Video Article

Intramyocardial Cell Delivery: Observations in Murine Hearts

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Abstract

Previous studies showed that cell delivery promotes cardiac function amelioration by release of cytokines and factors that increase cardiac tissue revascularization and cell survival. In addition, further observations revealed that specific stem cells, such as cardiac stem cells, mesenchymal stem cells and cardiospheres have the ability to integrate within the surrounding myocardium by differentiating into cardiomyocytes, smooth muscle cells and endothelial cells.

Here, we present the materials and methods to reliably deliver noncontractile cells into the left ventricular wall of immunodepleted mice. The salient steps of this microsurgical procedure involve anesthesia and analgesia injection, intratracheal intubation, incision to open the chest and expose the heart and delivery of cells by a sterile 30-gauge needle and a precision microliter syringe.

Tissue processing consisting of heart harvesting, embedding, sectioning and histological staining showed that intramyocardial cell injection produced a small damage in the epicardial area, as well as in the ventricular wall. Noncontractile cells were retained into the myocardial wall of immunocompromised mice and were surrounded by a layer of fibrotic tissue, likely to protect from cardiac pressure and mechanical load.

Video Link

The video component of this article can be found at http://www.jove.com/video/51064/

Introduction

Various cell delivery protocols have been tested in murine and rat models of cardiovascular diseases with the aim of translating the efficiency, efficacy and safety of this experimental procedure in human patients. In small rodent hearts, intramyocardial cell delivery is the most feasible method of cell delivery[1,2], whereas in the rat heart antegrade[3] and retrograde[4] intracoronary cell infusion can also be used. Both methods have limits and advantages. Cell delivery via the intracoronary route has theoretical advantages over direct intramuscular injection in promoting global cell dissemination[3], but it also has the risk of causing coronary embolism[3,5]. Limitations on intramyocardial delivery are associated with mechanical injury, acute inflammation, and myocardial damage[6,7]. In humans, cells for cardiac repair are delivered by intramyocardial injection through an endocardial or surgical epicardial approach or by intracoronary arterial route[8]. Injection by transvascular routes is suited in patients with acute infarct and reperfused myocardium, but may not be possible in case of total occlusions or poor flow within the vessels of the effected territory[9]. Direct injection into the ventricular wall by transendocardial or transepicardial injection is technically feasible depending on the patient health status. Indeed, it has been shown that this technique is safe[10,11], although for transepicardial injection an open chest surgery is necessary and for transendocardial approaches an electrophysiological mapping for each patient is required to differentiate sites of viable ischemic or scarred myocardium[9].

Importantly, in cell therapy studies the choice of the best cell to be transplanted is still under investigation. Short-term analyses (4 weeks) showed that injection of cardiac stem cells defined as cardiospheres[12] or side population cells from bone marrow[13] induced cardiac functional recovery in murine[14] and rat[15] models of myocardial infarct by decreasing scar size and cell death. Allogeneic transplantation of cardiospheres in a rat myocardial infarct model without immunosuppression was found to be safe, promoted cardiac regeneration, and improved heart function through stimulation of endogenous repair mechanisms[15]. Lin-/c-kit+ adult progenitor cells in the heart were shown to be self-renewing, clonogenic, and multipotent *in vitro* and *in vivo*, and when injected into an ischemic rat heart reconstituted large portions of the injured myocardial wall[16] and had the ability to form conductive and intermediate-sized coronary arteries[17]. These promising data fuelled phase I and II clinical trials in humans: injection of autologous and allogeneic mesenchymal stem cells (MSC)[18], cardiospheres[19], or c-Kit positive cardiac stem cells (CSC)20 in ischemic human hearts each showed beneficial effects in cardiac function in long term studies. Nevertheless, extensive long-term follow-up and retrospective meta-analysis demonstrated that stem cell therapy provides significant benefit to some patients, but not in others with a range of unpredictable outcomes[21]. It is possible that these limitations will necessitate the design of specific protocols of cell delivery for each individual and each disease.

In mouse and rat models, long-term studies revealed that cell injection did not further improve cardiac function (12 months). Indeed, grafts of human embryonic stem cell-derived cardiomyocytes (hESC-CM) were largely isolated from the host myocardium by a layer of fibrotic tissue[22,23]. Similar results have been observed after intramyocardial transplantation of skeletal myoblasts into the heart of infarcted mice[24]. Furthermore, the long-term ability of allogeneic MSCs to preserve function in the infarcted heart has been limited by transition from an immunoprivileged to an immunogenic state after differentiation[25].

