Int J Clin Exp Pathol 2016;9(3):2915-2926
www.ijcep.com /ISSN:1936-2625/IJCEP0020868

Original Article

Intramyocardial injection of heart-derived extracellular matrix combined with cardiosphere-derived cells improves myocardial structure and function in rats with acute myocardial infarction

Da-Qing Jiang, Tian-Xiang Gu, Guang-Wei Zhang, Shuang Liu, Xue-Yuan Li

Departments of Cardiac Surgery, Cardiovascular Ultrasound and Cardiology, The First Hospital of China Medical University, Shenyang, China

Received November 30, 2015; Accepted January 31, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: This study aimed to investigate the cardioprotective effects of intramyocardial injection of heart derived extracellular matrix (ECM) and cardiosphere-derived cells (CDCs) in a rat model of myocardial infarction (MI). ECM and CDCs were collected from the rat heart, and the proliferation, apoptosis and morphology of CDCs maintained on ECM were characterized *in vitro*. MI model was established in rats (n=24) and intramyocardially injected (10^6/150 μl). When delivered into infarcted rat hearts, ECM solution proceeded with CDCs showed best cardiac structural and functional benefits, CDCs differentiation, retention and survival benefits as compared to ECM, CDC and vehicle alone at 3 weeks after acute myocardial infarction. There were similar benefits between ECM and CDC groups. Heart-derived ECM may promote CDCs to differentiate toward endothelial cells, cardiomyocytes and smooth muscle cells both *in vitro* and *in vivo*, increase the proliferation *in vitro*, improve the survival *in vitro* and suppress the apoptosis of CDCs *in vivo*. Intramyocardial transplantation of CDCs+ECM significantly improves the cardiac structure and function as compared to CDC or ECM alone in MI rats.

Keywords: Myocardial infarction, cardiosphere-derived cell transplantation, heart-derived extracellular matrix, cardiac function

Introduction

Myocardial infarction (MI) may lead to the permanent loss of cardiac tissues and ultimately cause heart failure. Despite the improvement of pharmacological and invasive treatments, the mortality and morbidity remain high. However, current therapies only delay the progression of MI. The patients who survive from MIs without prompt reperfusion may develop large scars and severe impairment of left ventricular (LV) function. Thus, it is imperative to develop new therapies to regenerate the damaged hearts and then improve the prognosis of MI patients.

Treatment of MI with cardiac tissue engineering and biomaterials rely mainly on the use of synthetic or biological matrix materials to reconstitute contractile myocardium-like tissues and support the injured heart. Among the biomaterials, injectable biomaterials are particularly attractive as their delivery is minimally-invasive [1]. Dai et al [2] found that treatment with heart-derived decellularized matrix could thicken the LV wall, prevents paradoxical LV systolic bulging, and improves LV EF after MI in rats.

Cardiosphere-derived cells (CDCs) are a heart-derived cell population rich in cardiac progenitor cells and belong to the supporting cells. They are able to self-renew and differentiate into cardiac specific cells such as cardiomyocytes and vascular cells, either *in vivo* and *in vitro* [3]. They can promote myocardial regeneration and improve heart function in a mouse model of MI [4]. Allogeneic CDCs transplantation without immunosuppression is safe, and

can promote the cardiac regeneration and improve cardiac function in a rat MI model [5]. Malliaras et al also revealed that allogeneic CDCs attenuated myocardial remodeling, improved global and regional function, decreased scar size, and increased viable myocardium at 2 months post-treatment in a Yucatan minipigs model of MI [6]. CDCs from advanced heart failure patients exhibit augmented potency in ameliorating ventricular dysfunction post-MI [7]. However, the retention and long-term survival of transplanted cells are limited within the injured myocardium. The washout and squeezing out of transplanted cells may contribute to the low survival rate within the host myocardium [8, 9]. Injectable biomaterials can be used as a delivery vehicle for the cell transplantation to increase the retention and survival of transplanted cells [10-13].

On the basis of above findings from preclinical studies and highly encouraging results from trials autologous transplantation [14, 15], two ongoing clinical trials are conducted with allogeneic heart-derived cellular products (ALLSTAR, CAREMI) for acute MI. The preliminary results from a trial on the allogeneic cardiac-derived stem cells are promising.

However, whether the addition of biomaterials may improve the therapeutic efficacy of CDCs is still poorly understood. In the present study, the CDCs seeded on heart-derived ECM were intramyocardially injected in acute MI rats and the cardioprotection was evaluated. Our results showed this treatment could promote the proliferation and differentiation of CDCs *in vitro* and *in vivo*, thicken the LV wall, improve the LV function and increase the retention and long-term survival of transplanted CDC in the myocardium of MI rats.

Materials and methods

The whole protocol was approved by the Liaoning Administrative Committee for Laboratory Animals and performed in accordance with the "Guide for the Care and Use of Laboratory Animals" of the National Institutes of Health in 2011.

Isolation and culture of CDCs from rats

CDCs were collected from the heart of WKY male rats (n=) aged 8 weeks according to the previously described [4]. In brief, rats were intraperitoneally anesthetized with ketamine at 75 mg/kg and xylazine at 5 mg/kg. Under an aseptic condition, the heart was separated, harvested, washed with Dulbecco's phosphate buffered saline (DPBS; Invitrogen) and cut into pieces in 0.05% trypsin-EDTA (Invitrogen). Heart tissues were plated on the fibronectin-coated dishes containing 1.5 ml of complete explant medium (CEM), Iscove's modified Dulbecco's medium (IMDM; Invitrogen) supplemented with 20% fetal bovine serum (FBS; Invitrogen), 1 U/ml penicillin, 1 μg/ml streptomycin and 0.2 mM L-glutamine (Gibco). After incubation in 5% CO_2 at 37°C for 3-4 days, small, round and phase bright cells grew out from the explants over a bed of stromal-like cells. Once they reached 80-90% confluence, these explant-derived cells (EDCs) were collected using trypsin and plated on poly-D-lysine coated 12-well plates at a density of 50000 cells/well in cardiosphere growth medium (CGM) containing 65% Dulbecco's modified eagle medium (DMEM/F12), 35% IMDM, 10% FBS, 2% B27 (Invitrogen), 25 ng/ml cardiotrophin (Peprotech EC), 10 ng/ml epidermal growth factor (EGF; Peprotech EC), 20 ng/ml basic fibroblast growth factor (FGF; Promega) and 5 U of thrombin (Sigma). EDCs were harvested once every 7 days for 4 weeks. Cardiospheres formed after 2-3 day culture. They were harvested by mechanical trituration and plated in CEM in fibronectin or rat heart-derived ECM coated flasks. Cardiosphere-derived cells (CDCs) were passaged once every 3 days.

