EXPERIMENTAL STUDY

A Novel Rabbit Model for In-Stent Neoatherosclerosis
Insights from Optical Coherence Tomography

Gang Wang,1* MD, Xing Luo,1* MD, Ruoxi Zhang,1 MD, Shuyuan Chen,1 MD, Jingbo Hou,1 MD and Bo Yu,1 MD

Summary
In-stent neoatherosclerosis is an important problem after percutaneous coronary intervention. To explore the mechanisms and treatment of in-stent neoatherosclerosis, an animal model is needed. To avoid the disadvantages of current animal models, such as excessive use of X-rays and a high mortality rate, we attempted to develop an improved animal model. We explored a method that uses a short time interval to establish a rabbit model of in-stent neoatherosclerosis with a high survival rate and to evaluate its indicators. Sixty rabbits were divided into three equal groups: group A, the traditional method; group B, the standard intervention method; and group C, the improved method. In group C, we made two small incisions in each rabbit’s neck, separated the common carotid, punctured it, and implanted a stent. The incision was then sutured. Four weeks later, we used optical coherence tomography (OCT) to scan all rabbits for neoatherosclerosis. We found no significant differences in OCT data between our new animal model and the traditional and interventional groups (P > 0.05). The technological success rate was higher in the new animal model (P < 0.001). We developed a new method to establish an animal model of neoatherosclerosis, which had similar results to the traditional and interventional methods.

Key words: Cardiovascular disease, Percutaneous coronary intervention, In-stent restenosis

Cardiovascular disease is the most common cause of death in modern times.3-5 Coronary atherosclerotic heart disease is the most common type of heart disease. Currently, percutaneous coronary intervention (PCI) is an important therapeutic option to treat coronary atherosclerotic heart disease. However, in-stent restenosis (ISR) and late stent thrombosis affect patients’ prognosis after PCI and have a serious impact on patients’ health. Much literature reveals that in-stent neoatherosclerosis is a common pathological feature in ISR.2-6) According to Park’s study, both drug-eluting stents (DES) and bare metal stents (BMS) tend to develop ISR.5) BMS has the tendency to develop into ISR in the early stages. DESs typically develops ISR after two years, and the higher the loss of luminal diameter.7) The pathological feature of plaques in about 52% of neoatherosclerosis cases, similar to a vulnerable plaque, is a thin cap fibroatheroma (TCFA). TCFA is characterized by a thin fibrous cap (< 65 μm), high lipid levels, and eccentric lesions.8) As a result, neoatherosclerosis is prone to rupture, which increases the probability of a deadly event.

With the help of imaging technology, such as optical coherence tomography (OCT) and intravenous ultrasound, researchers have developed two different potential mechanisms for ISR, one being inflammation. Many researchers hold the opinion that the intima and media on the vascular wall are destroyed by the stent and balloon during PCI so the plaque is squeezed out, which causes acute inflammation and induces the release of tissue factors. As a result, local inflammation of the coronary artery occurs. The clustering of inflammatory cells contributes to luminal loss.9,10) The other potential mechanism is delayed endothelial healing. According to this theory, in patients with DES, ISR occurs because the drug-covered stent inhibits repair of the damaged intima, contributing to poor endothelial coverage. Long-term inhibition of endothelial coverage causes the endothelium to lose homeostatic balance, and the endothelium will have a greater tendency to absorb white blood cells and platelets.10) Moreover, lipids would adhere more easily to the vascular wall. Once the drug on the stent was released completely, the vascular in-

---

*These authors contributed equally to this study.

From the 1Department of Cardiology, The 2nd Affiliated Hospital of Harbin Medical University, The Key Laboratory of Myocardial Ischemia, Chinese Ministry of Education, Harbin, PR China.