Taking into consideration the challenges and prospects outlined above, we show here how to deliver cells by intramyocardial injection in mice. We observe that cells without cardiomyocyte contractile properties are not connected with the host myocardium and form a cohesive mass with a thin fibrotic barrier. Although in some cases this outcome may be advantageous, the following analysis may be useful to understand how cell engraftment may be modulated to generate functionally connected myocardial structures as well.

Protocol

All animal studies were performed in compliance with international (Directive 2010/63/EU of the European Parliament) and national (UK Home Office, Act 1986) regulations. The procedures herein described are part of our plan of work under UK license authorities and have not been undertaken for the purpose of recording.

1. Preparation of Cells

This protocol describes the preparation of a specific cell line (Human Embryonic Kidney, HEK293 cells) for demonstration purposes. Cell-specific protocols must be employed for growing and eventually differentiating pluripotent or adult stem cells into specific cell lineages.

1. Grow cells in DMEM media (high glucose, 4,500 mg/L) containing 1% sodium pyruvate, 2 mM glutamine, 1% antibiotic Pen/Strep and 10% Fetal Bovine Serum (FBS). One 10 cm plate should be normally split at 1:3 and media supplemented fresh every two days.
2. Treat cells mildly with trypsin 1x and resuspend in DMEM medium as described in 1:1.
3. Count cells in a hemocytometer chamber and collect 3 x 10^6 cells into a separate tube.
4. Centrifuge in a swing bucket rotor at 100 g for 5 min.
5. Discard the supernatant and wash the cell pellet with sterile phosphate buffer 2x.
6. Resuspend in PBS 1x at a concentration of 10^6/50 ul for injection into the left ventricle of immunodepleted mice.

2. Presurgical Conditions

1. Maintain immunodepleted mice (NOD.CB17-Prkdscid/JHliHSD) with sterile food pellets and water in a temperature-controlled isolator (22 °C; **Table 1**) before each surgery.
2. Perform surgery of immunodepleted mice under a laminar flow hood. Clean the working area with 70% Ethanol and autoclave surgical tools (forceps and scissors).
3. Turn on heating pads for the surgery and for the recovery. Heating pads are important to block the decrease of body temperature occurring during and post-surgery.
4. Place a clean and autoclaved cage on the recovery-heating pad.
5. Transport the mice in their original cages from the isolator to the surgery room into a sterile autoclaved bag.
6. Weigh the mouse and according to the body weight inject subcutaneously (s.c.) a solution containing medetomidine and ketamine hydrochloride diluted in 0.9% sterile saline solution to anesthetize the mouse, as well as to relax the musculature (medetomidine 1 mg/kg; ketamine hydrochloride 75 mg/kg).
7. Remove the mouse from the cage when fully anesthetized (within 1-2 min, no toe-pinch reflex after stimulation)
8. Apply hair removal cream on the left side of the chest and the throat area. After an appropriate amount of time (approximately 2-5 min), wipe away the loose hairs with a water-wet tissue.
9. Place the mouse on the surgery panel in a supine position. From the surgeon's viewpoint the position is vertical, tail down-head up.
10. Inject analgesic solution at 0.1-0.2 µg/g diluted in sterile saline solution (buprenorphine) into the hind limb subcutaneously and cover the shaved area with the aseptic solution povidone iodine using a cotton tip applicator.
11. Focus the microscope (2.5X objective) on the throat area.

3. Surgical Conditions

1. With blunt scissors make a midline ventral skin incision of about 0.5-1 cm length slightly below the cricoid cartilage. Separate the skin from connective tissue to visualize the salivary glands.
2. Split the salivary glands on their natural midline division by simultaneously pulling each part sideward with forcep and magnetic chest retractor and expose the trachea.
3. Pull the tongue gently to the side to expose the larynx. Slide the intubation tube carefully into the trachea. The tube tip is visible through the displayed larynx and trachea. Importantly, if the tube is in, but not clearly visible, it is in the esophagus. Remove it and change the position of the tube tip.
4. Connect the tube to the ventilation machine. Set the stroke volume at 200 µl and 150 stroke/min. Secure the ventilation tubing on the surgery panel with a tape.
5. With blunt scissors and forceps, make a vertical tail to head 1- to 1.5-cm-long skin incision over the left thorax area.
6. Loosen the skin from the connective tissue/muscle layers and the major and minor pectoral muscles without making incisions.
7. Perform thoracotomy between the third and fourth ribs as follows:
    1. Perforate the intercostal muscle layer by pinching and scratching with small, rounded forceps.

