of the patient's life. As Ware et al explain it, *"there are eight main scales derived from the 36 questions: Physical functioning (10 items), role limitation due to physical function (4 items), role limitation due to emotional problems (3 items), social functioning (2 items), mental health (5 items), energy/vitality (4 items), pain (2 items), general health perception (5 items), and change in health (1 item). The scores for each dimension can vary from 0 -100; the higher the scores the better the quality of life"*.[13] The score of 50 was considered normal. The clinical characteristics of patients are summarized in Table 1.

*The cells*

The adult stem cells used in this study are *"Angiogenic Cell Precursors (ACPs)"* developed by VesCell technology (VesCell™ , TheraVitae Co. Ltd., Ness Ziona, Israel).[10] The ACPs are obtained from the patient's own blood, avoiding immunological concerns. Peripheral blood of 250 ml was collected from the patients using the same technique for general blood donation and sent for cell expansion. Blood cultures for aerobic and anaerobic bacteria were collected at the same time as blood collection and confirmed negative result during the process.

**Table 1.** The clinical characteristics of patients.

| Clinical characteristics | Dilated Cardiomyopathy (n = 52) | Ischemic Cardiomyopathy (n = 74) |
|---|---|---|
| Age (years) | 55.1 ± 13.0 | 62.1 ± 11.1 |
| Gender - Male | 38 (73.1%) | 71 (95.9%) |
| NYHA Functional Class (Mean ± SD) | 3.0 ± 0.6 | 2.8 ± 0.6 |
| Diabetes | 12 (23.1%) | 36 (48.6%) |
| Hypertension | 16 (30.8%) | 51 (68.9%) |
| Dyslipidemia | 22 (42.3%) | 61 (82.4%) |
| Renal Failure | 4 (7.7%) | 6 (8.1%) |
| Chronic pulmonary obstructive disease | 2 (3.8%) | 5 (6.8%) |
| Cerebrovascular accident | 7 (13.5%) | 12 (16.2%) |
| Pulmonary hypertension | 14 (26.9%) | 17 (23%) |
| AICD/CRTD Implantation | 37 (71.2%) | 32 (43.2%) |
| Mitral regurgitation | | |
| Trivial | 5 (10.2%) | 6 (8.6%) |
| Mild | 20 (40.8%) | 29 (41.4%) |
| Moderate | 13 (26.5%) | 29 (41.4%) |
| Severe | 6 (12.2%) | 1 (1.4%) |
| Distance walked in 6 mins. (meters) | 372.8 ± 102.1 | 362 ± 123.1 |
| Preoperative left ventricular ejection fraction (LVEF, %) | 22.0 ± 7.3 | 23.2 ± 7.7 |
| NT-ProBNP ($pg.ml^{-1}$) | 3512.9 ± 3027.0 | 3878.1 ± 4583.3 |

NYHA = New York Heart Association
AICD = Automatic implantable cardioverter defibrillator
CRTD = Cardiac resynchronization therapy with defibrillator
NT-ProBNP = NT-ProB-type natriuretic peptide

The multipotent progenitor cells were isolated from the blood, rich in CD45, $CD31^{Bright}$, $CD34^{+}$ $CD45^{-/Dim}$ and $CD34^{Bright}$ cells. The cells at a concentration of 1.5-3.0 x $10^{6}$ cells/ml were then cultured with vascular endothelial growth factor (VEGF, R&D Systems, Minneapolis, MN, USA) and 5 IU/ml heparin (Kamada, Beit-Kama, Israel). The process of cell expansion took 5 days. Number and viability of cells were checked and passed the following quality control before their use. The product consisting of at least 1.5 ± 0.5 million autologous endothelial progenitor cells that had been isolated from the patient's blood and then expanded ex vivo under sterile conditions was suspended in 15 ml sterile cell culture medium. Acceptable culture parameters as assessed by microscopy and flow cytometry were in accordance with the following specifications: Cell viability = 75% and Morphology-spindle-shaped, large cells forming long thread-like structures (Figure 1).

Sterility tests were performed according to 21 Code Federal Regulation (CFR) 610.12. Assessment of cell culture sterility was performed on a sample of the cell fraction supernatant or phosphate buffered saline (PBS) following cell washing. Interim negative sterility results of all samples taken at different stages of the culture were compulsory for the release of the final product. The Bacterial Endotoxin test was performed according to United States Pharmacopeia (USP) 23. The Limulus Amebocyte Lysate (LAL) test was performed on a sample of supernatant taken from the cell culture. Endotoxin levels below the acceptable limits were compulsory for the release of the final product. Gram stain was used as a rapid and qualitative method for assessing bacterial contamination of tissue culture samples. Negative results of the Gram stain performed on samples taken from the washing medium of cells before vialing was compulsory for the release of the final product. Mycoplasma contamination was tested and a negative result of the test was also obtained. The product phenotype was assayed by immune staining, as well as for angiogenic potential (tube formation assay) and cytokine secretion. All cell preparations complied with pre-defined release criteria of safety and potency.