This work was supported by the National Natural Science Foundation of China (Grant No. 81671794 to Jingbo Hou; Grant No. 81330033 to Bo Yu) and the Key Laboratory of Myocardial Ischemia, Chinese Ministry of Education, Harbin, Heilongjiang Province, China (Grant No. KF201811 to Ruoxi Zhang); the General Undergraduate Colleges and Universities Young Innovative Talents Training Plan, Heilongjiang Province, China (Grant No. UNPYSCT-2018075 to Ruoxi Zhang).

Address for correspondence: Jingbo Hou, MD, Department of Cardiology, The 2nd Affiliated Hospital of Harbin Medical University, The Key Laboratory of Myocardial Ischemia, Chinese Ministry of Education, Harbin, 150086, PR China. E-mail: jingboHou@163.com

Received for publication December 28, 2017. Revised and accepted July 13, 2018.

Released in advance online on J-STAGE September 4, 2019.

doi: 10.1536/ihj.17-737

All rights reserved by the International Heart Journal Association.

1154
A novel rabbit model for ISR

Pharmacological inhibition also disappeared. With the stimulation of the stent, proliferation of the endothelium and smooth vascular muscle may be an important factor leading to ISR caused by DES. Above all, this would explain why ISR occurred earlier in patients with BMS than in patients with DES. Besides, other researchers reported that irregular protrusion post-DES implantation and stent fracture were also associated with subsequent neatherosclerosis formation. However, certain mechanisms of neatherosclerosis are still unclear, so we should attach more importance to the further study of neatherosclerosis, and an appropriate animal model that allows clear scanning of the arterial wall is required.

Researchers have already designed different animal models to study the mechanisms and treatment of neatherosclerosis. The traditional method separates the common carotid artery and branches, creates a large incision on the rabbit’s neck from the mandible to the sternum to separate the carotid artery and its branches, and clips all the branches except the left external carotid artery. The stent is then introduced through the first branch of the left external carotid artery and is passed into the left common carotid artery. Finally, the two ends of the incision are ligated to stop the bleeding.

Another method is to separate the common carotid artery and perform aortic arteriography on the bilateral common carotid arteries by transcatheterization of the right femoral artery with a cut-down technique.

However, both of these models have disadvantages. The former method destroys the normal anatomical structure and greatly affects the hemodynamics. As a result, this type of animal model has a high rate of mortality. The latter model requires X-rays, which harm the experimenters, and it requires many experimenters. Therefore, the aim of the present study was not only to establish an animal model of neatherosclerosis with morphological and structural characteristics similar to those seen in human neatherosclerosis but also to develop a convenient method that causes less trauma.

Methods

Ethical approval of the study protocol: The study protocol was approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University (Harbin, China).

Experimental Protocol: Sixty adult New Zealand white rabbits (2.5-3.5 kg), 3-4 months of age, were fed a normal lipid diet for four weeks. Animals were given free access to water and food; rabbits were housed continuously at the open animal care facilities of the Second Affiliated Hospital of Harbin Medical University. The rabbits were divided into the following groups: (1): group A: 20 rabbits had stents implanted the traditional way (2): group B: 20 rabbits had stents implanted via transcatheterization and aortic arteriography (3): group C: 20 rabbits had stents implanted using our improved method.