2. Once the intercostal muscle layer is perforated, place the chest retractor to maximally open the chest cavity. The upper and middle parts of the left ventricle (LV) with its overlying auricle (atrium) are now visible.

8. Prepare a precision syringe with cells. The syringe should be able to deliver an amount of volume of 10 ul for each injection. Cast a 30 G needle at the tip of the syringe.
9. Fix with tape a small plastic cannula (from an introcan-W 20 G) on the needle, leaving exposed only 1 mm including the open tip of the needle. This will prevent the needle from perforating the entire left ventricular wall and injecting the cells into the left ventricle cavity.
10. With the help of a 5X objective, visualize at this magnification the left ventricle and inject the cells into the left ventricular wall in 5 different locations (each injection will deliver 10 ul for a total of 106 cells injected). If the injection is successful, a white area and the absence of major backwash of the cells will be visible (if the injection was not successful, the animal would be excluded from functional analysis).
11. Remove the retractor. Close the thoracic wall by enclosing the two separated ribs with two stitches of 6-0 silk suture.
12. Moisturize the pectoral muscles and skin with a cotton tip drenched in PBS 1x and gently put the muscles back into the original position with the forceps.
13. Close the skin with 3-4 stitches of 6-0 silk suture. Suture the throat incision with 2-3 stitches to close the skin.
14. Inject atipamezole (1 mg/kg final concentration) to revert the anesthetic effects.
15. As soon as the mouse starts breathing autonomously, take out the tube from the trachea and put the mouse on its right side. The mouse is expected to recover within minutes.
16. When the mouse starts to turn and walk, place it in the warm recovery cage (small IVC cage with closed filtered top) for an hour.
17. Leave the animals overnight in a controlled heated box (37 °C).
18. Fill a dish with water and pellet food to support recovery during the first 2 days after surgery.

4. Post-surgery Analyses

Ten hearts injected with cells and 10 control not injected hearts are analyzed histologically as follows:

1. Perform analysis of the grafted cells 1 week after injection (**Figures 1** and **3**).
    1. After euthanasia by overdose of isoflurane, perfuse the hearts with 20 ml of 4% paraformaldehyde (PFA) to fix the tissue. Dehydrate the fixed tissue in increasing percentages of ethanol (from 70-100%) and embed in paraffin blocks.
    2. Section the hearts in paraffin with a microtome at 5 µm and stain with hematoxylin and eosin for morphological analysis.
    3. Visualize connective tissue by using Masson's Trichrome stain.
    4. Analyze the images under a slide scanner microscope (**Figures 1A, 2A, 3A, 3B,** and **3C**) and visualize the entire heart in short axis view using a digital slide scanner microscope (**Figures 1B** and **1C**).

2. After the above analyses, deparaffinize the heart tissues in xylene, rehydrate by submerging specimens in decreasing percentages of ethanol (from 100% to water) and process for Scanning Electron Microscopy (SEM) as follows:
    1. Incubate deparaffinized blocks with 1% tannic acid in 0.1 M phosphate buffer for 1 hr.
    2. Dehydrate specimens in increasing percentages of ethanol (from 25-100%).
    3. Dry samples with HMDS (hexamethyldisilazane).
    4. Mount the specimens on Stubbs with Acheson Silver Dag and coat them with gold-palladium.
    5. View samples in an analytical scanning electron microscope (**Figure 2B**).

Representative Results

We injected HEK293 cells, which are distinguishable from the heart cells by their different morphology (**Figure 1**), with a cobblestone shape compared to the elongated cardiomyocytes (**Figure 1A**). HEK293 cells were more reactive to hematoxylin dye (blue color) compared to cardiomyocytes (pink color), likely because of their increased nuclear content (**Figure 1A**). To further distinguish the injected cells from the host tissue, HEK293 cells were labeled with DAPI 2 hours prior to injection. Labeled-cells were visible in the myocardial tissue as shown in **Figure 1E**. Control sham-operated mice showed no damage in the whole heart (**Figure 1C**) and integrity of the tissue (**Figure 1D**).

In a whole short axis view of the heart, injected cells were visible as a homogeneous group in the area of injection (**Figure 1B**). The grouped cells were surrounded by a thin layer of fibrotic tissue, which appeared as a blue area in the trichrome staining (**Figure 2A**). Cells were not connected with the host myocardial tissue, as shown by Scanning Electron Microscopy (**Figure 2B**), likely due to their noncontractile function and different cellular structure.

