Video Article

# Cell-based Therapy for Heart Failure in Rat: Double Thoracotomy for Myocardial Infarction and Epicardial Implantation of Cells and Biomatrix

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## Abstract

Cardiac cell therapy has gained increasing interest and implantation of biomaterials associated with cells has become a major issue to optimize myocardial cell delivery. Rodent model of myocardial infarction (MI) consisting of Left Anterior Descending Artery (LAD) ligation has commonly been performed *via* a thoracotomy; a second open-heart surgery *via* a sternotomy has traditionally been performed for epicardial application of the treatment. Since the description of LAD ligation model, post-surgery mortality rate has dropped from 35-13%, however the second surgery has remained critical. In order to improve post-surgery recovery and reduce pain and infection, minimally invasive surgical procedures are presented. Two thoracotomies were performed, the initial one for LAD ligation and the second one for treatment epicardial administration. Biografts consisting of cells associated with solid or gel type matrices were applied onto the infarcted area. LAD ligation resulted in loss of heart function as confirmed by echocardiography performed after 2 and 6 weeks. Goldner trichrome staining performed on heart sections confirmed transmural scar formation. First and second surgeries resulted in less that 10% post-operative mortality.

## Video Link

The video component of this article can be found at https://www.jove.com/video/51390/

## Introduction

Since the end of the 19$^{th}$ century, cardiovascular disease has remained the number one serial killer in industrialized countries. Among them, coronary artery disease represents the main etiology. The acute phase results in myocardial infarction (MI) and is followed by maladaptive remodeling that progressively develops toward a chronic phase and severe heart failure. Despite recent significant technological and therapeutic advances, the morbidity and mortality due to the progression of heart failure is still growing[1]. In this context, cell therapy has gained increasing interest as a new therapeutic option to stop the progression of the disease toward heart failure and to stimulate the recently identified regenerative capacity of the myocardium. Experimental and clinical investigations have provided compelling evidence of the beneficial effects obtained after cardiac transplantation of various cell types. Major outcomes included improved cardiac contractile function, decreased left ventricular remodeling, reduced infarct size, and increased vascular density in the infarcted area. However, the low cell number retention after cell injection remained an important drawback. Association of cells with a biomatrix to improve of cell delivery[2] have recently fostered researcher and clinical interests.

Ligation of the left anterior descending coronary artery (LAD) is a reference method for MI in small animal model that results in transmural infarction and a mature scar. Cell therapy applied in the chronic phase of MI requires a second surgical intervention. A median sternotomy is usually performed to allow intramyocardial injection of the cells or epicardial implantation of biografts. Such invasive surgical procedures increase the mortality rate, post-surgery recovery time, pain, and risk of infection. The minimally invasive approach presented here not only prevents such bias but also provides optimal accessibility of the heart for treatment application. MI and epicardial implantation of cells associated with a gel type biomatrix are performed on a beating heart *via* left intercostal thoracotomies.

## Protocol

NOTE: Lewis Male and Female rats, 200-220 g were housed under standard laboratory conditions (12 hr light and dark cycle, *ad libitum* water and food, IVC cage). All animals were treated in compliance with the recommendations of the FELASA and the Swiss Law on animal protection.

## 1. Cell Preparation: Mesenchymal Stem Cell Isolation from Bone Marrow

1. Anesthetize the rats with isoflurane 5% and $O_2$ 5 L/min in an induction chamber for 5 min. Place the animal's snout into a nose cone connected to the anesthesia system. Perform a toe or tail pinch to confirm sedation.

