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Enhanced Device for Cell Delivery to the Myocardium: Validation in Swine Hearts

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Abstract

Background: Endocardial infusion is a minimally invasive procedure for cell delivery with good selectivity to the target region. However, certain limitations to current devices could affect the precision of the procedure and the therapeutic outcome. Therefore, we developed an enhanced device for transendocardial cell infusion.

Methods and Results: Our device is based on an electrode-guided transendocardial bidirectional 75 cm long catheter and 0.5 mm diameter inner needle. The key advantages of our device are the slender catheter diameter (7 Fr), consistent needle tip length, regulation of the catheter angle and independence between the needle and catheter. Mesenchymal stem cells (MSCs) were obtained from the inguinal adipose tissue of six healthy swine and propagated through 2-3 passages. Using the catheter, pre-labeled MSCs were infused autogenously into the swine hearts. The MSCs-infused myocardial regions were harvested on the infusion day (day 0) or 2 days later, and histological analysis was performed. The MSCs were successfully infused into all six swine myocardia and distributed along the hole made by the needle. The spread area of MSCs was larger at 2 days after infusion than at day 0 (1.38 ± 0.26 *vs.* 0.51 ± 0.17 mm^2/infusion, p=0.013). No complications occurred during the procedure, such as cardiac tamponade or arrhythmia.

Conclusion: These results demonstrate that our enhanced device could be useful for delivering cells into the myocardium.

Keywords: Regenerative therapy; Cell infusion device; Mesenchymal stem cell

Abbreviations: MSCs: Mesenchymal Stem Cells; DMEM: Dulbecco's Modified Eagle's Medium; FBS: Fetal Bovine Serum; P/S: Penicillin and Streptomycin

Introduction

The treatment options for terminal heart failure due to myocardial infarction or cardiomyopathy are limited [1]. However, few patients are eligible or able to receive these treatments, although there are last-resort options, including insertion of a ventricular assist device and a heart transplant. Under these conditions, recent clinical studies [2-4] and animal experiments [5,6] have demonstrated the positive effects of stem cell transplantation on ischemic heart disease. Several methods have been proposed for transplanting stem cells into the heart, including transvenous infusion, interstitial retrograde coronary venous delivery, intracoronary arterial infusion, surgical transepicardial infusion using a cell sheet and transendocardial injection using a catheter [7-10]. Although clinical reports have shown that the left ventricular ejection fraction improved following transvenous infusion and intracoronary arterial infusion [11-14], the injected cells tend to be more heavily distributed over the lungs and only a few cells ultimately engraft into the heart in comparison with intramyocardial infusion [9,10]. In addition, surgical infusion is a rather invasive procedure, and recurrent operations are risky and challenging [9].

Endocardial infusion, which enables more precise selection of a target area, can retain more of the original injected cells than possible with intracoronary infusion [9]. Although there are currently a few devices available for the intramyocardial delivery of cells [14-18], there are several aspects that require improvement. A few endomyocardial cell infusion devices have already been used in animal experiments and clinical trials [2,5,8,16-18]. For example, the Myostar™ catheter [2] requires an 8-Fr guiding catheter and does not have a mechanism for precise adjustment of the length and angle of the needle tip. To improve upon these current devices, we developed a simple and versatile catheter for transendocardial cell infusion, and aimed to investigate the safety and operability of our device by infusing adipose tissue-derived mesenchymal stem cells (MSCs) in swine hearts.

Materials and Methods

Animals

Female domestic swine (mean body weight 20.0 kg) were used for the animal experiments. The animal study was approved by the Animal Care and Use Committee of Kanazawa University, and the experiments were conducted in accordance with the "Basic Guidelines for the Conduct of Animal Experiments" published by the Ministry of Health, Labor and Welfare of Japan.

Device design

Our cell infusion device consists of an electrode-guided bidirectional catheter with a platinum tip and a long inner needle. The length of the catheter is 75 cm, with a 25 cm flexible tip and a 50 cm rigid root. The

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outer diameter of the catheter is 7 Fr. Five electrodes can be placed along the catheter at every 2 mm from the tip, although these functions were not used in the present study. The needle lumen is located in the center of the catheter. The inner needle is 0.5 mm in diameter (Figure 1).

