Video Article

Apical Resection Mouse Model to Study Early Mammalian Heart Regeneration

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Abstract

Cardiovascular disease plagues the whole world due to intensive lifestyle changes. Heart regeneration holds great promise for repairing and restoring cardiomyocytes lost due to injury and disease. In contrast to the robust cardiac regeneration of certain lower vertebrates, adult mammalian hearts typically show minimal capacity for heart regeneration and repair. However, recent studies have sparked considerable scientific interest with the finding that, between postnatal day 1 to 7 (P1 to P7), the neonatal mouse heart retains significant regenerative potential after apical resection (*i.e.*, surgical amputation and exposure of left ventricular apex). One major controversy over this finding might be due to the diverse surgery-related procedures used in efforts to replicate or expand upon this important finding. These instructions dynamically present the materials and methodology for apical resection in a mouse model. The salient steps of this rodent survival surgery involve hypothermia anesthesia, thoracotomy, surgical amputation of heart ventricular apex, and suture and recovery of mice. The approach described could expand the application of the apical resection mouse model for cardiovascular research.

Video Link

The video component of this article can be found at https://www.jove.com/video/53488/

Introduction

Prolonged human life span leads to various aging- and lifestyle-related diseases, including heart failure, a leading cause of mortality. However, without replacement of lost or dysfunctional cardiac muscle cells, current therapeutics can only transiently improve cardiac function[1, 2]. Thus, it is necessary to discover and develop innovative strategies for cardiac regeneration and repair. The adult mammalian heart has limited regenerative potential. Studies from lower vertebrates, such as urodele amphibians and teleost fish, have provided unprecedented insights into the molecular and cellular mechanisms underlying heart regeneration[3, 4]. Recently, a neonatal mouse model of heart regeneration has emerged that might enable identification and characterization of more evolutionarily conserved pathophysiological events required for human heart regeneration[5].

Apical resection refers to the surgical removal of left ventricular apex. This procedure is restricted to 1- to 7-day-old (P1 to P7) mice due to its high lethality in older mice[6]. The cardiac regeneration process in neonatal mice after apical resection is expected to be as follows: (I) rapid and effective formation of a hematoma to seal the apex and prevent exsanguination; (II) cardiomyocyte regeneration and restoration of systolic function[5, 7]. Recent work has stimulated debate on the significance and efficiency of this model[8-11]. Thus, it is important to present the apical resection clearly and in detail. To this end, this protocol vividly and specifically describes a video of how I did apical resection based on a previous protocol[6].

Understanding the molecular mechanisms underlying cardiac regeneration is of importance for treatment of heart disease characterized by loss and/or injured cardiomyocytes, such as heart failure[1, 2]. Given the current and promising progress of apical resection in the research of cardiac regeneration, this study could promote the use of this technique and its uses in cardiac regeneration research.

Protocol

All mouse experiments were approved by the Animal Care and Use Program at the National Institutes of Health (NIH) with protocol number H0083R3. The NHLBI IACUC approved the protocol without analgesics.

1. Hypothermia Anesthesia in Neonatal Mice

1. Sterilize sponges and surgical equipment in an autoclave before surgery. Prepare all the surgical materials and switch on a hot bead sterilizer 15-20 min in advance to reach 240°C to 270°C.

2. Transfer all C57BL/6 pups (age P1) from their nursing mother to a clean mouse cage with fresh bedding and nesting materials. Once the pups are taken into a surgery room, perform the apical surgery promptly to minimize their time spent separated from the mother and to reduce the risk of maternal cannibalization.
3. Put sponges on an ice bed and then place one pup on the sponge for ~3 min to achieve hypothermia anesthesia. Confirm anesthesia by observing apnea and akinesia and pinching a rear foot. Check the status of neonates frequently because too little time will not make the pup akinetic and apneic, and an excessive duration of anesthesia can lower the survival rate[12].

2. Thoracotomy

1. Transfer the pup from the ice bed onto a surgical benchtop area and use tape to immobilize its arms, legs, and tail in a supine position.
2. Disinfect the chest using betadine and gently clean it using a 70% alcohol prep pad.
3. Make a transverse skin incision along the forth intercostal area of the chest cavity using a Vannas Spring Scissor, and then blunt dissect the forth intercostal muscles to facilitate access to the heart.
   NOTE: Survival rates improve when blood loss is minimized during the surgical process.

3. Surgical Amputation of the Heart Ventricular Apex

1. By hand, gently apply pressure on the abdomen to exteriorize the apex of the heart. Absorb blood around the surgical area with sterile cotton-tipped applicators for clear visualization. For sham-operated control neonates, proceed directly to Step 4 (Suture and Recovery of Mice).
2. Under a magnifying lamp and using iridectomy scissors, gently perform piecemeal resection of the left ventricle (LV) until the LV chamber is exposed. Be careful to minimize the resected portions of the LV. Approximately 15% resection is necessary to achieve optimum exposure of the LV chamber.
3. Ensure that the heart returns to the chest cavity once the LV chamber is exposed.