Preparation of rat heart-derived decellularized ECM

Rat heart-derived decellurized ECM was collected from the heart of female WKY rats according to previously reported [2]. Briefly, WKY female rats were intraperitoneally anesthetized ketamine at 75 mg/kg and xylazine at 5 mg/kg. Under an aseptic condition, the heart was harvested and the adipose tissues were removed. The heart was cut into 1-mm sections. Sections were placed in a 50-mL conical tube and rinsed in Dulbecco phosphate-buffered saline (PBS). Heart tissues were thoroughly homogenized with T10 basic ULTRA-TURRAX Disperser (IKA, Wilmington, North Carolina) in a 3.4 mol/L sodium chloride (NaCl) followed by

three 30-minute treatments in 3.4 mol/L NaCl. For decellularization, heart tissues were treated with PBS nuclease solution (50 U/mL DNAse and 10 mg/mL RNAse) for 1 h to remove residual nucleic. After 1-h treatment in 1% Triton X-100, the heart tissues were washed in PBS thrice (1 h for each). The washing and other procedures were conducted at 4°C with a constant shaking (130 rpm). Between two procedures, samples were centrifuged at 3000 rpm for 10 min, the supernatant was removed and the ECM was left in pellets. The heart ECM pellets were stored at -80°C until use. ECM was subjected to HE staining and observed under a light microscope.

CDC labeling

Rat CDCs of passage 2 were labeled with CellTrackerTM CM-DiI (C7000; Life Invitrogen) following the manufacturer's instructions on the day prior to transplantation. Briefly, 2 mg/ml stock solution was diluted with Dulbecco's phosphate-buffered saline (D-PBS). Then, 1.5-2.0 ml of 2 μM working solution was added into 60-mmdishespre-coated with FN, the cells were incubated in the working solution for 5 min at 37°C, and then for 15 min at 4°C. After labeling, cells were washed with PBS and resuspend in fresh medium or ECM solution.

Immunostaining of CDCs for c-kit

Rat CDCs of passage 2 were fixed with 4% PFA, and then stained with rabbit anti-rat c-kit (Santa Cruz) and FITC conjugated secondary antibodies (Abcam). Images were captured under a Leica TCS SP5 X confocal microscope.

Detection of proliferation, apoptosis and differentiation of CDCs on ECM vs. FN pre-coated 96-well plates or dishes

Rat CDCs were cultured on ECM or FN pre-coated 96-well plate and their proliferation was detected at 12 h, 1 d, 3 d, 7 d and 14 d with MTT assay. Cells at different time points were stained with Annexin V-FITC Apoptosis Detection Kit and apoptosis was measured by FACSCAN flow cytometry (Becton Dickison Company of USA). Rat CDCs were cultured on ECM or FN pre-coated 12-well plate for 14 days fixed in 4% PFA, and then treated with following antibodies: rabbit anti-rat von Willebrand factor (Abcam), rabbit anti-rat smooth muscle actin (Abcam), mouse anti-rat alpha sarcomeric actin (Abcam). The PE conjugated secondary antibodies were purchased from Abcam. Images were captured under a Leica TCS SP5 X confocal microscope.

Establishment of MI model, cell injection and cardiac function evaluation

WKY female rats aged 8 weeks were intraperitoneally anesthetized with ketamine at 75 mg/kg and xylazine 5 mg/kg, in tubated, and mechanically ventilated with room air. Under an aseptic condition, the hearts was separated after thoracotomy, and permanent ligation of the left anterior descending coronary artery was conducted. ECM and CDCs derived from WKY rats were then intramyocardially injected into the injured hearts with a 29-gauge needle at 4 sites of the peri-infarct region: CDCs (10^6 cells in 150 μl IMDM; n=6; IC group), medium (150 μl IMDM; n=6; I group), CDCs+ECM (10^6 cells in 150 μl of ECM; n=6; EC group) and ECM (150 μl of ECM; n=6; E group). Buprenex (0.001 mg/100 g body weight) was subcutaneously injected twice daily for 2 d for postoperative analgesia.

Echocardiography was performed in all the rats before surgery (baseline) and at 3 weeks after MI. Left ventricular posterior wall thickness (LVPW), LV end diastolic volume (LVEDV), LV end systolic volume (LVESV), left ventricular ejection fraction (IVEF), fractional shortening (FS%), left ventricular internal diameter in diastolic phase (LVIDd), and left ventricular internal diameter in systolic phase (LVIDs) were measured by small animal echocardiography (GE Vivid 7 Dimension ultrasound apparatus, M12S PRO, probe frequency range of 4-12 MHz).

Histological analysis

At 3 weeks after AMI, the heart (n=24; 6 hearts from each group) was harvested, cut into basal and apical halves and frozen in OCT (Tissue-Tek) over dry ice. Frozen tissues were cut into 10-μm sections (6 sections per heart with an inter-section distance of 100 μm) and stained with Masson's trichrome. The infarct border [16] was delineated and the integrated optical density (IOD) of positive staining area was measured using the Image Pro Plus 5.0 (Media