In addition to the above observations, we noted small areas of damage where the needle was injected (**Figure 3A**). These areas run from the external epicardial layer of the heart into the myocardial wall (**Figure 3A**). Injected cells (green arrow, **Figure 3B**) are present in proximity of the injured area (black arrows, **Figure 3B**). DAPI-positive cells were visible along the site of injection (**Figure 3C**, white arrows). One week after injury, Tunel assay did not show increased apoptotic cells at the site of injury, suggesting that necrosis rather than apoptosis has occurred after needle injection (**Figure 3D**).

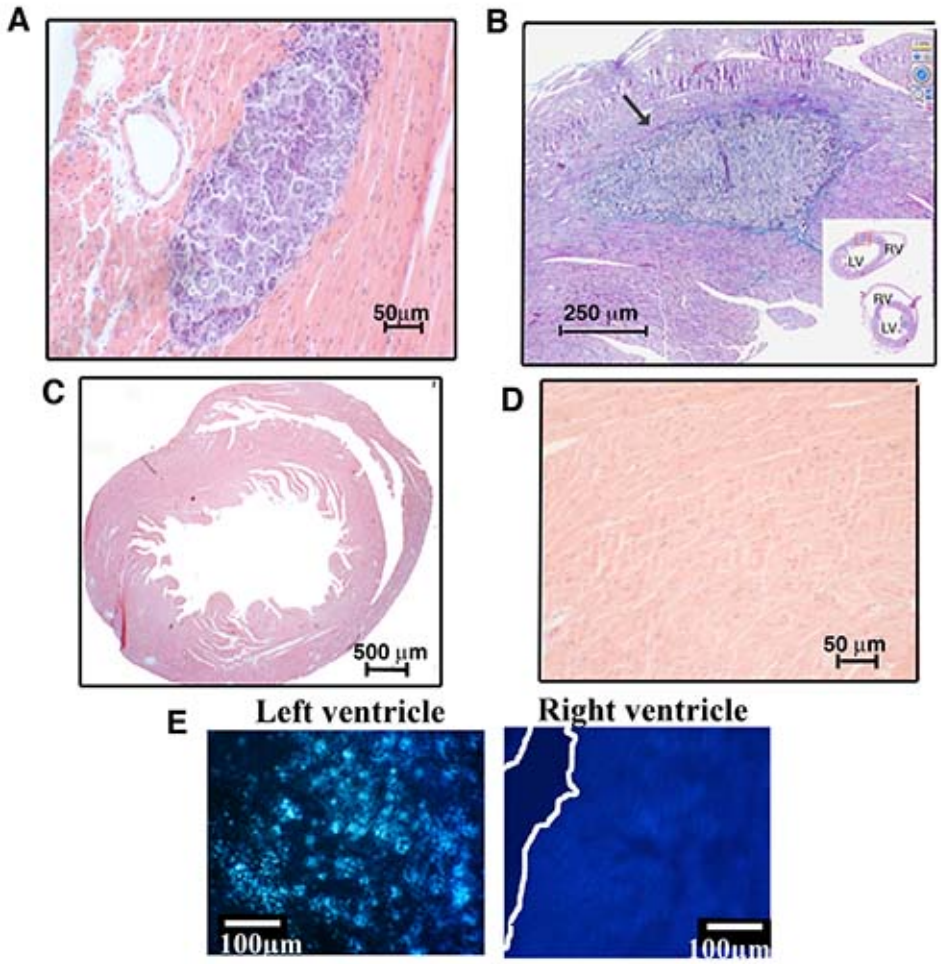

Figure 1. Histological analysis of injected cells. A) One week after cell injection hearts were embedded in paraffin and processed for hematoxylin and eosin staining. Injected HEK293 cells were present into the wall of the left ventricle. Images were obtained with a stereo fluorescence microscope. **B)** Trichrome stained tissues showed that the cells were grouped in the same area where they were injected (arrow). Right bottom panels show the short axis view with the right (RV) and left ventricles (LV). The red rectangle shows the area where cells are retained (1.5X). The images were recorded with a slide scanning microscope. **C)** Trichrome staining of control sham-operated hearts. Whole heart view with a 5X objective. **D)** Trichrome staining of sham-operated mice showing myocardial tissue integrity. **E)** HEK293 cells were stained with DAPI 2 hours prior to injection. One week after injection hearts were embedded in OCT and sectioned in a cryostat at 6 µm. Cells were visualized under UV in a fluorescent microscope. Left and right ventricles are shown. In the right ventricle, right panel, the white line marks the epicardial layer. Click here to view larger image.

