ORIGINAL RESEARCH

Preliminary evaluation of treatment efficacy of umbilical cord blood-derived mesenchymal stem cell-differentiated cardiac progenitor cells in a myocardial injury mouse model

Truc Le-Buu Pham, Tam Thanh Nguyen, Anh Thi-Van Bui, Ho Thanh Pham, Ngoc Kim Phan, My Thi-Thu Nguyen, Phuc Van Pham

Laboratory of Stem Cell Research and Application, University of Science, Vietnam National University, HCM city, Viet Nam
*Corresponding author: pvphuc@hcmuns.edu.vn

Abstract— Recently, stem cell therapy has been investigated as a strategy to prevent or reverse damage to heart tissue. Although the results of cell transplantation in animal models and patients with myocardial ischemia are promising, the selection of the appropriate cell type remains an issue that requires consideration. In this study, we aimed to evaluate the effect of cardiac progenitor cell transplantation in a mouse model of myocardial ischemia. The cardiac progenitor cells used for transplantation were differentiated from umbilical cord blood mesenchymal stem cells. Animal models injected with phosphate-buffered saline (PBS) and healthy mice were used as controls. Cell grafting was assessed by changes in blood pressure and histological evaluation. After 14 days of transplantation, the results demonstrated that the blood pressure of transplanted mice was stable, similar to healthy mice, whereas it fluctuated in PBS-injected mice. Histological analysis showed that heart tissue had regenerated in transplanted mice, but remained damaged in PBS-injected mice. Furthermore, trichrome staining revealed that the transplanted mice did not generate significant amount of scar tissue compared with PBS-injected control mice. In addition, the cardiac progenitor cells managed to survive and integrate with local cells in cell-injected heart tissue 14 days after transplantation. Most importantly, the transplanted cells did not exhibit tumorigenesis. In conclusion, cardiac progenitor cell transplantation produced a positive effect in a mouse model of myocardial ischemia.

Key words: cardiac progenitor cells; mouse model; myocardial ischemia; stem cell; transplantation.

INTRODUCTION

Over the past half-century, scientific advances in modifying risk factors, drug treatment, and surgical interventions have significantly improved the quality of life and lifespan of patients with damage to the cardiac muscle following myocardial ischemia. However, current treatment strategies fail to reverse scar formation or repair the injured ventricle wall after myocardial infarction (MI), which eventually leads to ventricular dysfunction and arrhythmia. Currently, stem cell therapy is considered as a strategy with great potential to prevent or reverse cardiac muscle damage and promote tissue regeneration.

The preclinical model of ischemic heart disease has raised awareness about the safety and efficiency of stem cell therapy (Quyyumi et al., 2011). Despite the promising results of cell transplantation in animal models and patients with ischemic cardiomyopathy, it remains to be determined which cell type is the most appropriate for this therapy. Current cell therapies for heart disease have shown remarkable developments compared with the first cell transplantation performed for heart damage more than 20 years ago (Marelli et al., 1992). Initially, scientists focused on using stem cells and skeletal muscle satellite cells because they were easily identified, and exhibited strong proliferation rates and high ischemia-withstanding ability

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(Marelli et al., 1992; Murry et al., 2002; Murry et al., 1996). However, these cell sources were later shown to be incapable of differentiating into myocardial cells, as well as exhibiting low incorporation with local myocardiocytes*in vivo* post-transplantation (Murry et al., 2005; Reinecke et al., 2002). Subsequent studies searching for appropriate cell sources capable of inducing positive therapeutic effects examined numerous cell sources, including bone marrow-derived mesenchymal stem cells (MSCs) (Mazo et al., 2012). Embryonic stem cells (ESCs) can be differentiated into mature cells and have been shown to regenerate damaged heart tissue in animal models. However, because of bioethical issues, legality (Blum and Benvenisty, 2008), and immunity (Zhu et al., 2009), their use in human trials have been substantially limited to date. In their place, numerous groups have investigated the use of induced pluripotent stem cells (iPSCs) (Wernig et al., 2007) or induced cardiomyocytes(Ieda et al.). Recently, the repeatability, reliability, and safety of cell reprogramming and genetic techniques have been examined in clinical trials (Kanashiro-Takeuchi et al., 2011; Mushtaq et al., 2011). Currently, heart muscle cells derived from adult stem cells, including bone marrow-derived mononuclear cells (Hare, 2011; Perin et al., 2012; Traverse et al., 2009; Williams et al., 2011), MSCs (Williams and Hare, 2011), adipose tissue stem cells (Mazo et al., 2012), and stem cells derived from cardiac tissue (Bolli et al., 2011; Makkar et al.) have been prioritized for clinical testing and evaluation.

