Intramuscular transplantation of pig amniotic fluid derived progenitor cells has therapeutic potential in a mouse model of myocardial infarction

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Running Header: pig AFPCs in myocardial infarction

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Abstract

Acute myocardial infarction (MI) is a fatal event causing a large number of deaths world-wide. MI results in pathological remodeling and decreased cardiac function which could lead to heart failure and fatal arrhythmia. Cell therapy is a potential strategy to repair the damage through enhanced angiogenesis or by modulation of the inflammatory process via paracrine signaling. Amniotic fluid-derived progenitor cells (AFPCs) have been reported to differentiate to several lineages and can be used without ethical concerns or risk of teratoma formation. Since pigs are anatomically, physiologically and genetically similar to humans and pregnant pigs can be an abundant source of AFCs, we used porcine AFPCs (pAFPCs) as our target cells. Intra-myocardial injection of AFPCs has been shown to cure MI in animal models. However, intramuscular transplantation of cells has not been extensively investigated. In this study, we investigated the therapeutic potential of intramuscular injection of pAFPCs on acute MI. MI mice were divided into 1) PBS control, 2) medium cell dose (1 x 10^6 cells per leg; cell-M), and 3) high cell dose (4 x 10^6 cells per leg; cell-H) groups. Cells or PBS were directly injected into the hamstring muscle 20 minutes after MI surgery. Four weeks after MI surgery, the cell-M and cell-H groups exhibited significantly better ejection fraction, significantly greater wall thickness, smaller infarct scar sizes and LV expansion index compared to the PBS group. Using in vivo imaging, we showed that the hamstring muscles from animals in the cell-M and cell-H groups had RFP positive signals. In summary, intramuscular injection of porcine AFPCs reduced scar size, reduced pathological remodeling, and preserved heart function after MI.

Introduction

Coronary artery disease (CAD) is one of the major causes of death world-wide. Acute myocardial infarction (AMI) is a CAD characterized by acute occlusion of the coronary artery. AMI often causes cell death in the ischemic area, results in pathological remodeling and decreasing cardiac function, and may even lead to heart failure eventually. There has been a recent focus on the use of cell therapy to treat myocardial infarction.

Of the different cell therapy strategies used, results from a phase I clinical trial and preliminary data from a phase II trial showed that mesenchymal stem cells (MSCs) had a low therapeutic efficiency when used to treat MI (25). When myocardial progenitor cells were transplanted, an irregular pulse was detected (26). Although transplantation of cardiac stem cells improved cardiac performance after AMI in a phase I trial (1, 2), obtaining sufficient cell numbers for transplantation was a major challenge. Induced pluripotent (IPS) cells were shown to possess great differentiation potential, but carried the risk of being tumorigenic.

Progenitor cells comprise the majority of cells in human amniotic fluid after 18 weeks of gestation and are a potential source of organ-specific precursor cells for clinical trials (6). Amniotic fluid derived progenitor cells (AFPCs) can be collected from various species including humans (17), pigs (32), mice (20). AFPCs have been shown to express mesenchymal cell-related markers such as CD44, CD105, and CD90, but do not express hematopoietic cells markers such as CD34, CD45, and CD31 (4, 17, 20). AFPCs have the potential to generate several lineages (4, 7, 20) and have been used to ameliorate liver fibrosis (24), myocardium infarction and acute tubular necrosis (29). AFPCs possess telomerase activity, maintain telomere length after serial passaging and do not form teratomas after transplantation (4, 7, 19). Furthermore, AFPCs express MHC class I, do not express MHC class II (17, 19), and are thought to modulate the immune system via secretion of IL-6, IL10, MCP-1 and MCP-2 (31, 27).

Studies investigating the mechanisms underlying cell therapy-mediated effects in the treatment of myocardium infarction reported that donor cells differentiate to myocardial cells, endothelial cells, and smooth muscle area to repair the lesion (1, 30). However, it has been suggested that donor cells cannot reside in the heart for a long time after transplantation (9, 16, 21), and some studies reported a paracrine signaling mechanism by which the donor cells reduced cell apoptosis and fibrosis and increased vasculogenesis to repair the injury (8, 35, 36).

