Video Article

Vein Interposition Model: A Suitable Model to Study Bypass Graft Patency

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Abstract

Bypass grafting is an established treatment method for coronary artery disease. Graft patency continues to be the Achilles heel of saphenous vein grafts. Research models for bypass graft failure are essential for a better understanding of pathobiological and pathophysiological processes during graft patency loss. Large animal models, such as pigs or sheep, resemble human anatomical structures but require special facilities and equipment. This video describes a rat vein interposition model to investigate vein graft patency loss. Rats are inexpensive and easy to handle. Compared to mouse models, the convenient size of rats permits better operability and enables a sufficient amount of material to be obtained for further diverse analysis. In brief, the inferior epigastric vein of a donor rat is harvested and used to replace a segment of the femoral artery. Anastomosis is conducted via single stitches and sealed with fibrin glue. Graft patency can be monitored non-invasively using duplex sonography. Myointimal hyperplasia, which is the main cause for graft patency loss, develops progressively over time and can be calculated from histological cross sections.

Video Link

The video component of this article can be found at https://www.jove.com/video/54839/

Introduction

Coronary artery diseases and their complications are among the leading causes of death worldwide. Current therapeutic strategies focus on re-establishing the blood flow, either by dilating the narrowed vessel or by creating a bypass. Coronary artery bypass grafting (CABG) using vein autografts was first described in 1968 and has been refined over the years. Apart from the revascularization of the left anterior descending coronary artery, saphenous vein conduits are most commonly used[1]. However, graft patency remains the Achilles heel of saphenous vein grafts (SVG). One year after surgery, graft patency is 85%, dropping to 61% after ten years[2,3]. Unveiling the pathophysiological mechanisms and causes of SVG patency loss is therefore an important task.

This video demonstrates a rat vein interposition model to investigate vein graft loss. The overall goals of this method are to explore the underlying pathobiological and -physiological processes during disease progression and to develop a suitable model for drug or therapeutic option testing. By transplanting the superficial epigastric vein into the arterial system, this model closely mimics the clinical setting of coronary artery bypass grafting. Surgical trauma, ischemia, and wall stress are important triggers of pathological vascular changes and are imitated in the model described.

Different models and species are available to investigate vein graft patency loss. Large animal models, such as pigs[4], sheep[5], dogs[6], and monkeys[7], resemble human vessel and anatomical structures and thus enable complex therapeutic strategies, such as bypass stenting or new surgical techniques, to be tested[8]. However, special housing, equipment, and staff are required. In addition, high costs and the need for an additional anesthetist during surgery impede their broader application. Small animals, including rats, are easy to handle, do not require special housing, and have manageable costs. Compared to mouse models[9,10], rat models have the advantage of better operability and therefore less variability in the outcome. Rats are physiologically and genetically more similar to humans than mice[11,12]. In addition, most wild-type mice only develop limited myointima[13], which make mouse models prone to type II errors. The histology of the main mouse veins, such as the inferior vena cava, only consists of a few cell layers and renders early evaluation difficult[13]. A further disadvantage is the small amount of tissue available for subsequent analysis after graft recovery.

The model described in this video is reproducible, inexpensive, and easy to perform, and it can be established quickly and reliably. It is especially suitable for evaluating expensive experimental therapeutic agents, such as viral vectors for gene therapy, in an economical fashion.

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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. Animal Care

1. Obtain Lewis rats (LEW/Crl) rats and ROSA/luciferase-LEW transgenic rats weighing 300-350 g from the Institute of Laboratory Animals.
2. Keep the rats under conventional conditions in ventilated cabinets and feed them standard rat chow and autoclaved water ad libitum.
3. Perform a graft transplantation using the ROSA/luciferase-LEW transgenic rats as the donors and the syngenic LEW/Crl rats as the recipients.