4. Suture and Recovery of Mice

1. Suture the ribs and muscles together to seal the chest cavity using sterile Proline 6-0 sutures, and then carefully close the skin incision site using skin glue.
2. Warm the neonate under a heat lamp for ~3 min for recovery, and then clean the blood and glue traces using a 70% alcohol prep pad before reintroducing it to its littermates. Try to complete the whole surgical procedure within 10 min because minimizing the time spent separated from the mother improves pup survival after apical resection.
3. Following each surgery, place the surgical tools in contact with the beads of the hot bead sterilizer for approximately 20 sec for complete sterilization. Allow the surgical tools to cool to RT before each surgery.
4. After completing surgeries for all pups in a litter, mix the pups with the mother's bedding and excrement before returning them to the mother's nest.
   NOTE: Generally, ICR/CD-1 mice are better foster mothers than C57BL/6, but even among nursing mothers of the same genetic background, fostering instincts vary. If changing a nursing mother is necessary to reduce mortality by maternal cannibalization, remove the P0 pups to a nursing mother and then perform apical resection at P1. Only perform either the sham operation or the apical resection in one litter of pups, because mixing sham and surgical pups could decrease the survival rates of the surgical group of pups[6].

5. Post-Surgical Analysis

1. One day after surgery, monitor the pups, ensure that there is no difference between sham and surgical groups, and count the number of pups to measure the survival rate. If the surgeries are performed properly, the survival rates should be similar and greater than 60% in both sham and surgical groups.
2. Heart isolation and fixation.
   1. Euthanize the pups at days 1 and 2 post-surgery via decapitation and day 21 post-surgery by CO2 with an assurance of death by cervical dislocation, then clean the chest using a 70% alcohol prep.
   2. Incise the midline skin and muscle of the chest and then open the chest.
   3. Excise the entire hearts from the thoracic cavity and fix the entire heart of each sample in 5 ml of 4% paraformaldehyde O/N at RT.
3. On the next day, transfer the samples to 70% ethanol. Samples can be stored up to one week before paraffin embedding.
4. Slice 5-µm-thick paraffin sections through the entire ventricle and perform standard hematoxylin and eosin (H&E) and Masson's Trichrome staining to examine the regenerative response[5, 7]. Specifically, H&E stain is used to examine muscle replacement and Masson's Trichrome stain is used to examine fibrotic responses[5].
5. Select the area of interest and set up at least three foci per sample manually using a slide scanner. Image and analyze the staining slides at 40× magnification according to the manufacturer's instructions with default parameters.

Representative Results

Mouse pups were euthanized 1, 2, and 21 days post-apical resection, and their hearts were collected for H&E and Masson's Trichrome stain. Blue color in Masson's Trichrome stain indicates the deposition of epicardial extracellular matrix[5]. With successful apical resection, a blood clot is effectively formed to seal the LV one day post-apical resection, as shown in **Figure 1A**. A gradual resorption of the blood clot and early cardiac fibrosis is observed, and replacement by myocardial tissue occurs until complete regeneration of myocardium takes place 21 days after apical resection (**Figure 1B and C**). Morphological analysis shows no difference between resected and sham-operated hearts at 21 days after surgical operation (**Figure 1C and D**).

