Therapeutic Approach for Global Myocardial Injury Using Bone Marrow-Derived Mesenchymal Stem Cells by Cardiac Support Device in Rats

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Abstract

Bone marrow-derived mesenchymal stem cells (BMSCs) have been considered a promising therapeutic approach to cardiovascular disease. This study intends to compare the effect of BMSCs through a standard active cardiac support device (ASD) and intravenous injection on global myocardial injury induced by isoproterenol. BMSCs were cultured *in vitro*, and the transplanted cells were labeled with a fluorescent dye CM-Dil. Isoproterenol (ISO) was injected into the rats; two weeks later, the labeled cells were transplanted into ISO-induced heart injury rats through the tail vein and ASD device for five days. The rats were sacrificed on the first day, the third day, and the fifth day after transplantation to observe the distribution of cells in the myocardium by fluorescence microscopy. The hemodynamic indexes of the left ventricle were measured before sacrificing. H&E staining and Masson's trichrome staining were used to evaluate the cardiac histopathology. In the ASD groups, after three days of transplantation, there were a large number of BMSCs on the epicardial surface, and after five days of transplantation, BMSCs were widely distributed in the ventricular muscle. But in the intravenous injection group, there were no labeled-BMSCs distributed. In the ASD+BMSCs-three days treated group and ASD+BMSCs -five days-treated group, left ventricular systolic pressure (LVSP), maximum rate of left ventricular pressure rise (+dP/dt), maximum rate of left ventricular pressure decline (-dP/dt) increased compared with model group and intravenous injection group ($P<0.05$). By giving BMSCs through ASD device, cells can rapidly and widely are distributed in the

myocardium and significantly improve heart function.

Keywords: Global myocardial injury, BMSCs, Active cardiac support device (ASD), Stem cell treatment, Epicardial delivery.

1. Introduction

Cardiovascular diseases (CVD) have the highest mortality rate throughout the world. Myocardial hypertrophy and fibrosis can cause the formation of cicatricial tissue in the heart, thus leading to the death of myocardium which can affect heart function and finally result in heart failure [2]. Therefore, prevention of myocardial fibrosis may slow the progression of heart failure. Recent studies have shown that mesenchymal stem cells (MSCs) can promote angiogenesis, inhibit cell apoptosis, and promote tissue regeneration [3, 4]. Thus, MSCs transplantation has become a promising alternative treatment to heart transplantation [1, 5]. There are many kinds of cells used in transplanting, among these are bone marrow stem cells (BMSCs) which show superiority to other cells for they can differentiate into multiple cells and released the growth factors into the damaged myocardium which will improve the symptoms of heart failure and increase the blood flow of infarcted area, thereby promoting myocardial regeneration [6-9]. The safety and feasibility of BMSCs transplantation have been studied and documented by some clinical trials [10, 11].

As a right donor cell for myocardial transplantation, MSCs are mostly injected through epicardium, endocardium with a catheter, intracoronary or intravenous, but they have some shortcoming that can't be avoided [12, 13]. This study aims to investigate whether an innovative

epicardial delivery method, active hydraulic ventricular attaching support system (ASD), is capable of increasing the storage and engraftment of stem cells and repair global myocardial injury.

The primary techniques for BMSCs administration include 1. Intravenous infusion: This is the simplest way to transplant cells with the ability of multiple infusions. 2. Epicardial injection: BMSCs are injected into the myocardial infarction area when the thoracic surgery is performed. 3. Endocardial injection: Cells are inserted into the endocardium through a catheter 4. Intracoronary injection: This method needs the application of the OTW balloon catheter to the infarcted coronary artery, then cells are sent through it to the ischemic myocardium [14-16]. However, current studies focus more on local delivery of stem cells in myocardial infarcted areas, but these local delivery methods will not be effective in global myocardium injury. Our research provided a novel approach to deliver BMSCs into the epicardial using ASD, which is a net cover made of silicone covering both ventricles of the heart. ASD can provide sufficient contact time between BMSCs and epicardium and can widely spread the cells.

In this research, we investigate whether delivery of BMSCs by ASD can improve cardiac function after ISO-induced global myocardial injury, compared with intravenous injection, the systemic delivery method which can also delivered cells multiple times.

2. Materials and methods

2.1. Ethical Statement

All procedures and experiments used in this study comply with guidelines of the local

institutional ethical committee of the China Pharmaceutical University (Jiangsu Province, Nanjing, China) regarding care and welfare of animals under study (T/CPU, 235-2018, 2018-09-09).

2.2. Experimental animals

Zhejiang Laboratory Animal Center provided a total of 40 male Sprague-Dawley (SD) rats weighing from 220-250g. Animal handling and disposal during the experiment conform to the criteria of the China Pharmaceutical University Institutional Animal Care and Use Committee. All animals were placed in the environment of free food and water for 1 week before the experiment. Isoproterenol was used to induce global myocardial injury, as described before with little modification [17]. Briefly, isoproterenol was administered with saline to a solution of 85 mg/ml and injected into the rats at 170mg/kg for two consecutive days. The control group was administered subcutaneously with 0.5 ml of saline. Forty rats were randomly divided into two groups, 8 rats were selected randomly as the (ⅰ) Control group, and the other rats were modeled with isoproterenol according to the method mentioned above. Two weeks later, the survived 30 rats were divided into 5 groups randomly, (ⅱ) ISO group (model group), (ⅲ) BMSC+ASD-one day treated group (ⅳ) BMSC+ASD-three days treated group (ⅴ) BMSC+ASD-five days treated group, (ⅵ) BMSC+IV treated group. BMSCs were given through the tail vein in IV-treated group and were delivered through the outside tube of ASD in the ASD-treated groups, once a day for 5 days.