A number of studies have investigated the efficacy of different transplantation methods to treat MI. Although intravenous infusion of progenitor cells is a convenient approach to treat MI, only a few cells were shown to migrate to the target site and the majority of the transplanted cells remained in the lung, causing increased risk of vascular occlusion (11, 21). Infusion of the cells from the coronary artery by cardiac catheterization is a common way to treat MI. However it causes occlusion of target vessels and increases the risk of interventions. Direct-heart injection is a technique for cell transplantation which has the advantages of low numbers of cells needed and a high retention rate in the target area. However, there is a risk of cardiac perforation (23). A recent study described a non-invasive transplantation strategy using intramuscular infusion, wherein the transplanted stem cells secreted SDF-1, IL6, VEGF, and HGF (paracrine effect) and enhanced angiogenesis in the injured heart tissue (34).

Since pigs resemble humans in terms of anatomy, physiology and genetics, and the pregnant pig is a rich source of AFPCs, we used pig-derived AFPCs (pAFPCs) as our target cells. In this study, we established a mouse acute MI model by ligating the left anterior descending coronary artery and studied the therapeutic potential of pAFPCs delivered via intramuscular injection.

Materials and methods

Animals

Male C57/B6 mice were acquired from the Laboratory Animal Center of National Taiwan University College of Medicine (Taipei, Taiwan). Animal maintenance and experimentation were performed under the guidelines of the Institutional Animal Care and Use Committee. Mice were fed the Laboratory Rodent Diet 5001 (PMI Nutrition Int., CA, USA). The temperature in the animal facility was maintained at $21\text{-}25^0C$, and humidity was maintained at 50-70%. The photo-period cycle consisted of 14 hours of light and 10 hours of darkness.

Establishment of Ds-red harboring pig amniotic fluid-derived progenitor cells

Ds-red harboring pigs were established and raised in our lab. In order to anesthetize Ds-red harboring pregnant 70 days pig (18 months old), 10ml Stresnil, 5ml Antopine muscularly and 300mg/10ml Citosol intravenously with $2\ O_2\text{:}1\ N_2$ and Halothane（2-Bromo-2-chloro- 1,1,1-trifluoroethane contains 0.01% Thymol）(All reagents: Janssen Pharmaceutica NV, CCPC, Belgium) were treated.

Abdomen and uterus were dissected to derive amniotic fluid. Utilize 70μm mesh (MiltenyiBiotec, Aubum, CA, USA) to filter the fluid and centrifuge the fluid in 1000g for 10 minutes. The pellet was re-suspended in α-MEM (Sigma-Aldrich, Aubum, CA, USA) and at a density of $2 \times 10^{\,5}$ cells/cm^2 to culture the cells in 10 cm dish (TPP, Trasadingen, Switzerland) in 37^0C in the presence of 5% CO_2. Until confluence, the supernatant was discarded and the cells were sub-cultured in the proportion of 1:2. These cells all express Ds-red protein and glow in red. Cells were cultured in 0.1 g∕ml 4,6 diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, Aubum, CA, USA) for 1 minutes and use phosphate buffered saline (PBS) to rinse the cells and eventually the cells can be observed under microscopic. In order to verify if the cells obtained were progenitor cells, the cells were harvested,

resuspended at a density of $5x10^5$ cells/100μl and incubated with CD31-PE, CD4a-PE, CD44-PE, CD90-PE (eBioscience, San Diego, CA,USA), and then subjected to flow cytometry (FACS scan flow cytometer, FACSAria Becton-Dickinson).

Establishment of myocardial infarction (MI) mouse model

We investigated whether xenogenic transplantation of pAFPCs could reduce the infarcted area while maintaining the geometry. The mouse model was established as the following. Briefly, 8 week old C57/B6 mice were anesthetized with an intraperitoneal injection of 2,2,2-tribromoethanol (1ml/10g) (Sigma-Aldrich, Aubum, CA, USA). The hair around the mouse breast was shaved and the mouse was intubated using the pressure mode of a small animal breathing machine SAR-830/AP (CWE, Ardmore, PV, USA) to maintain breathing for the duration of surgery. The surgery for ligation of the left descending coronary was performed as previously described (10). The mouse left breast was dissected, fourth rib muscle was separated, and the heart was exposed. The left descending artery was ligated at a 2mm distance from the atrium using 6-0 thread (UNIK, Taipei, Taiwan). A successful procedure was indicated by the blood supply turning pale.

