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TITLE:

Murine Myocardial Infarction Model using Permanent Ligation of Left Anterior Descending Coronary Artery

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KEYWORDS:

Myocardial, infarct, mouse, permanent, ischemia, LAD, coronary artery, ligation

SUMMARY:

Herein we describe a surgical procedure showing how to achieve permanent ligation of the left-anterior descending coronary artery in mice. This model is of high relevance to investigate the pathophysiology of myocardial infarction and the concomitant biological processes.

ABSTRACT:

Myocardial infarction (MI) and acute coronary diseases are among the most prominent causes of death in population with western lifestyle. The murine models of MI with permanent ligation of left-anterior descending (LAD) coronary artery closely mimics MI in humans. Murine models benefit from the extensive genetic engineering available nowadays. Here we propose a reproducible murine surgical model of myocardial infarction by permanent LAD coronary ligation. Our technique comprises anesthesia with ketamine/xylazine that can be rapidly reversed by administration of an antagonist, intubation without tracheotomy for mechanical-assisted ventilation, ventilation with application of extrinsic positive end-expiratory pressure (PEEP) to avoid alveolar collapse, a thoracotomy method limiting to the minimum surgical lesions made to skeletal muscles, and lung inflation without thoracentesis. This method is sparsely invasive, reproducible and reduces post-surgery mortality and complications.

INTRODUCTION:

Acute myocardial infarction (MI) is the most severe expression of ischemic heart diseases (IHD). IHD are the leading cause of morbidities and death worldwide, especially in western countries[1]. Consequently, it has an enormous economic impact on healthcare systems[2]. MI is characterized by the occlusion of a coronary artery by atherosclerotic plaque and the subsequent arrest of blood flow in large parts of the myocardium. Lack of oxygen supply in the myocardium leads to ischemic death of cardiomyocytes. This pathological condition triggers responses in the ventricular tissue that ultimately leads to deficiencies in ventricular functions, remodeling and heart failure[3]. MI is a complex pathophysiological condition that involves multiple and intricate

biological processes comprising regulated cell death, response to oxidative stress, inflammation, wound healing, fibrosis and ventricular remodeling. Some of these biological responses are modeled as individual processes in vitro like necrosis-induced release of damage-associated molecular patterns and associated inflammatory responses[4]. These simplified models are essential to understanding MI. However only an in vivo model can provide a realistic image of the biological processes complexity engaged in response to MI.

Even though models of MI in larger animals like swine may more closely relate to human pathophysiology of MI, the power of the murine models resides in the possibilities offered by genetic engineering that is more advanced than in any other mammal species. Other non-negligible aspects are the relative low cost and the simplicity of the surgical setup.

It is worth to mention that models of ischemia-reperfusion of the myocardium can exhibit different outcomes than permanent MI models. Biological processes like the type of cell death engaged, quality/amplitude or kinetics of inflammatory and wound healing responses in the myocardial tissue might vary according to the model[5-7]. However, this protocol of permanent coronary occlusion can easily be adapted to obtain an ischemia-reperfusion model.

This method is relevant for studies related to the physiopathology of MI without reperfusion and allows monitoring of pathological processes occurring from coronary occlusion (minutes) to late stage heart failure (weeks) at the local heart tissue and systemic levels.

PROTOCOL:

Animal experiments described in this protocol were reviewed and approved by Animal Ethics Committee of Canton of Vaud.

NOTE: For these experiments, we used male C57Bl/6J mice weighing between 25 g and 30 g and an age of 8–12 weeks. Mice were fed chow pellets and water ad libitum and bred under conventional conditions. Surgical equipment was previously sterilized. The experimenter should wear sterile surgical gloves and a surgical mask to limit contamination and post-operative infections.

1. Anesthesia and tracheal cannulation.

1.1. Weigh the mouse to determine the dosage of anesthetic drugs, post-operative analgesic medication and tidal volume of the ventilator. Pre-warm the heating pad at 37 °C. The surgical setup is depicted in **Figure 1**.

1.2. Inject mouse intraperitoneally with a mix of ketamine and xylazine at a dose of 80 mg/kg and 10 mg/kg respectively.

1.3. Quickly shave the mouse fur on the throat and the left side of the rib cage using an electric razor.

1.4. Check depth of anesthesia by pinching tail and/or hind feet and settle the animal in a supine position on the heating pad. Place a small gauze compress under the head of the animal to avoid overheating of the eyes. Apply ocular gel to avoid eye dryness.

1.5. Secure the four limbs with adhesive tape on the surface of the heating pad. Pass a loop of 5-0 silk suture under the upper incisors and stick the extremity of the loop with adhesive tape onto the heating pad. This will keep the mouth of the animal open and facilitate cannulation.

1.6. Apply hair removal cream on the pre-shaved areas and gently massage with a cotton swab for 1 min. Wipe the excess of fur and cream with a gauze. Use drops of 0.9% saline solution and gauze to clean the incision areas. Apply pieces of sterile gauze to the shaved throat and thorax and soak them in iodopovidone.