2. Preparation of the Donor Rat

1. Use an induction chamber to anaesthetize a rat with isoflurane (2.5-3%).
2. Place the rat on its back and maintain the anesthesia with a facemask covering the mouth and nose. Check for sufficient depth of anesthesia by pinching the hind feet and verifying the absence of reflexes. Apply some vet ointment to the eyes to prevent dryness while under anesthesia.
3. Spread the hind legs and fix their position using tape.
4. Shave the inguinal hair with a hair trimmer and disinfect the entire area using povidone-iodine followed by 80% ethanol. Repeat the disinfection step twice.
   NOTE: The surgical area, gauze, and surgical instruments should be sterilized. Maintain a sterile field throughout the procedure and wear single-use, sterile surgical gloves, masks, and caps.
5. Under a microscope, perform a vertical incision along the linea inguinalis. Use two forceps to gently separate the subcutaneous tissues and expose the superficial epigastric vein from its origin on the femoral vein. Carefully isolate the superficial epigastric vein from the surrounding tissues.
6. Stop blood flow in the superficial epigastric vein using two micro clamps.
7. Harvest an approximately 0.5 to 1 cm segment of the vein by carefully lifting the isolated vein with forceps and cutting through the vessel with microscissors. Leave the micro clamps on the vessel stump to prevent the loss of blood. Place the removed piece of vein on sterile gauze. Carefully place a 30 G needle inside one end of the harvested vein and flush the vessel with heparin (50 units/ml).
   NOTE: Handle the vein with care and avoid damage during lifting, cutting, and flushing. Make sure to flush the graft with the proper amount of heparin.
8. Keep the vessel segment in 1% lidocaine on ice until transplantation into the recipient rat to prevent a vessel spasm.
9. Euthanize the donor rat by increasing the anesthesia to 5% isoflurane. After 2-3 min, open the abdomen along the linea alba, cut through the diaphragm, and remove the heart to stop circulation.

3. Preparation of the Recipient Rat

1. Anesthetize and fix the recipient rat in the same way as the donor rat.
2. Shave the medial side of the legs with a hair trimmer and disinfect three times using povidone-iodine and 80% ethanol.
3. Monitor the depth of anesthesia and ensure that it is sufficient by verifying the absence of reflexes when pinching the hind feet.
4. Perform a median femoral incision from the knee to the inguinal fold. Under a microscope, use 2 forceps to separate the femoral artery from its surroundings.
5. Use micro clamps to stop the flow of blood. Place the proximal clamp first, followed by the distal clamp.
6. Cut out a short segment of the clamped femoral artery with microscissors and discard it. Shorten the remaining arterial stump with microscissors, creating a gap that is 1-2 mm larger than the vein graft. Flush the arterial stump with heparin using a 30 G needle.
   NOTE: If the adventitia protrudes slightly beyond the vessel stump, use forceps to pull it slightly over the end of the vessel and remove a piece.
7. Place the harvested vein from step 2.8 between the arterial stumps and adjust the length so that it fits suitably into the gap. Note the direction of the vein.
8. Perform the proximal anastomosis first using a 10-0 prolene suture. Conduct single stitches in the order shown (**Figure 1D**). Start with a suture on each lateral side before adding three more sutures on the ventral side. Afterwards, place three stitches on the dorsal side of the vessel to complete the anastomosis.
9. Connect the distal vessels with the graft using the same technique as for the proximal anastomosis described in step 3.8. Again, start with a suture on each lateral side, and then place three sutures on the ventral side and the dorsal side.
10. Load two 1-mL syringes with fibrin glue component 1 and 2. Carefully lift the graft with forceps and drop approximately 100 µl of fibrin glue component 1 under the graft, followed by component 2.
    NOTE: Make sure that components 1 and 2 are applied in a 1:1 ratio.
11. Place the graft back in its position and drop an additional 100 µl of components 1 and 2 on top of the graft. Be sure that the glue covers both the graft and the anastomosis in order to prevent anastomotic insufficiency and over-distension of the vein graft.
12. Carefully open the distal clamp, followed by the proximal.
13. Confirm a successful surgery by checking for a visible pulse in the transplanted vein and distal artery of the graft.
14. Remove excessive glue, which impedes skin closure. Use forceps to lift the cured glue and remove the excess with microscissors. Close the skin layers with 5-0 prolene sutures.

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15. Inject 4-5 mg/kg Carprofen subcutaneously before allowing the rat to wake up. Do not leave the animal unattended until it has regained sufficient consciousness to maintain sternal recumbency. Keep the animal in a single cage until it is fully recovered.
16. Add Metamizole to the drinking water (50 mg Metamizole per 100 ml) as pain medication for the following 3 days and monitor the animal daily.

4. Duplex Sonography

NOTE: Use duxplex sonography to visualize blood flow non-invasively in rats[14].