2.3. Isolation of BMSCs

3 to 4 weeks old rats with the weight of 60-80g were sacrificed. BMSCs were isolated from tibia and femur under sterile conditions, and cultured in low glucose DMEM (Genview, USA) containing 10% FBS (Hyclone, USA) and 1% penicillin-streptomycin (Gibico, USA) in plastic culture flasks with the concentration of 1×10^9 at 37℃ in 5% CO_2 saturated wet incubator. Four days later, the whole culture solution was changed, the unattached cells were washed off, and the adherent cells propagated for 3 to 4 passages before transplantation. Then the culture solution was changed every two days. When the cells grow to 80%-90% confluence, trypsinized (0.25% trypsin/1Mm EDTA, Solarbio, China), and expanded in a ratio of 1:2.

2.4. Identification of BMSCs

Harvesting the third generation BMSCs, and analyzing them by flow cytometry. Trypsinize and prepare the single-cell suspension in phosphate-buffered saline (PBS) per 1×10^6 cells, 2.5μg/ ml, 2 antibodies including FITC anti-rat CD45 antibody, APC anti-mouse/rat CD29 antibody (Biolegend, USA) were added and incubated on ice for 30 min in the dark. Cells were washed with 2ml PBS twice and centrifugated at 350rpms for 5 minutes. Flow cytometric analysis was performed, and the quantitative analysis results were obtained.

2.5. Labeling of BMSCs

To track the BMSCs after transplantation, cells were labeled with cell tracker CM-Dil (Invitrogen, USA). Cells were suspended at a density of 1×10^6/ml in the serum-free culture medium, then 5μl cell labeling solution was added into the suspension with very gentle mixing. The labeled cells were incubated at 37 degrees for 20 minutes, then washed twice with 2ml PBS

at 1000 rpms for 5 minutes. The labeled cells were observed under fluorescence microscope and analyzed by flow cytometry, then cultured in expansion medium for transplation.

2.6. Designing and Manufacturing of ASD

Active hydraulic ventricular attaching support system (ASD) is a net cover made of silicone a biocompatible material with a heart-shaped configuration consisting of flexible interconnected hollow tubules; it is surgically fixed to the outer wall of the heart and covers both ventricles of the heart providing physical support [18]. ASD is manufactured as previously described method [19, 20]. Briefly, the 3D printed wax model of ASD was made at first, and covered it with silicone evenly, then heated it at 100°C for one hour to melt the wax and obtain the pure silicone ASD **(Fig. 1a, b,c)**.

2.7. Implantation of ASD

Two weeks after the last ISO injection, rats were sedated with 10% chloral hydrate by intraperitoneal injection (0.03ml/kg). The small animal ventilator was used to keep breathing. After confirming the ventricular position, the pericardium was separated, and the heart was exposed. ASD was fixed on the surface of the heart with suture and cover both ventricles, and with a connection of silicone tube which entered the chest wall from the second intercostal space, and pierced the skin from the back of the ear **(Fig. 1d, e)**.
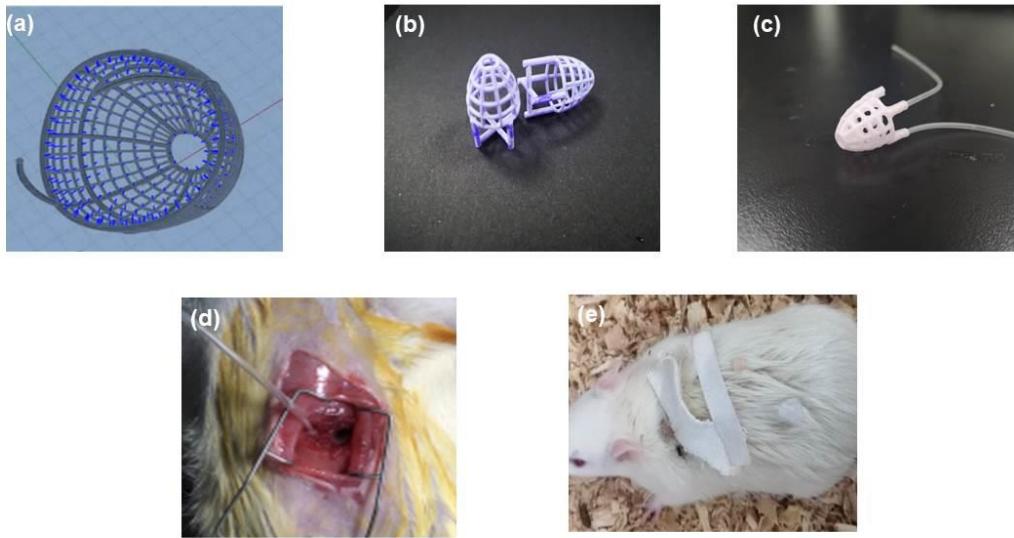
Figure 1. (a): 3D structure of ASD, **(b)**: 3D printed wax model of ASD, **(c)**: Pure silicone ASD, **(d):** Implantation of ASD, **(e):** Catheter back of the ear for cell delivering.