2. Remove the skin from the leg with scissors by cutting the skin from ankle to the hip. Remove the muscles and cut the femoral artery for animal exsanguination. Expose the hip joint and dislocate the femur head. It is important not to damage the femur head. Place the bones in sterile PBS in a 50 ml plastic tube.
3. Remove all muscles and ligaments from the bones without breaking them. NOTE: It is important that the bone remains intact to avoid linkage of flushing solution or contamination with ethanol. Rinse the peeled bones in 70% ethanol for 5 min.
4. Under the sterile laminar flow hood, cut both tips of the bones with scissors. Flush the bone marrow by injecting sterile PBS from the bone extremity. Collect marrow into a 15 ml tube. Centrifuge 7 min at 300 x g.
5. Remove the supernatant. Suspend the pellet in 3 ml of Red Blood Cell Lysis buffer. Incubate the suspension for 1 min at room temperature before spinning again for 7 min at 300 x g. In a 150 ml culture flask, add sterile cell culture medium (395 ml IMDM medium, 5 ml pen/strep, 100 ml FBS) and seed the cells.
6. Change medium the second day to remove nonadherent cells.
    1. Perform medium changes every second day for 2-3 weeks until desired cell quantity is obtained.
    2. Alternatively, prepare the biograft by seeding the cells onto the solid matrix as needed and described elsewhere[3].
7. The day of cardiac implantation, prepare the cells or harvest the biograft just before epicardial application.
    1. Collect the cells using Accutase cell detachment solution. Count the cells. Fill a 1.5 ml centrifuge tube with the volume of cell suspension calculated to obtain desired cell quantity. Centrifuge for 7 min at 300 x g and remove the supernatant.
    2. Alternatively, harvest the biograft from cell culture medium, rinse in sterile PBS, and keep in fresh culture medium without serum.

## 2. First Thoracotomy and LAD Ligation

1. Weigh the rat. Turn on the heating pad at 37 °C. Anesthetize the rat with 5% isoflurane and 5 L/min 100% $O_2$ in an induction chamber for 5-7 min. Perform a toe or tail pinch to confirm sedation. Place the rat on the heating pad.
2. Intubate the animal with a 14 G i.v. catheter. Connect the intubation catheter to a rodent ventilator programmed for 2.5 L/min oxygen, 2.5% isoflurane, tidal volume of 2 ml, and a breathing frequency of 90 breaths/min.
3. Prepare a buprenorphine solution at 0.1 mg/kg. Inject subcutaneously one third of the solution. Shave the left part of the thorax. Disinfect with a 1% Betadine solution.
4. Incise the skin perpendicular to the sternum at the fourth intercostal space. Separate the 3 layers of thoracic muscle (pectoralis major, ascending pectoralis, and external oblique pectoralis). Open the fourth intercostal space (between ribs 4 and 5).
5. Use a small retractor to spread the ribs and to expose the heart. Open the pericardium carefully. Locate and ligate the left anterior descending coronary artery (LAD) with a 7.0 suture 4 mm below the atrium.
6. Close the intercostal space with two stitches using 3.0 sutures. Position the two sutures proximally and distally from the sternum. First, tighten the distal suture. Clamp the ventilator exhaust tubing for 2 sec in order to inflate the chest and avoid any pneumothorax. Tighten the second suture. Position the muscles layers back in place (no sutures are needed). Close the skin with a 5.0 suture.
7. Disinfect the suture with 1% Betadine solution. Inject the rest of the buprenorphine solution. Turn off the anesthesia system. Remove intubation catheter.
8. Keep the rat in the cage under a warm Lamp for 1-2 hr. Keep a thermometer in the cage and control the distance between the warm lamp and the cage in order to avoid over heating. Return the rat in the IVC unit. Inject subcutaneously Buprenorphine 0.05-0.1 mg/kg post – operatively every 6-12 hr for 48 hr.

## 3. Epicardial Administration of the Treatment *Via* a Second Thoracotomy

1. Repeat steps 2.1-2.3.
2. Incise the skin perpendicular to the sternum at the fifth intercostal space. Separate the 3 layers of thoracic muscle (pectoralis major, ascending pectoralis and external oblique pectoralis). Open the fifth intercostal space (between ribs 5 and 6).
3. Use a small retractor to spread the ribs and to expose the heart. If there is some adherence, carefully remove them with fine forceps.
4. Locate the infarct area that appears as a pale area below the ligature. NOTE: When necessary, use a 10 cm piece of suture (7.0) inserted at the apex to better visualize the left ventricle. Pull gently to the suture maintained loosely by a clamp to expose the heart.
5. Apply one of the following treatments:
    1. Position the biograft at the surface of the heart. Use the prefilled fibrin sealant syringe prewarmed at room temperature for 10 min. Apply a drop (50-100 µl) of fibrin sealant under the biograft using a 25 G Luer needle. Verify that the biograft is perfectly sealed.
    2. Alternatively, apply the cell pellet collected with a pipette tip at the surface of the heart onto the pale area below the ligature. Apply a drop (50-100 µl) of fibrin sealant onto the cell pellet.
6. Remove the piece of suture from the apex.
7. Repeat steps 2.6-2.8.

