Published in final edited form as:

Atherosclerosis. 2010 April ; 209(2): 359–366. doi:10.1016/j.atherosclerosis.2009.09.071.

A Novel Mouse Model of In-Stent Restenosis

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Abstract

Background and aims—In-stent restenosis (ISR) is the major complication that occurs after percutaneous coronary interventions to facilitate coronary revascularization. Herein we described a simple and cost-effective model, which reproduces important features of ISR in the mouse.

Methods and results—Microvascular bare metal stents were successfully implanted in the abdominal aorta of atherosclerotic ApoE-null mice. Patency of implanted stents was interrogated using ultrasound biomicroscopy. Aortas were harvested at different time points after implantation and processed for histopathological analysis. Thrombus formation was histologically detected after 1 day. Leukocyte adherence and infiltration were evident after seven days and decreased thereafter. Neointimal formation, neointimal thickness and luminal stenosis simultaneously increased up to 28 days after stent implantation. Using multichannel fluorescence molecular tomography (FMT) for spatiotemporal resolution of MMP activities, we observed that MMP activity in the stented aorta of Apo-E null mice was 2-fold higher than that of wild-type mice. Finally, we compared neointimal formation in response to stenting in two genetically different mouse strains. In-stent neointimas in FVB/NJ mice were 2-fold thicker than in C57BL/6J mice ($p = 0.002$).

Conclusion—We have developed a model that can take advantage of the multiple genetic resources available for the mouse to study the mechanisms of in-stent restenosis.

Keywords

In-Stent Restenosis; Mouse; Stent; Animal Model

1. Introduction

In-stent restenosis (ISR) is the major complication that occurs in 5-25% of cases after percutaneous coronary interventions (PCI) [1-3]. ISR is a pathologic inflammatory and proliferative response to the vascular injury and stenting procedure that leads to vascular re-occlusion [4-6]. The molecular mechanisms underlying the development of ISR have not been fully characterized. Identifying these mechanisms may represent a breakthrough that provides new therapeutic targets and save lives.

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Reliable animal models of ISR are required to dissect the mechanisms underlying the exaggerated arterial response to injury. ISR experimental models in rabbit [7,8], dog [9], pig [10,11] and rat [12,13] have been developed and used in preclinical studies. Although these models reproduce aspects of the human vascular response to PCI and stenting, they have some limitations [14]. In particular the experimental animals used for the study of ISR usually lack the phenotypes for acquired heritable metabolic disorders which preclude studies of important risk factors for the disease such as diabetes, obesity, and systemic hypertension.

On the other hand, mice have become the premier animal model for the study of numerous complex human diseases [15]. Mouse models are cost effective and have a favorable generation time for cross breeding experiments. They allow the use of genetically modified strains such as the ApoE and LDLR knockout mice that are relevant to atherosclerosis and other vascular diseases [16-18]. Recently, Ali et al. reported the first mouse model of ISR [19]. The authors described neointimal development after grafting a balloon injured and stented aorta to a recipient carotid by sleeving the aortic ends over artery cuffs.

In this study, we describe a novel, relatively simple and reproducible model of ISR in the mouse. We successfully deployed microvascular stents in the abdominal aorta of atherosclerotic mice. We also evaluated the vascular response to stent implantation in two genetically different mouse strains. The mouse model of ISR herein described should assist researchers in the finding of molecular and biochemical mechanism that leads to vascular re-occlusion after stent implantation.

2. Materials and Methods

2.1 Animals

Animal procedures were approved by the University of Miami Institutional Animal Care and Use Committee (IACUC) and conform to the guide for the care and use of laboratory animals published by US National Institute of Health (NIH publication No. 85-23, revised 1996). Experimental mice (C57BL/6J, FVB/NJ and ApoE-null) were obtained from Jackson Laboratories (Bar Harbor, Mn). Only males over 30g body weight (12-16 weeks old) were used. Atherogenic diet (1.25% cholesterol, no cholate; Research Diets, New Brunswick, NJ) was given to mice 8 weeks before surgery until sacrificed. Aspirin (100 mg/L in the drinking water) was given one week pre-surgery until sacrificed to prevent acute vessel closure. Stented vessels of ApoE-null mice were harvested 1, 7, 14, and 28 days post-operatively.

