Video Article

Imaging In-Stent Restenosis: An Inexpensive, Reliable, and Rapid Preclinical Model

Tobias Deuse[1], Fumiaki Ikeno[2], Robert C. Robbins[1], Sonja Schrepfer[1]

[1]Department of Cardiothoracic Surgery, Stanford University School of Medicine

[2]Stanford University School of Medicine

Correspondence to: Sonja Schrepfer at schrepfer@stanford.edu

URL: https://www.jove.com/video/1346
DOI: doi:10.3791/1346

Keywords: Medicine, Issue 31, stent, rats, restenosis, OCT, imaging

Date Published: 9/14/2009

Citation: Deuse, T., Ikeno, F., Robbins, R.C., Schrepfer, S. Imaging In-Stent Restenosis: An Inexpensive, Reliable, and Rapid Preclinical Model. *J. Vis. Exp.* (31), e1346, doi:10.3791/1346 (2009).

Abstract

Preclinical models of restenosis are essential to unravel the pathophysiological processes that lead to in-stent restenosis and to optimize existing and future drug-eluting stents.

A variety of antibodies and transgenic and knockout strains are available in rats. Consequently, a model for in-stent restenosis in the rat would be convenient for pathobiological and pathophysiological studies.

In this video, we present the full procedure and pit-falls of a rat stent model suitable for high throughput stent research. We will show the surgical procedure of stent deployment, and the assessment of in-stent restenosis using the most elegant technique of OCT (Optical Coherence Tomography). This technique provides high accuracy in assessing plaque CSAs (cross section areas) and correlates well with histological sections, which require special and time consuming embedding and sectioning techniques. OCT imaging further allows longitudinal monitoring of the development of in-stent restenosis within the same animal compared to one-time snapshots using histology.

Video Link

The video component of this article can be found at https://www.jove.com/video/1346/

Protocol

Aortic Stent Deployment

1. Male Sprague-Dawley rats weighing 550-600 g are purchased from Harlan (Indianapolis, IN, USA). House rats under conventional conditions, fed standard rat chow and water ad libitum.
2. Anesthetize rat with isoflurane (2%) and ketamine (25 mg/kg). Under microscopic view, perform an upper median mini-laparotomy to expose the infrarenal aorta.
3. Dissect the abdominal aorta from the surrounding tissue, from the level of the renal arteries down to the bifurcation. CAUTION: No need to dissect the aorta from the IVC.
4. Use microclamps to stop the aortic blood flow. Place the proximal clamp first, followed by the distal clamp.
5. Open the aorta with a small transverse incision and flush the aorta with heparin (200 units).
6. The aortic endothelium is denuded by the passage of a 2-french Fogarty arterial embolectomy catheter (Baxter Healthcare, Deerfield, IL, USA).
7. Use any human stent size between 8mm and 12mm in length, and 2.5mm-3mm in diameter. CAUTION: The diameter of the stent should not exceed the vessel diameter by more than 10% to avoid pre- and post-stent stenosis. CAUTION: Don't switch stent length within the same study.
8. Deploy the stent using the appropriate balloon pressure to achieve the desired diameter.
9. The small aortic incision is closed with 9-0 Prolene sutures (Ethicon, Norderstedt, Germany).
10. Close the abdominal incision in layers with 4-0 Vicryl running sutures (Ethicon, Norderstedt, Germany).
    The skin sutures should still be removed within 7-14 days even when Viacryl is used.
11. Animals received Carprofen (6mg/KG) intraoperatively, and Metamizol for 7 more days in the drinking water.

Optical Coherence Tomography (OCT) Imaging

OCT images are obtained with the M2 OCT imaging system (LightLab Imaging, Inc., Westford, MA, USA). ImageWire is an imaging probe to deliver the light to the tissue and collect the signals. The ImageWire consists of 0.006"(0.15 mm) fiber-optic core, inside a sheath with a maximum O.D. of 0.019" (0.48 mm).