1. Anaesthetize a rat in an induction chamber (isoflurane 2%). Place the rat on its back and maintain anesthesia with a facemask covering the nose.
2. Use hair clippers and hair removal cream to remove the hair around the area of the thigh.
3. Apply ultrasound gel to the thigh. Make sure that there are no air bubbles. Acquire duplex sonography images using an MS 400 transducer (center frequency: 30 MHz) with a frame rate of 230-400 frames/sec.

5. Histopathology

NOTE: Harvest and stain the vessel with Masson's trichrome staining for morphometric analysis[15].

1. Fix the harvested vessel in 4% paraformaldehyde overnight and dehydrate it in increasing concentrations of ethanol. Embed the sample in paraffin and cut it into 5 µm thick slices using a microtome.
   NOTE: Paraformaldehyde is toxic and should be handled with special care.
2. Deparaffinize the slides before staining them with trichrome staining solution. Dehydrate the stained slides, clear them with xylene, and mount them in mounting medium. After drying the slides, view the samples with a microscope.

6. Bioluminescence Imaging (BLI)

NOTE: The postoperative graft was tracked over time in vivo by measuring bioluminescent signal[16].

1. Dissolve 1 g of D-Luciferin potassium salt in 22 ml of PBS and inject it intraperitoneally into the rat (375 mg/kg body weight). Wait 15 min for the luciferin to circulate in the animal.
2. Place the rat into a real-time bioluminescent quantification system and access the bioluminescence signal.

Representative Results

The rat vein interposition model is suitable to study the development of myointima hyperplasia and vein graft failure. Animals recover well from the surgery and show excellent physical condition post-operation. **Figure 1** shows the key surgical steps. After the skin incision along the linea inguinalis, the epigastric superficial vein and femoral artery are identified (**Figure 1A**). Harvesting of the graft should be performed carefully, without damaging the graft (**Figure 1B**), as this can lead to early graft failure and patency loss. After positioning the graft in the recipient animal (**Figure 1C**), anastomosis stitches are performed in the order shown in **Figure 1D**. The completed venous interposition graft appears pale (**Figure 1E**) and should become red and show pulsation after reperfusion (**Figure 1F**).

The successful integration of the vein into the femoral artery and graft patency after transplantation can be confirmed non-invasively using duplex sonography (**Figure 2A**). By transplanting the vein of a Luc-positive rat into a syngeneic Luc-negative rat, bioluminescence imaging can be used to monitor graft presence over time (**Figure 2B**).

Myointima hyperplasia develops progressively in the graft over time. Histological staining with Masson's trichrome demonstrates myointima formation inside the internal elastic lamina (**Figure 2C**). The calculation of luminal obliteration, (dividing the cross-sectional area of the lumen by the area within the internal elastic lamina) revealed a gradual loss of graft patency from day 7 to day 28 post-surgery, thus confirming the reproducible dynamics of myointima hyperplasia in this rat model (**Figure 2D**).

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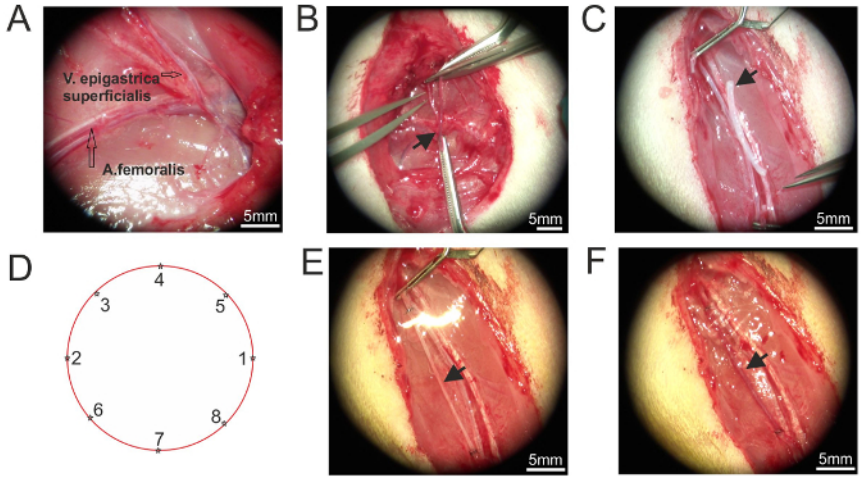


Figure 1: Detailed Scheme of the Surgical Procedure. (A) Anatomy of the inguinal region. (B) Harvesting the epigastric superficial vein graft. (C) Placing the vein graft between the arterial stumps of the recipient animal. (D) Order of anastomosis stitches. (E) Site after the venous graft construction. (F) Site after the reperfusion of the venous interposition graft. Black arrows mark the epigastric superficial vein. The Scale bar = 5 mm. Please click here to view a larger version of this figure.