## Representative Results

All animals recovered within 1 hr after thoracotomies. The wound healing was rapid. No infection or edema was observed.

The double left thoracotomy allowed optimal access to the heart (**Figure 1**). Pain and post-surgery mortality were low. The animal recovered quickly from the surgery and gained weight (**Figure 2**). Kaplan Meir survival percent was 96% for the first thoracotomy. Four rats over 104 died within the 24 hr following treatment application *via* a second thoracotomy. Kaplan-Meir survival percent varied from 100% when animal received a second thoracotomy but no treatment (sham), 96% for MSC and fibrin sealant group, or 86% when animal received MSC directly injected within the myocardium (**Figure 3**).

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M-mode echocardiographic evaluation performed 2 weeks post LAD ligation showed a reduction in fractional shortening (FS) from 44 ±3% to 22 ±4%. The frequency representation of the FS recorded on 104 animals 2 weeks post LAD ligation provided evidence of the repeatability of the method (**Figure 4**). The coefficient of variation (CV) was 17%. Furthermore, echocardiography performed at 2 and 6 weeks post LAD ligation showed a progression toward heart failure suggested by a reduction of the ejection fraction (EF) and an increase of LV volumes in diastole and systole (**Figures 4** and **5**). The development of a transmural infarct in the sham operated group was confirmed by Goldner staining of heart cross sections (**Figure 6**).

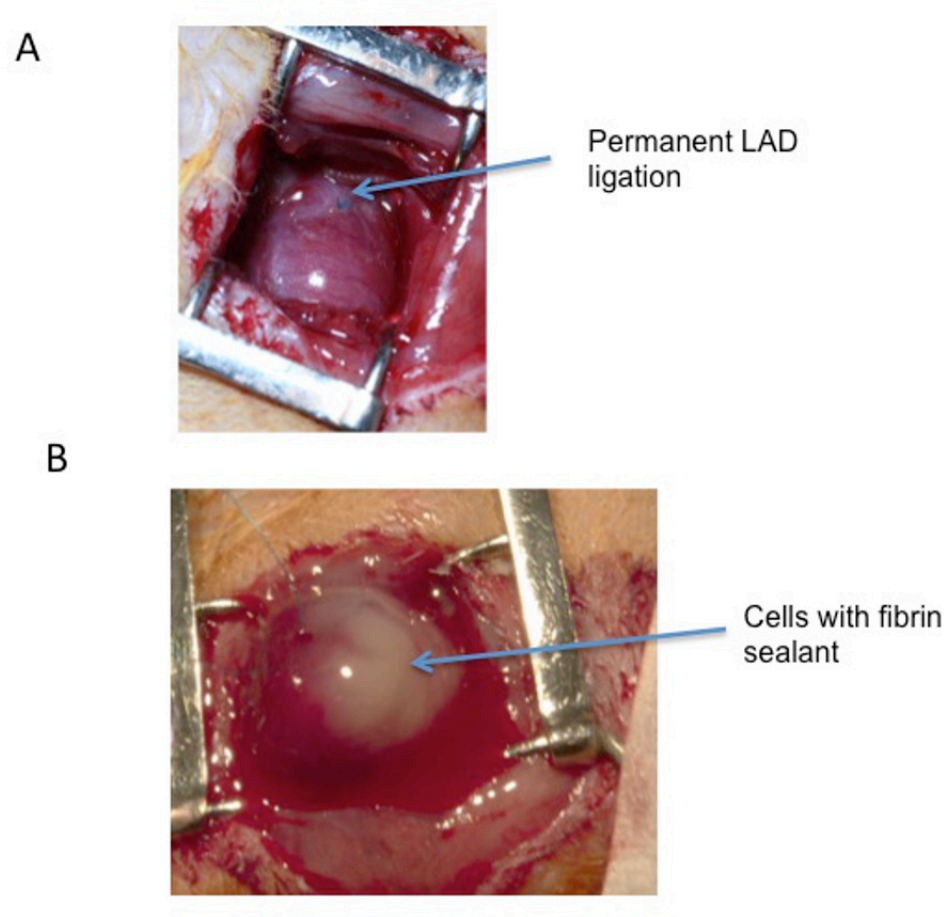


**Figure 1. (A) The use of retractor allowed access to the heart following thoracotomy.** LAD ligation was performed 2 weeks earlier and suture is visible (arrow). Epicardial treatment application was performed with minimum exteriorization of the heart. (**B**) The cells pellet fixed with fibrin sealant were maintained at the surface of the infarcted area.