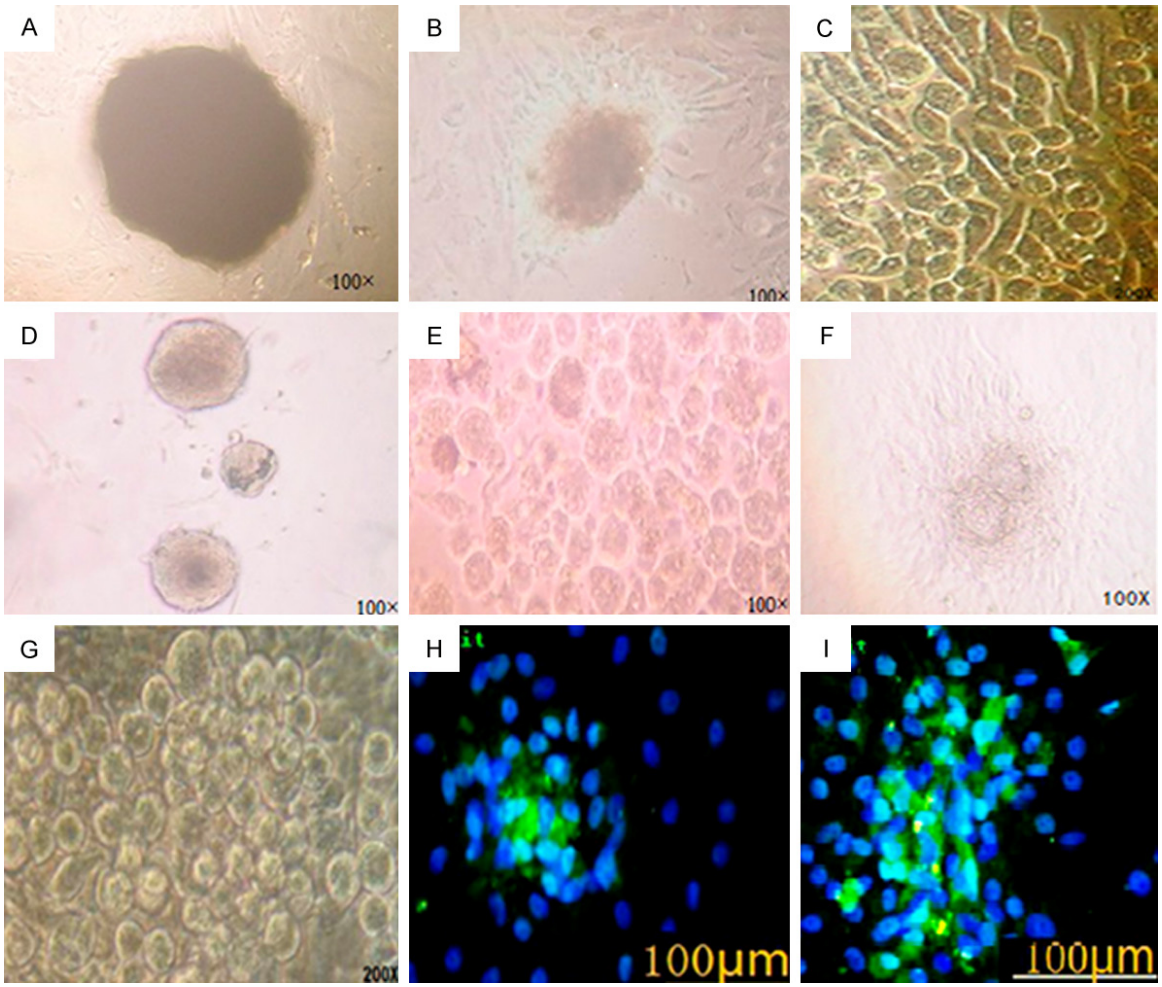

Figure 1. A-C. Small, round and phase bright cells grew out from the explants over a bed of stromal-like cells. D. Cardiospheres formed after 2-3 days. E. Cells were harvested on day 7, cardiospheres in 3 wells of 12-well plate were plated into a 60-mm dish pre-coated with FN or ECM; F. CDCs grew from cardiosphere on day 3; G. CDCs on day 5-6 of passage 2; H. CDCs expressing c-kit on day 3 before transplantation; I. CDCs expressing c-kit on day 6 before transplantation.

Cybernetics Inc, Bethesda, Md) image analysis software.

Heart cryosections were fixed in 4% PFA for 20 min at room temperature and blocked with Immunol Staining Blocking Buffer (Beyotime) at 4°C overnight. Sections were incubated with following primary antibodies at 4°C overnight: rabbit anti-rat von Willebrand factor (Abcam), rabbit anti-rat smooth muscle actin (Abcam) and mouse anti-rat alpha sarcomeric actin (Abcam). After rinsing, sections were incubated with FITC-secondary antibody for 1 h, and counterstained with DAPI. In negative control group, the primary antibody was replaced with PBS. Observation was performed under a Leica TCS SP5 X confocal microscope. The IOD of α-SA, α-SMA or v-WF positive area was quantified in at least 10 representative fields from 6 hearts (magnification: 100×).

Western blot assay for v-WF, α-SA and α-SMA in the heart

At 3 weeks after AMI, the heart tissues were collected from the infarct and per-infarct zone for Western blot assay. At least 6 animals per group were considered for each experimental setting (IC group vs. EC group).

Real-time polymerase chain reaction

Cells from male rats were injected into female rats and at 24 h and 3 weeks after MI, the rela-

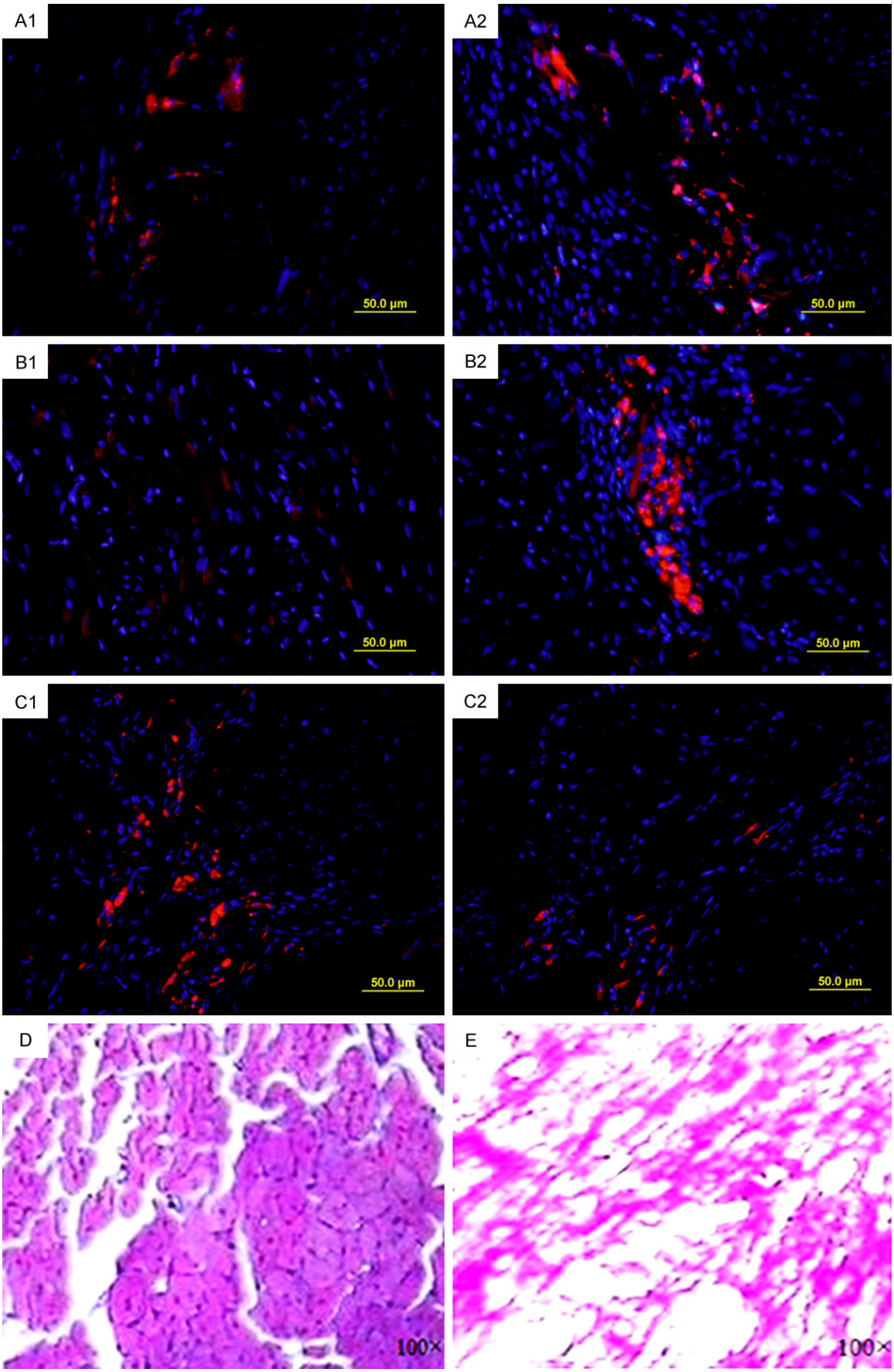
A1
50.0 μm
A2
50.0 μm
B1
50.0 μm
B2
50.0 μm
C1
50.0 μm
C2
50.0 μm
D
100×
E
100×