1.7. Set the ventilator at a tidal volume of 7 mL/kg and ventilation rate of 140 strokes/min.

NOTE: From now on work under a microsurgery stereomicroscope.

1.8. Hold the skin on the center of the throat and perform an incision of 0.5 cm following a caudal/cephalic line using small scissors. Separate the lobes of salivary gland, then gently separate fascia of sternohyoid muscle with curved dissecting forceps until larynx and trachea are visible. Secure edges of the opening with retractors attached to elastic bands.

NOTE: Do this step without incision of the muscles.

1.9. Hold gently the tongue sideways. With forceps, insert the blunted inner needle of a 16 G cannula into the trachea. Visualize correct insertion into the trachea through the throat incision.

1.10. Connect the cannula to the ventilator and ensure correct ventilation by placing the exhaust tubing into water. The presence of bubbles indicates correct intubation.

NOTE: In order to keep tissues wet during operation place sterile gauze soaked with 0.9% saline solution and iodopovidone on the throat incision. Control moisture during the procedure.

2. Ligation of LAD coronary artery

2.1. Release left anterior paw from duct tape and carefully move the mouse to right side decubitus position. Secure the left anterior limb once animal is in the correct position.

2.2. Identify the line between left pectoralis minor and major muscles and make an oblique skin incision on 1 cm with scissors following the line. With dissecting blunt micro scissors, separate fascia of pectoralis muscles without incision. Maintain pectoralis muscles separated with retractors attached to elastic bands.

2.3. Set the ventilator with a positive end-expiratory pressure (PEEP) of 3 cm H_2O.

2.4. Open the chest cavity by using blunt forceps at the 3rd intercostal space between 3rd and 4th ribs. Avoid touching internal thoracic artery as there is danger of bleeding. Do not touch heart or lung. Apply two retractors into the ribcage, one on each rib (**Figure 2A**).

2.5. With a curved fine forceps, carefully remove the pericardium and pull it apart without harming the heart and lungs.

2.6. Locate left anterior descending (LAD) coronary artery. LAD artery appears as a superficial bright red line running from the edge of the left auricle toward the apex.

2.7. Use a needle holder to pass a 7-0 silk suture under the LAD 2 to 3 mm below left atria. Pull the silk slowly to avoid a tearing of heart tissue. Tie the ligature with three knots. The lower left part of the left ventricle will instantly turn pale upon ligation (**Figure 2B-E**).

NOTE: It is important to not go too deep into the ventricular cavity or to stay too superficial. For sham-operated animals, pull the suture silk under the LAD and remove it slowly avoiding tissue tearing.

2.8. Release the rib retractors, hold the 3rd rib with forceps and make two passes with a 6-0 silk suture under the 3rd and 4th ribs.

CAUTION: Do not the perforate heart or lung. Do not tighten knots yet.

2.9. Put three drops of 37 °C 0.9% saline solution onto the opening and shut the expiration exhaust tube for 2 or 3 respiratory cycles to properly inflate lungs. Tighten the suture and secure with two throws.

2.10. Release retractors holding muscles and help them retrieve their correct place.

2.11. Close thoracic skin with two stitches of 5-0 suture silk and secure with two throws. Close throat skin with one stitch of 5-0 suture silk and secure with two throws.

3. Post-operative procedures and follow-up.

3.1. Remove adhesive tape bands from limbs. Put a compress on the heating pad on the right side of the animal.

NOTE: The overall procedure from anesthesia to this point should not take longer than 40–45 min. Optionally inject IP 0.2 mL of atipamezole at a concentration of 0.1 mg/mL to speed up the waking up process.

3.2. Intraperitoneally inject 0.3 mL of 5% glucose solution pre-warmed at 37 °C.

3.3. Carefully turn the animal on ventral decubitus onto the compress pad.

3.4. Stop ventilator; if the mouse spontaneously breathes, cautiously remove cannula.

3.5. Inject subcutaneous (SC) 0.1 mg/kg buprenorphine and put mice in a pre-warmed cage heated at 30 °C and ventilated with a 100% O_2 for a minimum of 1 h. Monitor mice for any life-threatening condition such as excessive dyspnea or hemorrhage.

3.6. During the two first days following surgery, monitor mouse twice daily. Inject SC 0.1 mg/kg buprenorphine twice daily. Intraperitoneally inject 0.3 mL 0 of 5% glucose solution twice daily. Provide mice with soft diet and water ad libitum. Warm up the animal if necessary.

3.7. From day three, inject SC 0.1 mg/kg buprenorphine twice daily if the animal exhibits any unusual signs concerning general appearance, respiration or behavior. Intraperitoneally inject 0.3 mL of 5% glucose solution twice daily if the animal is still losing weight. Warm the animal if necessary.