*Immune Staining*

Cell samples were washed in PBS and resuspended in 100 ml of PBS, stained with specific fluorochrome-conjugated anti-human antibodies or isotype-matched non-specific controls, and incubated in the dark for 30 minutes on ice. The following antibodies were used for staining:, -CD31-PE or -CD31-FITC (IQP, Groningen, The Netherlands); anti-CD34-APC (BD Bioscience, Franklin Lakes, NJ, USA), -CD117-APC (DakoCytomation, Glostrup, Denmark); anti -CD133-PE, -CD144-FITC, -KDR-PE, -Tie-2-PE (R&D Systems, Minneapolis, MN), and anti-vWF -FITC (Chemicon, Temecula, CA). Ac-LDL uptake was measured by incubating the cells with 0.8 mg/ml Ac-LDL (Alexa Fluor488 AcLDL-Invitrogen, Carlsbad, CA, or Ac-LDL-DiI-Biomedical Technologies, Inc., Stoughton, MA) for 15 minutes at 37°C. Non-viable cells were excluded by 7-Amino-Actinomycin D (7-AAD, eBioscience, San Diego, CA, USA).

Cell suspension triplicates of five hundred thousand cells each were stained, assessed by FACS (FACSCalibur, Becton Dickinson), and analyzed by CellQuest Pro software (Becton Dickinson). The percentage of each marker was determined in each test tube and the mean and % Coefficient of Variance (% CV) was calculated for each one. The results were expressed as mean ± Standard Error (SE) of the percentage of stained cells. The number of stained cells was calculated by multiplying the number of harvested cells by the staining percentages obtained using the FACS.

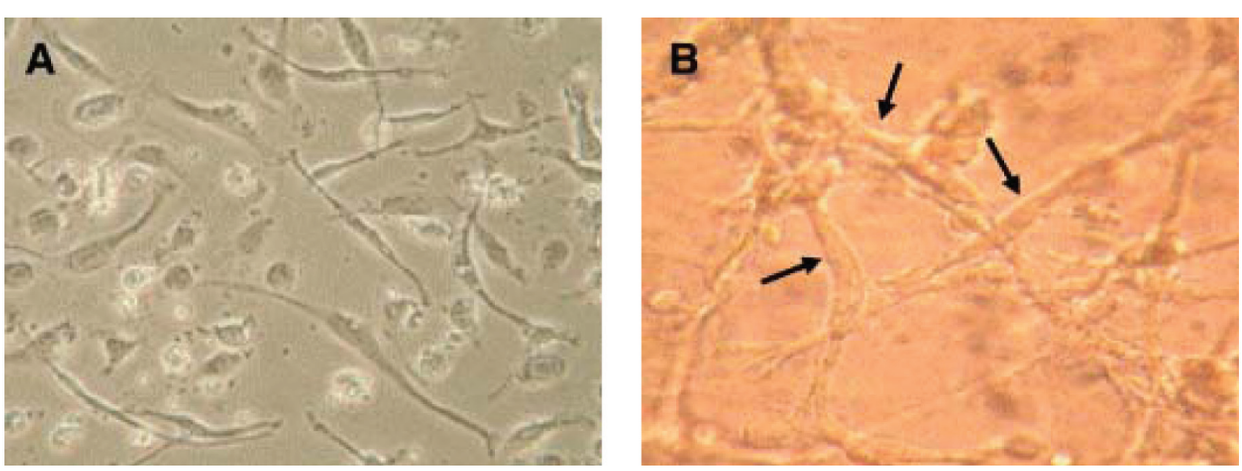


***Figure 1:*** *In Vitro Characteristics of angiogenic cell precursors (ACPs).*
*A. Photomicrographs illustrating the typical elongated, spindle-shaped morphology of cultured ACPs.*
*B. The angiogenic potential of ACPs indicated by organization into tube-like structures (arrows).*

*Tube Formation Assay*

The angiogenesis potential of the cells was measured by their ability to form three-dimensional tube-like structures according to a widely-used scale using an in vitro angiogenesis assay kit (Chemicon) and scoring under an inverted light microscope (Nikon ECLIPSE TS-100).[14]

*Analysis of Cytokine Secretion*

Samples of the harvested cells were washed in PBS and resuspended to one million in 1 ml X-vivo15 and grown for 24 hours in 24-well plates. Cytokine secretion in the supernatant was measured as compared to that of the medium only using flow cytometry and the BD™ CBA Human Angiogenesis Kit (Becton Dickinson).

Number of cells prior to injection was 46.1 ± 36.5 million (1.6 - 200) with 96.6 ± 3.7 % viability. Cells were injected into all areas of the left ventricle in the DCM. In the ICM patients, cells were injected into non-viable myocardium including interventricular septum and hypokinetic segments of the left ventricle.