2.8. Implantation of BMSCs

The BMSCs labeled by CM-Dil were harvested and suspended at a density of 2×10^5/ml with PBS. Rats in intravenous injection group and ASD groups received 2×10^5 cells once a day for 5 days. In this study, the rats in ASD group were sacrificed on 1, 3, and 5 days and IV injection group were sacrificed on 5 days.

2.9. Cardiac performance measurement

Before sacrificing, the rats were anesthetized with 10% chloral hydrate (0.3g/kg) by intraperitoneal injection. II electrocardiograph was used to monitor ECG continuously. In order to evaluate the left ventricular function, the right carotid cannula method was used, generally speaking, one end of the heparin saline (500U/ml)-filled polyethylene tube was inserted into the

left ventricle and the other end connected to the pressure sensor and then connected to the BL-420S biologic function experiment system and the hemodynamic indexes of left ventricle were measured including heart rate, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), maximum rate of LV pressure rise ($+dP/dt_{max}$) and maximum speed of LV pressure decline ($-dP/dt_{max}$).

2.10. Cardiac histopathology

After hemodynamic evaluation, the animals were sacrificed, and the heart was quickly removed. Washed in saline and cut into two transverse segments paralleling to the atrioventricular groove, then one was placed in 10% formalin for 24 hours, and the other was stored at -80℃ in OCT. For Masson's trichrome and hematoxylin and eosin (H&E) staining, the segment in formalin was made into a wax block and cut into slices with a thickness of 5μm. Digital pathology was used and the extent of fibrosis was quantified by ImageJ software. The other part was made into frozen section with a thickness of 5μm, observing labeled cells by fluorescence microscope.

2.11. Statistical analysis

All experimental data were presented as mean ±SD, analyzed by statistical package for the social sciences (SPSS21.0). The one-way analysis of variance (ANOVA) test was used between groups. *P* values <0.05 were considered statistically significant.

3. Results

In this experiment, three rats died after the injection of ISO in 24h; one died after the ASD implant surgery. Therefore there were six rats left in each group.

3.1. Morphology and characterization of MSCs

After the cells were inoculated into the culture flask, the cells were round and of different sizes, suspended them in culture medium (**Fig. 2a**). At 72 hours after the extraction, the number of cells was increased, with the shape of round or spindle (**Fig. 2b**). After 14 days of culture, the cells increased in a large number, which was uniform spindle-shaped (**Fig. 2c**).

BMSCs were labeled with CM-Dil at 3 passage and detectd by fluorescence microscope before delivery (**Fig. 2d**). The flow cytometry results showed CM-Dil labeling rate was >90% (**Fig. 2e**), and the isolated cells expressed the MSC marker CD29 but didn't have the pan leukocyte marker CD45 (**Fig. 2f**).
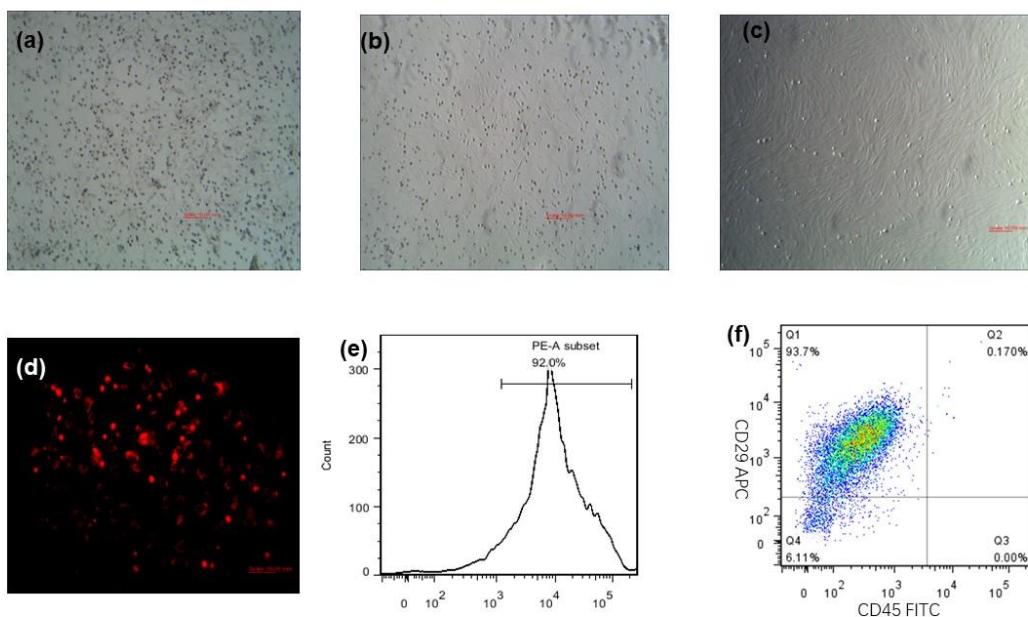
Figure 2. Morphology of BMSCs at different stages (40× magnification). **(a)**: The first day of cell inoculation, **(b)**: 4 days after initial plating, **(c)**: 14 days after initial plating. BMSCs were collected from rat femurs and tibias and expanded in culture with an irregular shape, three or

four days later, the cells appeared fibroblast-like in morphology, 14 days later, the cells formed a confluent monolayer. **(d):** BMSCs were labeled with Chloromethylbenzamido-1,1´-Dioctadecyl-3,3,3´3´Tetramethylindocarb cyanine Perchlorate (CM-Dil) 100× magnification. **(e):** Flow cytometric analysis showed the labeling rate of CM-Dil was >90%. **(f):** Flow cytometric analysis of adherent. Most cultured BMSCs expressed CD29. In contrast, the majority of MSCs CD45 negative.

