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A Murine Model of Femoral Artery Injury: Tricks of the Trade

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ABSTRACT

Mammals is a powerful tool to study pathophysiologic process of cardiovascular disease, but for a long time it is just limited to large animals, like pigs, rabbits, and big rats. The mice with reproduction fast cycle, lower costing, and close to human genes, can be showed about some phenomenon quickly, also more easy to form model of atherosclerosis. Thus, a mouse model of vascular injury is vitally important for researching the pathophysiological mechanisms to restenosis after percutaneous transluminal coronary angioplasty (PTCA) as well as translational approaches. In this Experiment, we established a unique murine model of femoral artery injury through a home-made wire. The mouse model of vascular injury may be used to explore the molecular mechanism of post-angioplasty restenosis with lowest costing.

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Authors' Contributions
XW and JH conceived and designed the study. JW and JX bred the mice and collected vascular samples. HZ and YX performed the surgery of mice. XC collected and analyzed the data. JH wrote the article. XP assisted in manuscript preparation and interpretation of data.

Key words
Banna Mini-pig Inbred Line, Prokaryotic expression, Nucleoplasmin, Polyglutamic acid, Embryo development.

INTRODUCTION

Restenosis is a serious complication for the treatment of coronary atherosclerosis with percutaneous coronary intervention (PCI). However, the mechanism of restenosis is still not clear, and the control measures of restenosis is a hot spot for researcher (Takagi *et al*., 2002; Farooq *et al*., 2011; Touchard and Schwartz, 2006). So, the mouse model of vascular injury is useful for the mechanistic study of the vascular response to injury, which is usually technically challenging to perform due to their small size (Chamberlain *et al*., 2010; Keshi *et al*., 2014). On the other hand, the straight spring wire is very expensive that is inserted into the femoral artery of mice in this experiment, and the way of purchase is not convenient, caused laboratories of many countries are difficult to implement the animal model. So we specially made a very simple wire tool and found a simple approach for a surgical technique that induces endothelial denudation.

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MATERIALS AND METHODS

Wire making

A diameter of 0.02 mm of ordinary steel wire, surface is sandpapered to be coarse (Fig. 1A), then with a mosquito hemostatic (straight) of specifications 12.5 centimeters continuous pinch, forming bent (Fig. 1B). When inserted into the femoral artery of mice, holding the end of wire with hemostatic forceps, not with the hand directly, because of poor stability, also it is not easy to find when breaking off in the process of experiment.

Animals

10 to 14 week old C57BL/6J (wild-type, n=20) mice, weighing between 25 and 35 g, purchased from Xinjiang Medical University. All animals were maintained in the Animal Facility of the Shihezi University. For all surgical procedures, the mice were anesthetized by intraperitoneal injection of 50mg/kg Nembutal (Abbott Laboratories, North Chicago, IL, USA) diluted in 0.9% sodium chloride solution. Performed a pinch test of the mouse's foot to confirm that it was fully anesthetized. Ensured the animal does not move when the pinch test was administered. All procedures involving experimental animals were

performed in accordance with protocols approved by local institutional guidelines for animal care of The Huazhong University of Science and Technology.

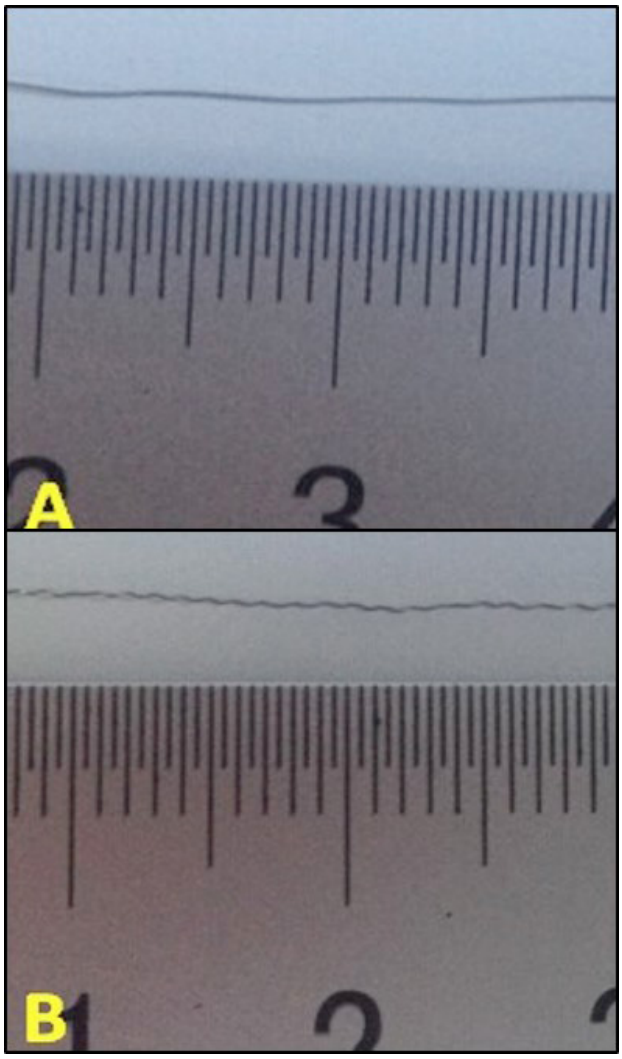

Fig. 1. Wire making. **A,** a diameter of 0.02 mm of ordinary steel wire; **B,** making the wire bent.