1. Anesthetize rat with isoflurane (2%) and ketamine (25 mg/kg). Under microscopic view, perform a median redo laparotomy to expose the infrarenal aorta.
2. Clamp the proximal aorta and both iliac artery.
3. Perform a transverse arteriotomy at the distal end of the aorta, flush with 1ml PBS using a 28G catheter and insert the OCT catheter and forward it into the aorta.
4. Motorized pullback OCT imaging is performed at a pullback rate of 1.0 mm per second.
5. Acquire images at 15 frames per second; images are displayed with a color look-up table and digitally archived.
6. Close the arteriotomy.
7. Close the abdominal and incisions with 4-0 Vicryl running sutures (Ethicon, Norderstedt, Germany).
   The skin sutures should still be removed within 7-14 days even when Viacryl is used.
8. The maximum neointima formation will develop within 6 weeks after stent implantation.

Analyzing OCT images

OCT measurements are performed using the LightLab OCT imaging proprietary software with a rat-based interface.

1. Calibrate the system to the reflection of the OCT imaging wire, which is the standard calibration technique for this system.
2. Trace lumen and stent cross sectional areas (CSA) manually at each 1.0 mm intervals.
3. Calculate plaque cross sectional area as stent CSA subtracted from the lumen CSA. Calculate percent plaque area as plaque CSA divided by stent CSA (%). Calculate average percent plaque areas.

Validation of the OCT technique

OCT results correlate well with histopathology (Figure 1). Histologic plaque CSAs are calculated as described above. Histology reveals intimal hyperplasia with high density of spindle-shaped cells and only few mononuclear inflammation cells. After 6 weeks, stents are completely covered with neointimal granulation tissue and the plaque CSA measures 1.3±0.4 mm^2 in a 2.5mm stent.

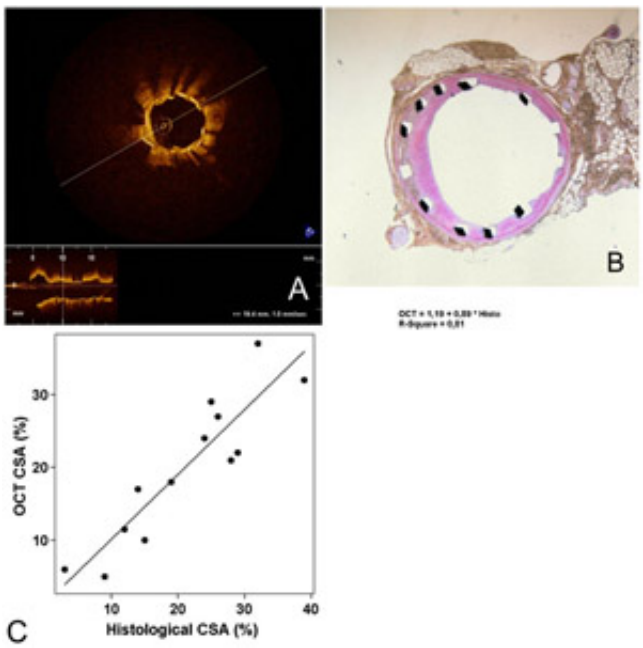

Figure 1: OCT (A) and histological (B; magnification 16x) images of the stent 6 weeks after deployment. Plaque CSA results obtained from OCT images correlate well with histopathology. Please click here to see a larger version of figure 1.

Discussion

Although the rabbit iliac artery and the pig coronary artery models are the most frequently used for stent placement [1], a combination of radiological and surgical equipment is required, animal housing capacity is limited, and the costs of purchase are high. Limitations of the rat stent

model is the necessary use of specifically designed stents for rats, the metal-to-artery ratio resulting in more vascular injury[2], and the artificially high incidence of thrombosis[3].

The rat stenting model is a simple, inexpensive, rapid, and accurate preclinical model [4]. After the initial report of direct stenting of the rat aorta by Lowe et al. [5], feasibility and suitability of this model for the evaluation of the pathophysiology of in-stent restenosis has been thoroughly shown [5,6]. The diameter of the rat aorta is adequate to allow expansion of commercially available stents without disruption of the physiologic vessel architecture. It has been shown that pathophysiological mechanisms, such as thrombus formation, inflammation, and SMC proliferation, develop in these rat models as they do in the rabbit and pig. Therefore, these models are good representations of the actual process of restenosis.