The root of the needle contains a marked scale, which enables determining the position of the tip of the needle. In addition, a scale of 0-3 is labeled on the catheter grip that corresponds to the catheter angle: when the scale of the grip is +3, +2, +1 or 0, the degree of the catheter is 0°, 30°, 60° or 90°, respectively. In general, a needle protrudes more when the catheter is bent than when operated in a straight state. However, in our device, the extent of protrusion of the needle could be maintained at a constant length by matching the scale of the needle to the scales of the grip increasing by +1 (Figures 2A-2L).

Cell preparation

After being anaesthetized with ketamine hydrochloride (20 mg/kg intramuscularly; Daiichi Sankyo Propharma, Tokyo, Japan), the swine were maintained on general anesthesia with 2% sevoflurane (Inhalation; Mylan, Canonsburg, PA, USA) and oxygen. Adipose tissues were harvested from both sides of the inguinal region of the six swine. Harvested adipose tissues were minced with scissors and washed with Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA). After incubation with 0.1% collagenase type 1 (Worthington Biochemical Corporation, Lakewood, NJ, USA) at 37°C for 60 min, MSCs were separated by centrifugation at 400 *g* for 10 min. MSCs were cultured in DMEM containing 15% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and 100 U/mL penicillin and 100 μg/mL streptomycin (P/S; Invitrogen). A small number of cells developed into visible symmetric colonies by days 5-7. Non-adherent hematopoietic cells were removed, and the medium was replaced. The adherent, spindle-shaped MSCs were expanded to passage 2-3 after the cells were first plated.

Fluorescent labeling

After 2-3 passages, the cultured cells were labeled using the PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich) according to the manufacturer protocol. In brief, after washing with serum-free medium, the cells were treated with 4 mL/dish TrypLE (ThermoFisher Scientific, Waltham, MA, USA) and incubated at 37°C until the cells came off the dish. The cell suspension was mixed with DMEM containing 15% FBS and P/S and centrifuged. The supernatant was aspirated and suspended with Diulent C (Sigma-Aldrich). PKH26 (4 μL) was then added to the cell suspension mixed with 1 mL Diulent C. After incubation of the cell and dye suspension at 37°C for 5 min, FBS was added to stop the staining. The cells were centrifuged and washed several times. Before infusion, we confirmed that the cells had been successfully labeled with PKH using a fluorescent microscope (BZ-9000, KEYENCE, Osaka, Japan).

Preliminary experiment

As a preliminary experiment to validate the functionality of the device, we infused 0.4% trypan blue solution (Wako Pure Chemical Industries, Osaka, Japan) into the extracted swine heart and observed the expansion of the dye. The procedure to extract swine hearts and to infuse *in vivo* was described later.

Cell delivery

The anesthesia status was carefully monitored throughout the experimental procedure to maintain appropriate sedation. The electrocardiogram and heart rate were continuously monitored by a polygraph recording system (OptiPlex755, Nihon-Kohden, Tokyo, Japan) throughout the procedure.

Arterial access was obtained via the left carotid artery with a cut-down technique and a 7-Fr vascular sheath was used to cannulate the artery. The cell infusion device was inserted through the sheath into the left ventricle. In the case that the tip of the device made contact with an obstacle in the left ventricle, an infusion needle was inserted through the lumen of the device. Approximately 5.0×10^6 MSCs per point were infused at 2-9 points in the apex of the left ventricle.

Histological analysis

Three of the swine were anesthetized with ketamine hydrochloride and 2% sevoflurane and sacrificed with infusion of 20 mEq potassium chloride solution (Terumo Corporation, Tokyo, Japan) in the left atrium on the day of infusion (day 0) and the other three swine were sacrificed two days after infusion (day 2). The myocardium tissues of the cell infusion regions were harvested as blocks and fixed with 4% formaldehyde (Wako Pure Chemical Industries) for more than 24 h and embedded with 10%, 20% and 30% sucrose solutions (Wako Pure Chemical Industries). Cryosections were prepared using a cryostat (Leica CM1950, Leica Biosystems, Nussloch, Germany). By the observation with microscopy (KEYENCE), we measured the area in acknowledgment of PKH-labeled MSCs per slice.

Safety evaluation

The safety of the technique was evaluated according to the mortality, fatal arrhythmia during the procedure, bloody pericardial fluid, and damage to the cardiac structures (e.g. aortic and mitral valve structures, coronary and great vessels) caused by the device [19].