The results of recent clinical trials support the notion that stem cell therapy is safe and is capable of repairing heart structures, as well as restoring heart function (Karantalis et al., 2012; Williams et al., 2011).

The above results help to confirm the safety and efficiency of MSC transplantation for cardiovascular disease. However, the majority of clinical studies have used autologous bone marrow-derived MSCs. Because the properties of bone marrow-derived MSCs tend to decline with age, studies continuing to examine if alternative cell sources are necessary. Of these cells, human umbilical cord blood-derived stem cells have proven to be a valuable candidate (Goldstein et al., 2007). In animal models, cord blood stem cells reduced infarct size, increased heart wall thickness, reduced inflammation at the lesion site, and improved the ejection fraction of the left ventricle (Henning et al., 2004; Henning et al., 2006; Henning et al., 2008). However, before proceeding to clinical studies and applications, further studies are still required.

Until now, the exact mechanism of the effects produced by stem cell transplantation in the treatment of MI remain unknown. However, cell therapy is believed to regenerate myocardial tissue damages through either one or both of the following mechanisms: (i) creating new myocardiocytes and inducing angiogenesis to supply nutrients for myocardial cell mass, and/or (ii) releasing paracrine factors to stimulate cell regeneration, induce the directed-differentiation of stem cells, prevent scarring, and stimulate angiogenesis (Mazo et al., 2012).

In this study, cardiac progenitor cells, differentiated from human umbilical cord blood-derived MSCs (UCB-MSCs), were transplanted into a mouse model of myocardial ischemia to determine whether they could be induced to differentiate into mature heart muscle cells, which could replace dead tissue and reduce the infarct lesion area.

MATERIALS AND METHODS

This study was approved by the Institutional Ethical Committee of Laboratory of Stem Cell Research and Application, University of Science, Vietnam National University, Vietnam.

Cell source for transplantation

Third- to fifth-generation human UCB-MSCs were induced to differentiate into CPCs using 5-Azacytidine (5-Aza)-containing medium, according to the process of Pham *et al.*(Pham et al., 2014). Briefly, the cells were induced in Dulbecco's-modified Eagle's medium supplemented with 10% fetal bovine serum (FBS); 1% penicillin/streptomycin; 10 μM 5-Aza; 50 ng/mL activin A; 0.1 mM ascorbic acid. After 24 h induction, cells were washed twice with phosphate-buffered saline (PBS) and cultured in Dulbecco's-modified Eagle's medium supplemented with 15% FBS, 1% penicillin/streptomycin, 50 ng/mL activin A; 0.1 mM ascorbic acid, without 5-Aza. The medium was replaced every 3 days until day 30.

Cell transplantation

Male albino mice (25–30g) were anesthetized by thigh muscle injection with 0.022 g/mL ketamine until the ratio of ketamine anesthetic reached 87 mg/kg of body weight (Kanno et al., 2002). Mice were intubated and mechanically ventilated, with pump speed 120–130 cycles/min, pump volume per cycle was 1 mL/100 g

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body weight. After left thoracotomy, ligation of the left anterior descending artery (LAD) was performed at the distal 1/3 of the coronary artery from the aorta to the heart apex (Degabriele et al., 2004). After 3 min, CPCs were infused into the area of infarction, at a density 10^6 cells per 30 μL for each mouse. Then, the chest was closed, and the muscle layer was sewn tight and applied with antiseptic. The mouse was placed carefully into a clean case. Normal mice were used as positive controls, while LAD ligation plus PBS injection was used as a negative control.

Transplantation efficacy was assessed according to criteria, including blood pressure fluctuation, and the survival, migration, and influence of CPCs on the grafted heart tissue.

Blood pressure monitoring methods

Mice blood pressure changes were monitored using a 58500 Blood Pressure Recorder (Ugo BasileSrl, Italy), 1–2 weeksbefore and after cell transplantation.

To facilitate with stress, mice were trained to be familiar with tail-cuff blood pressure measurements. The animals were anesthetized by placing them in an anesthesia closed chamber, which provided continuous supply of 5% pure oxygen at a rate 1 L/min of isoflurane, until coma was induced. The mouse tail was inserted into the blood pressure measuring ring with signal sensor. Systolic (SYS) and diastolic (DIA) blood pressure of the animals were measured and recorded following the manufacturer's instructions (58600 Blood Pressure Recorder). Mean arterial pressure (MAP) was calculated by the following formula:

$$MAP = [(2 \times DIA) + SYS] / 3$$

Heart tissue harvesting and cryosectioning

The animal heart was washed with PBS and fixed in 4% paraformaldehyde overnight. After sucrose cryoprotection, the tissue was moulded with optimum cutting temperature compound and cryosectioned into 10-μm-thick slices. The obtained slices were then treated with hematoxylin and eosin (H&E) stain, Trichrome stain, and immunohistochemistry.