3.2. Effects of BMSCs transplantation on heart function

In this experiment, there was no significant difference in heart rate. Two weeks after the last ISO injection. Compared with the ISO group, LVSP, LV+dP/dt$_{max}$ and LV-dP/dt$_{max}$ ($P<0.05$) were significantly increased in ASD-three days treated group and ASD-five days treated group. As compared to the BMSCs+iv group, the ASD-five days group also showed significantly increased LVSP and LV+dP/dt$_{max}$ ($P<0.05$) (**Table 1, Fig. 3**).
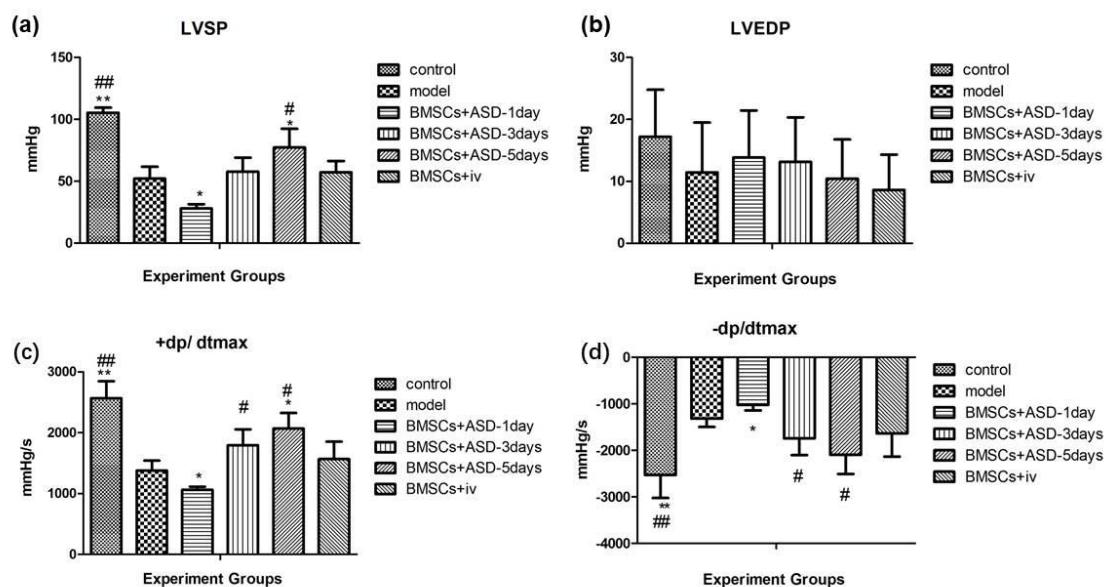
Figure 3. Effects of BMSCs transplantation on left ventricular (LV) hemodynamic parameters in the experiments. #significant difference vs. model group, ##*P* value <0.01, #*P* value <0.05, *significant difference vs. BMSCs+IV group, ***P* value <0.01, *P* value <0.05 (Each group n = 6). HR, Heart rate; LVSP, Left ventricular systolic pressure; LVEDP, Left ventricular end-diastolic pressure; +dP/ dt$_{max}$, Maximum rate of left ventricular pressure rise; -dP/dt$_{max}$, Maximum rate of left ventricular pressure decline.

Table 1. The effects of BMSCs transplantation on HR, LVSP, LVEDP, and ±dP/dt$_{max}$

Groups	HR	LVSP	LVEDP	+dP/dt$_{max}$	-dP/dt$_{max}$
		mmHg	mmHg	(mmHg/s)	(mmHg/s)
control	380.83±30.82	105.34±4.06 ##**	17.18±7.16	2566.94±279.73 ##**	-2528.8±495.23 ##**
model	423.67±11.41	52.12±9.56	11.45±8.04	1378.91±162.38	-1317.87±181.75
BMSCs+A SD-1day	421.17±21.65	29.58±4.96*	13.84±7.59	1062.22±52.23*	-1019.61±109.9*
BMSCs+A SD-3days	415.16±20.67	57.64±11.47	13.13±7.21	1794.03±261.66#	-1741.13±360.46#
BMSCs+A SD-5days	425.5±34.56	77.34±15.03#*	10.44±6.32	2082.03±283.48#*	2093.75±413.74#
BMSCs+iv	411.33±51.89	57.16±9.18	8.6±5.70	1566.77±287.43	1634.32±500.77

Data are presented as mean ±SD, #significant difference vs. model group, ##*P* value <0.01, #*P* value <0.05, *significant difference vs. BMSCs+IV group, **P* value <0.01, *P* value <0.05 (Each group n = 6). HR, Heart rate; LVSP, Left ventricular systolic pressure; LVEDP, Left ventricular end-diastolic pressure; +dP/dt$_{max}$, Maximum rate of left ventricular pressure rise; -dP/dt$_{max}$, Maximum rate of left ventricular pressure decline.