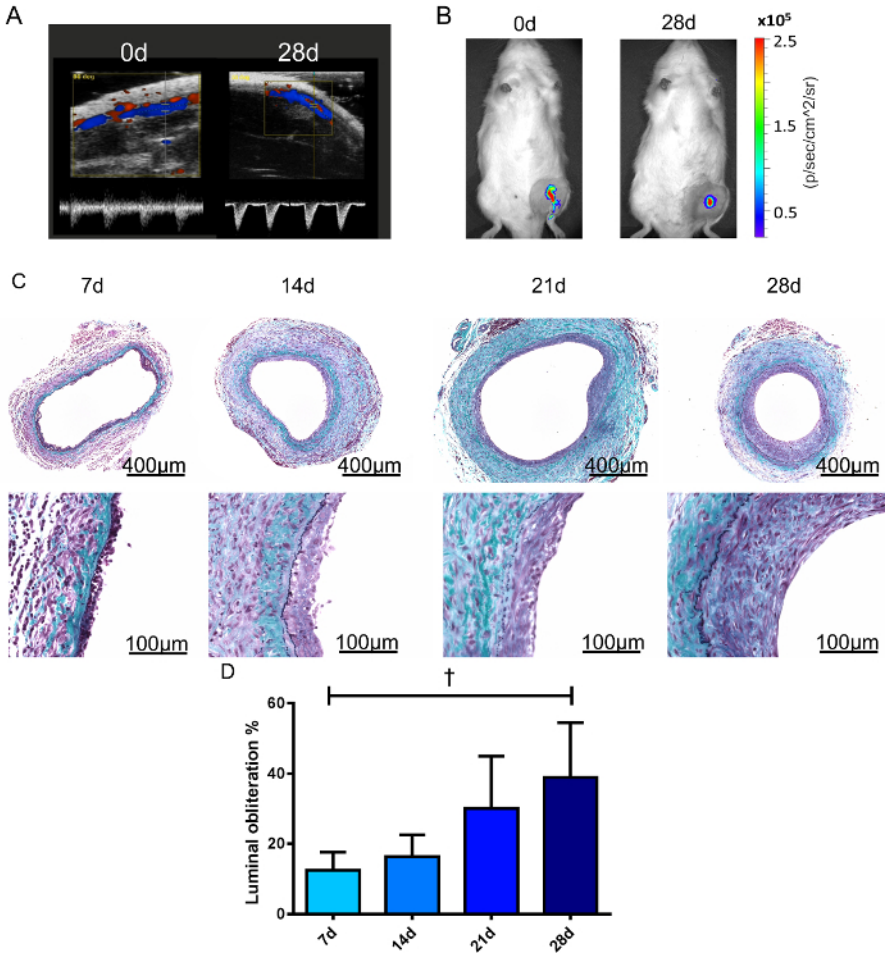
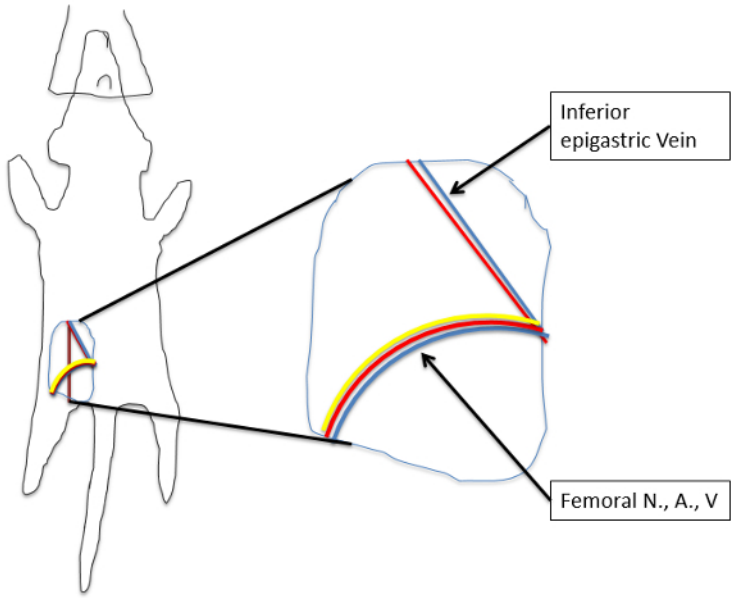