Hematoxylin& eosin staining

H&E staining was performed to evaluate tissue and cell structures. Slices were immersed in a graded series of alcohol solutions as follows: 100% for 2 min, 90% for 2 min, 70% for 1 min, 50% for 1 min, and double-distilled water for 2 min. The surrounding tissue area was dried with absorbent paper, followed by an addition of a drop of hematoxylin stain onto the tissue slice. After 2 min, the slice was washed with distilled water. Two drops of eosin stain were added to the tissue slice for 30 s and the specimen was successively embedded again with: 50% alcohol for 1 min, 70% alcohol for 1 min, 90% alcohol for 1 min, and 100% alcohol for 1 min. Immersion oil was dispensed onto the slice followed by lamelle fixation.

Trichrome staining

Masson's Trichromedye is commonly used to distinguish collagen in pathological cases. Three dyes are used to identify collagen, fibrin, and erythrocytes. Tissue was initially stained with Biebrich Scarlet acidic dye (light red), which has a binding preference to acidic components in the tissue. When the tissue was treated with phosphomolybdic acid, the red color was displaced from the collagen component. In addition, phosphomolybdic acid also generated links between collagen and light green molecules that tainted the collagen green.

Tissue specimens were immersed in a graded series of alcohol solutions as follows: 100% for 1 min, 90% for 1 min, and 80% for 1 min. Samples were washed gently twice with distilled water, followed by immersion in Bouin-containing solution overnight at 56 °C. Samples were rinsed with water to remove traces of piric acid (yellow) and soaked in Weigert's iron hematoxylin solution for 10 min. Samples were gently washed continuously with distilled water for 30 s. Next, they were embedded in Biebrich scarlet solution for 1 min. The slices were washed quickly again with distilled water and immersed in 5% phosphomolybdic acid solution for 30 min. Then, samples were transferred and bathed in the light green solution for 10 min. The samples were rinsed quickly with distilled water and immersed in 0.5% acetic acid solution for 2 min. The samples were immersed in a series of graded alcohol solutions as follows: 95% for 1 min × 2, followed by 100% for 1 min × 2. Immersion oil was dropped onto the slices, followed by lamelle fixation. Trichrome-stained sections were identified and collagen structures were evaluated by ImageJ software.

Immunocytochemistry staining

Cells grown on coverslips (Santa Cruz Biotechnology, Dallas, TX, USA) were prepared for immunocytochemistry as follows: cells were fixed in 4% paraformaldehyde solution for 15 min; permeabilized with 0.1% Triton X-100; washed three times in PBS; blocked with BSA; and incubated with a primary human anti-

body alpha-actinin (Abcam 1:400) or human Troponin T (Abcam 1:400) overnight at 4 °C. After washing, the samples were treated with goat anti-rabbit IgG secondary antibodies (Abcam 1:400) and Hoechst 33342 (Sigma 1:400) for 45 min at room temperature. The samples were rinsed three times with PBS, mounted, and observed under a fluorescent microscope (Zeiss Axiovert).

Immunohistochemistry staining

Tissue sections were fixed and stained with Hoechst 33342 (Sigma, 1:400) and antibodies specific for human proteins of interest, Troponin T (Abcam, 1:200) and alpha-actinin (Abcam, 1:200), according to the manufacturer's protocol. Slides containing tissue sections were washed briefly for 2 × 5 min in PBS. Tissue sections were blocked with 5% BSA at room temperature for 30 min and incubated with primary antibodies at room temperature for 60 min. After washing, slides were then treated with secondary antibodies at room temperature for 60 min in the absence of light to avoid optical bleaching. The samples were washed 4 × 5 min in PBS, coated with polyvinyl alcohol solution, and covered by lamelle. Their images were observed and recorded by fluorescence microscopy.

Reverse transcription-polymerase chain reaction

Before transplantation, CPCs were tested for the expression of human Sox2 and Oct4 genes, which are used to characterize tumor formation, using reverse transcription-polymerase chain reaction (RT-PCR). Human glyceraldehyde 3-phosphate dehydrogenase (hGAPDH) was used as an internal control.