Stent placement and tissue harvest: The rabbits in group C received oral aspirin (40 mg) in combination with oral clopidogrel (75 mg) 48 hours before surgery, followed by oral aspirin (40 mg) daily until the animals were euthanized; before the interventions, the rabbits were also given intravenous heparin (100 IU/kg), intramuscular xylazine 0.15 mL/kg to induce anesthesia, and 20% chloral hydrate, 0.5 mL/kg via intravenous injection to induce anesthesia. Each rabbit’s neck in the case group was epilated from the mandible to the sternum under local anesthesia induced by 1% lidocaine. On the left side of the trachea, we made two incisions about 2 cm in length, extending from the mandible (far end) to the sternum (proximal end). The two incisions were about 3-4 cm apart to allow room to implant the stent. Next, the common carotid artery was separated through the two incisions. The arterial sheaths around the common carotid artery were separated clearly, as this was important to stop the bleeding with artery clamps. The two parts of the separated artery were clipped, with the far end being clipped first so that the artery between the two clamps was full of blood, making arterial puncture easier (Figure 1A). After clipping, 0.5 mL heparin was injected into the ear vein. A 5-Fr introducer sheath (Terumo Co, Tokyo, Japan) was introduced into the far end of the artery. The guidewire was inserted into the artery until it approached the proximal end clamp, and another clamp was placed to hold the guidewire in place (Figure 1A). The stent delivery system (Partner, Lepu Medical, Beijing, China) was advanced into the
common aortic artery through the guidewire. The stent was deployed to achieve a stent-to-artery-size ratio range of 1.2:1.4. At the same time, the guidewire was withdrawn. Next, the balloon compressor on the stent delivery system was connected, and the pressure was maintained at 12 kPa for about 10 seconds. The balloon was removed. The near clamp was loosened for 1 second to protect from aeroembolism. The instrument for microsurgery was then used with a 7-0 suture to take three bites using the continuous suture closure technique (Figure 1B) to partially suture the tissue and skin. During the operation, the rabbits were visually monitored for signs of pain, distress, or morbidity (sudden behavioral change, poor posture, or ambulation difficulties). After the operation, 3 mL gentamicin was injected intramuscularly.

The rabbits in group A underwent an operation according to the traditional method. A cut was made from the mandible to the sternum, and we separated the common carotid artery and its branches. All the branches were clamped except the left external carotid artery. The stent was introduced through the first branch of the left external carotid artery and passed into the left common carotid artery. Finally, we ligated the two ends of the incision to stop the bleeding (Figure 2).

Group B underwent an operation with the interventional approach using a C-arm angiography unit (Innova 3100, GE Medical System). After anesthesia, aortic arteriography of the bilateral carotid arteries was performed via transcatheterization of the right femoral artery using a cut-down technique. The stent delivery system was advanced into the common carotid artery (Figure 3).

After four weeks, all rabbits in our study were humanely euthanized with an overdose of sodium pentobarbital. Stented vessels were harvested and stored in 4% formaldehyde.

**Acquisition of OCT images:** An OCT wire was inserted through the cut into the common carotid artery. OCT was obtained using a time-domain OCT system (Light Lab Imaging, Westford, MA, USA) with an imaging wire (crossing profile, 0.014 inches; Light Lab Imaging) at a pullback speed of 3 mm/second during intermittent flushing with 0.9% (physiological) saline through the guiding catheter to displace blood transiently.

After OCT scanning, the vascular stent was withdrawn. Samples were embedded in paraffin and cut into serial transverse sections of 5 μm for histopathological analyses.

**Histology:** Histological assessments were carried out using a previously validated methodology. After the follow-up stent imaging, rabbits were euthanized using an overdose of sodium pentobarbital; carotid specimens were excised, fixed in formalin, and then embedded in methyl methacrylate. Four 2-mm sections were obtained from each stent using a tungsten carbide knife. Sections (5-μm thickness) were then cut using an automated microtome and stained with hematoxylin and eosin. Specimens were then cut using a DMRAX2 photomicroscope (Leica Microsystems, Milan, Italy), and analyzed using Leica IM 500 image analysis software.

**Optical coherence topographic analysis:** Assessment of neoatherosclerosis included a determination of the presence of lipids within the stent (Figure 4). As reported previously, lipids were defined as a diffusely bordered, signal-poor region with rapid signal attenuation. Lipid-laden neointima was defined as neointima-containing lipids. Neoatherosclerosis was defined as the presence of lipid-laden neointima inside the stent. Neovascularization was defined as a small vesicular or tubular structure with a diameter > 50 μm but < 300 μm. TCFA-like neointima was defined as lipid-rich neointima having a cap thickness of ≤ 65 μm. OCT erosions were identified as an irregular lumen surface with an attached mural thrombus overlying a fibrous plaque. The OCT analysis included determination of the presence of lipid-laden intima, neovascularization, TCFA, plaque rupture, plaque erosion, thrombus, and severe stenosis.