Figure 2. CDCs cultured on ECM vs. TCP differentiated toward v-WF (A), α-SA (B) and α-SMA (C) cells; Normal heart (control) (D); No residual cellular ingredients in the decelluarized heart ECM, and intact ECM was reserved (E).

Table 1. Comparison among the 4 groups

Measurement index	Group I	Group IC	Group E	Group EC
LVPW (mm)	1.41±0.20	1.43±0.14	1.41±0.22	1.41±0.22
LVEDV (ml)	0.65±0.05	0.65±0.06	0.65±0.05	0.65±0.06
LVESV (ml)	0.08±0.02	0.08±0.02	0.08±0.03	0.08±0.02
LVEF (%)	87.33±3.72	86.67±3.83	87.67±4.08	87.67±3.33
LVIDd (mm)	5.65±0.91	5.67±0.82	5.64±0.92	5.63±0.94
LVIDs (mm)	2.68±0.55	2.63±0.52	2.8±0.3	2.67±0.35
FS% (%)	53.83±1.60	54.17±3.97	53.17±2.71	52.83±2.23

Notes: IMDM group (I), IMDM+C group (IC), ECM group (E), ECM+C (EC) in LVPW (thickness of left ventricular posterior wall, mm); LVEDV (left ventricular end diastolic volume, ml); LVESV (left ventricular end systolic volume, ml); LVEF (left ventricular ejection fraction,%); LVIDd (left ventricular internal diameter in diastolic phase, mm); LVIDs (left ventricular internal diameter in systolic phase, mm) and FS% (left ventricular fraction shortening, %) before acute myocardial infarction (AMI). No significant difference appears among the 4 groups, $P > 0.05$.

Table 2. Comparison among the 4 groups

Measurement index	Group I	Group IC	Group E	Group EC
LVPW (mm)	0.70±0.02	0.77±0.04*	0.765±0.038*	0.93±0.09**
LVEDV (ml)	1.76±0.09	1.31±0.24*	1.227±0.149*	0.66±0.08**
LVESV (ml)	1.22±0.10	0.74±0.15*	0.672±0.073*	0.24±0.05**
LVEF (%)	30.83±3.31	43.17±2.14*	42.33±1.75*	62.83±3.54**
LVIDd (mm)	9.55±0.18	8.42±0.50*	7.77±0.10*	6.55±0.25**
LVIDs (mm)	8.38±0.18	6.92±0.40*	6.33±0.13*	4.53±0.27**
FS% (%)	12.33±1.21	18.00±1.67*	18.17±1.94*	30.83±2.32**

Notes: IMDM group (I), IMDM+C group (IC), ECM group (E), ECM+C (EC) for LVPW (thickness of left ventricular posterior wall, mm); LVEDV (LV end diastolic volume, ml); LVESV (LV end systolic volume, ml); LVEF (left ventricular ejection fraction, %); LVIDd (left ventricular internal diameter in diastolic phase, mm); LVIDs (left ventricular internal diameter in systolic phase, mm) and FS% (left ventricular fraction shortening, %) 3 weeks after acute myocardial infarction (AMI). 3 weeks after AMI, thickest LVPW, smallest EDV and ESV, highest LVEF, smallest LVIDd and LVIDs, highest FS% in EC group. There was no significant difference between group E and group IC. *Indicates $P < 0.05$ when compared to control (I); **Indicates $P < 0.05$ when compared to all other 3 groups. n=6, $\bar{x} \pm s$.

tive cell engraftment was quantified with the species-specific SRY gene using the $2^{-\Delta\Delta Ct}$ method [17] with StepOne modification (β-actin as an internal control). Rat SRY served as a template (forward primer: 5'-GGA GAG AGG CAC AAG TTG GC-3', reverse primer: 5'-TCC CAG CTGCTT GCT GAT C-3').

Statistical analysis

All the data are expressed as means ± standard deviation (SD), unless specified. Student t test and One Way analysis of variance (ANOVA) were used for the comparisons between two groups and among groups, respectively. A value of two tailed $P < 0.05$ was considered statistically significant.

Results

Characteristics of CDCs cultured on TCP and ECM

After 3-5 day culture in 5% CO_2 at 37°C, small, round and phase bright cells grew out from the explants over abed of stromal-like cells (**Figure 1A-C**). Once they reached 80-90% confluence, these EDCs were collected using trypsin and plated on poly-D-lysine coated 12-well plates at a density of 50000 cells/well. Cardiospheres formed after 2-3 days (**Figure 1D** and **1E**). Then, they were harvested by mechanical trituration and plated in CEM in fibronectin or rat heart-derived ECM coated flasks. CDCs were passaged once every 3 days (**Figure 1F** and **1G**). Immunofluorescence staining of CDCs was performed to detect the c-kit expression. c-kit expression was observable at 3 and 5-6 days (**Figure 1H** and **1I**).