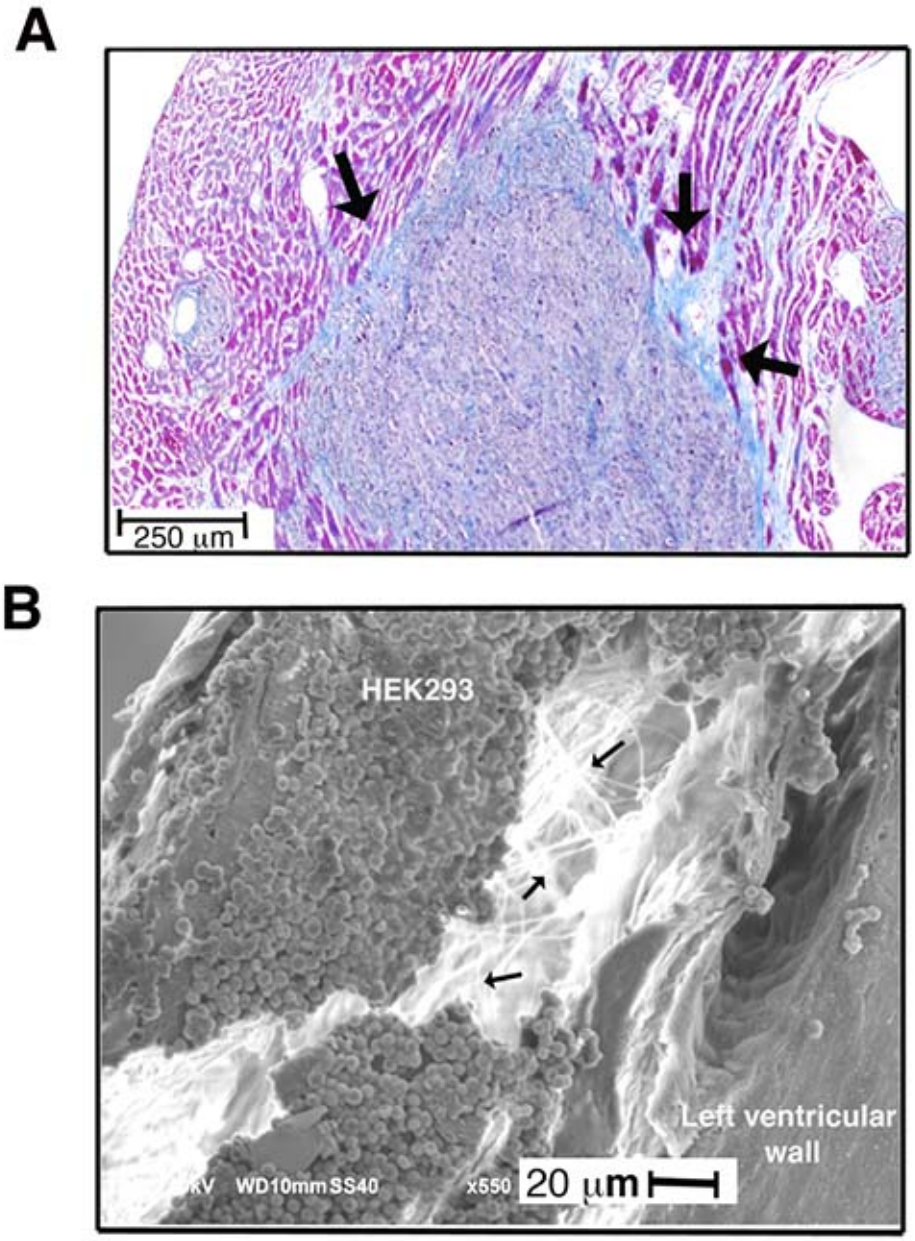

Figure 2. Formation of a fibrotic layer between injected cells and host tissue. A) Trichrome staining show collagenous tissue formed between HEK293 cells and murine myocardial tissue (arrows). The images were recorded with a digital scanning microscope. **B)** Hearts were deparaffinized and processed for Scanning Electron Microscopy. Images were recorded with a scanning electron microscope and show collagen fibers (arrows) between the injected HEK293 cells (left side) and the host myocardium (right side). Click here to view larger image.