*Surgical Techniques*

All DCM had intramyocardial cell injection alone by thoracoscopic technique or microthoracotomy. Most patients underwent microthoracotomy approach due to better exposure, control and shorter operative time than the thoracoscopic technique. For ICM, 49 (66.2%) had ACPs injection alone and 25 (33.8%) had combined CABG plus ACPs injection.

*Microthoracotomy for ACPs Injection*

Under general anesthesia with one-lung ventilation, the patient was placed in the right lateral decubitus position. A 10-cm incision was made in the left chest at the 5$^{th}$ intercostal space on the posterior axillary line. The chest cavity was examined and the pericardium was opened longitudinally anterior to the phrenic nerve. Pericardial traction stitches were placed appropriately to assist in reaching all regions of the left ventricular wall.

The cells were injected with the 23-guage butterfly needle with home-made guard. The needle was brought to the heart and then the injections were done manually outside the chest with the extension line. There were 30 sites of injections, 0.5 ml/ injection. Cells were injected into non-viable myocardium predetermined by CMR or myocardial nuclear scan including interventricular septum and hypokinetic segments. After adequate hemostasis the microthoracotmy was closed with small chest drainage left in the 7$^{th}$ intercostal space opening.

*Off-Pump coronary artery bypass grafting (OPCAB)*

OPCAB approach was carried out and can be summarized as follow. After 1 mg/kg of heparin was given, deep pericardial traction stitches were applied to verticalize the heart. The heart was then stabilized with an Octopus III or Octopus IV stabilizer (Medtronic, Inc., Minneapolis, MN 55432) without using any cardiac positioning device. The systemic systolic blood pressure in both groups was kept above 100 mmHg and central venous pressure/pulmonary artery diastolic pressure in the 20's to maintain adequate perfusion. All operative maneuvers were carried out in routine fashion.

Anastomoses was usually performed to the left anterior descending (LAD) and diagonal arteries first; or to the highest grade or totally obstructed arteries when the LAD system had less severe obstruction. After the measuring was completed the anastomosis was performed in the usual fashion using side to side anastomosis for sequential and end to side for distal end anastomosis with 4-8 interrupted stitches of 7-0 prolene. Intra-coronary shunt was used only in the large dominant right coronary artery or when patient was unstable or exhibited significant and persisting EKG changes after the occlusion. One right ventricular temporary epicardial pacing wire was inserted in these high risk patients. Protamine was given. Normothermia was maintained with sterile warm blanket throughout the procedure. The intramyocardial cell injections were performed after protamine was given.

*Statistical Analysis*

Statistical analyses were carried out with SPSS™ for Windows version 10.0 (SPSS Inc, Chicago, IL). Continuous variables are expressed as the mean ± SD unless otherwise indicated. The categorical data was reported as proportion. Paired T-test was used to compare the mean difference of LVEF, NYHA class, scores of quality of life and CMR parameters between pre and post treatments. Independent sample t-test was used to compare the mean difference of the LVEF between patients who underwent OPCAB plus intramyocardial cell injection and patients who underwent intramyocardial injection alone. A $p$ value of less than 0.05 was considered significant.

## Results

There was no new ventricular arrhythmia. 30-day mortality rate was 3.8% (2/52) and 4.1% (3/74) in DCM and ICM, respectively.

*New York Association Functional Class*

NYHA class improved from 3.0 ± 0.6 to 2.0 ± 0.9 at 485.8 ± 370.3 days ($p < 0.001$) in DCM and improved from 2.7 ± 0.6 to 1.9 ± 0.8 at 419.6 ± 345.5 days ($p < 0.001$) in ICM (Figure 2).

*Left Ventricular Ejection Fraction*

LVEF increased from 23.3 ± 7.0% to 27.7 ± 11.3% at 409.7 ± 352.4 days ($p = 0.03$) in DCM and increased from 23.6 ± 7.7% to 31.5 ± 10.0% at 400.6 ± 350.1 days ($p < 0.001$) in ICM (Figure 3). For the ICM, the LVEF was improved 11.4 ± 12.2% in patients who underwent OPCAB plus intramyocardial cell injection and LVEF was improved 5.7 ± 7.5% in patients who underwent intramyocardial cell injection alone. This was no statistically significant difference ($p = 0.056$).

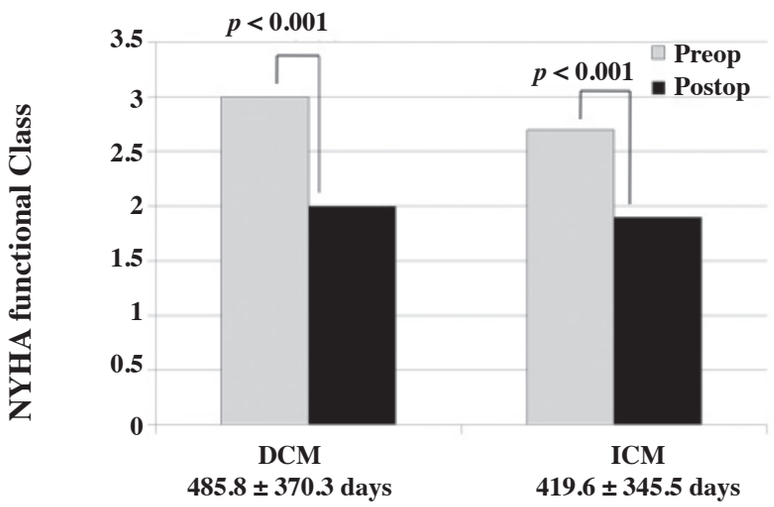


***Figure 2 :*** *New York Heart Association (NYHA) functional class: Preoperative and postoperative periods in dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM).*