At 14 days post-transplantation, total RNA from hearts of control animals or test animals was collected to examine the tumor formation capacity of infused cells in vivo.

Total RNA was extracted from CPCs prepared for transplantation, human MCF7 breast cancer cells, and heart tissue samples 14 days post-transplantation using Easy Blue Reagent (Intron), following the manufacturer's instructions. cDNA was synthesized from RNA using the Superscript II Reverse Transcriptase (Invitrogen), according to the manufacturer's procedure. Next, PCR was performed using Taq DNA Polymerase (Takara, Shiga, Japan). The primers of the examined genes are listed in **Table 1**.

Gel electrophoresis was performed on RT-PCR products on 2% agarose gels, at 100 V, for 60 min. A 100-bp DNA ladder was used for all products. The results were analyzed by electrophoresis using the Gel Doc IT system (UVP).

Table 1. Primers of the examined genes

| Gene	Primer	Ta	Cycles	Product size (bp)
hSox2	F: CAACGGCAGCTACAGCA R: GGAGTGGGAGGAAGAGGT	60°C	35	283 bp
hOct4	F: TGGGGGTTCTATTTGGGAAGG R: GTTCGCTTTCTCTTTCGGGC	60°C	35	193 bp
hGAPDH	F: GTCAACGGATTTGGTCGTATTG R: CATGGGTGGAATCATATTGGAA	60°C	35	139 bp
mGAPDH	F: GCATGGACTGTGGTCATGAG R:CCATCACCATCTTCCAGGAG	60°C	35	322 bp

Statistical Analysis

Data were statistically interpreted usingGraphPad Prism software version 6.

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# RESULTS

UCB-MSC differentiation into CPCs

UCB-MSCs were induced in medium containing 5-Aza. The induced cells were checked by ICC staining with antibodies specific for human cardiac muscle cells, including human alpha-actinin and human troponin T. The results indicated that a phenotypic change occurred in the induced cells. Originally, the population exhibited the morphology of MSCs, which are mononuclear and spindle shaped; then, they transformed into multinuclear cells, which are similar to those of cardiac muscle cell lineages. Additionally, they also expressed both specific markers of cardiac muscle cells, human alpha-actinin and human troponin T (**Fig. 1**).
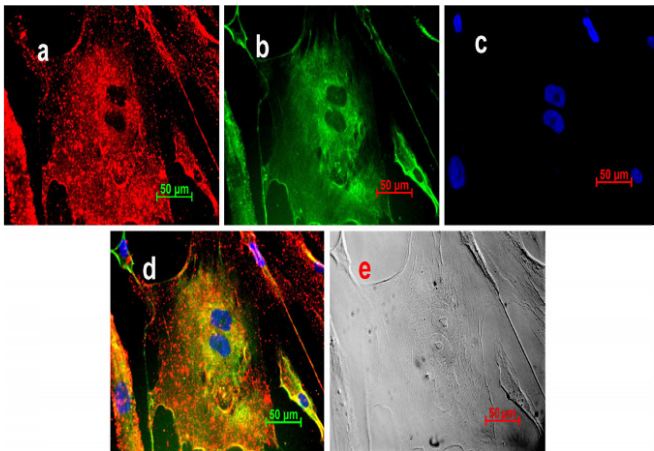
Figure 1. Immunocytochemistry results of induced cells. (a) Induced cells were positive for human alpha-actinin; (b) induced cells were positive for human troponin T; (c) cell nuclei were stained with Hoechst 33342; (d) overlay image of images a, b, and c; (e) induced cell image was captured under white light. Scale bar, 50 μm.

Blood pressure changes

To assess the effect of cell transplantation in the mouse model of myocardial ischemia, changes in blood pressure before and after transplantation were recorded through a dedicated system (**Fig. 2**). The collected data were processed using statistical software.
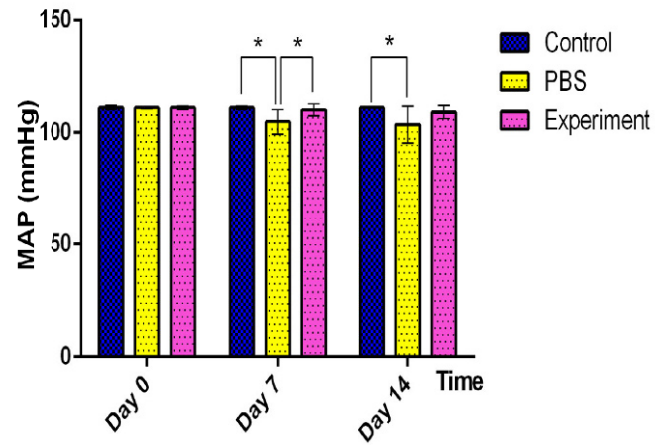