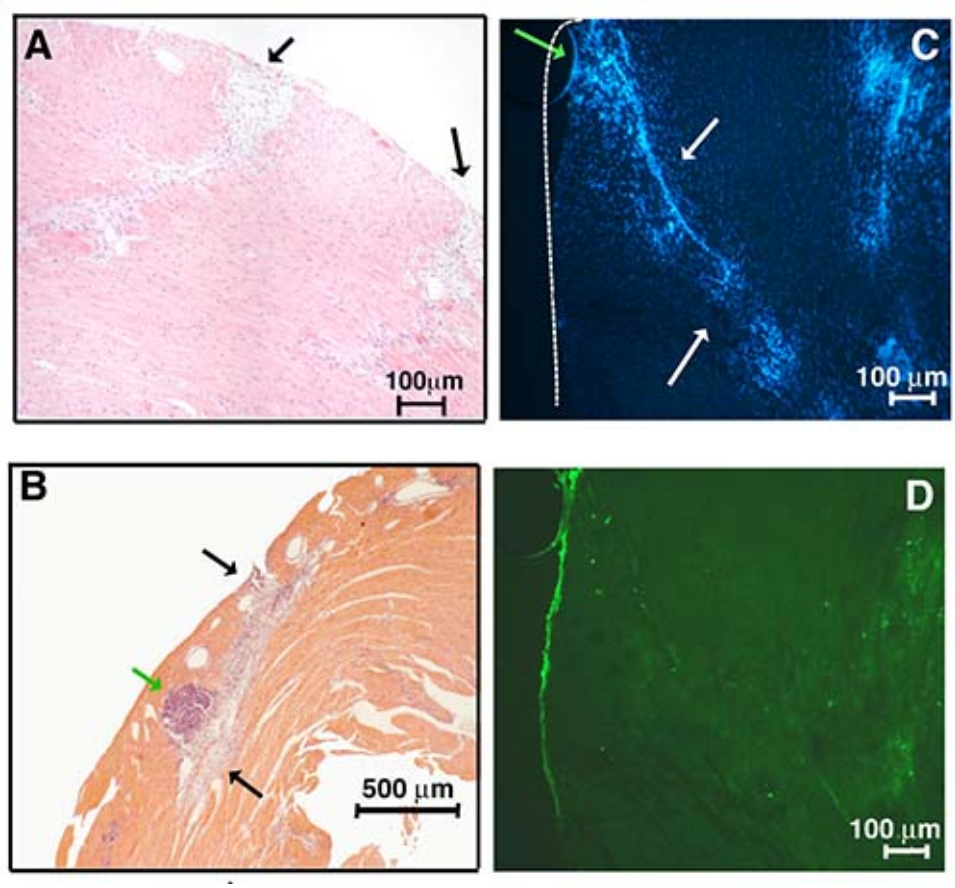

Figure 3. Cell injection produced small areas of damage. A) Trichrome staining shows small areas of damage where needle was injected (blue color; arrows). **B)** Paraffin sections were stained by Trichrome staining and the area where the cells were injected is indicated by black arrows. Injected cells (green arrow) were visible in proximity to the injured area. **C)** HEK293 cells, stained with DAPI 2 hours prior injection, were present along the injection site (white arrow). The site of injection is indicated with a green arrow and the white open line marks the pericardial layer. **D)** Tunel Assay, performed on paraffin sections, did not show relevant fluorescent positive nuclei. Click here to view larger image.

Discussion

In this manuscript, we have shown how to perform intramyocardial injection of cells in murine hearts. As a proof of this methodology, we have used HEK293 cells. It is important to emphasize that HEK293 cells are not used in any cell therapy study and therefore the findings of this manuscript are not appropriate for direct translation to a therapeutic approach. However, the fact that HEK293 cells are not contractile cells and do not transdifferentiate in other cell types focuses attention on technical aspects such as the properties of the cells to be delivered and the route of delivery.

It has been reported that an intramyocardial route tends to cause cell clusters embedded in the cardiac tissue[24,26], whereas the intracoronary route could provide more homogeneous distribution at the site of injury. For cardiomyocyte replacement, the ideal donor cell should exhibit the electrophysiological, structural and contractile properties of cardiomyocytes and should be able to integrate structurally and functionally into the host tissue, suggesting the necessity for a cell differentiation program to be employed before transplantation.