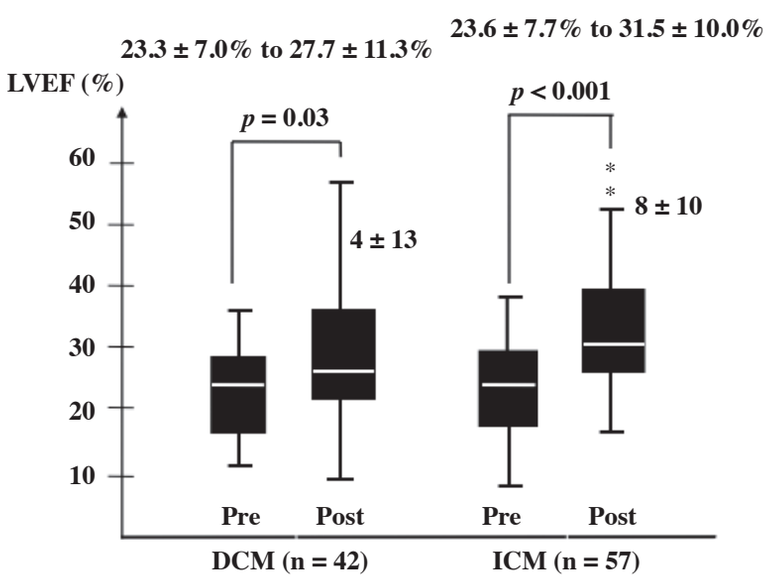


***Figure 3 :*** *Boxplot of the left ventricular ejection fraction: before (pre) and after (post) treatment in dilated cardiomyopathy (DCM) and ischemic cardiomyopathy (ICM). The lower and upper edges of the "box" demonstrate the first and third quartile of the data (50% of the data lie within the box). The "median" is shown as a line inside the box. The ends of the vertical lines or "whiskers" indicate the minimum and maximum values, unless outliers are present in which case the whiskers extend to a maximum of 1.5 times the inter-quartile range. *represents outlier, LVEF = left ventricular ejection fraction.*

*Cardiac Magnetic Resonance Imaging*

A number of ICM had preoperative and postoperative CMR. The infarction volume, end diastolic volume and end systolic volume of the left ventricle were significantly reduced at the 474.1 ± 279.8 days follow-up (Table 2, Figure 4 ). Only a few patients in the DCM group had preoperative and postoperative CMR, therefore their results were not analyzed.

*Quality of life*

Quality of life postoperatively evaluated at 3 months has significantly improved for physical function, role-physical, general health and vitality domains ($p = 0.001$, 0.014, 0.001 and 0.002 respectively) in DCM. For ICM, physical function, role-physical, general health and social function domains ($p = 0.037$, 0.005, 0.001 and 0.026 respectively) were improved (Figure 5).

**Table 2.** Preoperative and postoperative cardiac MRI (CMR) results in ischemic cardiomyopathy (IMC) patients.

| CMR parameters | Preoperative | Postoperative | *p* value |
|---|---|---|---|
| Infarction volume (%) | 39.8 ± 29.3 | 25.9 ± 21.1 | 0.03 |
| End-diastolic volume (ml) | 269.5 ± 66.5 | 232.0 ± 77.9 | 0.03 |
| End-systolic volume (ml) | 211.5 ± 65.6 | 173.0 ± 72.0 | 0.008 |
| Stroke volume (ml) | 56.9 ± 24.4 | 59.0 ± 23.4 | 0.7 |
| Cardiac output (l/min) | 4.5 ± 1.9 | 3.9 ± 1.4 | 0.2 |
| Left ventricular mass (g) | 156.9 ± 45.6 | 164.1 ± 40.6 | 0.3 |

