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Video Article

Balloon-based Injury to Induce Myointimal Hyperplasia in the Mouse Abdominal Aorta

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Abstract

The use of animal models is essential for a better understanding of MH, one major cause for arterial stenosis.In this article, we demonstrate a murine balloon denudation model, which is comparable with established vessel injury models in large animals. The aorta denudation model with balloon catheters mimics the clinical setting and leads to comparable pathobiological and physiological changes. Briefly, after performing a horizontal incision in the *aorta abdominalis*, a balloon catheter will be inserted into the vessel, inflated, and introduced retrogradely. Inflation of the balloon will lead to intima injury and overdistension of the vessel. After removing the catheter, the aortic incision will be closed with single stiches. The model shown in this article is reproducible, easy to perform, and can be established quickly and reliably. It is especially suitable for evaluating expensive experimental therapeutic agents, which can be applied in an economical fashion. By using different knockout-mouse strains, the impact of different genes on MH development can be assessed.

Video Link

The video component of this article can be found at https://www.jove.com/video/56477/

Introduction

Arterial stenosis in coronary and peripheral arteries has a large effect on the morbidity and mortality of patients[1]. One underlying pathological mechanism is myointima hyperplasia (MH), which is characterized by increased proliferation, migration, and synthesis of extracellular matrix proteins from vascular smooth muscle cells (SMC)[2]. SMC are located in the media layer of the vessel and migrate upon stimulation to the surface of the lumen. Stimulatory signals include growth factors, cytokines, cell-cell contact, lipids, extracellular matrix components, and mechanical shear and stretch forces[3,4,5,6]. Injuries of the vessel wall, pathological or iatrogenic, cause endothelial cell and smooth muscle cell damage and stimulate inflammatory reactions, and thus lead to MH[7].

Different animal models are currently available to study arterial injury and myointima hyperplasia. Large animals like pigs or dogs have the advantage of sharing a similar artery and coronary anatomy with humans and are especially suitable for studies investigating angioplasty techniques, procedure, and devices[8]. However, pig models have the drawback of higher thrombogenicity[9,10], while dogs only have a mild response to vessel injury[11]. In addition, all large animal models require special housing, equipment, and staff, which are connected with high costs and are not always available at an institution. Small animal models include rats and mice. Compared to rats, mice have the advantages of lower cost and the existence of a variety of knock out models. The model described in this video can be combined with ApoE-/- mice fed with a western diet to closely mimic the clinical setting of angioplasty in atherosclerotic vessels[12]. Previous models induced vascular injury via wire injury[13], fluid desiccation[14], spring[15], or cuff injury[16]. Since the nature of the injury will greatly impact the development and constitution of MH, using a balloon catheter to induce vessel injury is the best way to mimic the clinical setting.

In this article we describe a novel method to induce MH with a balloon catheter in mice. The use of a balloon catheter (1.2 mm x 6 mm) with a RX-Port (**Figure 1A**) allows the scraping of the intimal layer and, at the same time, the induction of an overdistension of the vessel. Both of these factors are important triggers for the development of MH. The observation time for this model is 28 days[17].

Protocol

Animals received humane care in compliance with the Guide for the Principles of Laboratory Animals, prepared by the Institute of Laboratory Animal Resources, and published by the National Institutes of Health. All animal protocols were approved by the responsible local authority ("Amt für Gesundheit und Verbraucherschutz, Hansestadt (Office for Health and Consumer Protection) Hamburg").

1. Catheter Preparation

NOTE: Refer to the **Table of Materials** for information regarding the catheter.

1. Take catheter from the catheter holder.
2. Pull the guidewire from the lumen through the distal port out.
3. Put in a drop of cyanoacrylate adhesive on the distal end of catheter.
   Caution: Wear latex or nitrile gloves.
4. Place the guidewire at the RX port of the catheter and advance it through the lumen to the distal port. Afterwards, pull the guidewire back slightly to leave a space of ~5 mm to the end.
5. Wait 5 min to allow the adhesive to dry.
6. Move the guidewire, it should be fixed. If it is still mobile, repeat steps 1.3 - 1.6.
7. Fill a 3-mL syringe with 1.5 mL 0.9% saline and connect it to the balloon inflation port.
8. Push the syringe plunger to test balloon inflation. Leave 0.6 mL saline in the syringe.