Figure 2. Blood pressure changes. Blood pressure stabilized in normal healthy mice and tended to decline in PBS-injected mice. During the survey, the blood pressure of transplanted mice was decreased, but not significantlycompared with PBS-injected mice. Results are means ± standard deviation; Student's t-test, $P < 0.05$: * indicates significant difference**.**

In normal mice, the MAP was approximately 111 mmHg (Tiemann et al., 2003). Healthy mice (n = 10) were used as a positive control with stable blood pressure (D0 = 111.1 mmHg; D7 = 111.0 mmHg; D14 = 111.1 mmHg). However, MAP in the LAD ligation and PBS-injected mice (PBS group) (n = 10) tended to decrease over the survey period (D0 = 111.0 mmHg; D7 = 104.8 mmHg; D14 = 103.5 mmHg). This result suggested that heart activity was decreased in the PBS group. Meanwhile, the MAP in the CPC-transplanted group (n = 10) was decreased compared with the control group (D0 = 111.0 mmHg; D7 = 110.1 mmHg; D14 = 109.1 mmHg); however, the decrease was evidently less than that of the PBS group.

CPC transplantation reduced heart tissue damages

To assess the effects of grafted CPCs in the mouse model, animals were divided into three groups. The control group consisted of normal healthy mice; the PBS group consisted of mice receiving LAD ligation and PBS injection; and the CPC group comprised mice receiving LAD ligation and CPC transplantation.
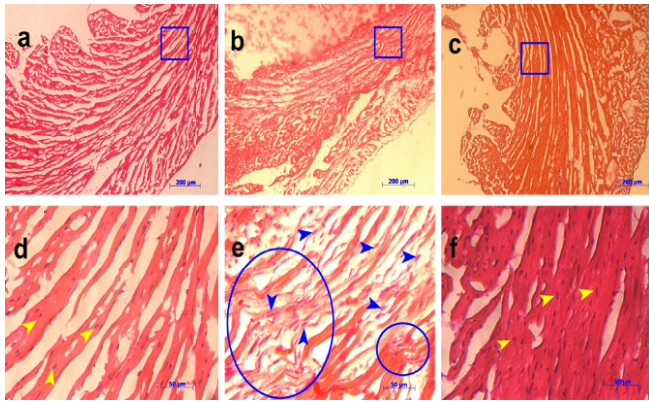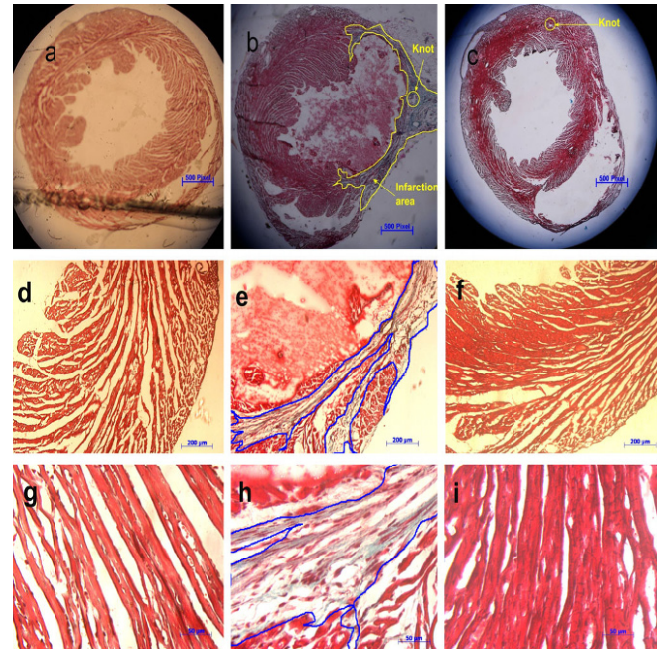
Figure 3. Results of myocardial tissue H&E staining. (a, d) heart tissue of control group was healthy and unbroken; (b, e) heart tissue of the PBS group was fractured, rippled (blue circled area), and exhibited karyolytic nuclei (blue arrow); (c, f) heart tissue of the CPC group was not rippled and the nuclei were intact in the center of the cell (yellow arrow).