NOTE: Strictly apply predefined interruption criteria when necessary to avoid excessive suffering. Usually mice lose weight up to day 3 and 4 and then gain weight. After seven days, mice usually retrieve pre-operation weight.

REPRESENTATIVE RESULTS:

Mice were euthanized seven days after surgery. Animals were anesthetized with 80 mg/kg ketamine and 10 mg/kg xylazine. Under anesthesia, blood was drawn from vena cava and heart was sampled. Atria were removed, myocardium were washed in ice-cold PBS. For measurements of ischemic areas, hearts were frozen at -20 °C for 40 min, then sliced and stained for 20 min at 37 °C in PBS containing 2% triphenyltetrazolium chloride (TTC). Heart slices were fixed overnight in 4% buffered paraformaldehyde solution at room temperature. Ischemic areas remained unstained whereas live tissue was stained in red due to the presence of dehydrogenases. Ischemic areas were calculated as percentage of white area of the left ventricle (LV) with an imaging software (**Figure 3A, B**). For biochemical and molecular biology analyses, hearts were frozen in liquid nitrogen. After grinding hearts on liquid nitrogen the organ powder was used for protein and mRNA extraction. The extent of fibrosis in the myocardial tissue of infarcted hearts was assessed by western blot analysis of alpha smooth-muscle actin (αSMA) and SMAD2 phosphorylation, which are respectively major read-outs of myofibroblasts and of TGFβ signaling activation (**Figure 3C**). mRNA expression of *Tgfb*, and downstream targets *Ctgf*, *Postn* and *Il11* are all indicators of myocardial fibrosis. This was shown by real-time polymerase chain reaction (PCR) analysis (**Figure 3D**).

Pro-inflammatory signaling pathways and expression of pro-inflammatory genes were typically found activated within the first week following myocardial infarction. Phosphorylation of NF-κB p65 transcription factor is a hallmark of inflammation and was observed in whole myocardium extracts of the MI mice (**Figure 3E**). mRNA expression of pro-inflammatory genes *Il1b*, *Il6* and *Cxcl10* (**Figure 3F**) and monocytes/macrophages markers *Cd14* and *Mertk* were analyzed by real-

time PCR (**Figure 3G**). Note that there was a variability in the extent of NF-κB p65 and SMAD2 phosphorylation (**Figure 3C**,**E**, lanes 4–7). This variability depends largely on the size of the infarct.

FIGURE AND TABLE LEGENDS:

Figure 1: Description of the surgical setup. (**A**) Surgical setup comprises a modified heating pad, a ventilator and retractors attached to elastic bands. (**B**) Set of scissors, forceps and needle holder used during the surgery. (**C**) Close-up of the mini-retractors. Not shown: surgical stereo microscope.

Figure 2: Representative images of the surgery and LAD ligation. (**A**) Opened chest with retractors. The left ventricle was apparent. Top, left and bottom retractors held the ribcage and right retractor held the pectoralis muscle. (**B**) The needle was passed under the LAD. (**C**) Suture silk was passed under the LAD, into the left ventricle. (**D**) Single stitch on the LAD. (**E**) End of the ligation procedure, the suture was secured with three knots. (**F**) Representation of an anterior view of the heart. The position of LAD ligation was 2–3 mm below left atria and above diagonal branch of the LAD.

Figure 3: Fibrosis and inflammation in whole myocardium extracts seven days post-surgery. (**A**) Representative images of TTC staining of a sliced infarcted heart seven days post-surgery. Pale ischemic areas remained unstained and white whereas live tissue was stained red. The ligation was visible on the third slice from the left. (**B**) The size of the ischemic areas of five infarcted hearts were measured using TTC staining technique. Results were the percentage of white area of the left ventricle (LV). (**C**) Western blot analysis of SMAD2 phosphorylation and alpha-SMA expression in whole myocardium as indicators of fibrosis. (**D**) mRNA expression of *Tgfb*, *Ctgf*, *Postn* and *Il11* in whole myocardium extracts. (**E**) Western blot of NF-κB p65 phosphorylation in whole myocardium extracts. (**F**) mRNA expression of pro-inflammatory genes *Il1b*, *Il6* and *Cxcl10* in whole myocardium extracts. (**G**) mRNA expression of *Cd14* and *Mertk* as indicators of the presence in the myocardium of monocytes/macrophages and phagocytic macrophages respectively. N = 3 in sham and N = 4 in MI group. For mRNA expression analysis, expression was relative to the endogenous control Rps18 and group comparisons were unpaired Student's T-tests, *$p \leq 0.05$, **$p \leq 0.01$, *** $p \leq 0.001$. In panels B, D, F and G error bars represent standard deviations.