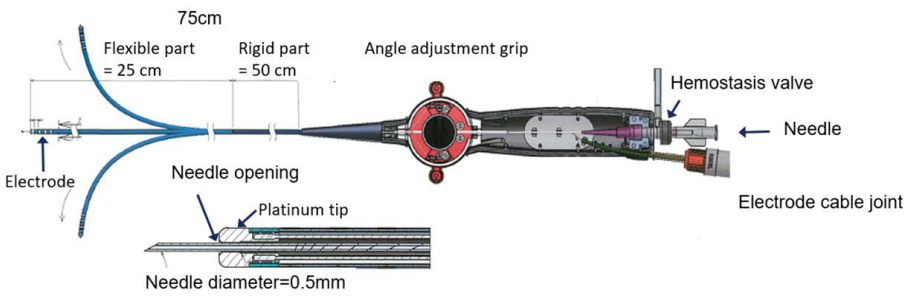

Figure 1: Design and appearance of the device. Our device consists of an electrode-guided bidirectional catheter with a platinum tip and long core needle. There is a 25 cm flexible catheter tip and a 50 cm rigid root; the outer diameter of the catheter is 7 Fr. There is a grip at the root of the device to adjust the angle. The core needle is 0.5 mm in diameter.

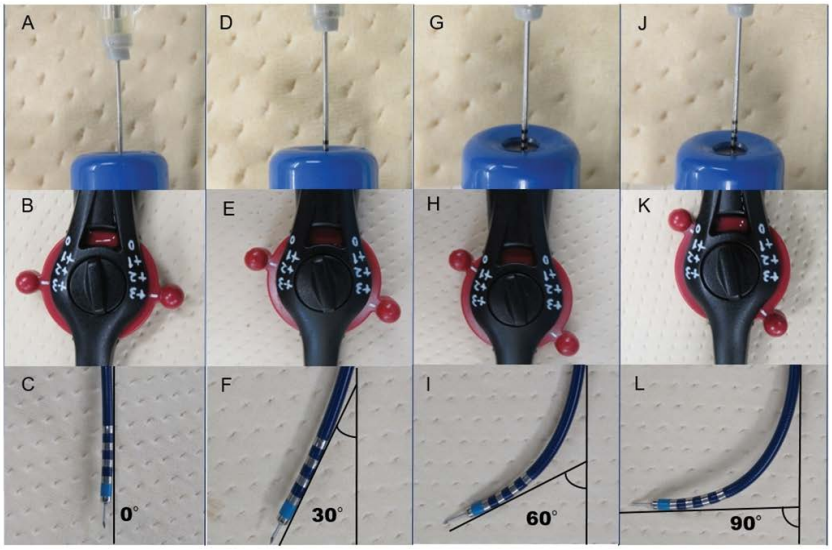

Figure 2: Length maintenance of the needle from the tip. (A–C) When the catheter is kept straight, the length of the tip of needle can be maintained at 3 mm by keeping the proximal marker at the entrance of the catheter. (D–L) When adjusting the angle of the catheter from 30° to 90°, the length of the needle tip can be kept to be 3 mm by pulling back the needle according to markers.

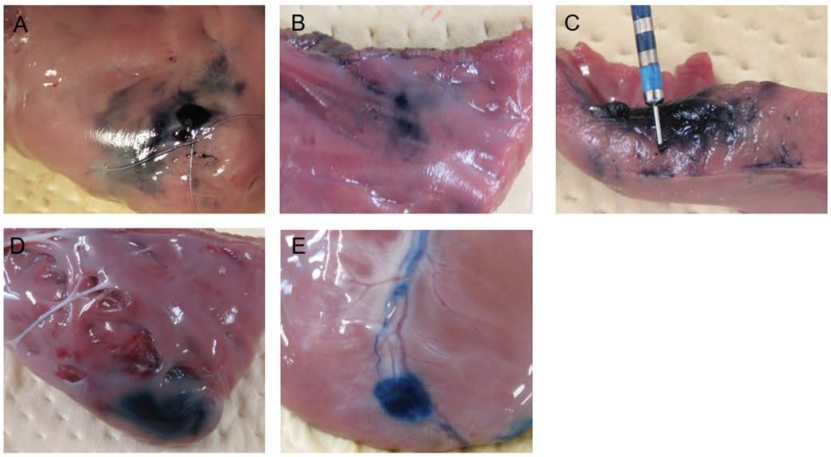

Figure 3: *In vitro* and *in vivo* preliminary experiments. A 0.4% trypan blue solution was poured into the myocardium using the device. *In vitro* injection the dye appeared on both the (A) endocardium and (B) epicardium sides. (C) Transverse section evaluation showed that the dye was distributed in a fan form along the hole made by the needle. When the length of the needle was 3 mm, the needle was inserted about halfway into the myocardium. *In vivo* infusion, the dye was observed in both the (D) endocardium and (E) epicardium sides.