The heart tissue was fixed and cryo-sectioned onto slides (10-µm-thick slices). Slides were then stained with H&E for tissue damage assessment. Results showed that heart tissue in the control group remained intact and exhibited no sign of inflammation. The left ventricle wall of the control group was normal, while it was thinner in the PBS group. Heart tissue of the PBS group exhibited more loss of cross-striations, with significantly shrunken bands and nuclear karyolysis in heart cells (green arrow, **Fig. 3**). Some neutrophils infiltrated the heart muscle and fibers appeared rippled in the PBS group (area circled in blue, **Fig. 3**). However, no fracture or damage of the heart tissue was observed on the CPC group. In addition, the nucleus was located in the center of the cell (yellow arrows, **Fig. 3**), pale pink plates were also observed and the left ventricle wall was not as thin as the PBS group (**Fig. 3**).

To assess scar formation in the damaged heart tissue and effects of CPCs on the process, mice hearts were collected for Trichrome staining. Results showed that the myocardium of the control group did not experience fibrosis (**Fig. 4a, 4d, 4g**), while the PBS group formed broad blue regions of fibrosis when stained with Trichrome (**Fig. 4b, 4e, 4h**). No sign of fibrosis was observed in the myocardium of the CPC group compared with the PBS group (**Fig. 4c, 4f, 4i**).


Figure 4. Results of myocardial tissue Trichrome staining. (a, d, g) Heart tissue of the control group exhibited no scar formation; (b, e, h) heart tissue of the PBS group formed a large scar; (c, f, i) heart tissue of the CPC group also exhibited no scar formation, at 14 days post-implantation.

Post-transplantation evaluation of infused CPCs in heart tissue

To investigate the fate of the infused CPCs in the damaged heart tissue of experiment animals, mouse heart tissue samples were collected, frozen, and sectioned. Slices were then stained with antibodies, which were specific for human heart cells, including human alpha-actinin and human troponin T.

The results of tissue staining indicated that heart tissues of the control and PBS groups were negative for staining with these specific antibodies (**Fig. 5a, d, g, j, b, e, h, k**). Meanwhile, some areas of CPC-transplanted heart tissue exhibited positive staining (**Fig. 5c, f, i, l**), indicating that grafted CPCs survived and expressed both human Troponin T (green) and human alpha-actinin (red). These cells not only did not form scar tissue but they also prevented or reduced tissue damage caused by ischemia. Therefore, the left ventricular walls of CPC-transplanted mice were relatively thick compared with normal mice. In contrast, mice in the PBS group exhibited thin ventricular walls and scar formation.
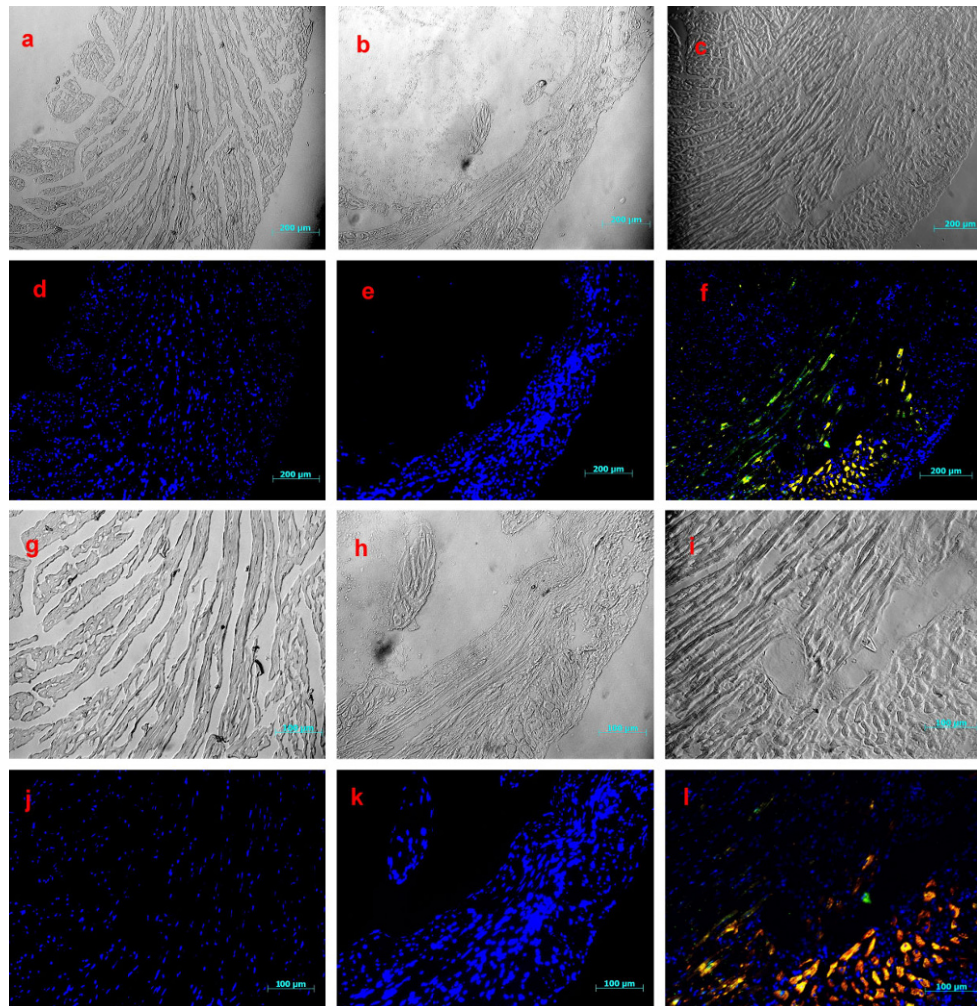
Figure 5. Heart tissue immunohistochemistry results of the three groups. (a, d, g, j) Myocardial tissue of normal mice did not stain with any of the specific antibodies for human cardiac muscle at various magnifications; (b, e, h, k) heart tissue in the PBS group was also negative for these specific antibodies; (c, f, i, l) transplanted CPCs existed in the mouse heart tissue and were positive for both human alpha-actinin and human troponin T. FL (Flourescein); WF (white field).