2.2 Arterial Stenting Procedures

Operative procedures are shown in Figure 1A-F. Mice were anaesthetized with isoflurane (Forane, Baxter Healthcare Corporation, Deerfield, IL). A midsagittal incision was made to access the abdominal aorta with standard surgical techniques. Two microhemostatic clamps (Aesculap AG, Center Valley, PA) were placed onto the aorta, one distal to the renal arteries and one proximal to the aortic bifurcation. A small longitudinal arteriotomy was made in the distal abdominal aorta and the balloon catheter was advanced after topical application of 3–5 drops of Lidocaine HCL (10 mg/ml, Hospira INC, Lake Forrest, IL). The balloon catheter (NIC Nano 0.85 ULP CTO – Balloons SIS-Medical AG, Winterthur, Switzerland) was inflated at 2 atmospheres to dilate the artery (balloon-to-vessel ratio of 1.9:1). Arterial dilatation damages the endothelium and causes enough arterial injury to stimulate vascular smooth muscle cell proliferation and neointimal formation. A plastic cuff containing a five-side stainless steel stent, 2.5 × 0.6 mm in dimension (Figure 1G; NanoInterventions, St. Paul, MN) was guided into the dilated artery. The cuff was retrieved and the stent delivered at the site of implantation. The balloon catheter was passed through the stent and the stent was deployed using a single inflation at 2 atmospheres for 10 seconds (stent-to-vessel-ratio of 1.1:1). After deflation and removal of

the balloon, the aortic incision was closed with a 10-0 suture using continuous sutures. Reperfusion was established in both procedures by removing the clips and the abdomen was closed with 4-0 absorbable sutures. The median total procedure time was approximately 40 minutes.

2.3 Tissue processing, and histopathological analysis

Stented vessels were perfusion-fixed *in situ* with 10% neutral buffered formalin solution (Sigma-Aldrich, St. Louis, MO). Stent proximal and distal aortic sections (1 mm, “sham controls”) were paraffin-embedded and processed for histopathological analysis. Stented arteries were sent to Charles River Laboratories, Pathology Associates (Frederick, MD) for histology. Arteries were processed according with Rippstein et al. (2006) with some modifications [20]. The specimens were embedded in methylmethacrylate: dibutyl thalate at 7:3 ratio with Perkadox 16 as the activator. The stents were sectioned using a rotary microtome at approximately 5 μ M. Five independent sections were cut from proximal, middle and distal parts of the stent. Sections were deplastified (see below) and stained with hematoxylin-eosin.

Histomorphometric analysis was performed as previously described [19]. Total vessel area, and the area of the lumen (LA), media, and neointima (NA) were measured using Image Pro Plus (Media Cybernetics, Inc., Bethesda, MD). Stent expansion was calculated as the area inside a polygon connecting the midpoint of each stent strut. Neointimal thickness was the perpendicular distance from lumen to internal elastic lamina at the midpoint between stent struts on each section. The percentage of stenosis was defined as the percentage of lumen occupied by the neointima $[(NA*100)/LA]$.

Total cell density in the neointima was measured in H&E stained sections using computerized threshold segmentation of colors (Image Pro Plus, Media Cybernetics, Inc.) and expressed as numbers of cells (nuclei) per mm^2 . Macrophages, leukocytes and VSMCs were counted manually in 200X fluorescence microscopy pictures.

2.4 Injury and Inflammation Scores

Injury and inflammation scores after stenting were determined as described by Schwartz et al. [11] and Kornowski et al. [21] respectively.