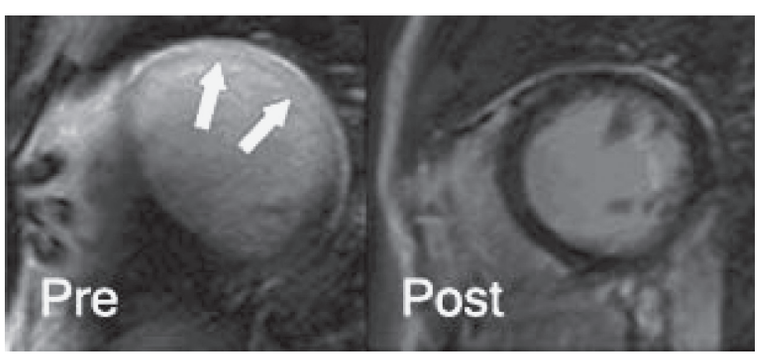


***Figure 4:*** *Cardiac magnetic resonance imaging with gadolinium contrast;* ***Pre:*** *infarction seen as a bright signal (arrows) at the left ventricular anterior and lateral walls, before stem cell transplant. Left ventricular ejection fraction was 13.2% with end-diastolic volume of 296 ml.* ***Post:*** *3 months after stem cell transplant, there was no myocardial scar and ejection fraction was 20% with end-diastolic volume of 268 ml.*

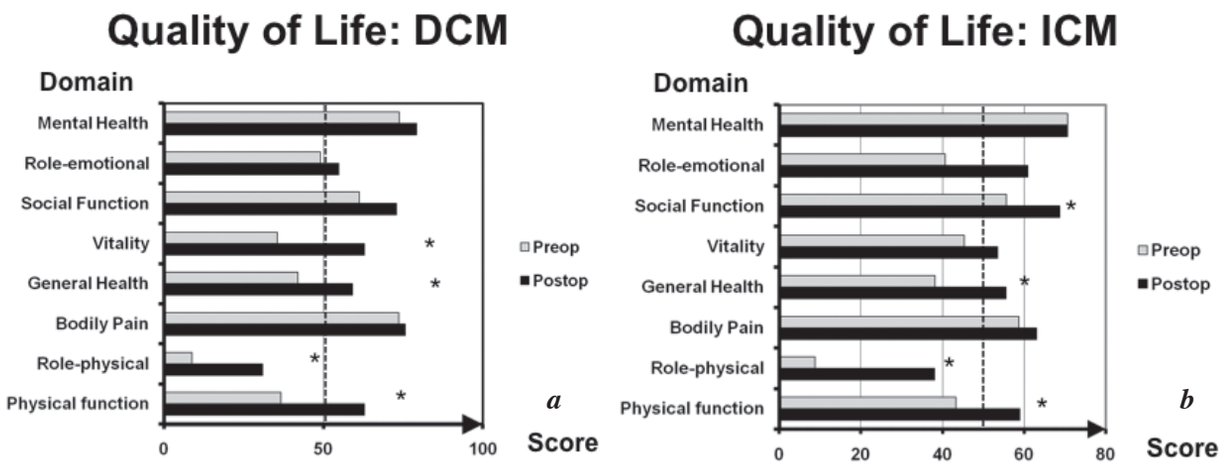


***Figure 5:*** *Quality of life evaluated by the Short Form 36 at 3 months follow-up.*
*a. Dilated cardiomyopathy (DCM). b. Ischemic cardiomyopathy (ICM).*
** p value < 0.05*

## Discussion

In the present study, we have demonstrated the safety of the intramyocardial peripheral blood stem cell so called *"angiogenic cell precursor (ACP)"* transplantation in patients with DCM and ICM. The 30-day mortality was about 4%. With increasing clinical experience, mortality could be lowered in both groups. The efficacy of the cell injections was demonstrated by comparing postoperative and preoperative NYHA functional class, LVEF, CMR parameters (ICM) and quality of life. NYHA functional class, LVEF and half of the domains in quality of life questionnaire were significantly improved in both DCM and ICM. Infarction volume, end diastolic volume and end systolic volume were significantly improved in the ICM patients demonstrated reverse remodeling of the left ventricle after the intramyocardial ACPs injection.

Although this study was a nonrandomized study, it did include all spectrums of the common types of heart failure patients. The data were prospectively collected and followed-up. The intention of this report is to give results of a larger group of patients than in previous reports. We did not have control group in this analysis. However we had reported our case-match studies for both DCM and ICM previously. Those patients who had undergone intramyocardial ACPs injection tended to have improvement in NYHA functional class and LVEF unlike the controls.[15, 16]

The research results on outcomes of stem cell injection for DCM were limited.[17-22] Roura S, et al., demonstrated defective vascularization and impaired vasculogenesis (the de novo vascular organization of mobilized endothelial progenitors) and angiogenesis in dilated cardiomyopathy patients. The defective vascularization was associated with reduced myocardial expression of vascular β-catenin, an important angiogenic regulator.[23] Werner L, et al., also showed that endothelial progenitor cell transfer is effective in attenuating myocardial damage in a model of dilated cardiomyopathy.[24] Chu VF, et al., demonstrated an angiogenesis effect of the mechanical puncture of myocardium. However, this was controversial.[25] These are the rationale used for intramyocardial ACPs injection in our study. Vrtovec B, et al., investigated the effects of intracoronary transplantation of $CD34^+$ cells in patients with DCM. Twenty eight patients were randomized to have intracoronary peripheral blood $CD34^+$ cells injection which were mobilized by granulocyte-colony stimulating factor and collected via apheresis compared with control. At one year, intracoronary peripheral blood $CD34^+$ cells injection was associated with an increase in LVEF (from 25.5 ± 7.5% to 30.1 ± 6.7%; $p = 0.03$), an increase in 6-minute walk distance and a decrease in NT-proBNP.[26]