CPCs did not induce tumorigenesis post-transplantation

To test for the tumor-forming ability of the transplanted cells prior to transplantation, CPCs were evaluated for human cancer gene expression, including human Sox2 (hSox2) and human Oct4 (hOct4). The human breast cancer MCF7 cell line was used as a positive control (**Fig. 6**) and distilled water was used as a negative control. The results showed that the CPCs did not express hOct4 and hSox2 compared with MCF7 cells (**Fig. 7a**).

To assess whether CPC survival and tumorigenesis in mouse cardiac tissue post-transplantation, CPC-transplanted tissue was collected. Total RNA was extracted from heart tissue for further analysis of hSox2 and hOct4 expression. RNA from normal mouse heart was used for comparison. MCF7 cells and distilled

water controls were treated with the same procedure. The results showed that transplanted CPCs existed in grafted tissues and the tumor genes hSox2 and hOct4 were not expressed (**Fig. 7b**).
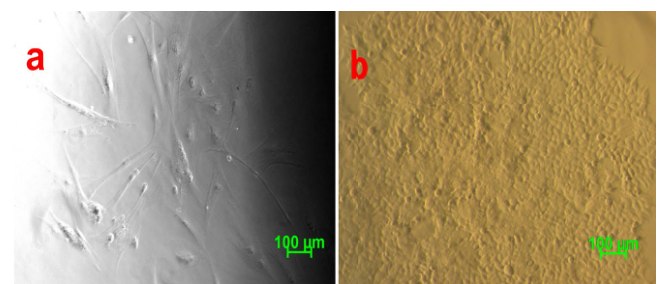
Figure 6. Cardiac progenitor cells (a) and MCF7 human breast cancer cells (b).
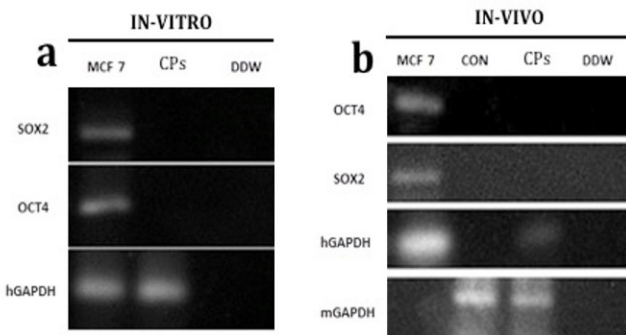
Figure 7. Cardiac progenitor cells (CPCs) did not express hSox2 and hOct4. (a) CPCs did not express hOct4 and hSox2 when cultured *in vitro*; (b) CPCs existed in transplanted tissue and did not express hSox2 and hOct4 14 days post-transplantation*in vivo*

