In Vivo Comparison of a Polymer-Free Biolimus A9-Eluting Stent With a Biodegradable Polymer-Based Biolimus A9 Eluting Stent and a Bare Metal Stent in Balloon Denuded and Radiated Hypercholesterolemic Rabbit Iliac Arteries

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Objectives: To evaluate the effect of a polymer-free Biolimus A9-eluting stent [BioFreedomTM (BF)], compared with that of a biodegradable polymer-based Biolimus A9-eluting stent [BioMatrix FlexTM (BMF)] and a bare metal stent (BMS) in balloon denuded and radiated hypercholesterolemic rabbit iliac arteries. Methods: Rabbits were fed with 1% cholesterol diet (n = 14) for 14 days, both iliac arteries were balloon denuded and radiated, and then rabbits were switched to 0.15% cholesterol diet. After 4 weeks, BF (n = 8), BMF (n = 8), and BMS (n = 8) were deployed in denuded and radiated areas. Four weeks later animals were euthanized, arterial segments were processed for morphometry. Results: The neointimal area in vessels implanted with BF stents was significantly less than that seen in vessels implanted with BMS (0.90 mm² ± 0.14 vs. 1.29 mm² ± 0.23, P < 0.01). Percent fibrin and fibrin score were higher with BMF stents compared to BMS (P <0.03 and <0.04) and giant cell number was significantly higher with both BMF and BF stents (P < 0.01 for both). Percent endothelialization was significantly higher and % uncovered struts were lower with BMS compared to either BMF or BF stents (P < 0.05 for both). Conclusion: This study demonstrates that compared to BMS, BF stents significantly decreased neointimal hyperplasia.

Key words: drug-eluting stent; polymer; biolimus A9; neointima

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Coronary stents have revolutionized the field of interventional cardiology, and stent implantation has become the standard of care in percutaneous coronary intervention. However, long-term success of coronary stenting is hampered by in-stent restenosis [1]. Drug-eluting stents (DESs) possess controlled release of antiproliferative agents from durable polymers and have reduced angiographic and clinical measures of restenosis compared with bare metal stents (BMSs) [2,3]. However, restenosis still occurs [4], and very late stent thrombosis is more germane to DES than to BMS [5,6], owing to delayed healing and re-endothelialization [7–9]. Although re-endothelialization is multifactorial in cause, it is partially attributed to inflammation possibly induced by durable polymers [10,11]. To curtail durable polymer-induced inflammation, new generation stents were designed with a focus on either utilization of biodegradable polymer coating [12–14] or on a completely polymer-free platform [15,16] to deliver the antiproliferative drugs.

We developed a balloon-denuded and radiated hypercholesterolemic rabbit model. The plaque developed in traditionally used balloon-denuded hypercholesterolemic rabbit models is rich in smooth muscle cells with few macrophages, whereas the plaque developed in our model is rich in macrophages expressing metalloproteinases with few smooth muscle cells, a characteristic of vulnerable plaque [17]. The proinflammatory characteristics of the plaque in this particular model could be attributed to increased oxidation of low-density lipoprotein following radiation [18]. Antioxidants [18], pioglitazone [19], and clopidogrel [20] could modify the plaque characteristics of this model. The purpose of this investigation was to evaluate the short-term effect of BMS (stainless steel BioFlex II™ stent, Biosensors International, Newport Beach, CA), the biodegradable polymer-coated Biolimus A9-eluting stent [BioMatrix Flex™, (BioFlex II™ platform with poly-lactic acid as biodegradable polymer with Biolimus A9) Biosensors], and the polymer-free Biolimus A9-coated stent [BioFreedom™, (BioFlex II™ platform, with a textured abluminal surface, onto which Biolimus A9 in a solvent is applied), Biosensors] on inflammation and in-stent restenosis. Thus, all three stents have the same platform and both DESs have the same drug (n = 14) for 14 days. On day 15, the animals were anesthetized with intramural injections of ketamine (35 mg/kg) and xylazine (5 mg/kg). During the experimental procedure, anesthesia was maintained by isoflurane and oxygen. A 5-F arterial sheath was introduced via the retrograde approach into the left carotid artery and advanced to the iliac artery under fluoroscopic guidance. An intra-arterial injection of 500 IU of heparin was given. A baseline angiogram was obtained and recorded; both proximal iliac arteries were balloon denuded. Following balloon denudation, a closed-end lumen radiation catheter was introduced with over the wire delivery into the left carotid artery with fluoroscopic guidance and advanced to the denuded area of the iliac artery. A 90Sr source ribbon was inserted into the delivery catheter, and its position was verified by cinefluoroscopic imaging. The prescribed dose was 15 Gy at 2 mm from the center of the source. Following the assigned treatment, the delivery catheter was removed, the carotid artery was ligated, and the cut-down site was closed with sutures.