DISCUSSION:

The first critical step of this procedure is certainly intubation. We use the blunted inner needle of a 16 G catheter as a tracheal tube. We do not recommend using this setup with mice that weight less than 22 g. With this setup, it may be difficult to intubate mice properly with smaller bodyweight without damaging the trachea. Another critical point is to limit incisions made to the muscle while exposing the trachea and ribcage. Reducing tissue damage is of major importance, especially when studying inflammatory processes subsequent to MI. That is why we prefer gentle spreading of muscle and ribs with forceps and retractors[8,9]. We do not use electric cauterizer to control bleeding[10]. This may cause iatrogenic burns and favor infections. Both trauma and infections may bias inflammatory read-outs. Application of an extrinsic PEEP of 3 cm H_2O by

plunging the ventilation exhaust into a water tube limits end-expiratory alveolar collapse during thoracotomy. Localization of LAD is another critical step and one should keep in mind that the anatomy of the coronary arteries may vary depending on the strain and the genotype of the mouse[11]. It requires some experience to visualize the LAD, however placing the suture directly 2–3 mm below the left atria as described in the procedure shall allow correct positioning of the ligation. Instant discoloration of large portions of the left ventricle under the suture confirm the accuracy. Finally, artificially applying auto-PEEP by blocking ventilation exhaust for 2–3 respiratory cycles during chest closure allows a transient hyperinflation of the lung that will help chase the air from thoracic cavity[12]. We purposely do not perform a thoracentesis as shown in [9,10]. This way, we limit the risk of lung and heart injuries and avoid excessive tissue damage or perforation.

Myocardial ischemia-reperfusion (I/R) is a related surgical model that mimics the restoration of coronary blood flow that is done to MI patients in clinics. During the I/R model a transient occlusion of the coronary artery is done by tightening a piece of tubing onto the LAD for a duration of 20 to 45 min[8,13]. Then the occlusion is released to allow reperfusion of the myocardium for the desired duration. This simple modification applied to our protocol can easily turn it into an I/R model[4,8,14,15]. The infarction can be confirmed by a blood test for cardiac troponin T[8,10] or by echocardiography[15].

MI differs from I/R model because reperfusion by itself induces an injury. MI induces more tissue necrosis and apoptosis is more pronounced in reperfused myocardium[5]. Kinetics of inflammatory cells infiltration is also different between in MI and IR with a delayed myocardial infiltration of immune cells in MI[7]. The size and the position of the infarcted area will also differ between permanent ligation and I/R models[15]. Keeping this in mind, one must be cautious to choose a relevant model since I/R and permanent MI models are not equivalent. Another murine model of myocardial infarction is the cryoinfarction model. Application of a cryogenic probe on the LV anterior wall induces the freezing of ventricular tissue and blood flow arrest in the LAD artery. This technique however differs from MI and I/R techniques regarding timing and amplitude of remodeling and inflammatory responses[16,17].

Variability is a limitation as for any surgical procedure. This variability relies on biological differences. A good example is the variation in coronary arterial arrangement in mice[11]. It also relies on experimenter skills. It is worthwhile mentioning that adequate training of the experimenters is mandatory in order to reach stable outcomes with this model. A well-trained experimenter can easily produce infarct sizes that are reproducible (**Figure 3A-B**). The mortality of the model depends on the position of the LAD, duration of the experiments (days, weeks), mouse strain and genotypes. The types of anesthetic and analgesic drugs may also affect the outcome of the experiments with putative cardioprotective or cardiodepressant effects. In our hands, this model has a global mortality rate of 25–30%. This mortality rate comprises spontaneous deaths and sacrifices before the end of the experiment, regardless strains and experiment duration. Most of the deaths or sacrifice are between the second and fourth days post-surgery. Applying a strict pain management and follow up of the animals can reduce mortality.

Here we present representative results of infarct size analyzed using TTC staining and expression of protein and genes involved in inflammatory or fibrotic processes in LV by western blot and real-time PCR respectively (**Figure 3C-G**). It is also possible to measure many of these parameters by enzyme-linked immunosorbent assay (ELISA) or enzymatic assays. Of course, in accordance with hypothesis that needs to be tested, this method can be followed by any functional analysis by ultrasound, MRI or intraventricular catheter measurement of pressure and volume. It is also possible to extract heart and further investigate cardiac cell biology on isolated cells. Overall, the MI model with permanent ligation of the LAD coronary artery is particularly useful to evaluate inflammatory and fibrotic processes, wound healing and changes in cardiac function subsequent to myocardial infarction.

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DISCLOSURES:

The authors have nothing to disclose.