2. Mouse preparation

1. Obtain male mice at the age of 14 weeks weighing approximately 30 g.
   NOTE: We used animals obtained from the Institute of Laboratory Animals. We used C57BL/6J here.
2. Use an induction chamber to anaesthetize the mouse with 2.0-2.5% isofurane (500 mL/min oxygen flow rate).
3. Place the mouse on its back on a heating pad and maintain anesthesia with a facemask covering the mouth and nose of the mouse. Check for sufficient depth of anesthesia by pinching the hind feet and tail to verify an absence of reflexes.
4. Remove the abdominal hair using a hair trimmer.
5. Spread the hind legs and fix their position using tape.
6. Disinfect the abdominal area using povidone-iodine, followed by 80% ethanol. Repeat this step two more times.
7. Use a surgical drape to ensure surgical site does not get contaminated. Open the skin and muscle layers along the *linea alba* with a scissor (or scalpel) to expose the abdominal organs.
8. Lay the intestines in a 0.9% saline moisturized glove and wrap to keep them moist.
9. Use two fine forceps to remove the fatty tissue above the abdominal aorta.
10. Using an insulin syringe (30G), inject 250 µL of heparin solution (50 U/mL) into the Inferior Vena Cava (IVC) and wait 3 min for its systemic distribution. Heparin will suppress hemostasis and prevent undesired clotting during surgery.
11. Using two forceps, dissect the infrarenal aorta down to its bifurcation and its outgoing branch vessels.
12. Ligate the side vessels, which are expected to be placed in the clamped area, with a high temperature cauterizer.
13. Stop blood flow by clamping the infrarenal aorta directly beneath the renal arteries.
14. Place a second clamp at a distal position just above the aortic bifurcation.
15. Perform a small horizontal incision using scissors at the midpoint between the clamps along the vessel.
    NOTE: The size of the incision should be equivalent to 1/3 of the circumference of the vessel.
16. Insert a syringe with a (30G) needle into the incision and flush the aorta with 250 µL heparin solution (50 U/mL).
17. Using 10-0 sutures, place one single knot on each side of the incision.
18. Dilate the aorta by inserting a vessel dilator into the incision and spread the vessel slightly. Repeat the dilation 2 to 3 times.
19. Moisturize the balloon-catheter with 0.9% saline.
20. Insert the flattened balloon-catheter into the aorta and advance it towards the proximal clamp on the aorta.
21. When reaching the proximal clamp, carefully open the proximal clamp and inflate the balloon to prevent blood leakage, by injecting ~0.6 mL of saline.
    NOTE: The ratio of the inflated balloon to vessel is 1.5:1.
22. Advance the catheter retrograde for approximately 2 cm.
23. Pull the expanded catheter back, while deflating the balloon slightly by releasing the syringe.
24. Reattach the proximal clamp when the catheter reaches the incision of the aorta. Deflate the balloon completely and remove it.
25. Rinse the aorta with 250 µL heparin solution (50 U/mL) using a 30G syringe.
26. Close the aortic incision using 10-0 sutures. Place interrupted stitches on each lateral side, followed by one or two stitches on the ventral side.
27. Open the distal clamp. In case of bleeding, close the clamp again and place additional stitches.
28. Open the proximal clamp carefully.
29. Place two swabs on the suture to support it and stop any bleeding.
30. Place absorbable hemostats on the suture to sustain it.
    NOTE: An aortic pulse should be visible distally from the incision.
31. Place the intestines back into the abdomen.
32. Rinse the abdominal cavity with sterile 0.9% saline, which has been pre-warmed to 37 °C.
33. Close the abdominal muscle layer using 6-0 running sutures.
34. Close the skin with 5-0 running sutures.
35. Inject 4-5 mg/kg Carprofen subcutaneously before allowing the mouse to wake up. Monitor the animal until it has gained consciousness, maintain sternal recumbency. Keep the animal alone in a cage until complete recovery.
36. Add Metamizole to the drinking water (50 mg/100 mL) as pain medication for 3 days and monitor the animal daily. Usually mice like the sweet taste of metamizole and start drinking immediately after the surgery. If preferred, sustained-release injectable agents can be used instead of metamizole.
    NOTE: The observation period for this model is 28 days.