The key points for a successful surgery can be summarized as follows:

1. Anesthetize mice with injectable compounds to slow heart rate and facilitate cell injection
2. Perform intratracheal cannulation of mice to favor lungs functionality.
3. Perform thoracotomy avoiding muscle incision to maintain pectoral muscle functionality for a correct breathing.
4. After exposing the heart, observe the left ventricle under a microscope to visualize whether injection has occurred (visible as a pale color).
5. Use a thin needle and a precision syringe to avoid extensive perforation of the myocardial tissue and to inject the desired amount of material respectively.
6. Sheathe the needle with a plastic tubing system exposing only the tip part. This would allow introduction of the needle to a specific depth into the left ventricle (0.5 mm), avoiding injection into the ventricular cavity.
7. Close the chest and the space between the ribs carefully to avoid exposure of lungs to external pressure.

This technique has a high level of success if post-operative health conditions are prioritized. Animals must be intensively monitored every day up to a week for signs of distress and pain. Injection of analgesic compounds to control pain must be administered as required post-operatively. Topical application of pain relief, such as lidocaine, may be also used. Bleeding should be prevented by careful surgical techniques. However,

if occurring, it can be stopped by compression or ligation of vessels. We have noted that animals recover better and faster if left in a heating box at 37 °C for 12-20 hr after surgery. This treatment will block rapid body temperature decrease, normally occurring after surgical procedure and administration of anesthesia. Postsurgical infections may occur but should be less than 1%. Aseptic procedures must be strictly followed to prevent infection. Local infection can be treated with application of antibiotics. Importantly, dehydration should be controlled by subcutaneous injection of saline.

It is preferable to administer injectable over inhaled anesthetic, such as isoflurane. Indeed, we observed that injectable anesthetic decreased heartbeat, allowing easier injection and less bleeding without causing animal discomfort.

Although invasive, injection of small volumes of cells (25-50 µl) in mice is feasible and safe. We had less than 1% mortality by injecting 50 µl of HEK293 cells in 5 different areas of the left ventricular wall (10 µl/injection). This methodology ensured the presence of the cells in several areas of the myocardial wall, which is important in stem cell therapy when extended areas of ischemic damage need to be reached by the delivered cells. To perform less invasive surgery, several protocols have been recently published, although their reproducibility in different laboratories has yet to be verified. For example, cell injection can be performed by close-chest technique using high resolution echocardiography[27]. The use of this system requires high operator skills to visualize clearly in a two-dimension image the wall of the left ventricle and to maintain the needle in a specific location during normal systolic/diastolic cycles. The advantage of this technique is the implantation of the cells into the mouse myocardium to desired locations in a clinically relevant time frame after myocardial infarct induction. However, this approach is not possible with surgery procedures such as myocardial infarct induction or transaortic banding because of the increased mortality of animals undergoing a second operation[27]. Interestingly, Hamdi and colleagues compared direct intramyocardial injection *versus* delivery of cells in a Gelfoam construct onto the epicardial area[28]. They observed that overlaying the cell construct onto the epicardium resulted in better graft functionality compared to intramyocardial cell injection and reported that this technique is reproducible, user-friendly, and less traumatic for the animals[28].

An important feature of cell injection experiments is the tracing of the cells and their survival. We have shown that HEK293 cells are easy to detect due to their distinguishable morphology compared to cardiac cells. These cells survived the injection (data not shown) and were retained in the tissue one week after surgery. To trace cells for long-term studies and analyze their engraftment in the tissue of delivery as well as in other tissues, several techniques are in use, including fluorescent labeling and FISH analysis in sex-mismatch transplantation experiments[29]. Importantly, *in vivo* imaging will play a major role towards *in vitro* analyses in future studies. Indeed, one advantage of *in vivo* imaging over histological methods is the tracking of the cells in longitudinal studies in the same animals without the need of euthanasia[29]. In this methodology, cells can be marked with bioluminescence reagents, iron particles, and specific reporter genes to be imaged with positron emission tomography (PET) and magnetic resonance (MRI).

Our analyses showed that noncontractile cells such as HEK293 cells preferentially grouped in the area where they were injected surrounded by a thin collagenous tissue. Although the pathophysiological mechanisms leading to the formation of the fibrotic tissue in an immunodepleted mouse are unknown, the collagenous tissue visible around the transplanted cells may be comparable to the endpoint of a tissue compatible implant. In this case, the exogenous material cannot be removed by the inflammatory cells and becomes encapsulated in a dense layer of fibrotic connective tissue, which isolates it from the surrounding tissues. Similarly to the end-stage phases of wound healing, this granular tissue is highly vascularized and ensures survival of the implanted material.

Technically the method described here was successful, although intramyocardial needle injection produced a small area of damage to the surrounding tissue. Although cardiac function measurements were not the aim of this review, future studies should investigate whether minor tissue damage can perturb cell therapy analyses. Furthermore, it would be important to analyze whether the injury produced in the injected area is caused by the needle, by the amount of cells injected or by a combination of both.

