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 mm²/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 prevent 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 transeptal cell 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 infusion 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...
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].

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**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.
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 $5 \times 10^6$ 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-labelled MSCs were clearly visible in all cryosections from samples harvested on day 0 and day 2 after infusion (Figure 5).
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 \((1.38 \pm 0.26 \text{ mm}^2/\text{infusion})\) greater than that on day 0 \((0.51 \pm 0.17 \text{ mm}^2/\text{infusion}, p=0.013)\). 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 \((5.0 \times 10^6 \text{MSCs/point})\). 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

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**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.
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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References