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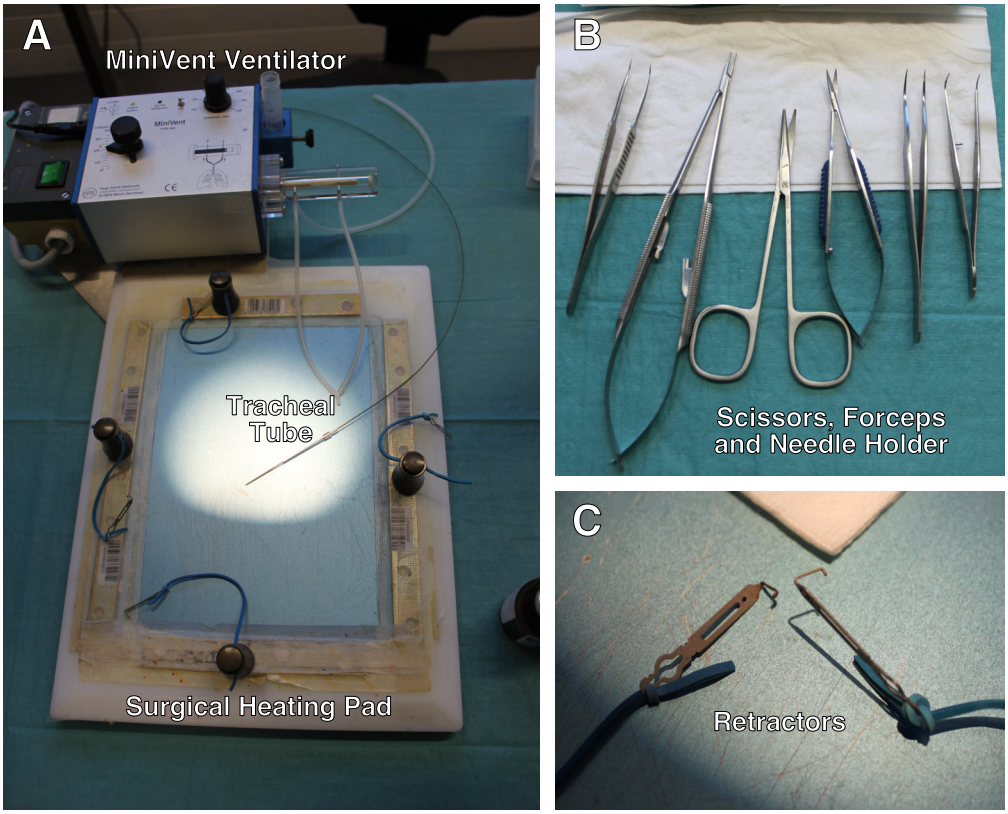