In support of our findings, it has been shown that transplantation of mixed populations of differentiated human embryonic stem cells (hESC) containing 20-25% cardiomyocytes (hESC-CM) into the healthy heart of immunocompromised mice (NOD-SCID) resulted in rapid formation of grafts in which the cardiomyocytes became organized and matured over time and the noncardiomyocyte population was lost[23]. Interestingly, the authors observed that hESC-CM were largely isolated from the host myocardium by a layer of fibrotic tissue which prevented the formation of an electrophysiological syncytium[22,23]. In a different study, Kehat *et al.* found that hESC-CM successfully paced the ventricle in swine with complete heart block. This study showed that the transplanted cells survived, functioned, and integrated with host cells, providing evidence for their ability to function as a biological alternative to the electronic pacemaker[30]. These two discordant outcomes can be reconciled by the difference in structure and function of host and donor species. Indeed, it is possible that the efficiency of coupling of transplanted cardiomyocytes may depend on the relative beating frequency of host and donor cells. In Van Laake's study[23], the formation of functional junctions could be impaired due to the different beating frequency of human cardiomyocytes (60-100 bpm) *versus* rodent cardiomyocytes (300-600 bpm). This would not be the case in human *versus* porcine transplantation experiments, as described in Kehat analysis[30].

The length of observation time is another confounding factor. hESC-CM transplanted into the rodent heart after myocardial infarction induced cardiac function amelioration only for short-term time points (4 weeks). However, at 12 weeks, cardiac function was not further sustained despite graft survival[23]. The authors observed that graft size (increased amount of cells) was not associated with improved long-term cardiac survival and functional improvement[31], indicating that long-term analyses *versus* short- or mid-term analyses should be performed in future studies without requiring increased numbers of cells for injection.

In the present study, several questions remain concerning the type of cells to be transplanted, the selection of the host-donor species to be tested and a detailed evaluation of the arrhythmic potential of the cells. Ideally, stem cells implanted into the myocardium for replacement or pacemaker function should functionally couple with the remaining myocytes. In a rat model of myocardial infarction, Fernandes *et al.*[32] used Programmed Electrical Stimulation (PES) to evaluate arrhythmia susceptibility, showing an increased inducibility of ventricular arrhythmias in myoblast-injected hearts compared with controls. In that same study, injections of bone marrow-derived autologous cells did not result in an increased inducibility of ventricular arrhythmias compared with controls, thereby leading the authors to conclude that myoblasts exhibited a specific arrhythmogenic risk. In a different study, bone marrow-derived cells (BMC) efficiently grafted in clusters within the infarct scar or border zone, but showed no electronically evoked Ca transients[33]. Interestingly, Wei *et al.* showed that mesenchymal stem cells (MSC) do not acquire

the electrophysiological properties of mature cardiomyocytes during the survival period in the infarcted hearts. However, they can alleviate the electrical vulnerability and do not promote ventricular arrhythmias[34].

The efficacy and arrhythmia occurrence of stem cell therapy depends on the cells as well as on the delivery routes. Indeed, in rat hearts after MI[35], intramyocardial BMC injections, although improving cardiac function, increased consecutive ventricular premature contractions and ventricular tachycardia for the initial 14 days. When the intracoronary route was used, ventricular arrhythmia occurrence was markedly decreased. In clinical studies, the pro-arrhythmic function of injected cells is difficult to assess as most of the patients receive beta-blocker agents as indicated for ischemic heart disease. Treatment with beta- blockers might mask a potential pro-arrhythmic effect of the transplanted cells. Animal studies are therefore crucial to assess the bases of pro-arrhythmic stimuli in stem cell therapy.

In summary, our data demonstrates the feasibility of intramyocardial cell-injection in mice but also emphasizes the need for a detailed analysis regarding the safety conditions of cell grafting. In humans, it has been shown that cell injection is associated with pro-arrhythmias[36-38], restenosis[39], accelerated atherosclerosis[40], and coronary obstruction[41]. Basic research will be important in future clinical applications to understand which cells should be delivered, the mode of delivery, the mechanisms of cell-mediated myocardial function repair, and the design of a allogeneic *versus* autologous cell transplantation protocol that benefit all the human population. Because of the high costs associated with large clinical trials, reproducibility of efficiency and efficacy of transplantation protocols needs to be addressed in preclinical models such as those described here.

Disclosures

The authors have nothing to disclose.

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