3.3. Distribution of CM-Dil labeled cells

After transplantation, labeled cells were observed in ASD+BMSCs -three days treated group and ASD+BMSCs -five days-treated groups, but not in other groups (**Fig.4**).
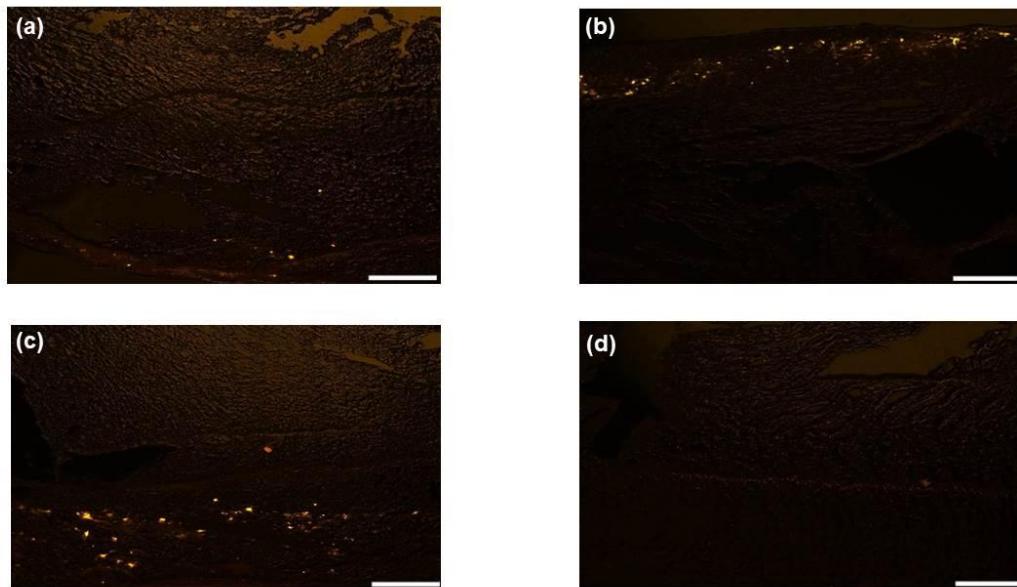
Figure 4. BMSCs engraftment in myocardium. The CM-Dil labeled BMSCs were detected under fluorescence microscopy. **(a):** BMSCs+ASD-1day group, **(b):** BMSCs+ASD-3days group, **(c):** BMSCs+ASD-5days group, **(d):** BMSCs+IV group, 40× magnifications, scare bar 100 μm.

The labeled cells in BMSCs+ASD-3 day's group were mainly distributed on the epicardium,

BMSCs were observed near the middle layer of the myocardium in BMSCs+ASD-5days group.

There were no BMSCs could be found in BMSCs+IV group.

3.4 Histopathological examination of heart tissues

We investigated the cardiac histopathology by H&E staining and Masson's trichrome

staining. In the group of rats undergoing myocardial injury, H&E staining showed there were a

significant loss of myocardial structure and the infiltration of inflammatory cells (**Fig. 5**).

Cardiac muscle fibers of the model group degenerated in BMSCs+ASD-1day group, BMSCs+IV

group (**Fig. 5c, 5f**). There were a large number of inflammatory cells infiltrated and distributed

diffusely. Myofibrils were arranged neatly near endocardium on BMSCs+ASD-3days group (**Fig.**

5d). In BMSCs+ASD-5days group, myocardium was arranged tightly, and less inflammatory

cells dispersed in the endocardium (**Fig. 5e**).
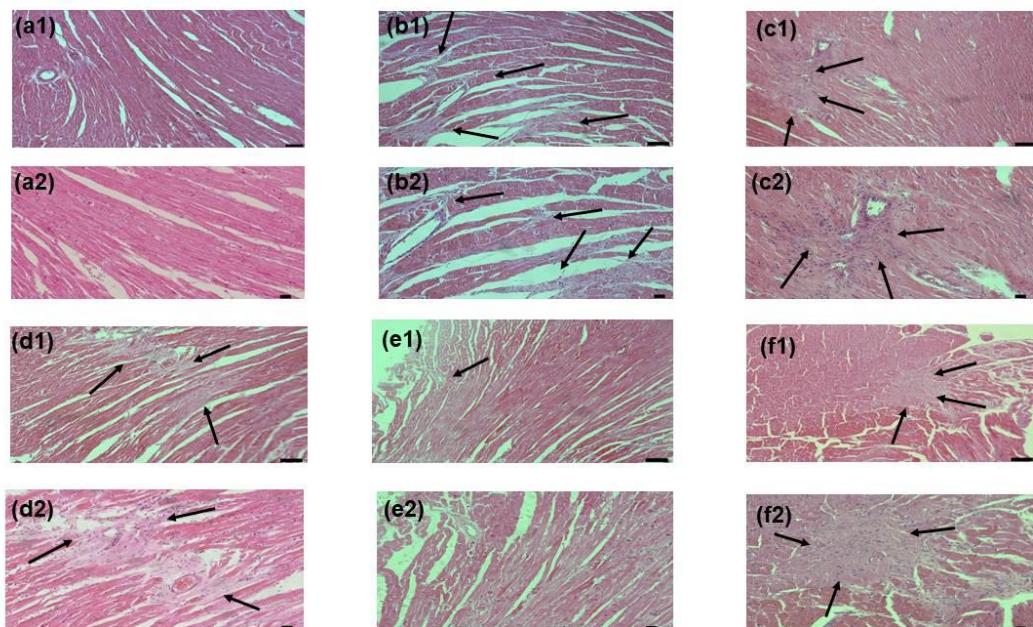
Figure 5. H&E staining of the ventricles **(a):** control group, **(b):** model group, **(c):**