Differentiation, proliferation and apoptosis of CDCs cultured on TCP vs. ECM

After 14-day culture on ECM, more CDCs differentiated toward v-WF, α-SA or α-SMA positive cells as compared to those cultured on TCP (**Figure 2A-C**). In addition, the proliferation of CDCs on ECM increased significantly when compared with those on TCP. However, the

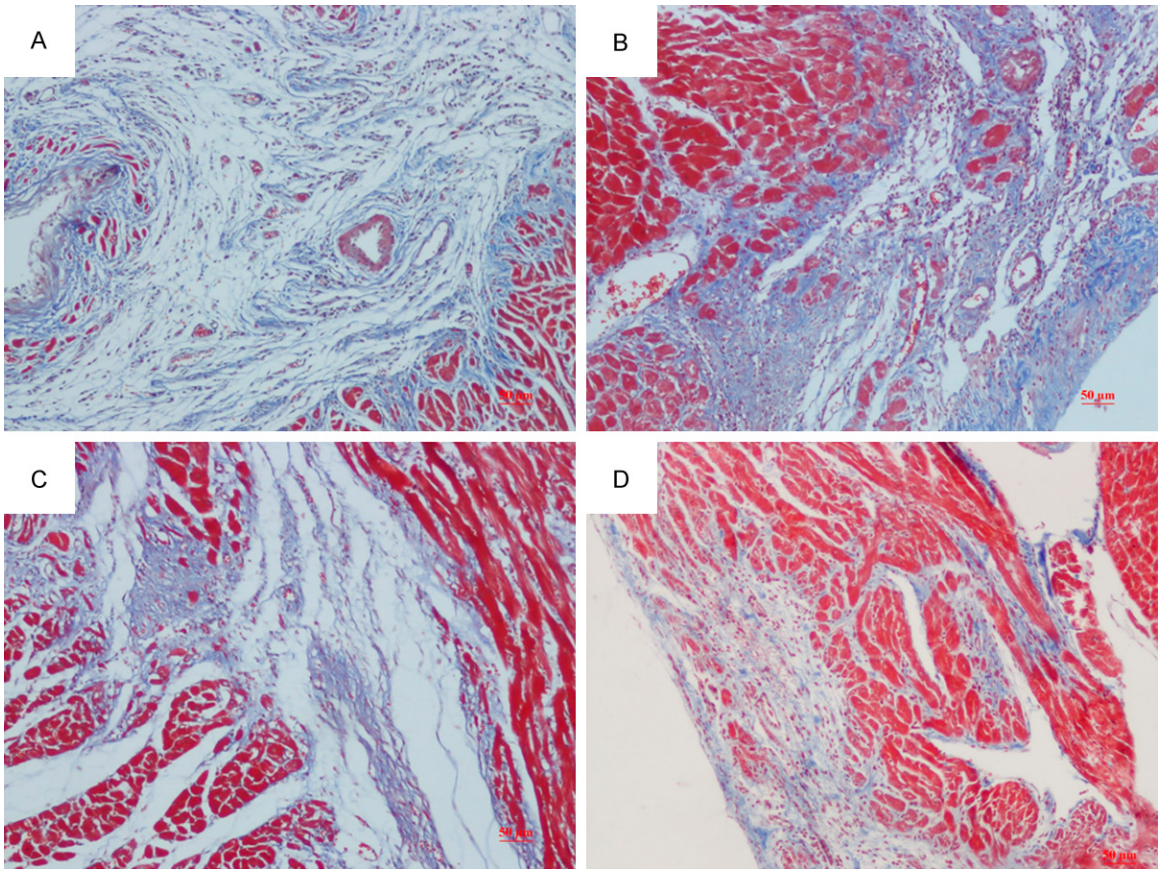

Figure 3. Positive staining area (%) and IOD in EC group at 3 weeks after AMI. A. I group, B. IC group, C. E group, D. EC group. *P < 0.05 vs. control (I); **P < 0.05 vs. other groups (n=6, $\bar{x}$±s).

apoptosis rate of CDCs on ECM was significantly lower than that on TCP. Collectively, ECM is able to significantly prompt the differentiation of CDCs toward endothelial cells, cardiomyocytes and smooth muscle cells, inhibit their apoptosis and increase their proliferation *in vitro*.

HE staining of rat heart-derived decellularized ECM

After decellularization of ECM pellet, HE staining was performed. There were no residual cells in ECM (normal heart tissues as a control). The intact extracardiac matrix structure was reserved (**Figure 2D** and **2E**).

Cardiac function and structure after injection of CDC+ECM

At baseline (I n=6, IC n=6, E n=6, EC n=6), there were no significantly differences among 4 groups (I group; IC group; E group; EC group) in the LVPW, LVEDV, LVESV, LVEF, LVIDd, LVIDs and FS% before AMI (**Table 1**). Three weeks after AMI (I n=6, IC n=6, E n=6, EC n=6), the thickest LVPW, smallest LVEDV and LVESV, highest LVEF, smallest LVIDd and LVID, and highest FS% were observed in EC group (**Table 2**). There were no significant differences in above parameters between E group and IC group.

Masson trichorome staining at 3 weeks after AMI

At 3 weeks after AMI, Masson trichrome staining was performed, and the positive staining area (%) and its IOD were determined and compared among 4 groups (I n=6, IC n=6, E n=6, EC n=6). The lowest positive staining area (%) and lowest IOD were found in EC group (**Figure 3**).

Immunostaining of the heart for v-WF, α-SA and α-SMA at 3 weeks after AMI (EC group vs. IC group)

Immunostaining of the heart was performed to detect the expressions of v-WF, α-SA and α-SMA on CDCs (EC n=6, IC n=6) at 3 weeks after AMI