Stem cells for treatment of myocardial infarction and ICM that are under-investigated clinically are mainly hematopoietic stem cells. The skeletal myoblasts injection failed to show an improvement of LVEF compared with controls in the randomized placebo-controlled myoblast autologous grafting in ICM patients (MAGIC trial).[27] There was also increased number of early postoperative arrhythmic events after myoblast implantation. The bone-marrow or peripheral blood derived stem cells have however shown positive in other clinical trials in both acute and chronic myocardial ischemia/infarction.[28-36] The range of improvement of the LVEF was 2.5-15 percentage points. There have also been some negative

studies in terms of LVEF.[37] The reasons for the disparity in results may be due to the differences in types of cells, e.g., CD34+, CD 133+ or unselected bone-marrow stem cells, dosage of cells, type of patients (acute or chronic myocardial ischemia) and delivery methods.

The advantages of using autologous peripheral blood derived stem cells in our study are as follows. Autologous cells (1) create no immunologic concern, (2) are easily harvested via blood donation, (3) have no systemic effect during the blood collection for progenitor cell selection and expansion,[38] (4) the cell populations harvested are not in the early phase of development, thus there are no tumor formation issues, and (5) ability of repeated procedure. However, the disadvantages are: (1) the cells may not be as potent as other embryonic or pluripotent stem cells to repair all the damaged areas, (2) cells had limited self-renewal process, therefore the improvement may not last forever, and (3) there was the limitation of patients with blood-borne infections such as hepatitis or patients with chronic immunosuppressant. In this current study, the reasons we delivered the cells by direct intramyocardial injection are: (1) intramyocardial injection is a simple method, (2) the target area of injection can be seen directly, (3) cell retention after implantation is maximized compared with transcatheter coronary artery infusion or retrograde coronary venous infusion,[39] and (4) do not effect coronary stents.

The proposed mechanisms of intramyocardial ACPs injection are paracrine effect, homing signal and possible transdifferentiation of ACPs to cardiomyocytes. Proving this in clinical trials has been difficult, although it was supported by basic science research.[40-42] The future researches are many: (1) the explorations of new types of cells, e.g., resident cardiac progenitor cell,[43] umbilical cord blood stem cells,[44] induced pluripotent stem cells[45] or combined stem cells, (2) non-invasive in vivo cell tracking,[46, 47] (3) pharmacologic manipulation[48] or combined gene therapy[49-51] and (4) heart tissue engineering.

*Limitation of the study*

This was not a randomized study. The follow-up was limited by the cardiologists and some patients came from overseas therefore they could not come for follow-up cardiac MRI. However, we received echocardiogram, NYHA functional class results and quality of life questionnaire from them.

## Conclusions

Intramyocardial ACPs injection is feasible and safe in both DCM and ICM. The NYHA, quality of life and LVEF had significantly improved in both DCM and ICM. The cardiac MRI in ICM demonstrated a reversed remodeling of the left ventricle. Large-scale placebo-controlled studies are needed to confirm that use of intramyocardial ACPs injection in cardiomyopathy is efficacious.

***Acknowledgement***

The authors thank Vibul Jotisakulratana, MD, Vitoon Pitiguagool, MD, Sujit Banyatpiyaphod, MD, Piyapan Pamornsing, MD, Chockchai Suwanakijboriharn, MD, Sawat Asavapiyanond, MD, Chayanin Vatcharasiritham, MD for their assistance with the surgery.

***Disclosures and Freedom of Investigation***

The *"Angiogenic Cell Precursors (ACPs)"* developed by TheraVitae Co. Ltd. Professor Michael Belkin was in the past an board member, a minor shareholder, and received a consulting fee from TheraVitae Co. However, the authors had full control of the study, methods used, outcome measurements, data analysis, and production of the written report.