Statistical analysis

The cell spread area was traced and measured under microscopy. Data are expressed as mean ± standard error of the mean. Comparison of the cell spread area was conducted with Welch's t-test. Differences were considered statistically significant at $p<0.05$.

Results

Dye injection

When the needle was stabbed 3 mm into the swine myocardium, the trypan blue solution overflowed from the side of the needle with an injection amount greater than 0.4 mL. Therefore, we decided to infuse 0.3 mL of the cell suspension, which corresponds to injection of 5×10^6 MSCs per point.

For both the *in vitro* and *in vivo* preliminary tests, the dye spread radially along the epicardium side and endocardium side (Figure 3). The *in vitro* results suggested that the needle had been inserted at approximately the halfway point into the myocardium. No pericardial effusion of the dye was detected in the *in vivo* model.

MSC characteristics

After 2 weeks of culture, spindle-like plastic-adherent cells increased in number, indicating the typical characteristics of MSCs (Figure 4A) [20,21]. Before cell infusion, we could observe that MSCs were labelled in PKH with microscopy (Figure 4B).

Observation of the harvested endocardium side of the myocardium showed clearly visible needle holes. Cryosections were successfully made of the myocardium regions containing the needle holes. PKH-labeled MSCs were clearly visible in all cryosections from samples harvested on day 0 and day 2 after infusion (Figure 5).

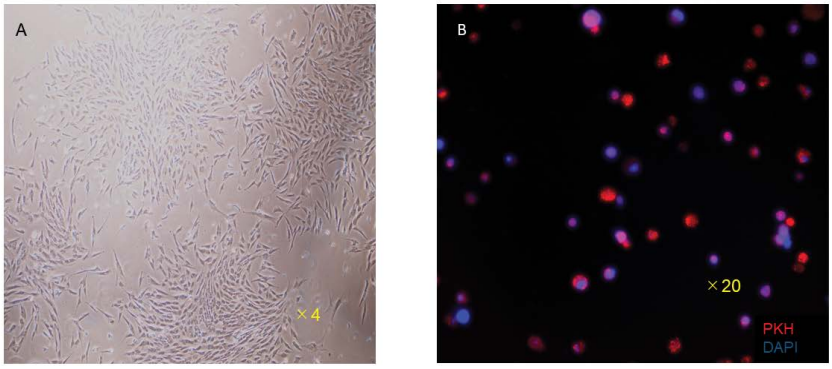

Figure 4: Isolated adipose tissue-derived mesenchymal stem cells (MSCs). (A) Spindle-like cells at 2 weeks after seeding. (B) Before intramyocardial delivery, the MSCs were labeled with PKH (red). Nuclei were stained with DAPI (blue).