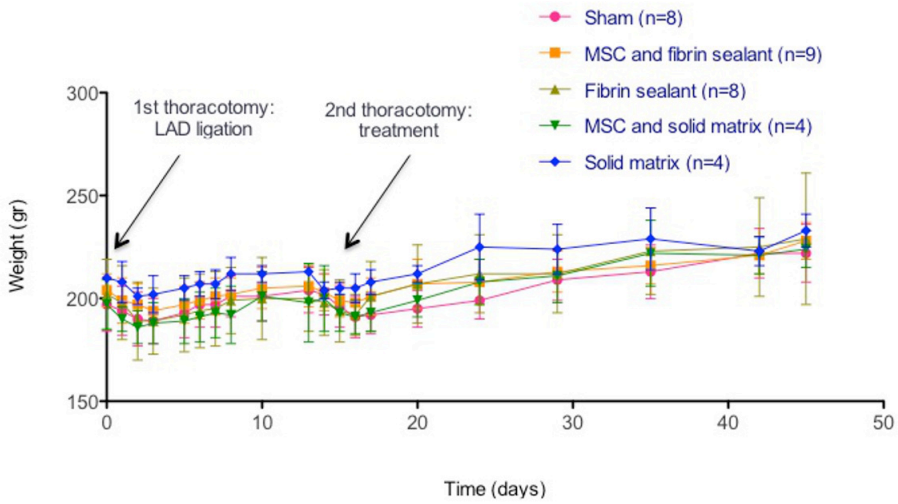


**Figure 2. Daily postoperative follow up showed weight loss for 2 days post-surgery.** Recovery was accompanied with gain of weight in all animals. Weight variations were independent of the type of treatment.