Following balloon denudation and radiation, animals were fed a diet supplemented with 0.15% cholesterol for 28 days. On the 42nd day, a 5-F arterial sheath was introduced retrogradely into the right carotid artery and advanced to the iliac artery under fluoroscopic guidance. An intra-arterial injection of 500 IU of heparin was given. The baseline angiogram was recorded, the balloon denuded and radiated area was verified by cinefluoroscopic imaging, and stents (BMS, n = 8; BioMatrix Flex™, n = 8; and BioFreedom™, n = 8 [Biosensors International, Newport Beach, CA]) were deployed in iliac arteries by high-pressure balloon inflation to achieve a 1:1.0 to 1.2:1.0 stent-to-artery ratio. Following the deployment of one stent in each iliac artery, the delivery catheter was removed, the common carotid artery was ligated, and the cut-down site was closed with sutures. Animals were allowed to recover and then returned to care facilities. Animals continued to be fed a diet supplemented with 0.15% cholesterol (Fig. 1).

Tissue Harvest

Twenty-eight days after stenting, animals were anesthetized and a follow-up angiogram of the iliac arteries was completed, followed by euthanasia and perfusion-fixation. Stented arteries were embedded in methylmethacrylate with sections (4.0 μm) taken from the proximal, medial, and distal portions of each stent. A 3-mm arterial segment just proximal and distal to the stents was processed and stained to evaluate edge effects. All sections were stained with hematoxylin and eosin, and

METHODS

Animal Preparation

Animal care and procedures were carried out in accordance with the guide for the care and use of laboratory animals. Male New Zealand White rabbits weighing 3.5 to 4.2 kg (Robinson Services, Inc. Clemmons, NC) were fed a diet supplemented with 1% cholesteroling 3.5 to 4.2 kg (Robinson Services, Inc. Clemmons, NC) were fed a diet supplemented with 1% cholesterol...
Movat pentachrome stain [19]. Sections were immuno-stained with RAM 11 to identify macrophages [19].

**Vessel Morphometry**

An independent observer blinded to the experimental groups performed morphometric analysis. The cross-sectional areas of proximal, middle, and distal stent sections were measured with computer assisted digital morphometry (IP Lab Spectrum Software, Scanalytics Inc, Rockville, MD) to determine the areas within the external elastic lamina (EEL), internal elastic lamina (IEL), or stent, and vessel lumen. The area within the IEL or stent was considered as the normal reference lumen area. The percent area stenosis was then defined as: \[ \text{(stent area - IEL area)/(stent area)} \times 100. \] Neointimal area was determined by subtracting the area of the lumen from the area within the stent wires. Neointimal thickness extending perpendicular from above the stent to the lumen surface was measured at each wire site. Inflammation, intimal fibrin, and endothelialization were evaluated using the following scales. Inflammation was graded as: 0 = none; 1 = scattered inflammatory cells; 2 = inflammatory cells encompassing 50% of a strut in at least 25–50% of the circumference of the artery; or 3 = inflammatory cells surrounding a strut in at least 25–50% of the circumference of the artery. The intimal fibrin content was graded as: 1 = focal residual fibrin involving any portion of the artery and for moderate fibrin deposition adjacent to the strut involving <25% of the circumference of the artery; 2 = moderate fibrin deposition involving >25% of the circumference of the artery or heavy deposition of fibrin adjacent to and between stent struts involving <25% of the circumference of the artery; or 3 = heavy deposition of fibrin involving >25% of the circumference of the artery. The number of giant cells per strut was counted. The stent endothelialization score was defined as the extent of the circumference of the arterial lumen covered by endothelial cells and was scored from 1 to 3 and expressed as percentages (1 = 25%; 2 = 25–75%; and 3 = >75%). Uncovered strut % was determined by dividing the number of stent struts without neointimal coverage/number of stent struts × 100. Macrophage positive area was expressed as the percentage plaque area stained positive for the RAM 11.