DISCUSSION

In this study, we evaluated the potential of CPCs to repair damaged myocardial tissue. CPC transplantation minimized blood pressure fluctuation in transplanted mice similar to the control group; and was more stable compared with the PBS group. The engrafted CPCs survived and integrated with the host cardiac tissue, improved tissue structure, and prevented the progression of fibrosis in the damaged heart muscle 14 days after transplantation. CPC transplantation did not induce teratoma formation and the expression of cancer genes, including hOct4 and hSox2, in CPC-transplanted mice. In other studies, the transplantation of undifferentiated ESCs into rat models of MI tended to generate teratomas(Nussbaum et al., 2007). In our study, the transplantation of CPCs derived from UCB-MSCs did not form teratomas in transplanted tissue **(Fig. 6, Fig. 7)**. This difference may be a result of the induction of different changes in the gene expression of CPCs and ESCs, whereby CPCs did not undergo oncogenic differentiation. This result was similar to the previous results of Huber *et al.*, where human ESC-derived cardiomyocytes (hESC-CMs) were infused into a mouse model of MI (Huber et al., 2007). The transplantation of these cells after MI did not lead to the formation of teratomas. However, the engrafted cardiomyocytes also failed to survive, proliferate, or integrate with host cardiac tissue (Huber et al., 2007; Klug et al., 1996). In this study, the transplanted CPCs survived and integrated with the host cells **(Fig. 5)**, which could be related to differences in cell sources, monitoring time, and cell differentiation

procedures. However, hESC-CM transplantation improved damaged heart function in a similar manner as CPC transplantation. Because cardiomyocyte loss plays a significant role in the development of heart failure, cardiomyogenesis is thus an important process in the regeneration of heart tissue (Laflamme and Murry, 2005, 2011). Interestingly, the results of CPC transplantation after 14 days showed that the left ventricular wall of the CPC-infused group was thicker compared with the PBS group (Figure 3). Trichrome staining results also confirmed the absence of scar formation from the heart tissue of the CPC group (Figure 4), while a large scar was observed in the heart tissue of the PBS group (Figure 4). This result suggested that grafted CPCs improved cardiac function deterioration in damaged heart tissue. Currently, there are several theories on how cell transplantation improves myocardial perfusion and cardiac function. The transplanted cells are capable of differentiating into myocardial cells after infusion and eventually improve cardiac function. In addition, the transplanted cells may possess the ability to release paracrine factors, such as vascular endothelial growth factor, insulin-like growth factor, hepatocyte growth factor, and fibroblast growth factor, which activate endogenous cardiac progenitors to protect and repair lost heart muscle. In this study, it was demonstrated that the delivered CPCs managed to survive in the grafted tissue and did not transform into muscle fibers, but maintained the functions of cardiac cells (**Fig. 4, Fig. 5**). In contract, the heart tissue of the PBS group was severely damaged (**Fig. 4, Fig. 5**). This result was similar to previous studies about the ability of cells to integrate with host cardiac tissue both *in vitro* and *in vivo*(Kehat et al., 2004; Xue et al., 2005). This effect was identical to the positive effect of menstrual blood-derived MSC (MMC) transplantation 2 weeks after LAD ligation (Hida et al., 2008); or similar to the results of porcine iPSC transplantation in a MI model (Li et al., 2013). The similarity between the grafted MMCs and iPSCs was that both exhibited the ability to differentiate into heart cells after transplantation and displayed positive effects in clinical trials. Conversely, cardiac MSCs formed a scar after transplantation (Carlson et al., 2011; Silva et al., 2014), hESCs formed teratomas after transplantation (Hentze et al., 2009), and BM-MSCs had no influential effects on the grafted heart tissue after infarction (Grauss et al., 2007; Hou et al., 2007). Meanwhile, these cell types lack the ability to differentiate into heart muscle cells after transplantation, whereas grafted CPCs were positive for specific

antibodies of myocardial cells before and after transplantation, which might explain why CPC transplantation was shown to have a favorable effect in animal models compared with other cell types. Although the initial results were encouraging, as well as showing significant potential for CPC application in animal models of damaged heart tissue caused by ischemia, further studies should be conducted using alternative methods, such as electrocardiography, or by increasing the experimental evaluation timeline to produce stronger results.

CONCLUSION

The CP transplantation therapy in the mouse model of damaged myocardial tissue caused by ischemia initially showed positive effects for the prevention of myocardial injury after LAD ligation.

Acknowledgments

Ethical standard statements

Umbilical cord blood samples collected from informed consent of the mother used to isolate MSCs. The collection was performed in accordance with the ethical standards of the local ethics committee Van Hanh General Hospital, Ho Chi Minh city, Vietnam. All procedures and manipulationsof animals have been approved by the Institutional Ethical Committee of Laboratory of Stem Cell Research and Application, University of Science, Vietnam National University, Vietnam.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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