**References**

1. Wexler RK, Elton T, Pleister A, et al. Cardiomyopathy: an overview. *Am Fam Physician* 2009;79:778-84.
2. Maron BJ, Towbin JA, Thiene G, et al. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. *Circulation* 2006;113:1807-16.
3. Aronow WS. Epidemiology, pathophysiology, prognosis, and treatment of systolic and diastolic heart failure. *Cardiol Rev* 2006;14:108-24.
4. Felker GM, Shaw LK, O'Connor CM. A standardized definition of ischemic cardiomyopathy for use in clinical research. *J Am Coll Cardiol* 2002;39:210-8.
5. Roger VL, Weston SA, Redfield MM, et al. Trends in heart failure incidence and survival in a community-based population. *Jama* 2004;292:344-50.
6. Meluzin J, Cerny J, Frelich M, et al. Prognostic value of the amount of dysfunctional but viable myocardium in revascularized patients with coronary artery disease and left ventricular dysfunction. Investigators of this Multicenter Study. *J Am Coll Cardiol* 1998;32:912-20.
7. Baker DW, Jones R, Hodges J, et al. Management of heart failure. III. The role of revascularization in the treatment of patients with moderate or severe left ventricular systolic dysfunction. *Jama* 1994;272:1528-34.
8. Allman KC, Shaw LJ, Hachamovitch R, et al. Myocardial viability testing and impact of revascularization on prognosis in patients with coronary artery disease and

left ventricular dysfunction: a meta-analysis. *J Am Coll Cardiol* 2002;39:1151-8.
9. Ragosta M, Beller GA, Watson DD, et al. Quantitative planar rest-redistribution 201Tl imaging in detection of myocardial viability and prediction of improvement in left ventricular function after coronary bypass surgery in patients with severely depressed left ventricular function. *Circulation* 1993;87:1630-41.
10. Porat Y, Porozov S, Belkin D, et al. Isolation of an adult blood-derived progenitor cell population capable of differentiation into angiogenic, myocardial and neural lineages. *Br J Haematol* 2006; 135:703-14.
11. Sun Z, Wu J, Fujii H, et al. Human angiogenic cell precursors restore function in the infarcted rat heart: a comparison of cell delivery routes. *Eur J Heart Fail* 2008; 10:525-33.
12. Arom KV, Ruengsakulrach P, Jotisakulratana V. Intramyocardial angiogenic cell precursor injection for cardiomyopathy. *Asian Cardiovasc Thorac Ann* 2008; 16:143-8.
13. Ware J, Kosinski M, Keller SD. A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. *Med Care* 1996; 34:220-33.
14. Kayisli UA, Luk J, Guzeloglu-Kayisli O, et al. Regulation of angiogenic activity of human endometrial endothelial cells in culture by ovarian steroids. *J Clin Endocrinol Metab* 2004;89:5794-802.
15. Arom K, Ruengsakulrach P, Jotisakulratana V. Efficacy of Intramyocardial Injection of Angiogenic Cell Precursors for Ischemic Cardiomyopathy: A Case Match Study. *Innovations* 2008;3:38-45.
16. Arom KV, Ruengsakulrach P, Belkin M, Tiensuwan M: Intramyocardial angiogenic cell precursors in nonischemic dilated cardiomyopathy. *Asian Cardiovasc Thorac Ann* 2009;17:382-8.
17. Sant'anna RT, Kalil RA, Pretto Neto AS, et al. Global contractility increment in nonischemic dilated cardiomyopathy after free wall-only intramyocardial injection of autologous bone marrow mononuclear cells: an insight over stem cells clinical mechanism of action. *Cell Transplant* 2010;19:959-64.
18. Olgunturk R, Kula S, Sucak GT, et al. Peripheric stem cell transplantation in children with dilated cardiomyopathy: Preliminary report of first two cases. *Pediatr Transplant* 2010;14:257-60.
19. Seth S, Narang R, Bhargava B, et al. Percutaneous intrcoronary cellular cardiomyoplasty for nonischemic cardiomyopathy: clinical and histopathological results: the first-in-man ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) trial. *J Am Coll Cardiol* 2006;48:2350-51.
20. Fischer-Rasokat U, Assmus B, Seeger FH, et al. A pilot trial to assess potential effects of selective intracoronary bone marrow-derived progenitor cell infusion in patients with nonischemic dilated cardiomyopathy: final 1-year results of the transplantation of progenitor cells and functional regeneration enhancement pilot trial in patients with nonischemic dilated cardiomyopathy. *Circ Heart Fail* 2009;2:417-23.
21. Wang JA, Xie XJ, He H, et al. A prospective, randomized, controlled trial of autologous mesenchymal stem cells transplantation for dilated cardiomyopathy. *Zhonghua Xin Xue Guan Bing Za Zhi* 2006;34:107-10.
22. Chen Y, Gao EM, Gao CY, et al. Effects of intracoronary autologous bone marrow mononuclear cells transplantation in patients with dilated cardiomyopathy. *Zhonghua Xin Xue Guan Bing Za Zhi* 2008;36:1087-91.
23. Roura S, Planas F, Prat-Vidal C, et al. Idiopathic dilated cardiomyopathy exhibits defective vascularization and vessel formation. *Eur J Heart Fail* 2007;9:995-02.
24. Werner L, Deutsch V, Barshack I, et al. Transfer of endothelial progenitor cells improves myocardial performance in rats with dilated cardiomyopathy induced following experimental myocarditis. *J Mol Cell Cardiol* 2005;39:691-7.
25. Chu VF, Giaid A, Kuang JQ, et al. Thoracic Surgery Directors Association Award. Angiogenesis in transmyocardial revascularization: comparison of laser versus mechanical punctures. *Ann Thorac Surg* 1999;68:301-7; discussion 307-8.
26. Vrtovec B, Poglajen G, Sever M, et al. Effects of intracoronary stem cell transplantation in patients with dilated cardiomyopathy. *J Card Fail* 2011;17:272-81.
27. Menasche P, Alfieri O, Janssens S, et al. The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 2008; 117:1189-200.
28. Pompilio G, Cannata A, Peccatori F, et al. Autologous peripheral blood stem cell transplantation for myocardial regeneration: a novel strategy for cell collection and surgical injection. *Ann Thorac Surg* 2004;78:1808-12.
29. Schachinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol* 2004; 44:1690-9.
30. Meyer GP, Wollert KC, Lotz J, et al. 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* 2006;113:1287-94.
31. Gao LR, Wang ZG, Zhu ZM, et al. Effect of intracoronary transplantation of autologous bone marrow-derived mononuclear cells on outcomes of patients with refractory chronic heart failure secondary to ischemic cardiomyopathy. *Am J Cardiol* 2006;98:597-602.
32. Perin EC, Dohmann HF, Borojevic R, et al. Improved exercise capacity and ischemia 6 and 12 months after transendocardial injection of autologous bone marrow mononuclear cells for ischemic cardiomyopathy. *Circu lation* 2004;110(11 Suppl 1):II213-8.
33. Patel AN, Geffner L, Vina RF, et al. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J Thorac Cardiovasc Surg* 2005;130:1631-8.
34. Stamm C, Kleine HD, Choi YH, et al. Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies. *J Thorac Cardiovasc Surg* 2007;133:717-25.
35. Strauer BE, Brehm M, Zeus T, et al. Regeneration of human infarcted heart muscle by intracoronary autologous bone marrow cell transplantation in chronic