Figure 2: Characterization of the Animal Model. (**A**) Duplex sonography of a venous interposition graft immediately after transplantation (0d) and 28 days post-operation (28d). (**B**) BLI from Luc-positive grafts transplanted into Luc-negative rats at 0 days and 28 days after surgery. (**C**) Representative graft cross sections harvested after days 7, 14, 21, and 28 and stained with Masson's trichrome. (Upper panel: the Scale bar = 400 µm; Lower panel: the Scale bar = 100 µm) (**D**) Luminal obliteration in percentage is calculated by dividing the new inner lumen from the former lumen. Intergroup differences were assessed by one-way analysis of variance (ANOVA) with Bonferroni's post-Hoc test. $p < 0.01$. The error bars are the standard deviation (SD). Please click here to view a larger version of this figure.



Supplemental File 1: Schematic Illustration of the Surgical Procedure. A vertical incision along the linea inguinalis is performed on the donor rat, exposing the inferior epigastric vein. Blood flow is stopped with two micro clamps, and a vein segment 0.5-1.0 cm long is harvested. In the recipient rat, a median femoral incision is performed, exposing the femoral vein, artery, and nerve. After clamping the femoral artery, a vessel segment is removed and replaced by the harvested vein graft. Please click here to view Supplemental File 1 (or right-click to download).

Discussion

This video demonstrates a rat vein interposition model to investigate vein graft loss and to allow for the exploration of the underlying pathological processes and the testing of new drugs or therapeutic options.

Anesthesia is a crucial aspect of surgical procedures. A continuous inhalative anesthesia system is recommended, as this is a safe and easy method, especially during prolonged operations. This can be of great importance during the training phase, when the operation takes more than 1 hr.

With respect to the surgical procedure, it is critical not to damage the vein graft during harvest and implantation[17]. Gripping the adventitia of the vessel can prevent damage to the vein graft and avoid subsequent thrombus formation and graft failure. Graft patency can be determined immediately after vein graft construction through the observation of blood flow and of pulsation in the vein graft or distal artery, as well as through duplex sonography.

Another critical aspect of the procedure is to prevent the over-distension of the vein graft. Sudden exposure of the vein graft to the arterial pressure system leads to increased wall tension, subsequent over-distension, and changes in flow pattern. These are sources of thrombosis, anastomotic insufficiency, and early graft failure. Supporting the vein graft with fibrin glue can prevent uncontrolled ballooning and protect the intima and media from mechanical destruction. Absorbable collagen covers are an alternative to fibrin glue and can be used as perivenous covers[18].

The most critical step within this protocol is undoubtedly the anastomosis between the vein graft and the artery. Care must be taken not to pierce the two walls of the vessel, as this will lead to the narrowing of the anastomosis, which may result in early failure of the graft. In addition, special attention must be paid to the localization of sutures. Congruency of the suture positions in the artery and vein ensures graft patency and prevents insufficiency. To facilitate this, sutures can be performed in the order shown in **Figure 1D**.

The degree of technical difficulty can be viewed as a limitation of the technique, because a novice must first become familiar with the microsurgical equipment and the small sizes of the anatomical structures. However, other models used exhibit the same difficulties, and we believe that this video will help novice surgeons to master this technique within a short time.

Numerous small animal models for venous graft failure have been described in the literature. However, most models only provide very small amounts of tissue for analysis[13]. An advantage of this method is the comparatively large amount of tissue that can be obtained. One graft can be divided into multiple parts and used for different assays, thereby reducing the number of experimental animals required.

Recent advances in zinc-finger nuclease technology enabled the generation of knockout rats[19]. By selecting suitable knock-out rats, graft patency loss can be studied in different disease conditions. For example, renin knockout rats can be utilized to study hypertension[20]. These genetic backgrounds can be combined with this animal model to glean information on the mechanisms of vein graft failure in different settings or on the impact of certain genes in the development of myointima hyperplasia.