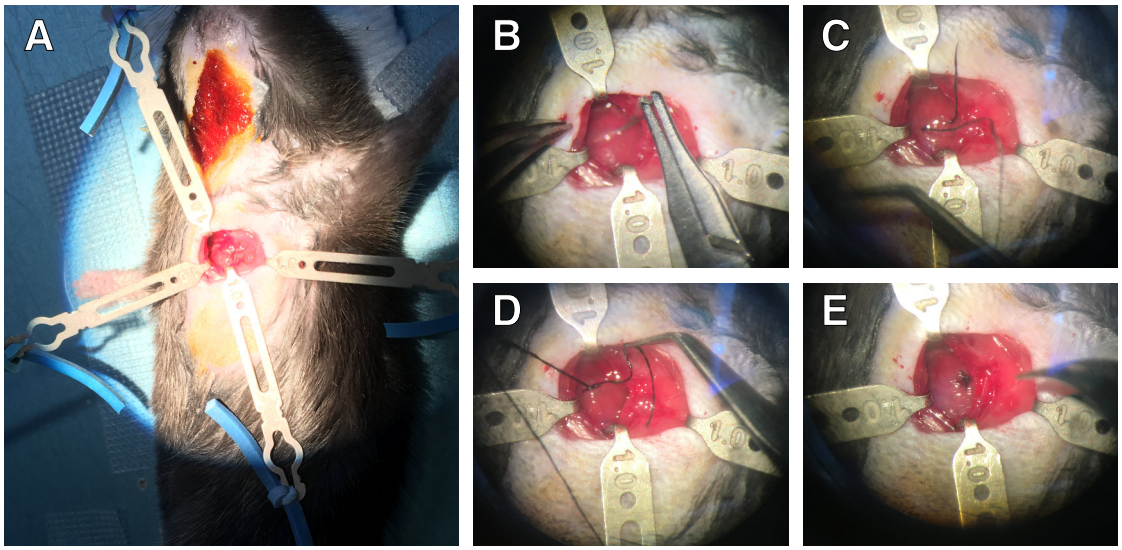
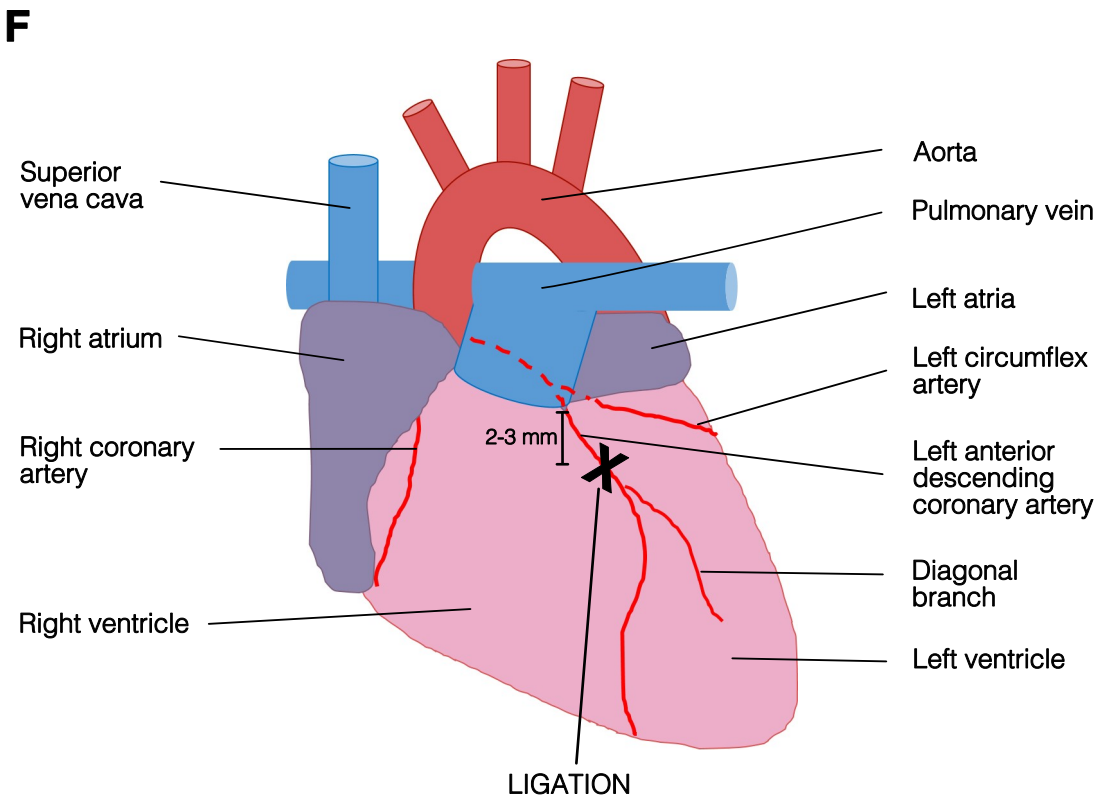
Figure 1