The OCT high-resolution imaging technology is useful to evaluate intimal hyperplasia. The penetration depth is only 1.5-2 mm, but its resolution is an order of magnitude greater than that of intravascular ultrasound (IVUS) [7,8]. Multiple studies comparing OCT with IVUS conclude that OCT is currently the preferred technique to evaluate neointimal hyperplasia after stent implantation [8-10]. Especially in small animals with small vessel diameters, the high resolution of OCT renders it the best imaging modality for the evaluation of restenosis.

In summary, this video shows that (1) rat aortic stenting is easily feasible, (2) rat abdominal aorta stenting is suitable for testing commercially manufactured stents and (3) OCT imaging is an accurate and elegant technique for longitudinal monitoring of in-stent restenosis.

Disclosures

All rats were housed in the animal care facility at Stanford University Medical Center (Stanford, Ca), under standard temperature, humidity, and lighting conditions, and were provided rat chow and water ad libitum. The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). The study protocol was approved by the Administrative Panel on Laboratory Animal Care, Stanford University.

Acknowledgements

Funding
The present study was supported by the Falk Research Fund for the Department of Cardiothoracic Surgery at Stanford University School of Medicine, Stanford, CA, USA. Tobias Deuse was funded by a research grant of the German Cardiac Society. Sonja Schrepfer has received a research grant from the Deutsche Forschungsgemeinschaft (DFG) (SCHR992/2-1).

References

1. Langeveld, B., Roks, A.J.M. Commentary: rat restenosis models: means for thorough restenosis research. *Journal of endovascular therapy.* 12 (3) : 343-345 (2005).
2. Roguin, A., Grenadier, E. Stent-based percutaneous coronary interventions in small coronary arteries. *Acute Card Care*. 8 : 70 - 74 (2006).
3. Post, M. J., and Waltenberger, J. Small Is Beautiful: A Miniature Stent Model.*Arterioscler. Thromb. Vasc. Biol.* 27 ; 701-702 (2007).
4. Langeveld, B., Roks, A.J.M., Tio, R.A., van Boven, A.J., van der Want, J.J.L., Henning, R.H., *et al.* Rat abdominal aorta stenting: a new and reliable small animal model for in-stent restenosis. *Journal of vascular research.* 41 (5) : 377-386 (2004).
5. Lowe, H.C., Chesterman, C.N., Khachigian, L.M. Rat aortic stenting: toward a simple model of in-stent restenosis. *The American journal of cardiology.* 88 (6) : 720-721 (2001).
6. Groenewegen, R., van der Harst, R., Roks, R., Buikema, R., Zijlstra, R., van Gilst, R., *et al*. Effects of angiotensin II and angiotensin II type 1 receptor blockade on neointimal formation after stent implantation. *International Journal of Cardiology.* 2007.
7. de Smet, B.J.G.L., Zijlstra, F. A look at drug eluting stents with optical coherence tomography. *European Heart Journal.* 28 (8) : 918-919 (2007).
8. Pinto, T.L., Waksman, R. Clinical applications of optical coherence tomography. *Journal of interventional cardiology.* 19 (6) : 566-573 (2006).
9. Matsumoto, D., Shite, J., Shinke, T., Otake, H., Tanino, Y., Ogasawara, D., *et al*. Neointimal coverage of sirolimus-eluting stents at 6-month follow-up: evaluated by optical coherence tomography. *European Heart Journal.* 28 (8) : 961-967 (2007).
10. Kawase, Y., Suzuki, Y., Ikeno, F., Yoneyama, R., Hoshino, K., Ly, H.Q., *et al*. Comparison of nonuniform rotational distortion between mechanical IVUS and OCT using a phantom model. *Ultrasound in medicine & biology.* 33 (1) : 67-73 (2007).