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In conclusion, the model described in this video is reproducible, inexpensive, and easy to perform, and it can be established quickly and reliably. Myointima hyperplasia, which is the main cause of vein graft failure, developed rapidly over four weeks, resulting in progressive luminal obliteration. Successfully tested treatment options in this model can be further confirmed in large animal models.

Disclosures

The authors have nothing to disclose.

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References

1. Sabik, J. F., 3rd. Understanding saphenous vein graft patency. *Circulation.* **124**, 273-275, (2011).
2. Goldman, S. *et al.* Long-term patency of saphenous vein and left internal mammary artery grafts after coronary artery bypass surgery: results from a Department of Veterans Affairs Cooperative Study. *J Am Coll Cardiol.* **44**, 2149-2156 (2004).
3. Fitzgibbon, G. M. *et al.* Coronary bypass graft fate and patient outcome: angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. *J Am Coll Cardiol.* **28**, 616-626 (1996).
4. Brien, J. E., Jr. *et al.* Wound healing around and within saphenous vein bypass grafts. *J Thorac Cardiovasc Surg.* **114**, 38-45 (1997).
5. El-Kurdi, M. S. *et al.* Ovine femoral artery bypass grafting using saphenous vein: a new model. *J Surg Res.* **193**, 458-469 (2015).
6. Yuda, A. *et al.* Angiotensin II receptor antagonist, L-158,809, prevents intimal hyperplasia in dog grafted veins. *Life Sci.* **68**, 41-48 (2000).
7. McCann, R. L., Hagen, P. O., & Fuchs, J. C. Aspirin and dipyridamole decrease intimal hyperplasia in experimental vein grafts. *Ann Surg.* **191**, 238-243, http://journals.lww.com/annalsofsurgery/abstract/1980/02000/aspirin_and_dipyridamole_decrease_intimal.18.aspx (1980).
8. Thomas, A. C. Animal models for studying vein graft failure and therapeutic interventions. *Curr Opin Pharmacol.* **12**, 121-126 (2012).
9. Hu, Y., & Xu, Q. New mouse model of vein bypass graft atherosclerosis. *Heart Lung Circ.* **11**, 182-188 (2002).
10. Salzberg, S. P. *et al.* Increased neointimal formation after surgical vein grafting in a murine model of type 2 diabetes. *Circulation.* **114**, I302-307 (2006).
11. Abbott, A. Laboratory animals: the Renaissance rat. *Nature.* **428**, 464-466 (2004).
12. Lindblad-Toh, K. Genome sequencing: three's company. *Nature.* **428**, 475-476 (2004).
13. Yu, P., Nguyen, B. T., Tao, M., Campagna, C., & Ozaki, C. K. Rationale and practical techniques for mouse models of early vein graft adaptations. *Journal of vascular surgery.* **52**, 444-452 (2010).
14. Olver, D. T., Lacefield, J. C., & Shoemaker, K. J. Evidence of bidirectional flow in the sciatic vasa nervorum. *Microvascular research.* **94**, 103-105 (2014).
15. Stubbendorff, M. *et al.* Inducing myointimal hyperplasia versus atherosclerosis in mice: an introduction of two valid models. *Journal of visualized experiments : JoVE.* (2014).
16. Conradi, L. *et al.* Immunobiology of fibrin-based engineered heart tissue. *Stem cells translational medicine.* **4**, 625-631 (2015).
17. Dashwood, M. R., & Tsui, J. C. 'No-touch' saphenous vein harvesting improves graft performance in patients undergoing coronary artery bypass surgery: A journey from bedside to bench. *Vascular Pharmacology.* **58**, 240-250 (2013).
18. Bekler, H. I., Rosenwasser, M. P., Akilina, Y., & Bulut, G. The use of an absorbable collagen cover (NeuraWrap) improves patency of interpositional vein grafts. *Acta orthopaedica et traumatologica turcica.* **44**, 157-161 (2010).
19. Geurts, A. M. *et al.* Knockout rats via embryo microinjection of zinc-finger nucleases. *Science.* **325**, 433 (2009).
20. Moreno, C. *et al.* Creation and characterization of a renin knockout rat. *Hypertension.* **57**, 614-619 (2011).

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