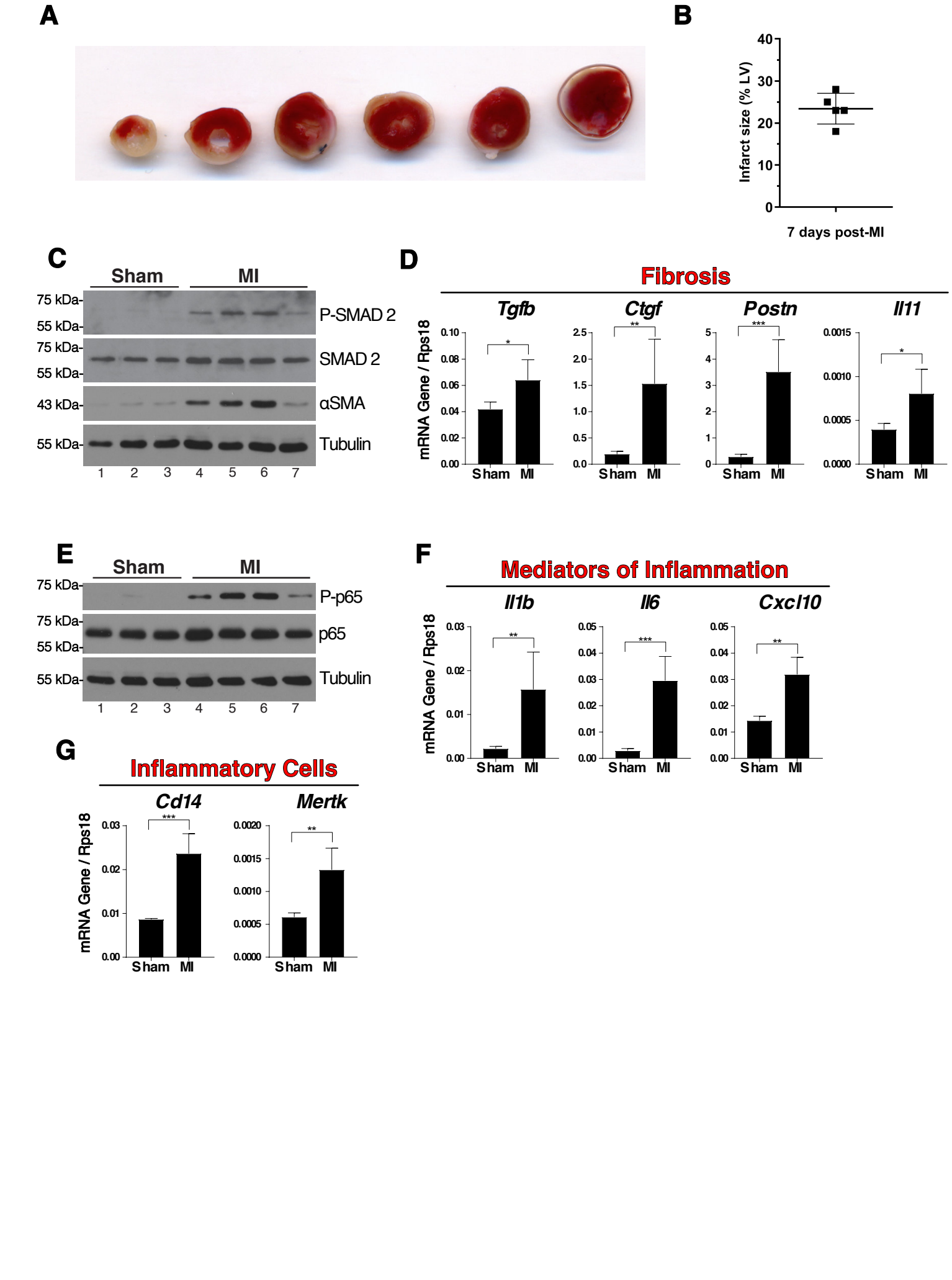


Table of Materials

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Name of Reagent/ Equipment	Company	Catalog Number	Comments/Description
1 CC Syringe, Omnifix-F	B. Braun	9161406V	
30G- Needle	BD Microlance 3	304000	
70% Ethanol			
Betadine 60 ml	MundiPharma		
Blunt Retractors	Fine Science Tools	18200-09	
Castroviejo Needle Holder Straight with Lock	Roboz	RS-6416	
Cotton Swabs	Applimed SA	6001109	
Dissecting Scissors, Curved	Aesculap	BC603R	
Electrical Razor	Remington	HC720	
Glucose 5% B.Braun	B. Braun	531032	
Hair Removal Cream, Veet	Silk & Fresh Tech.	8218535	
Iris Dissecting Forceps Full Curved	Aesculap	OC022R	
Ketasol 100 (100 mg/ml)	Dr. E. Graeub AG	QN01AX03	
Micro Scissors, Curved Blunt/Blunt	Aesculap	FM013R	
NaCl 0.9% B. Braun	B. Braun	534534	
Short Fixator	Fine Science Tools	18200-01	
Silk Suture 5-0, BB	Ethicon	K880H	
Silk Suture 6-0, P-1	Ethicon	639H	
Silk Suture 7-0,BV-1	Ethicon	K804	
Student Dumont #7 Forceps	Fine Science Tools	91197-00	

Student Fine Forceps-Angled	Fine Science Tools	91110-10
Surgical Gloves	Weitacare	834301
Surgical heating pad	Personalized setting	
Temgesic sol 0.3 mg/ml Buprenorphine	Indivior Schweiz AG	N02AE01
Tracheal tube inner needle of an 16G i.v. cat	Abbocath-T	G714-A01
Universal S3 Microscope, OMPIMD	Zeizz	
Ventilator, MiniVent Model 845	Harvard Apparatus	73-0043
Viscotears	Alcon	1551535
Xylasol (1mg/ml)	Dr. E. Graeub AG	QN05CM92

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Signature: Lucas Liaudet Date: 20th of December 2018

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Responses to Editorial and Reviewers Comments.

Editorial Comments:

• Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

Manuscript has been proofread. Typographical errors have been corrected. Some sentences have been rephrased to avoid grammatical errors.

• **Introduction:**
1) Line 41 needs a reference.

The first two sentences have been modified and a reference has been included.

• **Protocol Detail:** Please note that your protocol will be used to generate the script for the video, and must contain everything that you would like shown in the video.
1) 2.4: When and how was the skin on the chest incised?

Skin incision is described at the point 2.2. “Identify the line between left pectoralis minor and major muscles and make an oblique skin incision on 1 cm with scissors following the line”.

• **Protocol Numbering:** Please adjust the numbering of your protocol section to follow JoVE’s instructions for authors, 1. should be followed by 1.1. and then 1.1.1. if necessary and all steps should be lined up at the left margin with no indentations. There must also be a one-line space between each protocol step.

The numbering has been corrected to follow JoVE’s standards. All steps have been lined up at the left margin without indentation

• **Discussion:** JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

The discussion has been totally modified in order to take into account editor and reviewers concerns. Some sentences were rephrased for more clarity and grammatical accuracy. Discussion comprises now five paragrpahs

• **Figures:**
1) Fig 3: Define the units of the molecular weight markers in panels A and C. Define error bars in panels B,D,E.

Units of molecular weights are now visible in panels C and E of figure 3 according to the new numbering of the panels. The error bars of panels B, D, F and G are now defined as standard deviations in the figure 3 legend.