2.5 Fluorescence microscopy

Sections were brought through solutions of 2-methoxyethylacetate for 30 min twice, acetone for 10 min, and distilled water for 5 min twice. The slides were boiled in 10 mM sodium citrate buffer pH 6.0 for 25 minutes to retrieve the antigens. The blocking of non-specific binding sites was achieved with PBS-FBS 15 % for 20 minutes at room temperature (RT). The primary antibodies, mouse anti-human smooth muscle actin clone 1A4 (1:50, Dako), macrophage rat anti-mouse Mac-3 (1:50, BD Biosciences, Pharmingen), and pan-leukocyte rat anti-mouse CD45 (1:10, BD Biosciences, Pharmingen) were diluted in PBS-BSA 10% and incubated overnight at 4 °C. Unbound antibodies were washed with PBS- 1% Tween 20 twice for 3 min. Following a second incubation with PBS-FBS 15% for 20 minutes, tissue sections were incubated with either Alexa Fluor 546 goat anti-mouse (1:100 Invitrogen) or Alexa Fluor 488 goat anti-rat (1:100, Invitrogen) for 90 min at RT. Sections were DAPI counterstained (Sigma, St. Louis, MO) and mounted in Cytoseal (Richard-Allan Scientific, Kalamazoo, MI). The stained sections were examined with an IX71 inverted fluorescence microscope (Olympus, Center Valley, PA). Image analysis was performed using Image Pro Plus (Media Cybernetics).

2.6 Ultrasound Biomicroscopy

The abdominal hair was carefully removed by chemical depilation with Nair (Church & Dwight Co, Princeton, NJ). Ultrasound transmission gel was applied to ensure optimal image quality. To evaluate the position of the stent, endovascular images were acquired with a Vevo770 high-frequency ultrasound system (Visualsonics, Toronto, Canada). Imaging involved a 55-MHz scan head, with a 4.5-mm focus and an axial resolution of 30 μ m. First, on B-mode, a long axis view was used to visualize the stent in the aorta. Doppler mode was further used to measure the blood flow velocity at the site of interest in the stent.

2.7 Fluorescence Molecular Tomography

Mice were on low fluorescent chow (Harlan Teklad AIN-76A, Harlan Laboratories, Madison, WI) for one week before imaging as normal mouse chow contains alfalfa that is autofluorescent in imaging systems. Two days before imaging, mice drank a polyethylene glycol electrolyte solution (Golytely, Braintree Laboratories, Braintree, MA) to clean out the gut of chow remnants that may have inherently fluoresce and increase the background gut signal. Mice were scanned before probe injection to demonstrate the absence of confounding fluorescence signals. Then, twenty four hours before imaging, anesthetized mice received a single injection of 5 nmol of MMPsense-680 (VisEn Medical, Bedford, MA) via the tail vein. Proteolytic cleavage of the probe results in the release of fluorochromes and fluorescence generation. MMPsense-680 has excitation and emission wavelengths of 680 ± 10 nm and 700 ± 10 nm, respectively. Mice were imaged using a commercially available FMT imaging system (VisEn Medical). Quantitative reconstruction of 3-dimensional maps of fluorochromes was based on Born-forward algorithms [22]. After FMT imaging, mouse stented aortas were harvested and preserved in liquid nitrogen for RNA isolation.

2.8 RNA isolation and quantitative TaqMan RT-PCR

Stented aortas were pounded in liquid nitrogen using a Bio-Pulverizer (Biospec Products, Bartlesville, OK). Stent fragments were removed with microsurgery forceps. Remaining arterial tissue was suspended in 400 μ L of Trizol reagent (Invitrogen, Carlsbad, CA) and homogenized using an Ultra-Turra T 8 basic Disperser/Homogenizer (IKA Works, Wilmington, NC). Total cellular RNA was prepared according to the Chomczynski and Mackey method [23]. MMP-2, -9, -13 and β -actin mRNAs were quantified using the TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA). Real-time PCR was performed on an ABI Prism 7500 Fast Real-Time PCR System (96-well plate). Relative gene expression was determined using $\Delta\Delta$ CT method [24].