coronary artery disease: the IACT Study. *J Am Coll Cardiol* 2005;46:1651-8.
36. Losordo DW, Schatz RA, White CJ, et al. Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. *Circulation* 2007;115:3165-72.
37. Abdel-Latif A, Bolli R, Tleyjeh IM, et al. Adult bone marrow-derived cells for cardiac repair: a systematic review and meta-analysis. *Arch Intern Med* 2007; 167:989-97.
38. Kang HJ, Kim HS, Zhang SY, et al. Effects of intracoronary infusion of peripheral blood stem-cells mobilised with granulocyte-colony stimulating factor on left ventricular systolic function and restenosis after coronary stenting in myocardial infarction: the MAGIC cell randomised clinical trial. *Lancet* 2004;363:751-6.
39. Hou D, Youssef EA, Brinton TJ, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. *Circulation* 2005;112 (9 Suppl):I150-6.
40. Zhang S, Wang D, Estrov Z, et al. Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34-positive cells into cardiomyocytes in vivo. *Circulation* 2004;110:3803-7.
41. Schenk S, Mal N, Finan A, et al. Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. *Stem Cells* 2007;25:245-51.
42. Badorff C, Dimmeler S. Neovascularization and cardiac repair by bone marrow-derived stem cells. *Handb Exp Pharmacol* 2006;174:283-93.
43. Ott HC, Matthiesen TS, Brechtken J, et al. The adult human heart as a source for stem cells: repair strategies with embryonic-like progenitor cells. *Nat Clin Pract Cardiovasc Med* 2007;4 (Suppl 1):27-39.
44. Henning RJ, Burgos JD, Vasko M, et al. Human cord blood cells and myocardial infarction: effect of dose and route of administration on infarct size. *Cell Transplant* 2007;16:907-17.
45. Nakagawa M, Koyanagi M, Tanabe K, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol* 2008;26:101-6.
46. Ebert SN, Taylor DG, Nguyen HL, et al. Noninvasive tracking of cardiac embryonic stem cells in vivo using magnetic resonance imaging techniques. *Stem Cells* 2007;25:2936-44.
47. Doyle B, Kemp BJ, Chareonthaitawee P, et al. Dynamic tracking during intracoronary injection of 18F-FDG-labeled progenitor cell therapy for acute myocardial infarction. *J Nucl Med* 2007;48:1708-14.
48. Besler C, Doerries C, Giannotti G, et al. Pharmacological approaches to improve endothelial repair mechanisms. *Expert Rev Cardiovasc Ther* 2008;6:1071-82.
49. Kawamoto A, Murayama T, Kusano K, et al. Synergistic effect of bone marrow mobilization and vascular endothelial growth factor-2 gene therapy in myocardial ischemia. *Circulation* 2004;110:1398-405.
50. Cheng Z, Ou L, Zhou X, et al. Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. *Mol Ther* 2008;16:571-9.
51. Shujia J, Haider HK, Idris NM, et al. Stable therapeutic effects of mesenchymal stem cell-based multiple gene delivery for cardiac repair. *Cardiovasc Res* 2008;77:525-33.