BMSCs+ASD-1day group, **(d):** BMSCs+ASD-3days group, **(e):** BMSCs+ASD-5days group, **(f):** BMSCs+IV group, a1, b1,c1, d1, f1 100× magnification, a2, b2, c2, d2, e2 200× magnification, scare bar 100 μm. H&E staining showed that the myocardial fibers from the ventricles in the model group, BMSCs+ASD-1day group, BMSCs+IV group were thicker and myofibril ruptured. A large number of inflammatory cells (arrow heads) infiltrated and distributed diffusely. Myofibrils were arranged neatly near endocardium on BMSCs+ASD-3days group. Myocardium was arranged tightly on BMSCs+ASD-5 day's group.

Masson's trichrome staining showed a massive increase in the collagen fibers among the degenerating muscle fibers in the model group, BMSCs+ASD-1day group, BMSCs+IV group (**Fig. 6**). After 5 days' treatment, the collagen fibers decreased ($P <0.05$), especially in the medium and endocardium of the myocardium.
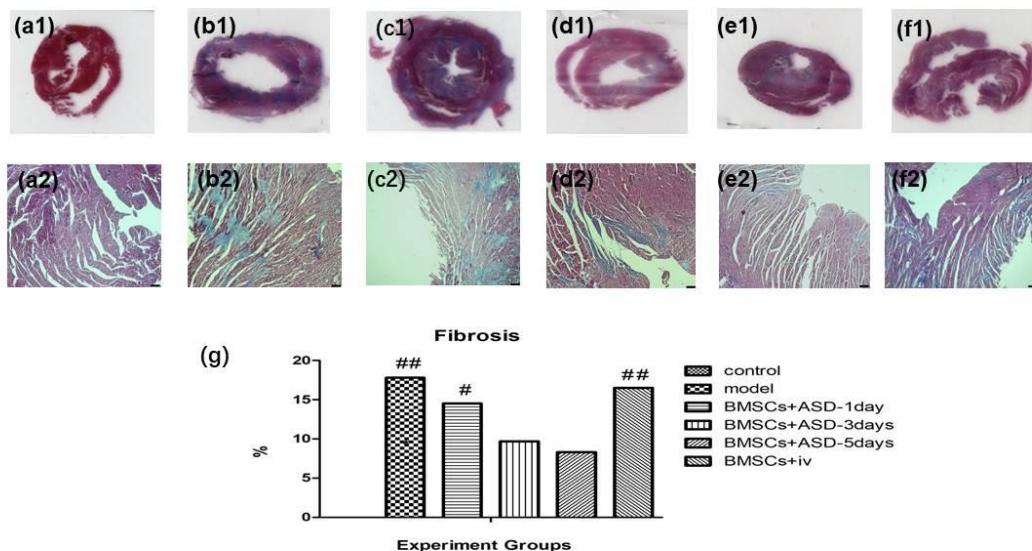
Figure 6. Masson's trichrome of ventricles **(a):** control group, **(b):** model group, **(c):**

BMSCs+ASD-1day group, **(d):** BMSCs+ASD-3days group, **(e):** BMSCs+ASD-5days group, **(f):**

BMSCs+IV group, **(g):** quantification of fibrosis of heart tissues. # significant difference vs.

BMSC+ASD-5 days group, ##*P* value <0.01, #*P* value <0.05 (Each group n = 6)); a1, b1, c1, d1,

f1 image scanning by digital pathology; a2, b2, c2, d2, e2, f2 in 100× magnifications, scare bar

100 μm. Masson's trichrome staining showed that large collagen fibers appeared in the model

group, ASD-1 day group, and IV group. But in the group of ASD-3 days and ASD-5 days, the

area of degenerating muscle fibers reduced.

4. Discussion

Teerlink *et al.,* [21] have confirmed that subcutaneous injection of ISO (> 85 mg/kg) can cause

diffuse myocardial fibrosis after 2 weeks, and gradually lead to heart failure. Compared with

ischemic myocardial infarction, this method can reflect the natural process of HF, which is

similar to that in patients [22]. In this study, we established the rat model of extensive myocardial

injury by subcutaneous injection of ISO at a dose of 170 mg/kg for 2 consecutive days. The

changes of hemodynamic parameters often used to evaluate the heart function [23, 24]. Two

weeks later, our results showed that in comparison with the control group, the left ventricular

function reduced in the groups received ISO injection (*P* <0.05).

Bone marrow-derived mesenchymal stem cells (BMSCs) are multipotent stem cells that exist in

the bone marrow and can differentiate into various mesodermal cells, such as bone cells, fat, and

matrix tissue cells. BMSCs were the kind of cells that can secrete a variety of hematopoietic

factors to support hematopoiesis and with the immune regulation ability [25].

BMSCs can express a variety of surface antigens, but no specific surface antigens. According to reports, CD29, CD44, CD59 are positive for surface antigen expression, while hematopoietic stem cell markers CD45, CD14, and CD117 are negative. In this experiment, CD29 and CD45 were selected, and it was found that adherent cells express $CD29^+CD45^-$, combining with the results of morphology, we confirmed that the isolated and cultured cells were BMSCs.