3. Histopathology

1. Harvest the balloon-injured aorta after 28 days by preparing the mice as described in steps 2.2 to 2.9.
2. Use scissors to remove the balloon-injured aorta (between the bifurcation and 0.3 mm above the renal vessels) and euthanize the mouse by cutting out its heart.
3. Flush the lumen of the vessel with 0.9% NaCl.
4. Fix the harvested vessel in 4% paraformaldehyde (PFA) overnight and dehydrate it in increasing concentrations of ethanol, starting with 70% ethanol for 2 hours, 80% ethanol for 1 hour, 95% ethanol for 2 hours, and 100% ethanol for 5 hours. Then, incubate the samples in xylene for 2 hours 3 times, before infiltrating the samples with paraffin.
   NOTE: Instead of flushing the harvested vessel with 0.9% NaCl, it can be flushed with 4% PFA.
   Caution: PFA and xylene are toxic and should be handled with special care.
5. Embed the sample in paraffin and cut into slices of 5 µm thickness using a microtome.
6. Deparaffinize the slides with xylene 3 times for 5 minutes.
7. Rehydrate tissue slides using a decreasing series of ethanol. Start with 100% ethanol 2 times for 5 min, followed by 3 minutes of 95%, 80% and 70% ethanol.
8. Stain the slides with Masson's trichrome staining as described[18].
9. Dehydrate stained slides in 100% ethanol 2 times for 10 min each. Clear with xylene 2 times for 10 min each and mount in mounting medium.
10. View the slides with a bright field microscope. Use a lens with 5x magnification and a numerical aperture of 0.12 for an overview picture or a lens with 20x magnifications with a numerical aperture of 0.35 for detailed observation.

4. Immunofluorescence Microscopy

1. Rehydrate tissue slides using a decreasing series of ethanol. Start with 100% ethanol 2 times for 5 min, followed by 3 min of 95%, 80% and 70% ethanol.
2. Perform antigen-retrieval by heating the slides in antigen-retrieval solution in a steamer for 20 min.
3. Let the slides cool down to room temperature.
4. After washing the slides for three times with phosphate buffered saline (PBS), apply antigen blocking solution on sections for 30 min.
5. Wash slides three times for 5 min with PBS.
6. Incubate sections with primary antibody diluted in primary antibody diluent.
   NOTE: The right concentration and incubation time should be chosen separately for each antibody.
7. Wash slides three times for 5 min with PBS to remove unbound antibody.
8. Incubate sections with a pre-conjugated secondary antibody diluted in secondary antibody diluent.
   NOTE: The right concentration and incubation time should be chosen separately for each antibody.
9. Remove unbound antibodies by washing the slides for 5 min three times.
10. Counterstain the cell nuclei using 4',6-diamidino-2-phenylindole (DAPI) for 15 min; the final DAPI concentration should be 350 nM.
11. Mount slides in immunofluorescence compatible mounting solution.
    NOTE: Using the wrong mounting solution can obscure the fluorescence signal.
12. View slides with a fluorescence microscope. Use a 40x magnification lens with a numerical aperture of 1.3.

Representative Results

Balloon denudation is a suitable model to study the development of MH in mice. Animals recover well from the surgery and show an excellent physical condition post-operation. We established this model in 50 mice with less than 3% death rate due to the surgical procedure. **Figures 1B-C** show the key surgical steps. After a skin incision along the *linea alba*, identify the *aorta abdominalis*. Place microsurgical clamps (**Figure 1B**). Make a small incision in the middle of aorta, set a balloon catheter into the vessel and slide it retrograde, against the direction of blood flow (**Figure 1C**). Movement of the inflated balloon leads to scraping of intima and, at the same time, overdistension of the vessel. The aortic incision will be closed with single stiches. An aortic pulse should be visible distally from the incision.

MH develops progressively in the graft over time. Histological staining with Masson's trichrome demonstrates myointima formation inside the internal elastic lamina (**Figure 2A**). Myointimal lesions consisted mainly of cellular components positive for SM22 and some extracellular matrix components (**Figure 2B**). Myointimal cells are further evaluated by immunofluorescence staining. The main population in the myointima consists of smooth muscle (smooth muscle actin (SMA) positive) cells and myofibroblasts (fibroblast activation protein (FAP) positive) cells (**Figure 2B**).