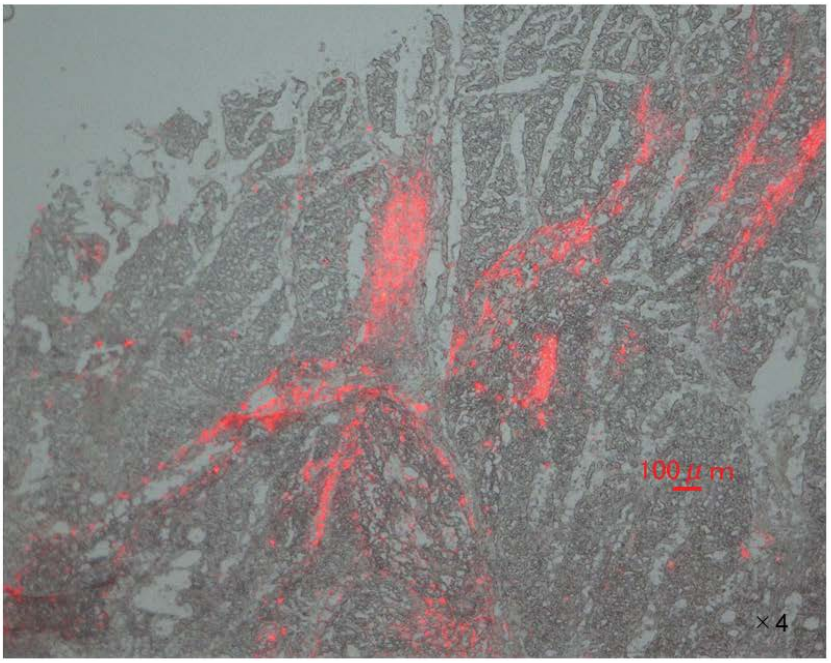

Figure 5: Overlay of the phase difference and fluorescence microscopy images on day 2. Note that most of the PKH-labeled MSCs were retained to stay along the hole of the needle in the myocardium.

Most of the MSCs were engrafted along an intramyocardial needle hole. However, some of the MSCs infiltrated into the myocardium from the needle hole. Importantly, the spread area of MSCs determined by planimetry on day 2 was (1.38 ± 0.26 mm^2/infusion) greater than that on day 0 (0.51 ± 0.17 mm^2/infusion, p=0.013). This suggests that using the present device delivered MSCs could retain to exist in the myocardium at least for 48 h after injection *in vivo*.

Safety of the protocol

The average number of infusion points was 4.5 (5.0×10^6 MSCs/point). There were no deaths resulting from the infusion of MSCs. In addition, no case of fatal arrhythmia was observed in the six swine at the time of cell infusion. Bloody pericardial effusion, suggesting penetration of the needle, was not detected at the time of harvest.

Discussion

In this study, we demonstrate that our new device can be effectively used for cell injection in the swine myocardium. There are four unique aspects to this device. The first is the slender catheter diameter; most existing devices require an 8-Fr sheath, and our device can insert through a 7-Fr sheath [14]. Second, this device enables stability in the length of the needle tip, regardless of the catheter angle. Third, the catheter angle itself can be precisely controlled by regulating the grip position. Finally, the device is not an integrated unit, and this independence between the catheter and the needle allows for simply replacing the needle rather than requiring an entirely new device in the case that a needle hole occludes with the cell solution or myocardium. Thus, our device can accurately adjust the projection of a needle on its tip, and the angle of the catheter was precisely adjustable. In addition, our device could be used in electromechanical mapping with the CARTO® system (Biosense Webster), which is commonly used in catheter ablation.

Recently, the beneficial effects of cell sheet-based myocardial regeneration therapy have been reported [22,23]. However, this is a highly invasive procedure. Although transendocardial cell infusion

is associated with a low level of cell engraftment, some methods have been proposed to increase the engraftment ratio of the transplanted cells [24-27]. MSCs are thought to be particularly effective for regeneration therapy owing to their paracrine effect even when the cell survival rate is low [28-30], because MSCs secrete various vascularization factors such as vascular endothelial growth factor and hepatocyte growth factor [31]. In addition, various other agents besides cells have been infused using endocardium infusion devices, such as proteins or genes [32,33].

One of the disadvantages of the needle injection for cell delivery seemed to be unstable distribution of the injected materials, although some studies demonstrated that approximately 10% of the infused MSCs remained in the heart [9] and the infused cells could survive for approximately 6 weeks [26]. It was quite interesting that the area of MSCs after infusion was even greater on day 2 after infusion than that on day 0. One might speculate that the infused MSCs kept spreading at least during these periods.

There remain several limitations of the present device that should be explored prior to clinical assessment. First, we infused the MSCs into healthy swine and therefore did not examine the effectiveness of the procedure and the optimal number of cells to infuse per point for treating a diseased heart. Second, we did not examine the ratio of retained cells and the long-term survival of the infused cells. To resolve these, further experiments with disease heart models for long-term observation are warranted to determine the utility of the present device.

Conclusion

We manufactured an enhanced device for cell delivery to the myocardium. Adipose tissue-derived MSCs were clearly detected in the swine myocardium on day 0 and day 2 after infusion. Our device overcomes some technical limitations of current devices, and shows promise for clinical applications in cell-based therapy for heart disease.

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