BMSCs therapy is a viable option for heart renovation. There are many ways to deliver BMSCs, but traditional methods of transportation always have some problems such as low storage rate, low cell survival rate, and inability to be administered multiple times [26]. Intravenous injection of BMSCs has been thought to be useful for diffuse myocardial disease, before engrafting in the infarcted myocardium, cells will go through the systemic circulation and receive the local signal of injured tissue [27]. In recent studies, epicardium delivery of BMSCs has been considered a promising method [28, 29], which can not only avoid myocardial injury and inflammation caused by intravenous injection. ASD is a kind of device that can deliver the drug or cells into the epicardium improving delivery efficiency [30, 31]. Besides, it can provide physical support to prevent ventricular enlargement. Our objective is to compare the effectiveness of multiple BMSCs deliveries through veins and ASD.

In this experiment, the increase of LVSP, and $\pm dP/dt_{max}$ in BMSCs+ASD-3 day's group and BMSCs+ASD-5 days' group indicated that the cardiac contraction improved. In our previous research, ASD can not only deliver drug but also provide pressure. For the BMSCs+ASD-1day group, the decrease of these indicators may be caused by the thoracotomy. There was no

difference in these indicators in the BMSCs+IV group compared with model group. The results indicated that although IV injection was a more convenient delivery method, but the treatment effect was not evident. According to other study, the cells would be caught by other organs such as liver, lung or spleen [32].

CM-Dil is a kind of lipophilic non-toxic live-cell dye that can track cells *in vivo* or *vitro* steadily [33]. The labeled cells could be injected into *vivo* to show the migration and differentiation of transplanted cells effectively. In this experiment, CM-Dil marked BMSCs was observed under a fluorescence microscope in BMSCs+ASD group. In ASD+BMSCs groups, as the time and the frequency of transplantation increased, the cells were more widely distributed and gradually migrated to the endocardial as the transplants increased, that will be beneficial for extensive myocardial damage.

In many cases of heart failure, increased content, and cardiac fibrosis can be detected, and they trigger a series of changes in ventricular function [34, 35]. The New York heart association (NYHA) reported that myocardial apoptosis in patients with heart failure of cardiac service III and IV accounted for 0.12%-0.70% of myocardial tissue, while that in normal myocardium accounted for only 0.001%-0.01% [36]. Masson's trichrome is one of the main methods for displaying fiber staining in tissues. It is mainly used for the differential analysis of collagen and smooth muscle and one of the classic methods for collagen fiber staining which is dyed blue. In our experiment, the collagen deposition area increased significantly after two weeks of ISO injection. We also observed that compared with model group and IV injection group, the

myocardial collagen area and the perivascular collagen area of the left ventricular endocardium in the BMSCs+ASD-treated groups were significantly decreased (P <0.01), indicating that the BMSCs grafted by the ASD can repair myocardial fibrosis effectively.

5. Conclusions

In this experiment, we firstly provide a kind of cell deliver method for global myocardial injury. This study demonstrated increased BMSCs distribution area and significant improvement in cardiac function compared with IV injection group. The results of this research indicate that multiple times the delivery of BMSCs by ASD is more effective for global injured myocardium. Further study should be focused on the integration of engrafted cells with the myocardium and enhancing cell survival by delivering with other substances.

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Conflict of Interest

All authors declare no conflict of interest.

References

1. Doppler SA, Deutsch MA, Lange R, Krane M. Cardiac regeneration: current therapies-future concepts. Journal of thoracic disease. 2013;5:683-97.
2. Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling.

The Lancet. 2006;367:356-67.

3.   Cai B, Tan X, Zhang Y, Li X, Wang X, Zhu J, et al. Mesenchymal Stem Cells and Cardiomyocytes Interplay to Prevent Myocardial Hypertrophy. Stem cells translational medicine. 2015;4:1425-35.

4.   Bernstein HS, Srivastava D. Stem cell therapy for cardiac disease. Pediatric research. 2012;71:491-9.

5.   Forte E, Chimenti I, Barile L, Gaetani R, Angelini F, Ionta V, et al. Cardiac cell therapy: the next (re)generation. Stem cell reviews. 2011;7:1018-30.

6.   Sawa Y. Current status of myocardial regeneration therapy. General thoracic and cardiovascular surgery. 2013;61:17-23.

7.   Heldman AW, DiFede DL, Fishman JE, Zambrano JP, Trachtenberg BH, Karantalis V, et al. Transendocardial mesenchymal stem cells and mononuclear bone marrow cells for ischemic cardiomyopathy: the TAC-HFT randomized trial. Jama. 2014;311:62-73.

8.   He Q, Ye Z, Zhou Y, Tan W-S. Comparative study of mesenchymal stem cells from rat bone marrow and adipose tissue 2018.

9.   Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Bone marrow stem cells regenerate infarcted myocardium. Pediatric Transplantation. 2003;7:86-8.

10. Seth S, Bhargava B, Narang R, Ray R, Mohanty S, Gulati G, et al. The ABCD (Autologous Bone Marrow Cells in Dilated Cardiomyopathy) trial a long-term follow-up study. Journal of the American College of Cardiology. 2010;55:1643-4.

11. Strauer B-E. The acute and long-term effects of intracoronary Stem cell Transplantation in 191 patients with chronic heARt failure: the STAR-heart study. European Journal of Heart Failure (2010.