The procedure is depicted in Figure 1. The mice were anesthetized with 2,2,2-tribromoethanol (0.1ml/10g) (Sigma-Aldrich, Aubum, CA, USA) (Fig.1.1). Then mice were intubated and their breathing maintained with a small animal ventilator (Fig.1.2). The skin was severed on the left chest and muscle layers were dissected (Fig.1.3, 1.4). A hole was made in the 4th intercostal space and the heart was exposed (Fig.1.5). In the MI group, the left descending coronary artery was ligated with silk suture (Fig.1.6). After ligation, a purse suture was made to close the skin and muscle layers (Fig.1.7). The operated mice were placed under a far red lamp for recovery (Fig.1.8). To confirm that ligation of the left descending coronary artery was successful, the color change of the ventricle wall was monitored. When ligation was successful, the wall of the infarcted region turned pale (Fig.1.9, 1.10).

Representative M-mode echocardiographs from the MI group and the sham group at 4 weeks after surgery are shown in Fig.1.11 and Fig.1.12, respectively.

Cell transplantation method and experimental design

Ds-red labeled pAFPCs were cultured to 7th passage and the cells were suspended by 2.5% trypsin-EDTA and the cells were centrifuged in 1000g for 5 minutes. The supernatant was discarded and the cell pellet was dispersed by PBS. The cells concentration was diluted to 1×10^6 cells in 50μl PBS and 4×10^6 cells in 50μl PBS. Among the research, we explored the curing efficiency of intramuscular injection of 7th passage of Ds-red labeled pAFPCs on acute MI. 8 weeks old Male C57/B6 MI mice were divided into 1) 50μl PBS control, 2) medium cell dose (1×10^6 cells in 50μl PBS per leg; cell-M), and 3) high cell dose (4×10^6 cells in 50μl PBS per leg; cell-H) groups. Cells or PBS were directly injected into the hamstring muscle by 29G insulin needle (BD, CA, USA) 20 minutes after MI surgery.

Echocardiography

Mice were anesthetized with inhalation of 1% Isoflurane (Sigma-Aldrich, Aubum, CA, USA) and echocardiography performed using Panoview β1500 and Coretronic Display Solution Corporation, Taiwan. M-mode was used to measure end-diastolic dimension (EDD), end-systolic dimension (ESD) and thickness of ventricular wall. The formula of ejection fraction percentage is (end-diastolic volume, EDV)：$[(EDD)^3 \times 7/ (2.4 + EDD)]$(ml), (end-systolic volume, ESV)：$[(ESD)^3 \times 7/ (2.4 + ESD)]$ (ml) and $[(EDV-ESV)/EDV] \times 100\%$. Left ventricle (LV) expansion index was assessed as previously described (15).

Histological assessment

Mice were anesthetized with 2, 2, 2-tribromoethanol and sacrificed using KCl (Sigma-Aldrich, Aubum, CA, USA). The heart was collected and fixed by soaking in 4% paraformaldehyde (PFA) (Sigma-Aldrich, Aubum, CA, USA) for 3 days. The tissues were embedded in methylmethacrylate (Sigma-Aldrich, Aubum, CA, USA) and sectioned longitudinally (5mm thickness) using a Leica ∕ Jung 2255 microtome (Leica, Wetzlar, Germany).

Masson trichrome stain

Sections were dewaxed with xylene (Sigma-Aldrich, Aubum, CA, USA) and rehydrated with different concentrations (100%, 95%, 90%, 80% and 70%) of alcohol. The biopsy specimens were fixed for 15 minutes in Bouin 'Solution (Sigma-Aldrich, Aubum, CA, USA) and washed with water until the yellow marks disappeared on the slice. The sections were then stained with Weigert's iron hematoxylin (Sigma-Aldrich, Aubum, CA, USA) for 10 minutes, rinsed with water for five minutes and then stained with Biebrich scarlet-acid fuchsin solution (Sigma-Aldrich, Aubum, CA, USA) for five minutes. The sections were rinsed with water for five minutes, stained with phosphomolybdic-phosphotungstic acid solution (Sigma-Aldrich, Aubum, CA, USA) for five minutes, washed with water and then placed in 1% acetic acid solution (Merck 100058, Germany) for a few seconds. This was followed by a dehydration step for a few seconds in 95% and 100% alcohol respectively. The sections were eventually soaked twice for 2 minutes each time in xylene and then mounted for observation.

Measurement of fibrosis scar size in the left ventricle of the heart

We measured scar size using the formula (Endo-diameter of left ventricle fibrosis scar area) + (exo-diameter of left ventricle fibrosis scar area)/(left ventricle endo-diameter + left ventricle exo-diameter) x 100% .

In vivo image system detection for Ds-red protein

In order to investigate the donor cell fate and destiny, the hearts were evaluated for the presence of Ds-red protein using the IVIS Imaging System 200 (Xenogen corporation, CA, USA).