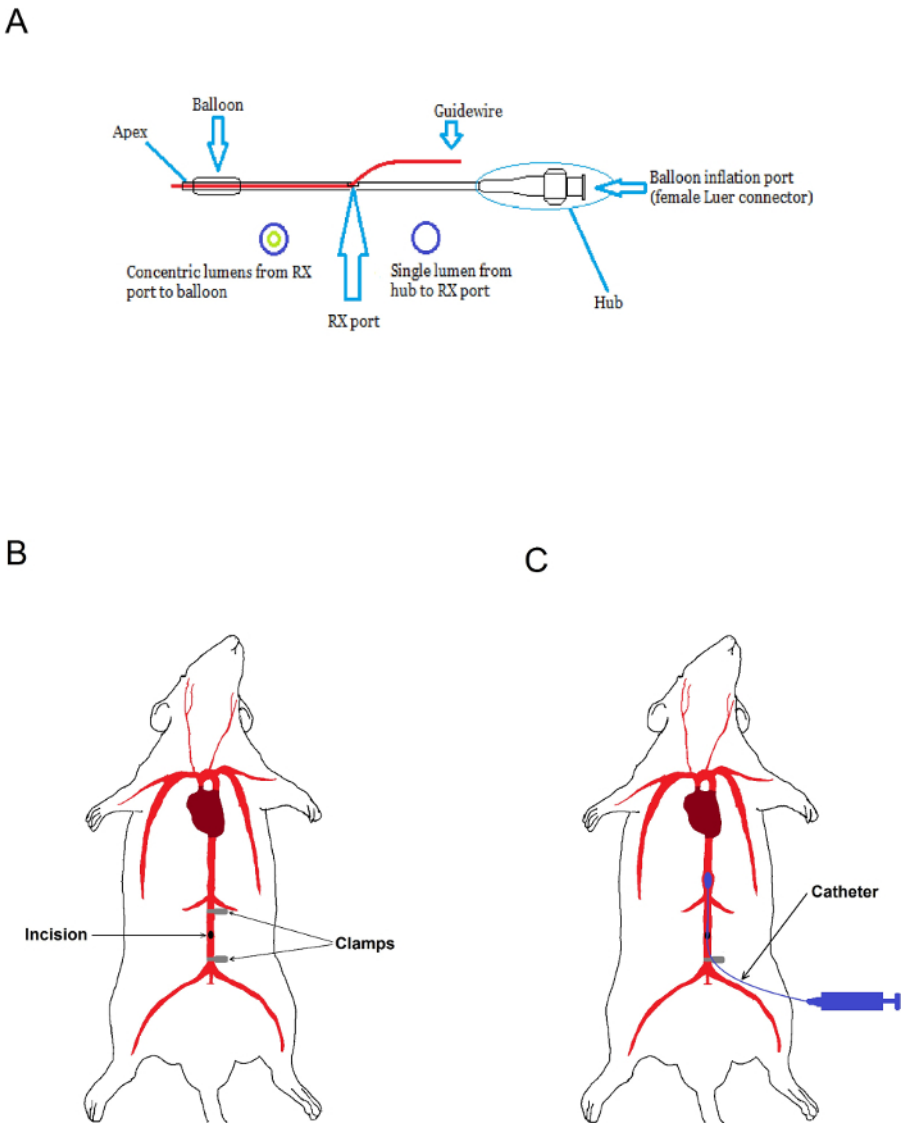

Figure 1. Schematics of the catheter and its implantation. (A) Detailed schematic of the catheter. Distal port, balloon, rapid exchange port (RX-port), guidewire, single lumen to RX-port, double lumens from RX-Port to balloon, hub, balloon inflation port. **(B)** Schematic illustration of surgical procedure. Blood flow of the *Aorta abdominalis* is stopped with two micro clamps and a small incision is performed. C. Inflated catheter inside *aorta abdominalis*. Please click here to view a larger version of this figure.