12. Campbell NG, Suzuki K. Cell delivery routes for stem cell therapy to the heart: current and future approaches. Journal of cardiovascular translational research. 2012;5:713-26.

13. Fukushima S, Coppen SR, Lee J, Yamahara K, Felkin LE, Terracciano CM, et al. Choice of cell-delivery route for skeletal myoblast transplantation for treating post-infarction chronic heart failure in rat. PloS one. 2008;3:e3071.

14. Dib N, Khawaja H, Varner S, McCarthy M, Campbell A. Cell therapy for cardiovascular disease: a comparison of methods of delivery. Journal of cardiovascular translational research. 2011;4:177-81.

15. Hristov M. Intracoronary infusion of autologous bone marrow cells and left ventricular function after acute myocardial infarction: a meta-analysis. Journal of Cellular and Molecular Medicine. 2006;10.

16. Strauer BE, Steinhoff G. 10 years of intracoronary and intramyocardial bone marrow stem cell therapy of the heart: from the methodological origin to clinical practice. Journal of the American College of Cardiology. 2011;58:1095-104.

17. John R. Teerlink JMP, Marc A. Pfeffer. Progressive Ventricular Remodeling in Response to Diffuse Isoproterenol-Induced Myocardial Necrosis in Rats. 1994.

18. Naveed M, Mohammad IS, Xue L, Khan S, Gang W, Cao Y, et al. The promising future of ventricular restraint therapy for the management of end-stage heart failure. Biomedicine &

Pharmacotherapy. 2018;99:25-32.

19. Yasmeen S, Liao X, Khan FU, Ihsan AU, Li X, Li C, et al. A novel approach to devise the therapy for ventricular fibrillation by epicardial delivery of lidocaine using active hydraulic ventricular attaching support system: An experimental study in rats. Journal of biomedical materials research Part B, Applied biomaterials. 2018.

20. Naveed M, Han L, Khan GJ, Yasmeen S, Mikrani R, Abbas M, et al. Cardio-supportive devices (VRD & DCC device) and patches for advanced heart failure: A review, summary of state of the art and future directions. Biomedicine & Pharmacotherapy. 2018;102:41-54.

21. J R T. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. Circulation research. 1994;1.

22. Li W, Gan R, Sun G. Chronic treatment of enbrel in rats with isoproterenol-induced congestive heart failure limits left ventricular dysfunction and remodeling. Chin Med J. 2002;115:1166-9.

23. Ren J, Li C, Ma S, Wu J, Yang Y. Impact of dexmedetomidine on hemodynamics in rabbits. Acta cirurgica brasileira. 2018;33:314-23.

24. Lee SK, Kim Y, Kim S-S, Lee JH, Cho K, Lee SS, et al. Differential expression of cell surface proteins in human bone marrow mesenchymal stem cells cultured with or without basic fibroblast growth factor containing medium. PROTEOMICS. 2009;9:4389-405.

25. Tropel P, Noel D, Platet N, Legrand P, Benabid AL, Berger F. Isolation and characterisation of mesenchymal stem cells from adult mouse bone marrow. Experimental cell research. 2004;295:395-406.

26. Hastings CL, Roche ET, Ruiz-Hernandez E, Schenke-Layland K, Walsh CJ, Duffy GP. Drug and cell delivery for cardiac regeneration. Advanced drug delivery reviews. 2015;84:85-106.

27. Barbash IM, Chouraqui P, Baron J, Feinberg MS, Etzion S, Tessone A, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. Circulation. 2003;108:863-8.

28. Wei K, Serpooshan V, Hurtado C, Diez-Cunado M, Zhao M, Maruyama S, et al. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature. 2015;525:479-85.

29. Smart N, Bollini S, Dube KN, Vieira JM, Zhou B, Riegler J, et al. Myocardial regeneration: expanding the repertoire of thymosin beta4 in the ischemic heart. Annals of the New York Academy of Sciences. 2012;1269:92-101.

30. Naveed M, Wenhua L, Gang W, Mohammad IS, Abbas M, Liao X, et al. A novel ventricular restraint device (ASD) repetitively deliver Salvia miltiorrhiza to epicardium have good curative effects in heart failure management. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie. 2017;95:701-10.

31. Y S. Cardiac support device (ASD) delivers bone marrow stem cellsrepetitively to epicardium has promising curative effects in advanced heart failure. Biomedical Microdevices. 2018.

32. Carolyn .C ea. Bone marrow-derived stromal cells home to and remain in the infarcted rat heart but fail to improve function: an in vivo cine-MRI study. Am J Physiol Heart Circ Physiol. 2008.

33. Staffend NA, Meisel RL. DiOlistic Labeling of Neurons in Tissue Slices: A Qualitative and Quantitative Analysis of Methodological Variations. Frontiers in neuroanatomy. 2011;5:14.

34. Li L, Zhang Y, Li Y, Yu B, Xu Y, Zhao S, et al. Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure. Transplant international : official journal of the European Society for Organ Transplantation. 2008;21:1181-9.

35. Whelan RS, Kaplinskiy V, Kitsis RN. Cell Death in the Pathogenesis of Heart Disease: Mechanisms and Significance. Annual Review of Physiology. 2010;72:19-44.

36. Orogo AM, Gustafsson ÅB. Cell death in the myocardium: My heart won't go on. IUBMB Life. 2013;65:651-6.