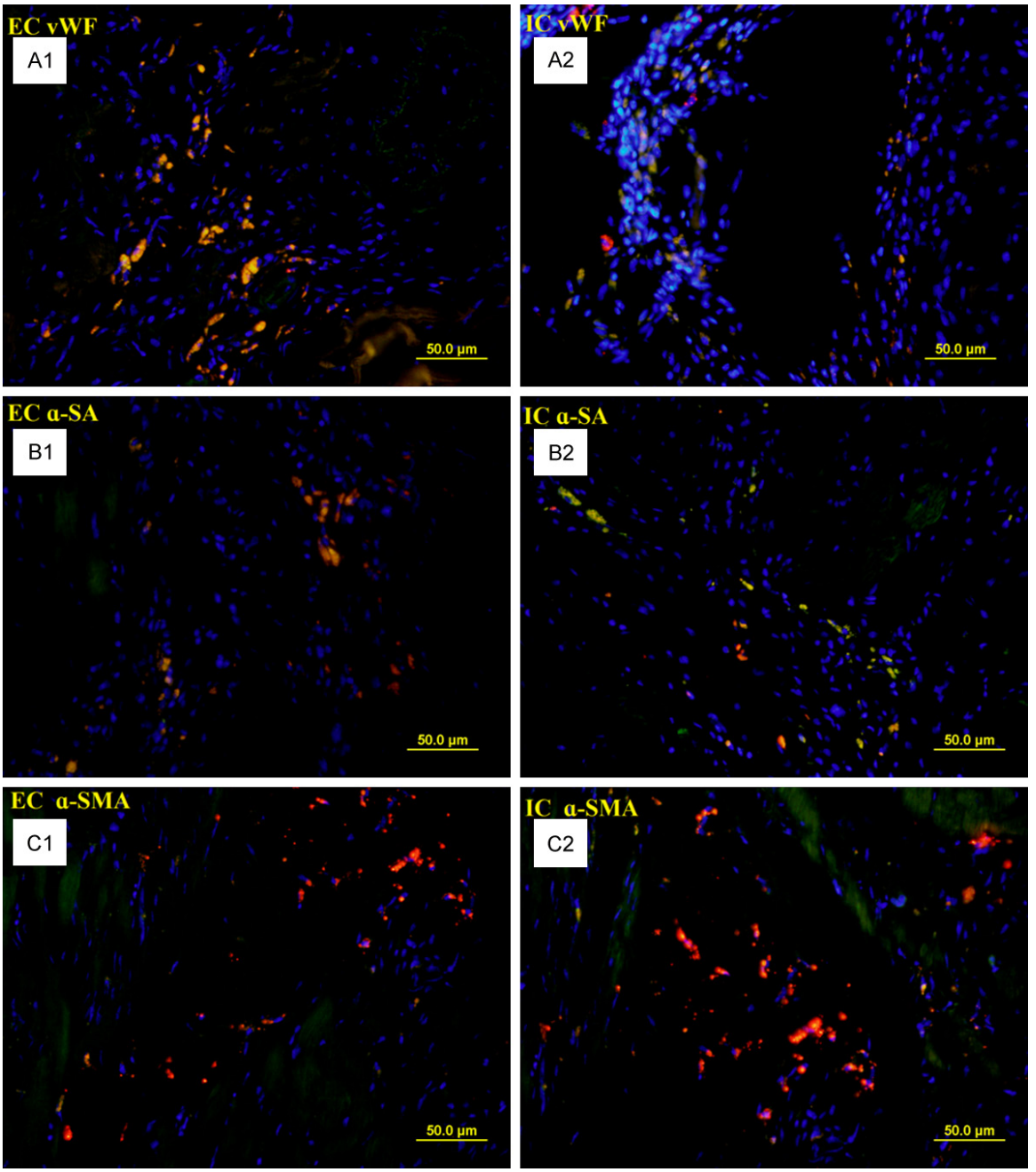

Figure 4. Expressions of v-WF, α-SA and α-SMA at 3 weeks after AMI in EC group increased significantly when compared with IC group *in vivo*. A-C. Before transplantation, CDCs labeled with CM-DiI showed red flurescene, CM-DiIlabled CDCs positive for v-WF, α-SA or α-SMA displayed yellow fluorescence *in vivo* (n=6, $\bar{x}\pm s$) *P < 0.05.

in EC group and IC group by measuring the positive staining area (%) and IOD. As shown in **Figure 4**, the expressions of v-WF, α-SA and α-SMA in EC group at 3 weeks after AMI were higher than in IC group. Before transplantation, CDCs labeled with CM-DiI showed red fluorescence under a fluorescence microscope. CM-DiI labeled CDCs positive for v-WF, α-SA or α-SMA were yellow under a fluorescence microscope (**Figure 4**).

Western blot assay for v-WF, α-SA and α-SMA in the heart

Western Blot assay (control group n=6, IC n=6, EC n=6) was performed for v-WF, α-SA and

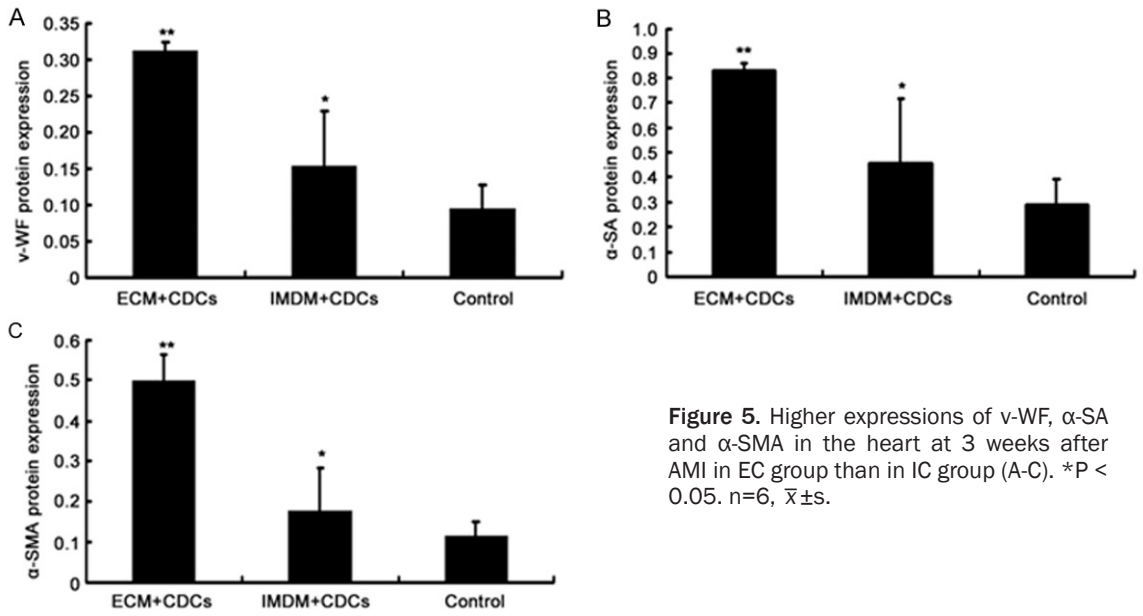

Figure 5. Higher expressions of v-WF, α-SA and α-SMA in the heart at 3 weeks after AMI in EC group than in IC group (A-C). *P < 0.05. n=6, $\bar{x}$±s.