OCT images were analyzed by two independent investigators who were blinded to subject information using proprietary OCT offline software (Light Lab Imaging, Westford, MA, USA). If there was discordance between
Figure 3. Aortic arteriography. Fluoroscopic images of iliac stent implantation via the carotid artery in a rabbit.

Figure 4. Representative cases with all tissue morphologies assessed with optical coherence tomography (OCT). A: Normal neointima is characterized by a homogenous signal-rich band (red asterisk). B: Lipid-laden intima (white asterisk) is observed as a signal-poor band region with a poorly delineated border. C: Intra-intima neovascularization (white arrow). D: Thin cap fibroatheroma-like intima (cap thickness, 40 μm). E: The OCT erosion is identified as an irregular lumen surface with an attached mural thrombus (arrows) overlying a fibrous plaque. F: OCT image of the disrupted fibrous cap (white arrow) and a cavity formation inside the plaque. G: OCT image of the red thrombus (white asterisk). H: OCT image showing severe stenosis.

the analyses, a consensus reading was obtained from a third independent investigator.

Correlation between OCT image and histopathology: The correlations between OCT and histological findings were analyzed using the stent edges as anatomical landmarks. Lumen area, average lipid arc, and average fibrous
cap thickness were compared between OCT and histopathology.

**Statistical analysis:** Numerical data are presented as mean ± standard error of the mean. Continuous variables were first checked for normal distribution by the Shapiro-Wilk goodness-of-fit test and analysis of variance or by the Wilcoxon rank-sum test, as appropriate. Dunnett’s correction post hoc adjustment was used to determine significant differences, and a P value of < 0.05 was considered statistically significant. Ordinal data were analyzed by the Wilcoxon rank-sum test. All analyses were performed using SPSS version 19.0 (IBM Corp., Armonk, NY).

**Result**

As shown in Table I, a total of 44 rabbits with 44 stents were scanned by OCT, and 16 rabbits did not complete the experimental protocol. Sixteen rabbits died from anesthesia, surgical accidents, or serious postoperative infections, seven from group A, eight from group B, and one from group C. The remaining rabbits underwent OCT and histological analysis.

**Vascular condition after stent implantation:** We scanned the vessels after the stents were implanted.

**OCT findings:** After the artery was harvested, we obtained OCT images randomly and measured the diameter, length, and area of the stents; the vessel diameter; the area of the lumen; the area and thickness of the intima; and the thickness of the fibroatheroma. The data from groups A, B, and C were compared (Tables I, II). The technical success rate in group C was higher than that in groups A and B, and C were compared (Tables I, II). The remaining data (mean stent length, mean stent diameter, stent area, mean lumen area, neointimal area, neointimal thickness, fibrous cap thickness, and lipid arc) were not significantly different between groups (P > 0.05).

**Agreement between OCT and histopathology:** Thirteen representative OCT images and their corresponding histological cross-sections were selected from 13 ISR lesions to ascertain the agreement between OCT and histological findings. Compared with histopathological findings, OCT measurements of the mean plaque area showed an acceptable correlation (Table III).

**Discussion**

In the present study, we described an improved method to establish an ISR animal model that reproduced the features of human ISR. We combined the fundamental techniques of the traditional method with microsurgery, which made the animal model more convenient and did not influence the hemodynamics.