2.9 Statistics

Data is expressed as means $\pm$ SEM. Statistical analyses were performed by one-way ANOVA followed by the Bonferroni test for multiple comparisons. Differences in neointima formation and stenosis after 28 days between C57BL/6J and FVB/NJ mice were compared with t test for independent samples.

3 Results

3.1 Surgical Procedures

After standardizing all surgical procedures, sixty-seven mice (36 ApoE-null, 12 C57B6/J and 12 FVB/NJ) weighing over 30g underwent stent placement in the abdominal aorta. A detailed operative procedure is shown in Figure 1A-F. The total procedure of stent implantation in the mouse took approximately 40 min. Seventeen (17) mice developed hind limb paralysis because of thrombosis and died post-operatively. Another seven animals died during surgery due to

bleeding from the abdominal aorta at the site of the suture. The procedural success rate was of 64.17%. All stented aortas were patent at the time of harvesting.

To explore noninvasive means of evaluating patency, implanted stents were visualized using ultrasound biomicroscopy on day 1, 7, 14 and 30 post-implantation. Figure 1I and J represent the data from a stent implanted in aorta at day 28. Using this technique, patency was confirmed in all stents. Some stents interrogated in this manner demonstrated some degree of stenosis as determined by an increase in the distal velocity, but no evidence of occlusion was noted (data not shown). These data are consistent with our physical examination.

3.2 Histomorphometry

Histomorphometric measurements at different time points after stent implantation in the aorta of ApoE-null mice are presented in Table 1. Stent expansion was similar through all time points indicating that in-stent restenosis was the result of the neointimal development. Stent expansion increased the total vessel wall area by approximately 25% compared with reference aorta. No differences were observed in the media areas. After 7 days, the neointima was limited to strut-associated thrombi and inflammatory tissue (Figure 2B). The neointimal hyperplasia increased progressively over a period of a month. The neointimal area increased from 0.02 ± 0.04 at day 7 to $0.09 \pm 0.04 \text{ mm}^2$ at day 28 after stent implantation. Similarly, neointimal thickness increased from 23.60 ± 9.42 to $60.38 \pm 19.38 \text{ }\mu\text{m}$. The neointima to media ratio increased by 3.2-fold ($p = 0.023$) over this period of time. Consistent with these findings, luminal area decreased between day 1 and day 28 by $11.53 \pm 1.78 \%$. Neointimal development was not detected in the stent's proximal and distal aortic segments (Figure I, Supplemental data). These segments were balloon dilated as the balloon was longer than the stent; yet they never got stented. It demonstrates that neointimal development in our model was due to stent deployment and not a simple healing response to the injury inflicted by balloon dilation. Injury scores were not different among time intervals.

3.3 Histology

Seven days after stent implantation, focal thrombus formation around the stent struts was observed (Figure 2B). At this time inflammatory cells were already infiltrated in the stented vascular wall. The inflammation scores are shown in Table 1. The inflammatory response reached its peak between 7 to 14 days post-procedure. The neointima completely covered the stent struts at day 28 (Figure 2A) and mainly consisted of smooth muscle actin positive cells ($63.23 \pm 16.87 \%$, Figure 2C) and extracellular matrix. Infiltrated cells around stent struts and in the vascular wall at day 7 were mainly of leukocyte origin as revealed by immunofluorescence microscopy with a specific pan antibody ($3.76 \pm 1.24 \%$, Figure 3C). However, only few of them were macrophages ($0.02 \pm 0.007 \%$, Figure 2C). Straightforward morphological identification of individual CD45 positive cells indicates that acute inflammatory cells (polymorphonuclear leukocytes) were present within the thrombus around the stent struts in 79% of arterial sections harvested prior to 14 days. In contrast, chronic inflammation (lymphocytes) was found infiltrated within the vascular wall and adventitia ($<90\%$) at any given time point after stenting. The total neointimal cell density significantly increased from $159 \pm 47.12 \text{ cells/mm}^2$ at day 7 to $1520.80 \pm 693.24 \text{ cells/mm}^2$ at day 28 post-stenting.