Surgical procedure

First, superficial subcutaneous muscle fascia was stripped to expose the femoral artery, vein and femoral nerve. The nerve was, gently separated from the vascular bundle using fine-tipped forceps (Avoided puncturing the vein, and did not damage the nerve). The nerve was pushed away from the bundle to avoid stimulating it. Because femoral artery was packaged by arterial sheath, it was very thin and not easy to seen.

Veins and connective tissues around the artery were carefully removed with microsurgery forceps, locating the femoral bifurcation. The region of the bifurcation was especially difficult to dissect. The exposed muscular branch artery was dilated by topical application of one drop of 1% lidocaine hydrochloride (Fig. 2A). Posterior to the bifurcation, looped a 6.0 silk suture under the femoral artery and secured with a hemostat. This proximal suture would be used to restrict blood flow in the artery. Distal to the bifurcation, looped 6.0 silk suture under the femoral artery and secured with a hemostat. This distal suture aided in the positioning of the artery. Looped two sutures under the muscular branch of the femoral artery, pre-tie them and secured with a hemostat. Remember to moisten the tissues with saline. Restricted blood flowing into the femoral artery by pulling the proximal suture. Slightly pulled the distal hemostat and secured the branch to expose the site for the arteriotomy. Ligated the muscular branch by tying the suture around it. Introduced the wire into the arteriotomy using hemostat slowly (Fig. 2B). Retracted and advanced the wire in a sawing motion four times to

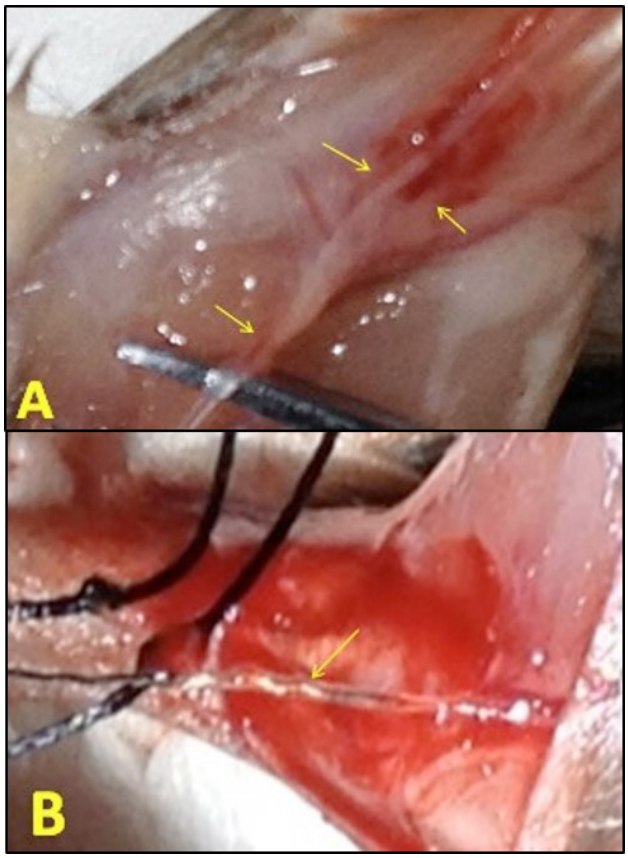

Fig. 2. Wire Injury Procedure. **A,** the common femoral artery, the superficial femoral artery and the deep femoral (yellow arrows); **B,** the wire is inserted via the deep femoral artery (long arrow), the deep femoral (short arrow) and the superficial femoral artery (dotted arrow).