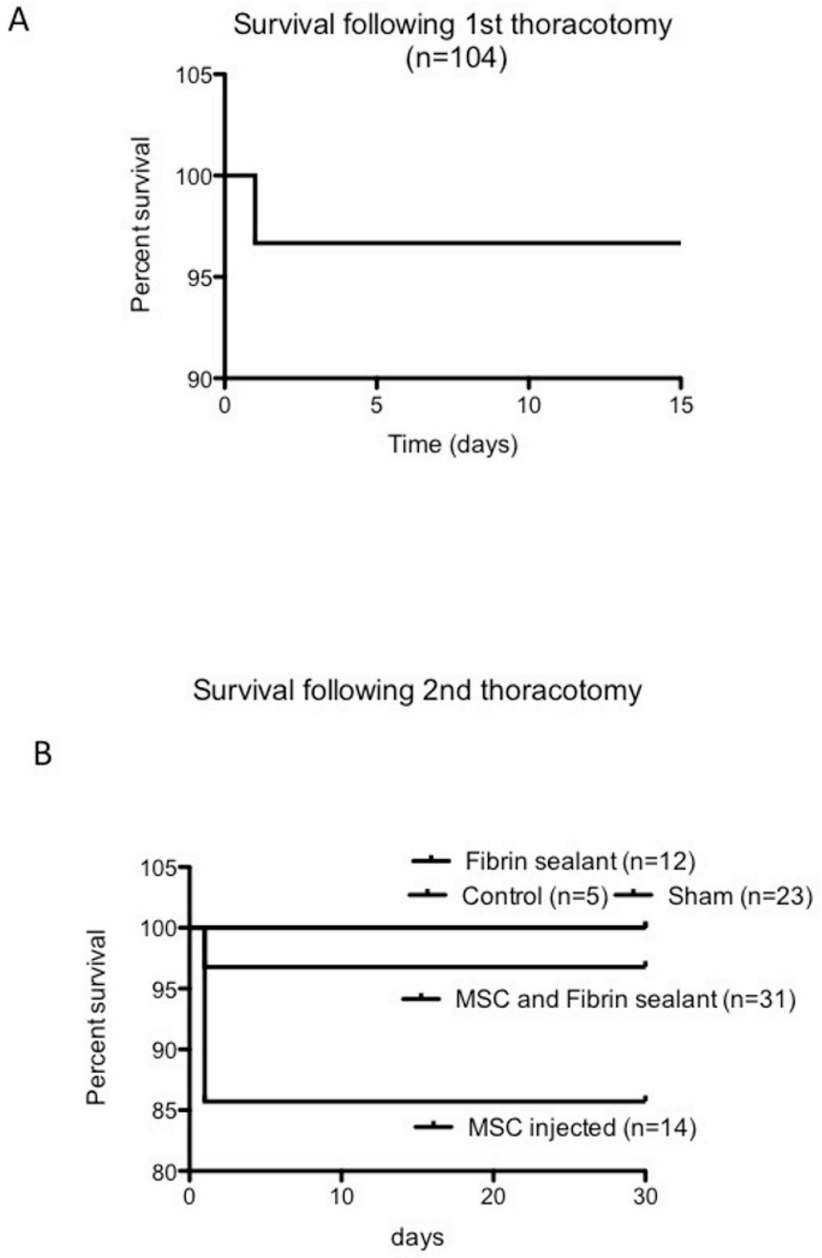


**Figure 3. Kaplan-Meir graph representing percent survival post LAD ligation (A) and post treatment *via* a second thoracotomy (B) showed that both surgeries resulted in reduced loss of animals.** Control group represents healthy animal that had no surgery. Two weeks after the first thoracotomy, animals received either a sham operation but no treatment (sham), fibrin sealant alone, cells fixed with fibrin sealant, or cells directly injected within the myocardium (MSC injected).