3.4 Noninvasive Fluorescent Molecular Tomography (FMT) imaging of MMP activity in stented aortas

MMPs (including MMP-9, MMP-13 and MMP-2) produced by inflammatory and vascular smooth muscle cells are involved in the progression and pathology of ISR [25-27]. We used a pan-MMP optical sensor that consists of near-infrared fluorochromes bound to polymers through specific protease peptide substrates to *in vivo* image MMP activity after stenting in the mouse. The bound fluorochromes are quenched, but cleavage of the scaffold by specific MMPs

releases fluorochromes and generates intense fluorescence. Each active MMP enzyme can activate multiple reporters, leading to signal amplification. We administered this MMP sensor in ApoE-null and wild-type (C57BL/6J) mice that had undergone aortic stenting 28 days before. No confounding fluorescence signal was found in the stenting aorta prior to probe injection. The injection of the sensor resulted in strong focal fluorescence on distal FMT slices of the abdomen. The FMT signal in the descending aortas co-localized with the regions where the stent was placed (Figure 3A and B). Noninvasive FMT analysis revealed a significant increase in MMP activity in stented aortas of ApoE-null mice with respect to the stented aortas of wild-type mice (10.00 ± 3.05 vs. 4.26 ± 0.96 , $p = 0.034$). Such increase in MMP activity in ApoE-null with respect to wild-type mice was further confirmed by TaqMan Real Time RT-PCR. Stented aortas in ApoE-null mice contained more transcripts encoding for MMP-2 (3.25 ± 0.72 fold), -9 (1.92 ± 0.08 fold) and -13 (1.64 ± 1.12 fold) than those in wild-type mice (Figure 3C). These *in vivo* FMT findings corroborated also with *ex vivo* histopathological analysis, which showed thicker neointimas in aortas of ApoE-null mice than in those of wild-type mice (neointima area: 0.09 ± 0.06 vs. 0.04 ± 0.01 mm², $p = 0.04$). The increase in MMP activity and neointimal thickness in stented vessels of ApoE-null mice was associated with higher numbers of in stent neointimal VSMC at the site of injury (1520 ± 693 vs. 837 ± 143 , $p = 0.043$). No significant differences were found between these two groups with respect to the number of macrophages and leukocytes at 28 days post-stent implantation. These results suggest that optical fluorescence tomography is a useful noninvasive technique that can be used to detect *in vivo* MMP activities associated with vascular remodeling after stenting in the mouse.

3.5 Comparison of the vascular response to stenting in C57BL/6J and FVB/NJ mice

We next sought to determine the vascular response to stent injury in two genetically different mouse strains (C57BL/6J and FVB/NJ). The comparative histomorphometric analysis is presented in Figure 4 and more extensively in Table II (Supplemental Data). Neointimas were thicker in aortas from FVB/NJ than in those from C57BL/6J mice (neointima area: 0.04 ± 0.01 vs. 0.09 ± 0.02 , $p = 0.021$) 28 days after stent placement (Figure 4). The neointima to media ratio of the FVB/NJ stented aortas was 2-fold higher than that observed in the C57BL/6J mice ($p = 0.002$). Consistently with these data, there was a significant luminal reduction in the aorta of FVB/NJ in response to stenting with respect to the aorta of C57BL/6J mice (35.71 ± 11.61 vs. 18.86 ± 5.43 , $p = 0.043$). Inflammatory response was also increased in FVB/NJ mice. Importantly, stent expansion was not different between the two strains indicating that in-stent stenosis was the result of neointimal hyperplasia and not due to differences in operative procedures.