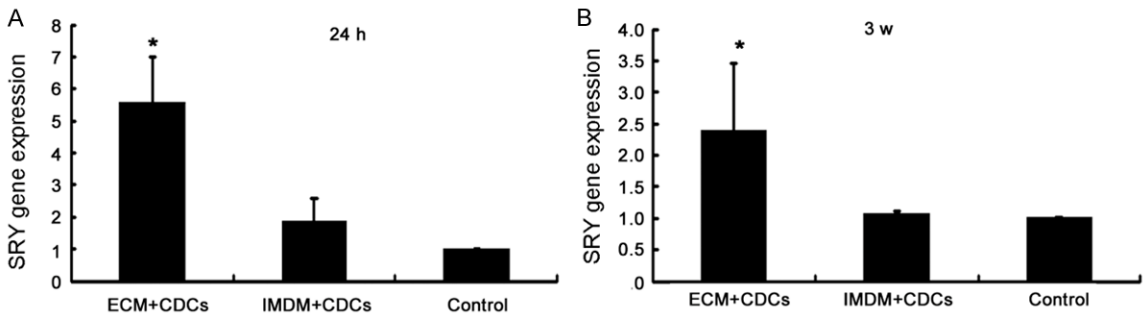

Figure 6. Engraftment and survival of CDCs in the heart of EC group increased significantly when compared with IC group at 24 h (A) and 3 weeks (B) after AMI. *P < 0.05, n=7, $\bar{x}$±s.

α-SMA at 3 weeks after AMI. Results showed the expressions of v-WF, α-SA and α-SMA in the heart of EC group increased significantly when compared with IC group (**Figure 5**).

Engraftment and survival of CDCs in the heart at 24 h and 3 weeks after AMI by real time PCR analysis, EC vs. IC group

At 24 h (control n=7, IC n=7, EC n=7) and 3 weeks (control n=7, IC n=7, EC n=7) after AMI, real time PCR was performed for sry gene in female rats. Results showed that more CDCs engrafted and survived in the heart of EC group than in IC group **Figure 6**.

Discussion

There is evidence showing that allogeneic CDC therapy without immunosuppression is safe and may improve the cardiac structure and function after MI [5]. CDCs represent an attractive cell type for the heart repair and regeneration, as CDCs are clonogenic and exhibit multilineage differentiation, thus meeting the criteria for heart-derived stem cells [18]. Importantly, the methodology is mature in studies on CDCs and the identity and utility of CDCs have been confirmed [19-25]. On the basis of findings from preclinical studies and the highly encouraging results from clinical trials on the autologous therapy [14, 15], two ongoing clinical trials are conducted with allogeneic heart-derived cellular products (ALLSTAR and CAREMI) for the therapy of acute MI. However, one of limitations for the cellular cardiomyoplasty is the poor and variable retention of transplanted cells [26-28]. Many injected cells are lost because of the blood flow (washing out cells) and cardiac con-

traction (squeezing out cells) [26]. Injectable biomaterials can be used as the delivery vehicle for cell transplantation to increase the retention and survival of transplanted cells [10-12].

In the present study, our results indicated that intramyocardial injection of CDCs combined with heart-derived ECM could enhance the retention and survival of transplanted CDCs at 24 h and 3 weeks after AMI as compared to IMDM plus CDCs (**Figure 6**). Heart-derived ECM promoted CDC proliferation, suppressed their apoptosis and necrosis of CDCs *in vitro*, promoted CDCs differentiation toward endothelial cells (v-WF), cardiomyocytes (α-SA) and smooth muscle cells (α-SMA) *in vitro* and *in vivo* (**Figures 2A-C**, **4A-C**). These cardioprotective effects may be ascribed to the preservation of intact heart ECM structure and ingredients (**Figure 2D**, **2E**) and the subsequent improvement of cardiac function and cardiac structure (**Table 2**; **Figure 3**). The improvement of cardiac structure and function was comparable between E group and IC group, which were consistent with previously reported [2]. The therapeutic effects may be related to both indirect (paracrine) and direct mechanisms of myocardial regeneration and recruitment of both injected and endogenous CDCs (**Figures 5** and **6**), which were consistent with previous findings [12].

In two ongoing clinical trials on the allogeneic heart-derived cellular products (ALLSTAR, CAREMI) for acute MI, results from the clinical trial on allogeneic cardiac-derived stem cell product were promising. Considering the simplicity of preparation of heart-derived ECM and its alone therapeutic effect), our findings provide evidence for the therapy of MI with CDCs on heart-derived ECM. However, the dose of ECM and number of CDCs used for treatment remain to be optimized.

Conclusion

Heart-derived ECM may promote CDCs differentiation toward endothelial cells, cardiomyocytes and smooth muscle cells *in vitro* and *in vivo*, increase CDCs proliferation and suppress CDCs apoptosis *in vitro*. Transplantation of CDCs on ECM may significantly improve the cardiac structure and function in MI rats as compared to CDCs or ECM alone. However, this therapeutic strategy is required to be further studied before its wide application.

Acknowledgements

This study was supported by the National Na-tural Science Foundation of China (No. 81400196), Science Technology Research Project of Education Department of Liaoning Province (No. L2011140, L2013297 and L20-13316) and Scientific Research Foundation of the First Hospital of China Medical University (fsfh1311). We thank Zhang Guang-Wei for the technical assistance in heart surgery.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tian-Xiang Gu, Department of Cardiac Surgery, The First Hospital of China Medical University, 155 Nanjingbei St, Shenyang 110001, China. Tel: 86-24-83283468; Fax: 86-24-83283468; E-mail: cmugtx@sina.com

References

[1] Ye Z, Zhou Y, Cai H and Tan W. Myocardial regeneration: Roles of stem cells and hydrogels. Adv Drug Deliv Rev 2011; 63: 688-697.

[2] Dai W, Gerczuk P, Zhang Y, Smith L, Kopyov O, Kay GL, Jyrala AJ and Kloner RA. Intramyocardial injection of heart tissue-derived extracellular matrix improves postinfarction cardiac function in rats. J Cardiovasc Pharmacol Ther 2013; 18: 270-279.

[3] Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MV, Coletta M, Vivarelli E, Frati L, Cossu G and Giacomello A. Isolation and expansion of adult cardiac stem cells from human and murine heart. Circ Res 2004; 95: 911-921.

[4] Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, Giacomello A, Abraham MR and Marban E. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. Circulation 2007; 115: 896-908.

[5] Malliaras K, Li TS, Luthringer D, Terrovitis J, Cheng K, Chakravarty T, Galang G, Zhang Y, Schoenhoff F, Van Eyk J, Marban L and Marban E. Safety and efficacy of allogeneic cell therapy in infarcted rats transplanted with mismatched cardiosphere-derived cells. Circulation 2012; 125: 100-112.

[6] Malliaras K, Smith RR, Kanazawa H, Yee K, Seinfeld J, Tseliou E, Dawkins JF, Kreke M,

Cheng K, Luthringer D, Ho CS, Blusztajn A, Valle I, Chowdhury S, Makkar RR, Dharmakumar R, Li D, Marban L and Marban E. Validation of contrast-enhanced magnetic resonance imaging to monitor regenerative efficacy after cell therapy in a porcine model of convalescent myocardial infarction. Circulation 2013; 128: 2764-2775.