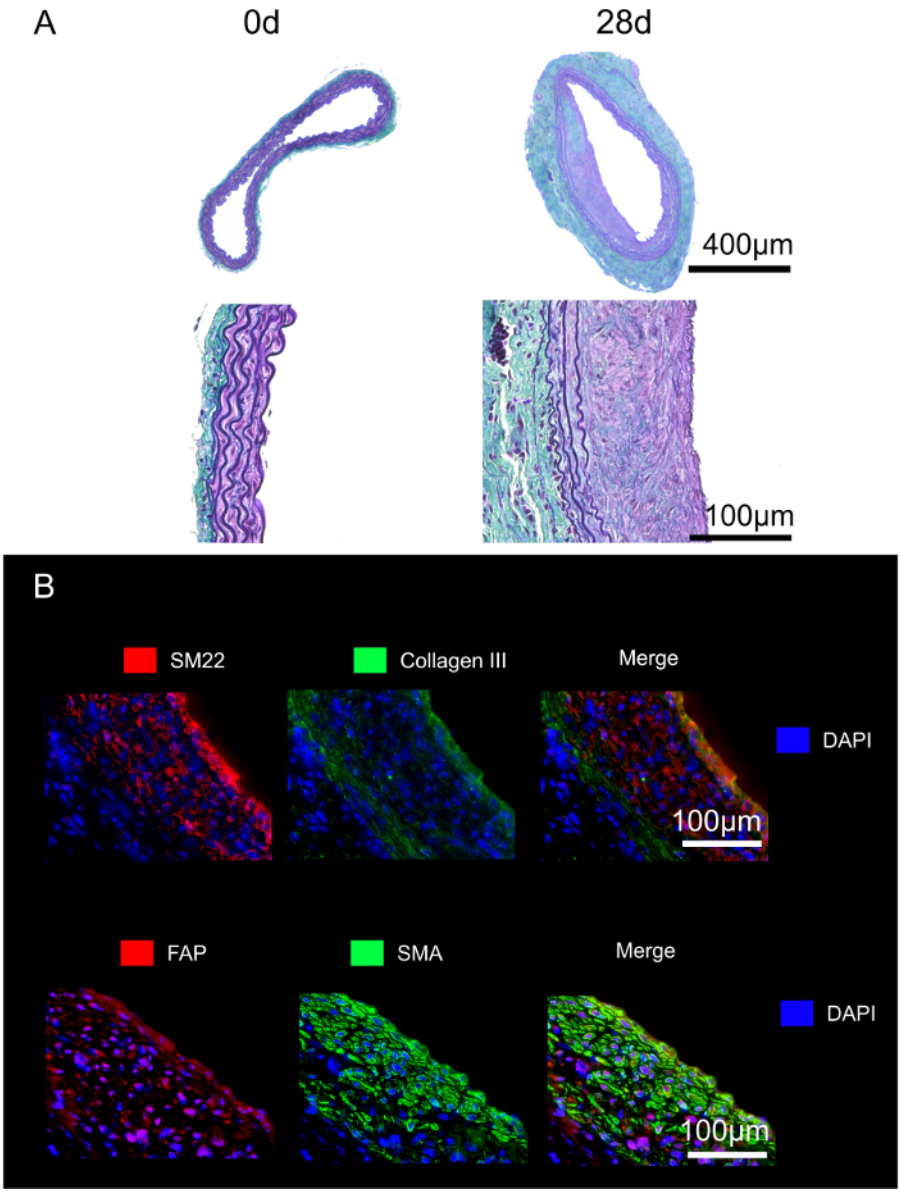

Figure 2. Myointima formation inside the internal elastic lamina. (A) Denuded mouse aortas are harvested, paraffin embedded, and a representative cross section is shown in trichrome staining. **(B)** Double immunofluorescence staining of balloon-denuded aortae is shown. The upper row depicts myointimal lesions stained for SM22 and collagen III. In the bottom row, vessels are stained for SMA and FAP. Please click here to view a larger version of this figure.

Discussion

This article demonstrates a murine model to study the development of myointimal hyperplasia and allows the exploration of the underlying pathological processes and the testing of new drugs or therapeutic options.

The most critical step in this protocol is the denudation of the aorta. Special care should be paid during this step as excessive denudation will lead to aneurysm formation and model failure. On the other hand, if denudation is performed insufficiently, too little myointima will develop. Therefore, the intensity of the denudation step is crucial for the outcome and success of this animal model.

With respect to the surgical procedure, it is critical the two walls of the vessel are not pierced by setting the stiches, which might result in early failure of the vessel patency. We have previously described a mouse model in which we induced vessel stenosis in the abdominal aorta of mice[18]. However, this and most other models only provide very small amounts of tissue for analysis. An advantage of this method is the comparatively large amount of tissue obtained (~1 cm vessel segment). A single vessel graft can thus be divided into multiple parts and used for various analyses, effectively reducing the number of experimental animals required.

Furthermore, suitable knock-out animals can be used to study the development of myointima hyperplasia in different disease conditions. The genetic backgrounds can also be combined with this animal model to understand the mechanisms of myointimal hyperplasia in a variety of settings or the impact of certain genes.

In summary, the model described here is reproducible, easy to perform, and can be established quickly and reliably. Successfully tested treatment options in this model can be further confirmed in large animal models[19].

Disclosures

The authors have nothing to disclose.

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