4 Discussion

In the present study, we have characterized a new model for ISR and evaluated the vascular response to PCI and stent implantation in the mouse abdominal aorta. A number of characteristics of this model are noteworthy. Neointimal formation and the percentage of luminal stenosis simultaneously increased up to 28 days after stent implantation. Although the absolute area of ISR formation in ApoE-null mice is modest, it is still robust, and significant enough to provide a model to evaluate anti-restenotic drugs and to carry out basic studies to determine the molecular mechanisms underlying the post-stenting neointimal formation.

For years, pre-clinical studies of ISR were limited to large animals including rabbits, dogs and pigs [7-11]. Although they are valuable, these large animal models have limitations. Firstly, these models, specifically the pig, are expensive. Secondly the potential for evaluating underlying pathophysiological mechanism is restricted as only a limited number of antibodies and transgenic or knockout strains are available in pigs and rabbits. Thirdly, the effect of important risk factors for ISR such as diabetes and systemic hypertension cannot be studied

because those phenotypes are unavailable in these species. In order to overcome these limitations, several groups have described the deployment of small-caliber human stents in the aorta and carotid artery of the rat [12,13]. The rat models of ISR still lack preexistent arterial pathology such as atherosclerosis that is available in knockout mouse strains. Consequently, a mouse model for ISR would greatly facilitate in-depth mechanistic studies of ISR because antibodies, genetic information, transgenic and knockout strains are readily available for this species.

The first mouse model of ISR was recently reported by Ali and associates in 2007 [19]. In this study, the thoracic aorta of ApoE-null mice was stented ex-vivo and transplanted into the carotid of a syngeneic recipient. In this model, the neointima in ApoE-null mice at day 28 was of 0.26 mm² - which is significantly thicker than the neointima developed by direct stenting of the mouse aorta (0.09 mm²) herein reported. Differences in neointimal development between the two models could be explained, at least in part, by differences in the ischemia time, and the degree of surgical trauma. In our model there is less vascular trauma due to surgical manipulations and ischemia and neointimal development occurs mainly as a result of the stent deployment without the need for transplantation and massive disruption of *vasa vasorum* and nerves in the adventitial layer.

We also evaluated pathophysiological mechanisms of ISR thrombus formation, inflammation and neointimal formation. Thrombus formation was present around the stent struts at 7 days after implantation. Leukocyte infiltration was also evident at that time. Adherence and infiltration of leukocytes peaked after 7 and 14 days, respectively. After 28 days the inflammatory response was significantly diminished. The neointima, which consisted mainly of smooth-muscle-like cells and extracellular matrix progressively increased in thickness up to 28 days after stent implantation. These histological findings resemble the events observed after stent deployment in porcine coronary [9] and rabbit iliac arteries [7,8]. However, it remains to be established whether our findings in the mouse abdominal aorta can be extrapolated to the changes observed in a stented human coronary artery.

Recently, research on the inflammatory components that cause ISR has moved from postmortem histological studies to less invasive virtual histology approaches using optical coherence tomography and intravascular ultrasound [28-30]. However, there is still a great need for state-of-the-art imaging methods to validate vascular re-endothelialization (healing), and stenotic progression over time to evaluate treatment responses. In this study we demonstrate that noninvasive molecular imaging of MMP-associated proteinase activity reflects ISR severity. We found that stented aortas of ApoE-null mice contained more MMPs and developed more ISR than those stented arteries in WT mice. As expected and accordantly to previous reports, detecting MMP activity in the stent using FMT agrees with the MMP gene expression levels measured by Real Time PCR [31]. The use of Fluorescence Molecular Tomography for *in vivo* molecular imaging may improve our fundamental understanding of cellular dynamics after PCI and stenting because the behavior of cells in tissues is dictated by local factors that often cannot be reproduced *in vitro*. Other studies to validate the use of pan-MMP sensors and FMT as a diagnostic tool for ISR are warranted.