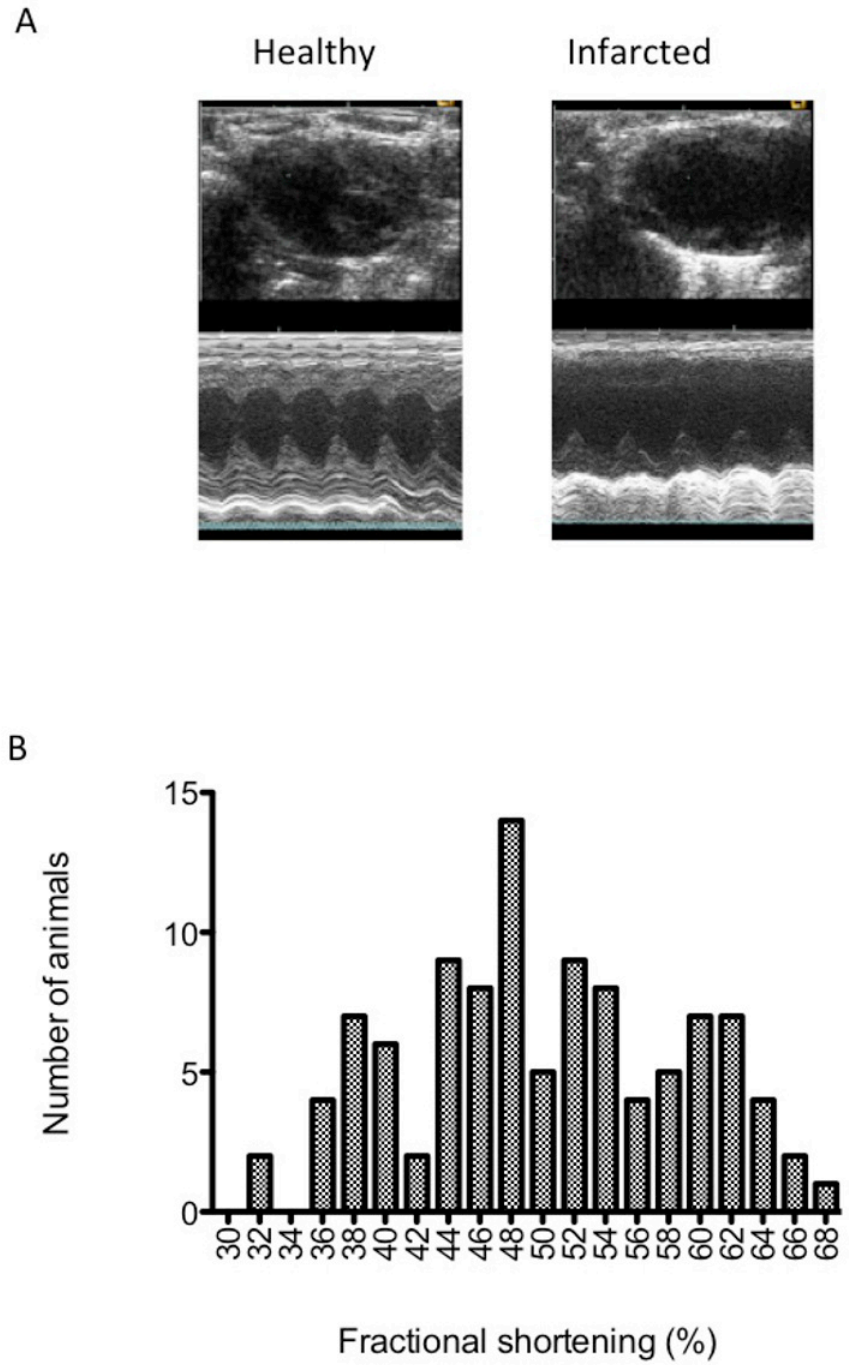


**Figure 4. (A) Representative M-mode echocardiography images registered pre (healthy) and 2 weeks post LAD ligation (infarcted).** (**B**) Frequency distribution of the fractional shortening values recorded two weeks post LAD ligation (n=104).