**Statistical Analysis**

Histopathology and immunohistopathology morphometric measurements were made in at least three sections per vessel. Results were expressed as mean ± standard deviation. Comparisons between the groups were performed by unpaired student’s *t*-test using the StatView software package (SAS Institute, Cary, NC). Significance was established by a value of *P* ≤0.05.
RESULTS

All animals (n = 14) appeared well and tolerated the 1% cholesterol supplemented diet. During the denudation and radiation procedures, one animal died due to technical problems and one died after the procedures. In the latter case, necropsy revealed a large trichobezoar and pronounced liver lesions. The remaining 12 animals tolerated the 0.15% cholesterol supplemented diet and went through the stenting procedure. Within 48 hr after stent implantation four animals died, two due to vessel dissection and two for unknown reasons. Sixteen vessels with BMS (n = 6), BioMatrix Flex (n = 4), and BioFreedom (n = 6) from the eight surviving animals were analyzed.

Morphometric Parameters:

From the eight surviving animals, six BMS stents were compared with four BioMatrix Flex stents and six BioFreedom stents. At 28-day follow-up, most of the stent struts in the BMS group were completely covered compared to the partial coverage in the other two groups. Among the groups, there were no statistically significant differences in EEL area, IEL area, medial area, stent area, and neointimal thickness. Neointimal area in the vessels implanted with BMS were greater than in those implanted with BioFreedom stents (1.29 ± 0.23 vs. 0.90 ± 0.14, P < 0.01, Table I, Fig. 2). Conversely, lumen area in the vessels implanted with BioFreedom stents was significantly larger compared to the vessels implanted with BMS (5.28 ± 0.68 vs. 4.06 ± 1.15, P < 0.03, Table I, Fig. 2). Percent area stenosis was lowest in BioFreedom stents followed by BioMatrix Flex stents; BMS had the highest, but it was not statistically significant (BMS 24.99 ± 7.01 vs. biodegradable polymer 18.14 ± 7.15 vs. polymer-free 15.00 ± 2.76, p = ns).

Inflammatory Parameters

Percent fibrin and fibrin score were higher in the vessels implanted with BioMatrix Flex stents compared to vessels implanted with BMS (55.20 ± 20.44 and 2.17 ± 0.79 vs. 19.74 ± 24.09 and 0.78 ± 0.98, P < 0.03 and < 0.04, respectively, Table I, Fig. 2). Compared to the vessels implanted with BMS stents, the percentage of struts with associated giant cells was significantly higher in the vessels implanted with BioMatrix Flex or BioFreedom stents (2.28 ± 0.83 vs. 5.83 ± 0.58 and 4.28 ± 1.22, P < 0.01 for both, Table I, Fig. 2); however, it was lower in the vessels implanted with BioFreedom stents when compared to vessels implanted with BioMatrix Flex stents (4.28 ± 1.22 vs. 5.83 ± 0.58, P < 0.03, Table I, Fig. 2). RAM 11 positive area was high in the BMS group compared to BioMatrix Flex and BioFreedom groups (1.48 ± 0.88 vs. 0.49 ± 0.38, 0.80 ± 0.49, P < 0.03, and < 0.11 Table I, Fig. 2).