Finally, we investigated the biological response to stenting in two distant mouse strains. Similar to previous studies, which demonstrate that genetic background plays an important role in the development of atherosclerosis [32] and arterial remodeling after injury [33], we found significant differences in the degree of neointimal formation after stenting between FVB/NJ and C57BL/6J mice. Neointimas developed in aortas of FVB/NJ mice were twice as thick as those that developed in the arteries of C57BL/6J mice. The cause of the exaggerated neointimal formation in FVB/NJ mice is unknown so far. Pilot studies from our lab have recently shown that FVB/NJ mice have a slower re-endothelialization rate after vascular injury than C57BL/

6J mice which could prolong arterial inflammation and favor the development of thicker neointimas (data not shown). Further studies to determine the causes of the exaggerated response of FVB/NJ mice to vascular injury and stenting are also warranted. However, these results confirmed previous studies using the wire-injury model in the mouse and support the hypothesis that intimal hyperplasia is influenced by genetic variations between strains [33]. Our animal model of aortic stent should serve as a valuable tool for the determination of candidate genes and biologic pathways underlying ISR susceptibility.

In conclusion, we have implemented a mouse model of in-stent restenosis in nonatherosclerotic and atherosclerotic inbred mouse strains. While the neointimal area was larger in C57BL/6J ApoE-null mice compared to wild-type mice, the neointimal development was also found exaggerated in FVB/NJ mice. These results clearly demonstrate that the presence of pre-existing lesions and the genetic background are independent factors that may significantly modify the vascular response to injury. However, we cannot say based on this study which one is the most important at determining the degree of intimal hyperplasia or if there are any interactions between them that may worsen in-stent restenosis. For that, it would have been necessary to include the FVB/NJ ApoE-null mice in our study. Unfortunately, to our knowledge this mouse is not commercially available.

Limitation of this study

The high shear stress conditions in the mouse aorta could affect the natural course of ISR, and favor stent patency compared with other models. Also, as discussed by others, most animal models of ISR including ours are simplistic representations of the actual clinical ISR. For instance, in the clinical setting, stents are placed onto atherosclerotic plaques while the animal vessels have much less disease. Although we used hypercholesterolemic mice with atherosclerotic lesions, we have not yet targeted the stent directly onto pre-existing plaque. Another limitation of this study could reside in the use of relatively low pressures for the intra vascular stent implantation. The use of a low pressure for stent deployment may on occasion lead to malapposition of stent with the subsequent acute in-stent thrombosis and animal death.

Conclusion

In this study we have demonstrated the feasibility of mouse model of in-stent restenosis, one of the main complications after the re-opening of atherosclerotic arteries, by deploying a bare metal stent directly into the abdominal aorta. Because this model is relatively simple and inexpensive it is attractive for high-throughput stent research. In addition, this model provides the opportunity to investigate molecular pathways and explore novel therapeutic targets for in-stent restenosis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant (5RO1 AG023073) and Florida Department of Health DOF Grant (11NIR06). We thank Irene Hung for her clerical assistance. We thank Dr. Levitt Roy's Laboratory and ViSen Medical Inc. for their support in FMT experiments.

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Figure 1. Stent Placement into the Mouse Abdominal Aorta

A) After the abdominal aorta was isolated, two microvascular clamps were placed distal to the renal arteries and proximal to the aortic bifurcation. A small arteriotomy was made in the distal abdominal aorta. **B)** A balloon catheter was advanced into the aorta (after topical application of 3–5 drops of Lidocaine HCL) and inflated to 2 atmospheric pressures to dilate the artery. **C & D)** A microvascular stent was loaded onto a plastic cuff made from a gauge angiocath and delivered into the mouse aorta. **E & F)** An angioplasty balloon was passed through the stent and inflated to open the stent. The catheter was removed, and the arteriotomy repaired. **G).** Microvascular stent (BMS). **H)** Illustrative microphotography of an implanted BMS in the mouse aorta. **I)** Visualization of the stent in the mouse aorta at day 28 using ultrasound

biomicroscopy. **J**) Color Dopler demonstrated blood flow through the stent and confirming stent patency. **Art**- arteriotomy, **MC**- microvascular clamps, **BC**- balloon catheter.