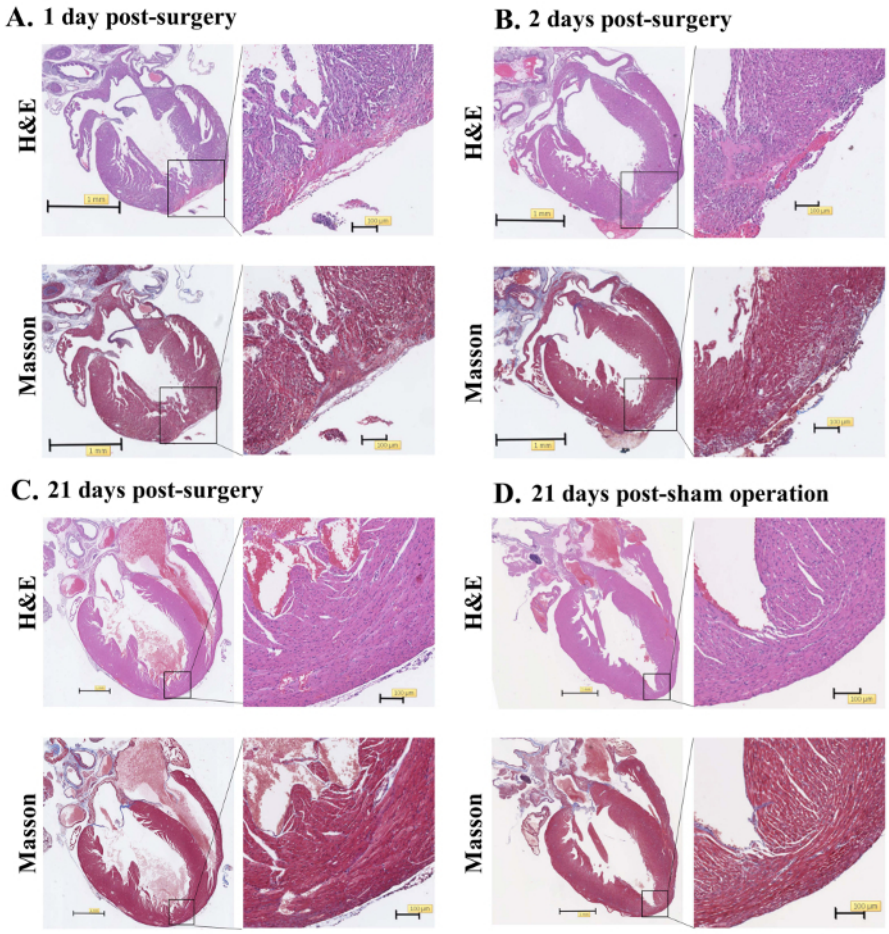

Figure 1. Representative H&E and Masson's Trichrome stain. Ventricular myocardium of neonatal mouse hearts 1 **(A)**, 2 **(B)**, or 21 **(C)** days after apical resection and 21 days after sham operation **(D)**. For each subfigure, the scale bar of the left panel is 1 mm and the scale bar of the right panel is 100 µm. Please click here to view a larger version of this figure.

Discussion

Cardiac regeneration shows potential for the treatment and prevention of heart failure[1, 2]. Animal models are indispensable and play a critical role in understanding how cardiac regeneration occurs[3-6]. Many amphibians and fish regenerate heart tissue in response to injury, providing insight into our understanding of human cardiovascular disease[13, 14]. In terms of evolution, however, the pathophysiology should be more conserved between a mouse model and humans. Heart regeneration in mammalian animal models is a relatively new but burgeoning research field for the therapeutics of heart failure[3-6].

Recently, two surgical approaches, apical resection and myocardial infarction, have been established in the study of heart regeneration in neonatal mice[6]. Although apical resection provides a relatively easy and direct model for loss and/or injury of cardiomyocytes, the main limitation of apical resection compared to myocardial infarction is that this surgical method cannot be performed beyond P7 due to high mortality[6]. There are, however, a number of ways to reduce mortality with this method. For apical surgery on P7 pups, the pups are cooled to 16°C for optimum hypothermia anesthesia. Generally, sterile conditions and surgical proficiency are critical for successful apical resection surgery. To improve survival, the surgery should be performed as quickly and gently as possible and a minimally invasive operation will reduce bleeding. Additionally, ICR/CD-1 mice are thought as good foster mothers and can effectively avoid of severe maternal cannibalization. For example, placing the P0 C57BL/6 pups with an ICR/CD-1 mother increases the survival rates of P1 pups with apical resection approximately from 60% to 80%[ref.6]. In addition, cryoinjury is an alternative procedure for study of cardiac regeneration. Compared to apical resection, cryoinjury model represents a different regenerative response and may be a more reproducible model[15, 16]. Thus far, a comparison of apical resection with other models warrants further investigations.

Using phospho-histone H3 and 5-bromo-2-deoxyuridine staining, Porrello and colleagues showed that cardiomyocyte proliferation contributes to the heart regeneration of neonatal P1 mice after apical resection response. Moreover, no significant cardiac hypertrophy and increase of cell size have been observed in mice with apical resection relative to sham-operated controls. After apical resection of 7-day-old mice, there is no regenerative response for myocardium[5]. A more detailed time course analysis of morphology and cardiac fibrosis after apical resection has been demonstrated[5]. Of note, the usefulness of apical resection in neonatal mouse models for heart regeneration research has been questioned by Anderson and colleagues[8]. Although many independent groups have demonstrated the reproducibility of apical resection, variations in the details of surgical procedures and different amounts of resected myocardium might be responsible for confusing results[10]. Quantification of the

genomic DNA in resected heart is helpful for control of minimal chamber exposition and reproducibility of this technique. Additional research will be needed to fully understand neomyogenesis and complete vs. incomplete heart regeneration in the apical resection model[8-11].

The main aim of this study is to present the procedures of apical resection in a visualized way. This video of apical resection in the neonatal mouse will be helpful to increase the use of this method and teach it to heart regeneration researchers. In addition, it may promote the pursuit of better approaches for mammalian heart regeneration in animal models and facilitate the discovery and translation of the molecular and cellular mechanisms responsible for heart regeneration in humans.

Disclosures

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