### Table I. Summary of Procedural Characteristics

<table>
<thead>
<tr>
<th>Feature</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P-value (A versus B)</th>
<th>P-value (A versus C)</th>
<th>P-value (B versus C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of rabbits</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Number of stents deployed</td>
<td>13</td>
<td>12</td>
<td>19</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Technical success rate (%)</td>
<td>65</td>
<td>60</td>
<td>95</td>
<td>&lt; 0.05</td>
<td>0.990</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Number of deaths</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Mean stent length (mm)</td>
<td>15.47 ± 1.12</td>
<td>15.52 ± 1.42</td>
<td>15.61 ± 1.40</td>
<td>0.806</td>
<td>0.946</td>
<td>0.641</td>
</tr>
<tr>
<td>Mean stent diameter (mm)</td>
<td>2.89 ± 0.36</td>
<td>2.97 ± 0.481</td>
<td>2.98 ± 0.38</td>
<td>0.948</td>
<td>0.607</td>
<td>0.255</td>
</tr>
</tbody>
</table>

NA indicates not available.

### Table II. Optical Coherence Tomography Analyses of the Neointima

<table>
<thead>
<tr>
<th>Feature</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P-value (A versus B)</th>
<th>P-value (A versus C)</th>
<th>P-value (B versus C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent area (mm²)</td>
<td>6.08 ± 1.00</td>
<td>6.01 ± 0.89</td>
<td>5.97 ± 0.81</td>
<td>0.439</td>
<td>0.731</td>
<td>0.787</td>
</tr>
<tr>
<td>Average lumen area (mm²)</td>
<td>4.66 ± 0.69</td>
<td>4.18 ± 0.81</td>
<td>3.84 ± 0.89</td>
<td>0.409</td>
<td>0.312</td>
<td>0.905</td>
</tr>
<tr>
<td>Neointimal area (mm²)</td>
<td>1.91 ± 0.82</td>
<td>1.96 ± 0.51</td>
<td>2.32 ± 0.89</td>
<td>0.313</td>
<td>0.147</td>
<td>0.212</td>
</tr>
<tr>
<td>Neointimal thickness (mm)</td>
<td>0.24 ± 0.08</td>
<td>0.28 ± 0.07</td>
<td>0.31 ± 0.08</td>
<td>0.248</td>
<td>0.725</td>
<td>0.122</td>
</tr>
<tr>
<td>Fibrous cap thickness (mm)</td>
<td>0.22 ± 0.06</td>
<td>0.22 ± 0.05</td>
<td>0.21 ± 0.06</td>
<td>0.127</td>
<td>0.6079</td>
<td>0.893</td>
</tr>
<tr>
<td>Lipid arc (°)</td>
<td>98 ± 8.64</td>
<td>109 ± 12.11</td>
<td>91 ± 9.21</td>
<td>0.487</td>
<td>0.211</td>
<td>0.367</td>
</tr>
</tbody>
</table>

### Table III. Agreement between Optical Coherence Tomography and Histology Findings

<table>
<thead>
<tr>
<th>Feature</th>
<th>OCT (n = 13)</th>
<th>Histology (n = 13)</th>
<th>ICC (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area (mm²)</td>
<td>3.45 ± 0.96</td>
<td>2.48 ± 1.22</td>
<td>0.665 (~0.046-0.815)</td>
<td>0.035</td>
</tr>
<tr>
<td>Average lipid arc (°)</td>
<td>139.54 ± 48.91</td>
<td>117.74 ± 47.92</td>
<td>0.939 (0.799-0.981)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Average FCT (mm)</td>
<td>178.52 ± 63.63</td>
<td>143.02 ± 64.41</td>
<td>0.933 (0.780-0.980)</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

Correlation coefficient (ICC) was used to evaluate the agreement between the OCT and histology findings. CI indicates confidence interval; and FCT, fibrous cap thickness.
PCI revolutionized the treatment of coronary atherosclerotic heart disease. However, the outcomes may be compromised by in-stent neoatherosclerosis or ISR through inflammation of the injured artery or delayed initial healing.\textsuperscript{8,13,15} With the development of DES, the risk of ISR has decreased significantly.\textsuperscript{12} After two years, the loss of lumen in patients with DES implantation is comparable to that in patients with BMS implantation,\textsuperscript{9} and the animal models we used in the past are inconvenient or have a high failure rate. However, researchers have reported several mechanisms for ISR, such as glucose fluctuation and stent fracture.\textsuperscript{1,11} This demonstrates that ISR is still problematic. An improved animal model is required.