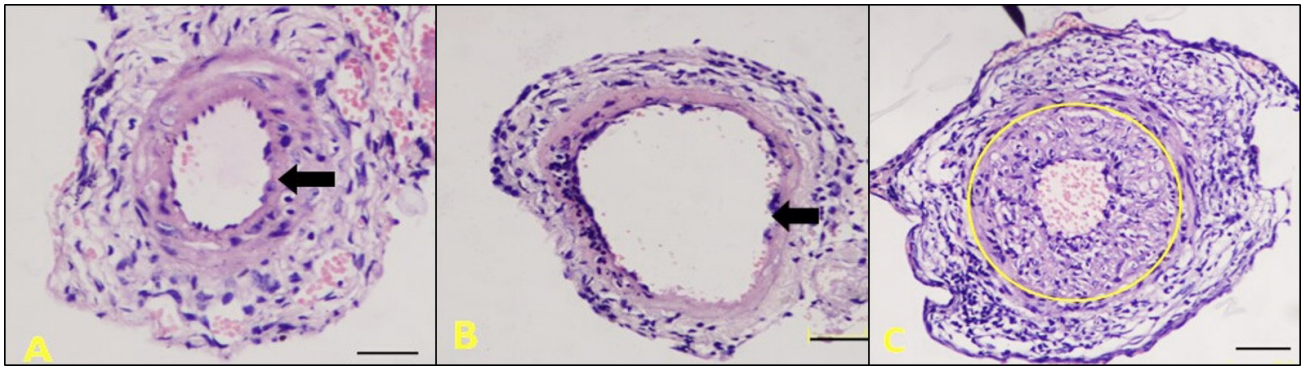

Fig. 3. Hematoxylin-eosin staining of wire-injured and uninjured femoral arteries. **A,** a cross-section of an uninjured femoral artery, Black arrow indicates intact arterial wall structures without any neointima formation; **B,** a cross-section of a wire-injured femoral artery at the first day. Black arrow indicates that endothelium was denuded, and the endothelium was not intact or disappeared; **C,** a cross-section of a wire-injured femoral artery at the seventh day. Yellow loop revealed a thick, highly cellular neointima plaque. Bar indicates 100 μm. performed in accordance with protocols approved by local institutional guidelines for animal care of The Huazhong University of Science and Technology.

injure and denude the endothelium of the femoral artery. Retraced the wire slowly and tightened the remaining suture on the muscular branch (Le et al., 2015).

The mice were killed by intraperitoneal administration of an overdose of Nembutal at the time points indicated. At death, the mice were perfused via the left ventricle with 0.9% NaCl solution followed by perfusion fixation with 4% formaldehyde in PBS (pH 7.4). The femoral artery was carefully excised, t fixed in 4% formaldehyde overnight at 4°C, and embedded in paraffin.

Histochemistry

After the first day and a week, for histological staining of the tissue specimens, hindlimb muscles were removed, formalin-fixed, and paraffin embedded. Three sections measuring 6 μm in thickness were taken from the paraffin-embedded specimens at 150 μm intervals, stained with hematoxylin and eosin (H & E), observed, and photographed with a microscope (Olympus BX40, Tokyo, Japan).

RESULTS AND DISCUSSION

The uninjured artery showed intact elastic lamellae and a normal thickness and circumference (Fig. 3A). The injured artery demonstrated endothelium was denuded, and the endothelium was not intact or disappeared at the first day (Fig. 3B) and revealed a thick, highly cellular neointima plaque at the seventh day (Fig. 3C).

The mice with reproduction fast cycle, lower costing, and close to human genes, can be showed about some phenomenon quickly, also more easy to form model of post-angioplasty restenosis (Lv *et al*., 2014). The ligation method for femoral and carotid arteries has been described in conventional methods papers and characterized extensively (Brouchet *et al*., 2001; Filipe *et al*., 2008; Feuls *et al*., 2003). In this experiment, we established a unique murine model of femoral artery injury through a home-made wire. It is very good replication process of vascular lesions, and its technology is not difficulty also with the reliability, repeatability and physiological relevance. People who without any microsurgery training can be very easy to grasp just after simple training. Although, it is a technical challenge for inserting into tiny artery by a coarse wire, but the success rate is more than 95% for all mice examined.

The major challenge including two sides, the first is the separation of the femoral artery from the femoral vein. Care should be taken at this stage, as it is easy to cause bleeding during the separation and the vein tears easily in comparison to the artery. Using forceps to blunt dissect and remove adventitia surround the artery and vein can help this process. Also, the artery may have branches underneath that can be torn if an overly aggressive technique is used.

The second challenge is that how to obtain vascular access and to restore flow to the injured artery following the wire injury. For this reason, simple arterial ligation models have been used to study neointimal hyperplasia in mice that do not require endovascular manipulations but are easier to implement. However, this type of surgical model differs substantially from the mechanical and biological aspects of a percutaneous intervention, lacking key aspects including arterial wall stretch, endothelial denudation and

luminal blood flow following injury.

In conclusion, we have established a simple way for the model of femoral artery injury with ordinary tool, and it can be performed safely and easily using the method presented here. This model may be widely used for researching the molecular pathways of post-angioplasty restenosis.

CONCLUSION

Restenosis is a complex process with major clinical implications of post-angioplasty. Laboratory animal models of femoral artery injury are available, but involve the challenging of surgical procedure and tools. Our article provides a detailed, illustrated manual with tips and tricks to guide a novice to master this technique and obtain reliable results with ordinary tool.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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