Analysis of statistics

Our data were presented in mean ± standard deviation. In order to measure the significance of the difference, the student's t-test was implemented. The p-values <0.01 were regarded as significant statistic value after Bonferroni correction.

Results

We successfully obtained pig amniotic fluid-derived progenitor cells (pAFPCs) which expressed Ds-red protein (Fig.2 a, b,c). We also showed that pAFPCs expressed CD44 (cell migration marker) (Fig.2 d) CD90 (stem like cell marker) (Fig.2 e), but did not express CD31 (endothelial marker) (Fig.2 f), or CD4a (lymphocyte marker) (Fig.2 g).

We showed that MI resulted in the formation of a scar with a size of about 40% (Fig.3a). Wall thickness of the MI and sham groups were measured and the black bar indicates the measurement positions of LV wall thickness (Fig.3b). There was a significant decrease in the wall thickness in the infarct region of animals in the MI group compared to the sham group (Fig.3b). There was also a significant decrease in the LV ejection fraction of animals in the MI group compared to the sham group at 2 weeks and 4 weeks after MI. Animals in the MI group had a significantly lower ejection fraction at 2W compared to the baseline ejection fraction. These animals also had a significantly lower ejection fraction at 4w compared to that at 2w (Fig.3c), suggesting that after MI surgery, heart function decreased with time (Fig.3c). Decreased wall thickness in the infarction region, and formation of a fibrosis scar within injury sites indicated that a suitable MI model has been established.

A comparison of the experimental groups at 4 weeks after MI surgery showed that the cell-M and cell-H groups had a significantly higher LV ejection fraction (43.16 ± 2.81%, 50.04 ± 5.37%) compared with PBS group (37.54 ± 3.98%; $p < 0.01$) (Fig.4a). The LV ejection fraction of animals in the cell-M and cell-H groups at 4 weeks after MI surgery were similar to those at 2 weeks after MI (Fig.4a). Animals in the cell-H group had a smaller LV infarction scar size compared to the PBS group (31.71±1.87% vs. 39.00±2.79%) (Fig.4b). However, the animals in the cell-M and cell-H groups had a higher ventricle wall thickness at 4 weeks after MI surgery compared to animals in the PBS group (0.58±0.04mm and 0.56±0.09mm vs. 0.49±0.05mm, respectively) (Fig.4c). Left ventricle (LV) expansion index was assessed as previously described (15). The data demonstrated that the LV expansion index was significantly lower in the cell-M and cell-H groups compared to the PBS group (0.72±0.09 and 0.63±0.02 vs. 0.88±0.13, $p<0.01$). Compared to animals in the PBS group, animals in

the cell-M and cell-H groups also had a smaller LV expansion index, which indicated a lower degree of LV dilation (Fig.4d). As a result, MI mice in the cell-M and cell-H groups showed an amelioration in the LV expansion index compared to the PBS group at 4 weeks after injection into the quadriceps. However, there was no significant difference between the cell-M and cell-H groups (Fig.4d). Representative short axis heart sections were prepared from normal mice (Fig.4.e1), MI mice treated with PBS (Fig.4.e2), MI mice in the cell-M group (Fig.4.e3), and MI mice in the cell-H group (Fig.4.e4), and stained with Masson's trichrome stain. There was an improvement in the MI hearts in the cell-M (B) and cell-H (C) groups (Fig.4e1-e4).

Using IVIS imaging system 200, we detected Ds-red fluorescent protein in the cell-M and cell-H groups (Fig.5). IVIS showed that both doses (1×10^6 or 4×10^6 cells/animal) were retained in the quadriceps for at least 4 weeks after injection (Fig.5), suggesting that the donor cells were not discarded by the host immune system.

Discussion

In this study, we successfully established a mouse MI model using left descending

coronary artery ligation. We obtained Ds-red-harboring pAFPCs and verified their identity by flow

cytometry. Porcine-derived bone marrow mononuclear cells were previously used to treat porcine MI

and were shown to elevate regional blood flow 4-fold and increase capillary densities 3-fold compared

to the control group. This was shown to be effected by paracrine signaling via mediators such as IL-

1b, TNF-a, bFGF, VEGF and Ang-1 (18). The fact that only 10% of the donor cells were maintained

for a period of 2 weeks strongly suggested that recovery of the lesion was mediated via the paracrine

effect (13). Donor cells were also previously shown to stimulate the proliferation and differentiation of

endogenous stem cells to myocardial cells and vessel structures (14, 33). Approximately 14% of donor

cells were shown to give rise to myocardial cells (30). A recent clinical trial showed recovery of heart

function in 6% of study patients after infusion of donor cells to the coronary artery (12). In this study,

we aimed to observe the therapeutic efficacy of high dose or medium dose xenogenic AFPCs injected

into the leg muscle of MI mice. Our data showed that both high dose and medium dose of donor cells

ameliorated the symptoms of MI. Our results were consistent with previous data showing that

injection of bone marrow-derived stem cells (BMSCs) caused a significant increase in myocardium

regeneration (28).