Healing Parameters

Percent endothelialization was significantly higher in the vessels implanted with BMS compared to the vessels implanted with either BioMatrix Flex or BioFreedom stents (97.28 ± 4.25 vs. 88.08 ± 7.36 and 89.44 ± 3.91, P < 0.05 and < 0.01, Table I, Fig. 2). On the other hand, % uncovered struts were significantly less in the vessels implanted with BMS compared to the vessels implanted with either BioMatrix Flex or
BioFreedom stents (10.69 ± 16.59 vs. 48.11 ± 22.38 and 43.45 ± 18.45, $P < 0.05$ and < 0.03, Table I, Fig. 2).

**DISCUSSION**

This study demonstrates that the BioFreedom stent is associated with a reduction in neointima formation with a concurrent gain in the lumen area when compared to BMS; however, no difference was seen when compared with the BioMatrix Flex stent in iliac arteries of balloon denuded and radiated hypercholesterolemic rabbits. Among the three stents, there were no statistically significant differences in EEL area, IEL area, medial area, stent area, neointimal thickness, and inflammation score. Percent fibrin and fibrin score were higher in the iliac arteries implanted with the BioMatrix Flex stent compared to BMS but not statistically different from BioFreedom stent. RAM 11 positive area was highest in the iliac arteries implanted with BMS when compared to those implanted with BioMatrix Flex or BioFreedom stents. Percent endothelialization was lower while % uncovered stent struts was higher in the iliac arteries implanted with BioFreedom or BioMatrix Flex stents compared to BMS.

At present, available DES consist of a metallic stent platform, a cytostatic or antimitotic pharmacological agent for suppression of neointimal hyperplasia, and a carrier vehicle, most often a durable polymer, serving for controlled drug delivery [21,22]. After initial enthusiasm, however, there has been controversial debate on the long-term safety of DES [2–4], with a focus on the potential for an increased risk of late stent thrombosis [5,6], particularly after discontinuation of thienopyridine therapy [23,24], as well as on delayed onset

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**Fig. 2.** Representative photomicrographs of movat pentachrome stained and RAM 11 immunostained sections of cholesterol fed balloon denuded and radiated rabbit iliac arteries. A, B, and C: Low-, high magnification, and RAM 11 immunostained photomicrographs of arteries implanted with bare metal stents. D, E, and F: Low-, high magnification, and RAM 11 immunostained photomicrographs of arteries implanted with BioMatrix stents. G, H, and I: Low-, high magnification, and RAM 11 immunostained photomicrographs of arteries implanted with BioFreedom stents. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
of restenosis or the DES “catch-up” phenomenon [25]. Based on animal and human pathological data, investigators have linked the above-mentioned concerns to the presence of durable polymers in DES, which have proinflammatory and thrombogenic potential [10,11], and may sometimes induce a hypersensitivity reaction [26,27].

Two strategies have been proposed to avoid the potential negative influence of durable polymers in DES. One of these consists of the utilization of biodegradable polymer as a means for storage and controlled release of incorporated drugs. The first reports have shown promising results with DES using biodegradable polymer in terms of their antirestenotic efficacy [12–14]. In this study, we used the BioMatrix Flex stent, which contains Biolimus A9 as the drug and poly-lactic acid and biodegradable polymer as the carrier. Biolimus A9 is a semisynthetic sirolimus analog with an alkoxyalkyl group replacing hydrogen at position 42-O. At a cellular level, Biolimus A9 forms a complex with intracellular FKBP-12, which binds to the mammalian target of rapamycin (mTOR) and reversibly inhibits cell-cycle transition of proliferating smooth muscle cells with a similar potency to sirolimus [28]. Compared to sirolimus, Biolimus A9 possess higher lipophilicity, which may enhance its cellular uptake and migration within the cellular membrane. Poly-lactic acid is degraded into carbon dioxide and water during a 6–9 month period. The drug and polymer are applied solely to the abluminal surface of the stent. In this study, though not statistically significant, a decreased neointimal area in arteries implanted with BioMatrix Flex stent is seen compared to arteries implanted with BMS.