[7] Cheng K, Malliaras K, Smith RR, Shen D, Sun B, Blusztajn A, Xie Y, Ibrahim A, Aminzadeh MA, Liu W, Li TS, De Robertis MA, Marban L, Czer LS, Trento A and Marban E. Human cardiosphere-derived cells from advanced heart failure patients exhibit augmented functional potency in myocardial repair. JACC Heart Fail 2014; 2: 49-61.

[8] Dow J, Simkhovich BZ, Kedes L and Kloner RA. Washout of transplanted cells from the heart: a potential new hurdle for cell transplantation therapy. Cardiovasc Res 2005; 67: 301-307.

[9] Cheng K, Li TS, Malliaras K, Davis DR, Zhang Y and Marban E. Magnetic targeting enhances engraftment and functional benefit of iron-labeled cardiosphere-derived cells in myocardial infarction. Circ Res 2010; 106: 1570-1581.

[10] Dai W, Hale SL, Kay GL, Jyrala AJ and Kloner RA. Delivering stem cells to the heart in a collagen matrix reduces relocation of cells to other organs as assessed by nanoparticle technology. Regen Med 2009; 4: 387-395.

[11] Wang H, Zhou J, Liu Z and Wang C. Injectable cardiac tissue engineering for the treatment of myocardial infarction. J Cell Mol Med 2010; 14: 1044-1055.

[12] Cheng K, Shen D, Smith J, Galang G, Sun B, Zhang J and Marban E. Transplantation of platelet gel spiked with cardiosphere-derived cells boosts structural and functional benefits relative to gel transplantation alone in rats with myocardial infarction. Biomaterials 2012; 33: 2872-2879.

[13] Hale SL, Dai W, Dow JS and Kloner RA. Mesenchymal stem cell administration at coronary artery reperfusion in the rat by two delivery routes: a quantitative assessment. Life Sci 2008; 83: 511-515.

[14] Bolli R, Chugh AR, D'Amario D, Loughran JH, Stoddard MF, Ikram S, Beache GM, Wagner SG, Leri A, Hosoda T, Sanada F, Elmore JB, Goichberg P, Cappetta D, Solankhi NK, Fahsah I, Rokosh DG, Slaughter MS, Kajstura J and Anversa P. Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO): initial results of a randomised phase 1 trial. Lancet 2011; 378: 1847-1857.

[15] Makkar RR, Smith RR, Cheng K, Malliaras K, Thomson LE, Berman D, Czer LS, Marban L, Mendizabal A, Johnston PV, Russell SD, Schuleri KH, Lardo AC, Gerstenblith G and Marban E. Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS): a prospective, randomised phase 1 trial. Lancet 2012; 379: 895-904.

[16] Edelberg JM, Lee SH, Kaur M, Tang L, Feirt NM, McCabe S, Bramwell O, Wong SC and Hong MK. Platelet-derived growth factor-AB limits the extent of myocardial infarction in a rat model: feasibility of restoring impaired angiogenic capacity in the aging heart. Circulation 2002; 105: 608-613.

[17] Winer J, Jung CK, Shackel I and Williams PM. Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Anal Biochem 1999; 270: 41-49.

[18] Davis DR, Ruckdeschel Smith R and Marban E. Human cardiospheres are a source of stem cells with cardiomyogenic potential. Stem Cells 2010; 28: 903-904.

[19] Aghila Rani KG and Kartha CC. Effects of epidermal growth factor on proliferation and migration of cardiosphere-derived cells expanded from adult human heart. Growth Factors 2010; 28: 157-165.

[20] Gaetani R, Ledda M, Barile L, Chimenti I, De Carlo F, Forte E, Ionta V, Giuliani L, D'Emilia E, Frati G, Miraldi F, Pozzi D, Messina E, Grimaldi S, Giacomello A and Lisi A. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. Cardiovasc Res 2009; 82: 411-420.

[21] Mishra R, Vijayan K, Colletti EJ, Harrington DA, Matthiesen TS, Simpson D, Goh SK, Walker BL, Almeida-Porada G, Wang D, Backer CL, Dudley SC Jr, Wold LE and Kaushal S. Characterization and functionality of cardiac progenitor cells in congenital heart patients. Circulation 2011; 123: 364-373.

[22] Takehara N, Tsutsumi Y, Tateishi K, Ogata T, Tanaka H, Ueyama T, Takahashi T, Takamatsu T, Fukushima M, Komeda M, Yamagishi M, Yaku H, Tabata Y, Matsubara H and Oh H. Controlled delivery of basic fibroblast growth factor promotes human cardiosphere-derived cell engraftment to enhance cardiac repair for chronic myocardial infarction. J Am Coll Cardiol 2008; 52: 1858-1865.

[23] Tang YL, Zhu W, Cheng M, Chen L, Zhang J, Sun T, Kishore R, Phillips MI, Losordo DW and Qin G. Hypoxic preconditioning enhances the benefit of cardiac progenitor cell therapy for treatment of myocardial infarction by inducing CXCR4 expression. Circ Res 2009; 104: 1209-1216.

[24] Zakharova L, Mastroeni D, Mutlu N, Molina M, Goldman S, Diethrich E and Gaballa MA.

Transplantation of cardiac progenitor cell sheet onto infarcted heart promotes cardiogenesis and improves function. Cardiovasc Res 2010; 87: 40-49.

[25] Koninckx R, Daniels A, Windmolders S, Carlotti F, Mees U, Steels P, Rummens JL, Hendrikx M and Hensen K. Mesenchymal stem cells or cardiac progenitors for cardiac repair? A comparative study. Cell Mol Life Sci 2011; 68: 2141-2156.

[26] Terrovitis J, Lautamaki R, Bonios M, Fox J, Engles JM, Yu J, Leppo MK, Pomper MG, Wahl RL, Seidel J, Tsui BM, Bengel FM, Abraham MR and Marban E. Noninvasive quantification and optimization of acute cell retention by in vivo positron emission tomography after intramyocardial cardiac-derived stem cell delivery. J Am Coll Cardiol 2009; 54: 1619-1626.

[27] Teng CJ, Luo J, Chiu RC and Shum-Tim D. Massive mechanical loss of microspheres with direct intramyocardial injection in the beating heart: implications for cellular cardiomyoplasty. J Thorac Cardiovasc Surg 2006; 132: 628-632.

[28] Al Kindi A, Ge Y, Shum-Tim D and Chiu RC. Cellular cardiomyoplasty: routes of cell delivery and retention. Front Biosci 2008; 13: 2421-2434.