Although pAFPCs were previously demonstrated to be capable of giving rise to spontaneously

beating myocardial cells in vitro (4), they could not differentiate to myocardial cells in vivo when

directly injected into the heart (32).Interestingly, secretion of MCP-1, IL-8, SDF-1, and VEGF by

AFPCs was shown to promote vasculogenesis, and injection of MI mice with conditioning medium

containing these factors ameliorated the symptoms and conferred the same therapeutic effect as direct

cell transplantation (3). These data suggested that AFPCs repaired the damage via paracrine signaling.

It was also previously shown that AFPC-secreted cytokines injected in the leg muscles were

transported to the damaged heart via the blood stream (22). Due to the risk of perforation when cells

are directly transplanted into the heart, we injected the donor cells into the quadriceps and showed that this area provided an optimal scenario for donor cell survival.

It was previously demonstrated that xenogeneic AFPCs rescued the infarcted heart either by means of differentiating to endothelial cell, smooth muscle cells and myocardial cells or via paracrine signaling in a rat model (3, 22). Since directed transfusion of cells to the injury site tissue presents challenges in clinical practice, a novel strategy was developed to implant the mesenchymal stem cells into the quadriceps, and the implanted cells were shown to preserve heart function in a paracrine manner (34). In the present study, we injected the Ds-red harboring pAFPCs to the mouse quadriceps and investigated whether these cells could improve the function of infarcted myocardium. We showed that Ds-red harboring pAFPCs reduced scar formation and possibly participated in remodeling after MI. Since the therapeutic efficacy of high dosage of implanted cells was shown to be higher than the low dosage (4, 17, 20), we investigated if the therapeutic effect was influenced by the number of implanted cells. We injected the quadriceps of the MI mouse with a low dose (1×10^6cells/animal) or a high dose (4×10^6cells/animal) of Ds-red-harboring pAFPCs. We utilized echocardiography, tissue sections and the non-invasive in vivo imaging system (IVIS) to evaluate therapurtic effects of the two doses and to observe whether the cells were maintained in the quadriceps.

Our echocardiography data demonstrated that cells injected into quadriceps could attenuate the reduction of left ventricle ejection fraction (EF). We also showed that implantation with a high cell dose was significantly more effective in the preservation of heart contractility and heart structure after myocardial infarction compared to the low cell dose. Our results were consistent with a previous study where cells were directly transplanted into the lesion site of the myocardium (3). Additionally, we also used IVIS to show that the donor cells could be detected in the quadriceps for at least 4 weeks after implantation and could attenuate the deterioration of heart contractility. Our

results suggested that more cells were retained at the site of implantation compared to previous studies where the cells were directly injected into the infarcted heart (5, 22).

Since AFPCs were previously shown to disappear 4 weeks after transplanting into the heart (22) and kidney (29), our data suggested that the quadriceps (leg muscle) provides the optimal environment for cell survival. Furthermore, localization of the Ds-red protein in the quadriceps supports a previous study showing the role of secreted cytokines in repair of the injured site (34). However, since IVIS has a limited sensitivity for detection of the fluorescent protein, it will be informative to use more sensitive methods such as PCR to detect the localization of Ds-red labeled cells in future studies.

In summary, intramuscular injection of pAFPCs can reduce scar size, reduce pathological remodeling, and preserve better heart function after MI. Intramuscularly injected AFSCs can potentially reside in the hamstring muscle, and play a role in recovery of heart morphology and function via paracrine signaling mediated by secreted factors.

Conclusion

Xenogeneic AFPCs derived from pig preserved heart function, and reduce scar formation which possibly play a role in remodeling after myocardial infarction. In addition, the high dose of implanted cells showed more efficacy than the low dose. The pAFPCs-transplanted group had a higher left ventricle ejection fraction compared to the PBS control group at 4 weeks after implantation. Importantly, data from short axis heart sections revealed that ventricle wall thickness in the cell-transplanted group was significantly thicker than in the control group, while heart expansion index and scar size in the cell-transplanted groups were significantly less than in the control group. In summary, pAFPCs improved heart function and retained structure in a mouse model of myocardial infarction.