The second strategy consists of the utilization of polymer-free DES platforms. In this regard, it was recently shown that polymer-free DES was effective in reducing neointimal hyperplasia and angiographic restenosis [15,16]. In this study, it was demonstrated that arteries implanted with the BioFreedom stent, a polymer-free Biolimus A9 releasing stent, had the least neointimal hyperplasia compared to both BMS and BioMatrix Flex stent implanted vessels.

Durable polymers present in DES have been shown to be associated with increased fibrin deposition, which could be secondary to delayed healing or impaired fibrin degradation [29,30]. In this short-term study, there was a significant increase in % fibrin and fibrin score in arteries implanted with the BioFreedom stent. In addition to plaque related factors, a significant inflammatory response to DES has been reported [10,11]. The likely explanation for the increased inflammation is a local hypersensitivity reaction to the polymer and perhaps to the drug as well [26,27]. Both BioMatrix Flex and BioFreedom stents are associated with increased inflammation compared to BMS.

Delayed healing and re-endothelialization are the hallmarks of DES [7–9]. Both drug and polymer could contribute to the delayed healing observed with DES. Sirolimus and paclitaxel, the most commonly used antiproliferative drugs in DES, are known not only to inhibit smooth muscle proliferation but also endothelial proliferation [31]. Apart from inhibiting the m-TOR pathway that plays an important role in regulating vascular smooth muscle cell proliferation and migration [32], sirolimus is also known to inhibit endothelial cell proliferation by deactivating the p70 S6 kinase pathway, an essential step for cell cycle progression [33]. Thus, there is potential for impaired endothelial regeneration by the antiproliferative drugs used on the stents. Excessive fibrin deposition [29,30] and increased inflammation caused by the polymer also contribute to delayed healing [26,27]. In agreement with these reports, in this study % endothelialization was lower and % uncovered stents was higher in the arteries implanted with BioMatrix Flex and BioFreedom stents.

Significant macrophage infiltration is a hallmark of the animal model used in this study [17–19]. The decreased macrophage infiltration as evidenced by decreased RAM 11 positive area in the vessels implanted with BioMatrix Flex and BioFreedom stents could be attributed to the anti-inflammatory properties of Biolimus A9. The parity between the BioMatrix Flex and BioFreedom stents could be due to the variation in the tissue drug levels. It is known that when the drug is delivered using polymer as a carrier, it is delivered over a longer period of time compared to when it is delivered as direct coating. Thus, the long-term drug presence in the vessels implanted with the BioMatrix Flex stent may have resulted in the greatest suppression of macrophage infiltration.

This study demonstrates that compared to BMS, the polymer-free BioFreedom stent significantly decreased neointimal hyperplasia in iliac arteries of balloon denuded and radiated hypercholesterolemic rabbits. Although delayed re-endothelialization and an increased inflammatory response were seen with both biodegradable polymer and polymer-free stents, it is possible that in the biodegradable polymer stent inflammatory response may go down after complete degradation of the polymer (typically 6–9 months) and in both of them after the completion of the drug elution process, thus removing the potential nidus for inflammation and thrombo-genicity over the medium- to long term.

**STUDY LIMITATIONS**

This study is limited to short-term observations with a small sample size seen in an experimental model of restenosis. However, compared to commonly used
native porcine coronary arteries or balloon denuded iliac arteries of hypercholesterolemic rabbits, we believe the plaque developed in this animal model has similar properties to diseased human arteries. However, as is the case with any animal model, and in particular with in-stent restenosis, extrapolation of data to the clinical situation should be viewed with caution. Despite the low number of stents, we believe the study provides evidence to conclude that stent based delivery of Biolimus A9 without using a polymer coating is feasible and effectively reduces in-stent neointimal formation.

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REFERENCES