Compared with the interventional method,\textsuperscript{13} our improved method requires less laboratory staff and less time. The interventional method requires radiography, which harms the staff and the rabbits. Moreover, the interventional method requires a large incision to separate the carotid artery and to suture, implant the stent, and ligate the hole. Additionally, rabbits require a long anesthesia time, increasing the probability of infection and reducing the blood supply to the brain, which can lead to mortality. As a result, the technological success rate in group C was higher than that in group B ($P < 0.001$). Our improved method only requires two small incisions and takes about 1.5 hours, which is shorter than the 4 hours required for groups A and B. The most important advantage is avoiding X-rays, greatly reducing the harm to the rabbits and experimenters. From a hemodynamic standpoint, microsurgery does not affect the arterial blood flow.

Compared with the traditional method,\textsuperscript{12} our method is a fundamental improvement; two incisions of only 3 cm each replace the large incision, reducing bleeding and infection. The traditional method requires the separation of all the branches of the carotid artery, and damage to the muscle tendons, thyroid gland, and parotid gland is difficult to avoid. During the operation, bleeding is also a cause of death, especially when separating the internal carotid artery, since the artery can only extend about 2 cm out of the incision. Therefore, it is difficult to separate the artery. Moreover, there may be too many clamps to clip the branch of the carotid artery successfully, and that may also increase the risk of bleeding.\textsuperscript{12,19,20} On the other hand, the new method decreases postoperative pain. The traditional method ligates the punctured artery, causing blood loss to the area nourished by this branch, and the new method avoids this. Moreover, in the traditional method, it is difficult to separate such a long artery and its branch, and finding the branch is not easy. The branch is so thin that much time is required to implant the stent from the artery. However, the technological success rate in group C was higher than that in groups A and B ($P < 0.001$).

The OCT images demonstrated that the new animal model we established clearly showed in-stent neoatherosclerosis.\textsuperscript{11,12} After the stent had been implanted for four weeks, we found endothelia-covered stents and atherosclerosis. The type of plaque was similar to a human plaque, including a fibrous cap and lipid core. The average fibrous cap thickness in group C was $0.21 \pm 0.06 \text{ mm}$, which did not exceed the thickness defined as TCFA (plaque with a thin fibrous cap, with the thinnest part $\leq 65 \text{ mm}$, overlying a large lipid pool of $> 2$ quadrants). However, in group C, we found some plaque with a thin fibrous cap of $\leq 65 \text{ mm}$, and this type of plaque has a greater tendency to rupture. Moreover, this plaque had ruptured because the thin fibrous cap had broken, and lipids had flowed out. Rupture is the most common reason for acute cardiac syndrome (ACS) after stent implantation, and the rupture of in-stent neoatherosclerosis is an etiology of ACS. As a result, an improved animal model is essential to study the mechanisms and treatment of neoatherosclerosis. Our improved method could be a useful animal model to study the mechanism and treatment of neoatherosclerosis.

**Limitations:** The limitations of this study need to be mentioned. First, no animal model completely mimics human neoatherosclerosis; thus, careful extrapolation of our results to humans is necessary. Second, the technology of microsurgery suturing requires much time to practice for new experimenters, and microsurgery tools are required. Third, the artery sutured by the 7-0 suture could have been narrower than normal arteries.

**Disclosure**

**Conflicts of interest:** None.

**References**


**Supplemental Files**

Supplemental Table
Please see supplemental files; https://doi.org/10.1536/ihj.17-737