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Figure Legends:

Fig 1. Establishment of the mouse MI (myocardium infarction) model. Mice were anesthetized with avertin, and the left chest shaved (1). The mice were then intubated and their breathing maintained with a small animal ventilator (2). The skin was severed on the left chest and muscle layers were dissected (3) (4).We made a hole in the 4th intercostal space in order to expose the heart (5). In the MI group, the left descending coronary artery was ligated with silk suture (6). After ligation, a purse suture was made to close the skin and muscle layers (7). The operated mice were kept under a far red lamp for recovery (8). Color change of the ventricle wall was monitored to assess if ligation of the left descending coronary artery was successful. When ligation succeeded, the wall of the infarcted region would turn pale (9) (10). Representative M-mode echocardiograph from the MI group and Sham group at 4 weeks after surgery (11) (12).

Fig2. Establishement and characterization of Ds-red pAFPCs. Morphology of Ds-red pAFPCs was evaluated under fluorescent film (a), DAPI staining for nucleus (b), and merge (a) and (b) picture (c). Surface antigen markers were detected by flow cytometry. Ds-red pAFSCs expressed CD44 (cell migration marker) (d) and CD90 (stem cell marker) (e), but did not express CD4a (lymphocyte marker) (f) and CD31 (endothelial marker) (g). Scale bar: 75um.

Fig 3. Comparison of scar size and LV ejection fraction in MI and sham control groups. Left ventricular (LV) infarction scar size was measured. The scar size was calculated as scar length divided by LV circumferenceand is depicted as a black line. After MI, a scar with a size about 40% formed (a).

Wall thickness of MI group and Sham group are shown and the black bar indicates measurement positions of LV wall thickness (b). Wall thickness in the infarct region was significantly reduced in the MI group (b). The MI group had a significantly lower LV ejection fraction compared to the Sham group at 2 weeks and 4 weeks after MI. In the MI group, the ejection fraction at 2W was significantly

lower than the baseline ejection fraction. The ejection fraction at 4w was also significantly lower than the ejection fraction at 2w. These results suggested that heart function decreased with time after MI surgery,(c). Here are representative short axis heart sections from the MI group and Sham group. Sections were stained with Masson's trichrome staining. Collagen was stained with blue color. *$p<0.01$ vs Sham. n=4 animals/group. Scale bars: 1mm.

Fig 4. Effects of transplantation of Ds-red pAFPCs on LV ejection fraction (a), LV infarction scar size (b), wall thickness (c), and LV expansion index (d) in MI mice.

Short axis of heart sections stained with Masson's trichrome from control mice (e1), MI mice treated with PBS (e2), MI mice treated with cell-M (e3), and MI mice treated with cell-H (e4). Animals in the cell-M and cell-H groups had a better LV ejection fraction compared to the PBS group, and animals in the cell-H had a better LV ejection fraction than animals in the cell-M group at 4 weeks. However, there was no significant difference between the three groups at 2 weeks (a). The LV infarction scar size was decreased in the cell-H compared to the PBS group 4 weeks after injection into MI mice quadriceps (b). Wall thickness in the cell-M and cell-H groups was thicker compared to that in the PBS group 4 weeks after injection into the quadriceps of MI mice (c). The LV expansion index was ameliorated in the cell-M and cell-H groups compared to the PBS group 4 weeks after injection into the quadriceps of MI mice. However, there was no significant difference between the cell-M and cell-H groups (d). Representative short axis heart sections stained with Masson's trichrome staining from normal mice (e1), MI mice treated with PBS (e2), MI mice in the cell-M group (e3) and MI mice in the cell-H group (e4) . There was a significant improvement seen in the cell-M (e3) and cell-H (e4) groups *$p < 0.01$ vs PBS group, # $p < 0.01$ vs Cell-M group (n= 5 animals/group). Scale bars: 1mm.

Fig 5. IVIS imaging to detect red fluorescent protein signals in hearts collected from the PBS, cell-M and cell-H groups. Two doses of AFPCs (2×10^6 and 8×10^6 cells/animal) were retained in the quadriceps for 4 weeks after injection. Scale bars: 5mm.

FIGURE 1

FIGURE 2

FIGURE 3

FIGURE 4

FIGURE 5