Figure 2.

Kinetics of vascular response to stent implantation in the mouse abdominal aorta. Stented aortas of atherosclerotic ApoE-null mice were harvested at day 1, 7, 14 and 28 days post-surgery. Arteries were plastic embedded and cross sections stained with hematoxylin and eosin (H&E). **A)** Cross sections from stented aortas at day 28. **B)** Local thrombus formation around the stent struts with infiltrated leucocytes was observed at day 7. Cell infiltration significantly increased at day 14 and an organized neointima was observed at day 28. **C.** Vascular Smooth Muscle Cells (VSMC), leukocytes and macrophages were detected in the neointima of stented vessels using anti- smooth muscle cell actin (**SMA**), anti- CD45 (**CD45**) and anti-Mac-3 monoclonal antibodies (**Mac-3**). Sections were visualized using fluorescence microscopy. Representative macrophages and leukocytes are indicated with white arrowheads. Magnification 200x.

Figure 3.

In vivo measurement of Matrix Metalloproteinase (MMP) activity in stented aortas by Fluorescent Molecular Tomography (FMT). **A)** Images of MMP activity (by FMT) in ApoE-null and wild-type mice 28 days after bare metal stent placement. **B)** Stenting induced a significant increase in MMP activity in ApoE-null mice with respect to that in wild-type (WT) animals, $p < 0.05$, $n=3$. **C)** Stented vessels in ApoE-null mice showed increased MMP-2,-9 and 13 mRNA levels versus those in WT as determined by TaqMan Real Time RT-PCR.

Figure 4.

FVB/NJ genetic background increases neointimal formation after stent implantation. **A).** Hematoxylin and eosin (H&E) stained cross-sections of stented aortas of C57BL/6J and FVB/NJ mice 28 days after stent implantation. Note the robust in-stent neointima formation in FVB/NJ mice. Minimal neointima formation was seen in the C57BL/6J mice after stenting. Magnification x100. **B).** Histomorphometric characteristics of neointimal response to stenting in C57BL/6J and FVB/NJ mice. $p < 0.05$, $n=6$.

Table 1

Time course of the vascular response to stent implantation in ApoE-null mice.

	Day 1	Day 7	Day 14	Day 30
Mice per group (n)	4	6	6	6
Total vessel area, mm ²	0.13	0.37 ± 0.10	0.57 ± 0.35	0.45 ± 0.09
Neointimal thickness, μm	-	23.60 ± 9.42	39.06 ± 27.77	60.38 ± 19.84
Neointimal area mm ²	-	0.02 ± 0.04	0.05 ± 0.02	0.09 ± 0.04
Medial Area, mm ²	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.02
N/M ratio	-	0.50 ± 0.07	1.25 ± 0.06	1.80 ± 0.06
Lumen Area, mm ²	0.26 ± 0.05	0.21 ± 0.04	0.26 ± 0.07	0.23 ± 0.08
% Stenosis	-	9.84 ± 5.49	19.48 ± 10.59	37.96 ± 10.79
Stent Expansion, mm ²	0.18 ± 0.08	0.17 ± 0.04	0.16 ± 0.02	0.21 ± 0.05
Injury Score	1.13 ± 0.71	1.80 ± 0.42	1.23 ± 0.41	1.37 ± 0.29
Inflammation Score	0.32 ± 0.2	2.20 ± 0.33	2.13 ± 0.30	0.80 ± 0.22