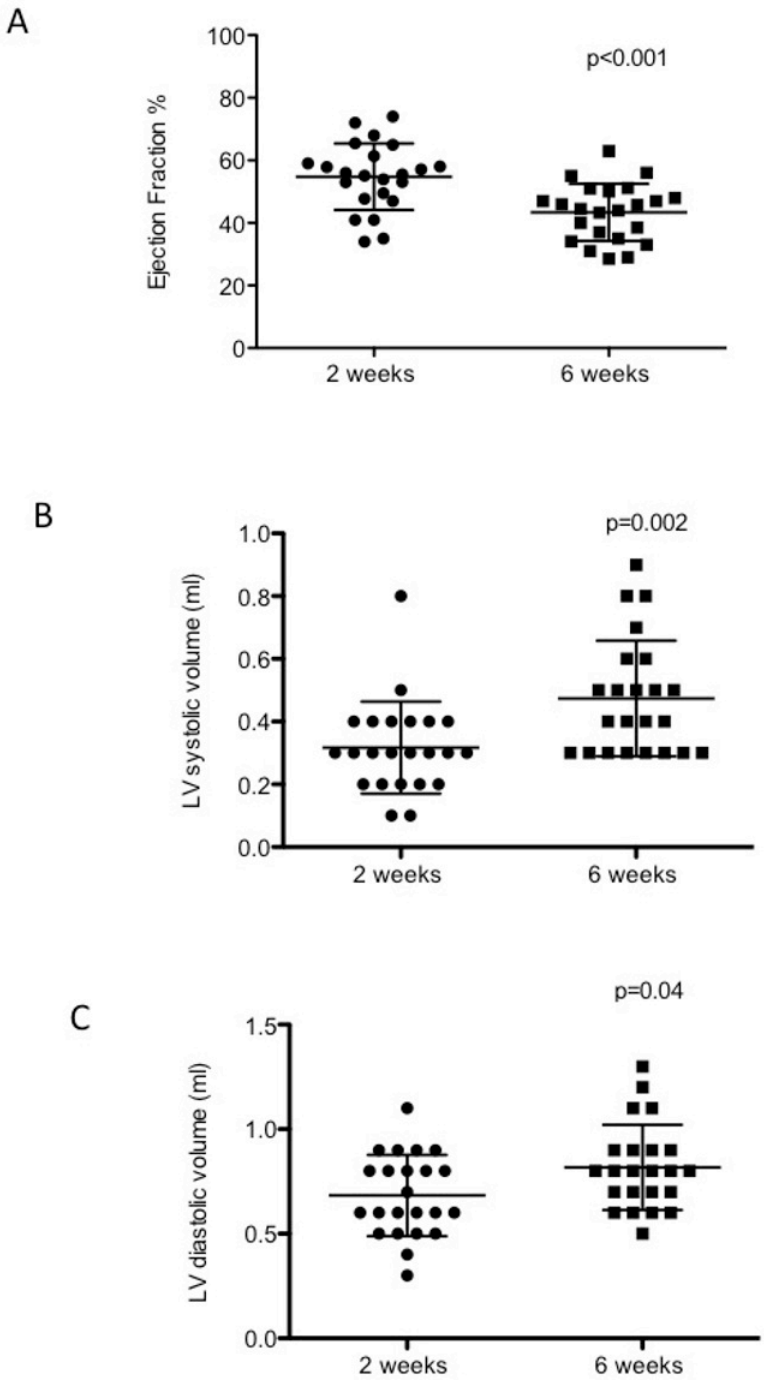


**Figure 5. Heart function was assessed by 2dimentional echocardiography (biplane Simpson's method) and recorded 2 and 6 weeks post LAD ligation.** Animal had two thoracotomies but received no treatment. Mean ±SD as well as individual values are represented for ejection fraction (**A**) LV volume in systole (**B**) and diastole (**C**). Statistical analyses were performed using a paired nonparametric Wilcoxon test.

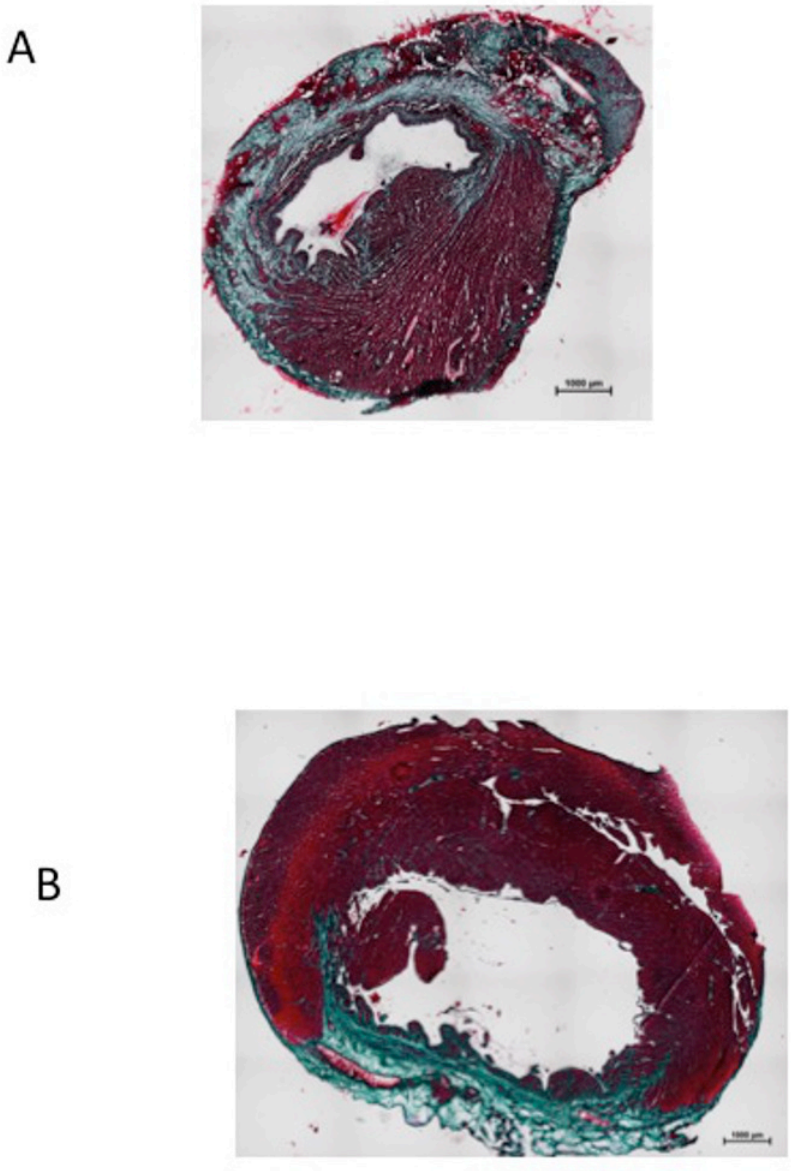


**Figure 6. Representative Goldner staining showing fibrotic scar (green) and viable myocardium (red) at 2 weeks (A) and 6 weeks (B) post LAD ligation.**