• **References:** Please expand the journal names.

References have been changed to JoVE's format.

• **Commercial Language:** JoVE is unable to publish manuscripts containing commercial sounding language, including trademark or registered trademark symbols (TM/R) and the mention of company brand names before an instrument or reagent. An example of commercial sounding language in your manuscript is Betadine. Please replace it with a generic alternative.

We have suppressed any commercial name. Iodopovidone replaces Betadine in the text. The brand Antisedan has been replaced by its active principle atipamezole.

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All figures and data are original.

Reviewer #1:
Manuscript Summary:
The investigators described a surgical procedure for how to produce an MI model in mice. This mouse MI model is useful for the pathophysiological and molecular studies of the heart.

Major Concerns:
None

Minor Concerns:
1) Regarding the title "Myocardial Infarction Model in Mice by Chronic Ligation of Left Anterior Descending Coronary Artery", "Chronic" is not appropriate to be used together with "ligation" here. "Chronic ligation" would be changed to "permanent ligation" as it is distinct from "transient"; or take "chronic" out (like this: Myocardial Infarction Model in Mice by Ligation of Left Anterior Descending Coronary Artery). "Chronic" usually indicates a disease persisting for a long time or constantly recurring.

Thank you for this kind review. As you suggested, the term "chronic" has been replaced by "permanent" in title and throughout the text.

2) What about percentage rates of mortality during MI surgery and post-MI surgery? The investigators mentioned that "This method is sparsely invasive, reproducible and reduces post-surgery mortality and complications".

We have a global mortality rate of around 25-30 % with this model. However, this may vary a lot according to the endpoints of the experiment since mortality peaks at days 2 to 4 post-operation. The mortality may also vary according to position of the ligation on the LAD. Usually for permanent ligation, the suture is done 2-3mm below left auricle. For ischemia reperfusion experiments, we do it higher on the LAD. We also observed anatomical variations of the position of the LAD branchings in the different genotypes that we use.

Reviewer #2:
Manuscript Summary:
This is adequate and clear.

Major Concerns:
None.

Minor Concerns:
The novelty of this JOVE paper should be better put into perspective. Specifically, a similar method was described in december 2014 in JOVE: Muthuramu I, Lox M, Jacobs F, De Geest B. Permanent ligation of the left anterior descending coronary artery in mice: a model of post-myocardial infarction remodelling and heart failure. J Vis Exp. 2014 Dec 2;(94). doi: 10.3791/52206. The authors should specify the differences between both methods in the Discussion section.

Thank you very much for this kind review.
We now pointed and discussed the differences with article by Muthuramu *et al*. published in JoVE in 2014. As aked by reviewer #3, we also discuss the articles published in JoVE by Xu et al. (2014), Reichert et al. (2017), Kolk et al. (2009), and Curaj et al. (2015).

Reviewer #3:
Manuscript Summary:
This manuscript by Liaudet et al. provided methodology for a mouse myocardial infarction (MI) model through permanent ligation of the left anterior descending (LAD) coronary artery. They describe the process of anesthesia, trachea cannulation, LAD coronary artery ligation, and post-operative procedures. Representative results included Western blotting of smooth-muscle actin, SMAD2 phosphorylation, and NF-κB p65 phosphorylation, as well as mRNA expression of several genes important for myocardial fibrosis and inflammation.
Overall, this is a useful method and fits the scope of the journal, but I would recommend several revisions prior to publication.

Major Concerns:
1) It would be useful to estimate the percentage of the heart affected by ischemia after this procedure, as well as the variability of this percentage.

Thank you for this comment. An experimental set of five animals was used to determine the size of the ischemic area seven days post-surgery. Data is now available as Fig. 3A and 3B. Variability is also discussed in the discussion paragraph related to the limitations of the method.

2) The positioning of the ligature on the LAD should be clarified

A scheme is now available as Fig. 2F in order to clarify the position of the ligature. The point 2.7 of the procedure now specifies the position of the ligature.

3) Sterilization of the instruments and sterile technique within the procedure should be more explicit.

A sentence concerning the sterilization of the instrument and sterile technique has been added to the first paragraph of the protocol.

4) Within the manuscript, a picture or diagram to locate the LAD coronary artery should be included.

A scheme is now available as Fig. 2F in order to clarify the position of the LAD coronary and the position of the ligation.

5) Other methodological publications regarding this technique should be compared to this method in the discussion, such as Xu et al. (2014), Reichert et al. (2017), Kolk et al. (2009), and Curaj et al. (2015).
Thank you for this comment, we now compare the articles you mentioned in the discussion. Procedures described in these articles are put in comparison with our manuscript together with the one from Muthuramu *et al.* (JoVE 2014) as asked by reviewer #2.

Minor Concerns:
1) Manuscript should be edited for typographical and grammatical errors

We are sorry for these errors. We hope that most of them are now suppressed.