## Discussion

The permanent ligation of LAD causes irreversible myocardial injury. The first animal model was described in 1960[4]. Since then, it has been considered as a standard and suitable model for chronic MI. Its stability and reproducibility allowed experimental evaluation of therapies for MI[5]. Improved procedures following the initial description reported an operative mortality rate of 35-13%[6].

As expected, LAD ligation induced impaired heart function observed within 2 weeks. A further decrease in heart function was recorded up to 6 weeks and associated with transmural fibrotic scar. In addition, LV remodeling including LV dilatation was clearly observed 6 weeks post ligation.

An interval of 2 weeks between the first and second surgery was chosen. In this condition, the second thoracotomy was facilitated by the absence of adherences, as they were too abundant after 1 week to perform a second surgery in optimal conditions. In addition, 2 weeks represented an optimal time frame for treatment application and allowed to avoid the early pic of inflammation induced by the ischemia and to apply the treatment before the formation of a dense fibrotic tissue and progression of the dilation.

Although lateral thoracotomy is nowadays the standard surgical procedure for LAD ligation on small animals, a median sternotomy is usually performed for cell therapy application[7]. The presented second intercostal thoracotomy for treatment application is a novel, minimally invasive approach; it allows access to the heart through the ribs without cutting bones and by splitting the muscle. The occurrence of adherence is

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reduced due to the small and limited scar following first thoracotomy and these do not interfere with the accomplishment of the treatment application. The approach resulted in very low risk of hemorrhage, fast postoperative recovery, low mortality, pain, and risk of infection. This second minimally traumatic procedure lasted 20 min for surgery and up to 20 min for matrix fixation. The rapid procedure and limited exposure of the heart minimized cardiac tissue drying. Post-operative ultrasound imaging resulted in good quality image acquisition for reproducible measurements analysis. There were no hematomas that could impair imaging.

The accessibility of the heart allowed optimal visibility of the infarcted myocardium as well as a good exposure of the heart for the application of epicardial treatments including solid and gel type biografts[8-10].

The use of fibrin sealant presented several advantages. (i) To fix solid patches, the use of fibrin sealant was less traumatic than sutures. (ii) When compared with other glue such as Bioglue (Cryolife), fibrin sealant was more compliant than Bioglue that formed rigid structure and impaired heart contractions. (iii) Its polymerization time was optimal for adequate scaffold or cell pellet positioning. (iv) Fibrin sealant allowed excellent initial adhesion of solid patch. The patch was tightly maintained on the surface of the heart for at least 4 weeks[10]. (v) Fibrin sealant was easy to process when used as a scaffold for epicardial cell delivery and fixed cells were maintained at surface of the infarcted area until the end of the surgical procedure. Although the retention time of the cells was out of focus of this study and would demand further investigations, it has been proposed that matrix allows improved myocardial retention of implanted cells[11-14]. Wash out of cells during cardiac contraction, as well as the hypoxic and inflammatory environment, may partly explain the rapid clearance. These drawbacks might be reduced when cells are associated with a matrix.

The main technical challenges concerned the surgical handling of a solid biograft. If the matrix is rigid and nonflexible, fixation with sutures should be favored. The following critical steps should be considered for successful procedure. First, a particular attention should be given during thoracotomy to avoid breaking the ribs. Second, excess of glue should be carefully removed to avoid adherence of others tissues. Finally, a standardized ligature positioning is important as it may reduce the variability in infarct size. A transmural infarct can be obtained when LAD is ligated 4 mm below the atrium.

In conclusion, double thoracotomy represented a safe surgical procedure with low mortality, adequate access to the beating heart for treatment application, and therefore will favor optimization of the experimental planning and reduced number of animal loss.

## Disclosures

The authors have nothing to disclose.

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