http://dx.doi.org/10.5012/bkcs.2013.34.6.1663

Comparison of the Neointima Inhibition Between Paclitaxel- and Sirolimus-Eluting Expanded Polytetrafluoroethylene Hemodialysis Grafts in a Porcine Model

Insu Baek,[a] AJin Cho,[a] Jinsun Hwang, Heasun Kim,[†] Jong-Sang Park,[†] and Dae Joong Kim[*]

Department of Internal Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 135-710, Korea. [*]E-mail: daejoongsmc.kim@samsung.com
[†]Department of Chemistry, College of Natural Science, Seoul National University, Seoul 151-747, Korea
Received September 13, 2012, Accepted March 7, 2013

Neointimal hyperplasia causes vascular access dysfunction in hemodialysis patients with synthetic arteriovenous (AV) grafts. Several studies have reported that paclitaxel- or sirolimus-eluting AV grafts inhibit neointimal hyperplasia and display lower rates of stenosis compared with control grafts. However, there have been few comparative studies of the efficacy of paclitaxel- and sirolimus-eluting grafts. We compared the neointimal hyperplasia of paclitaxel- and sirolimus-eluting grafts. AV grafts were implanted laterally between the common carotid artery and the external jugular vein in 12 female Landrace pigs. The animals were sacrificed six weeks after surgery. The neointimal hyperplasia at the anastomosis sites of the grafts was quantified using the ratio of the intragraft hyperplasia to the graft area (H/G ratio) at the graft–vessel interface. The area of intimal hyperplasia at the venous (paclitaxel 1.06 [0.72-1.56] *vs* sirolimus 2.40 [1.72-3.0] mm^2, P = 0.04) and arterial anastomosis sites (paclitaxel 0.93 [0.57-1.48] *vs* sirolimus 2.40 [1.72-3.0] mm^2, P = 0.04) was significantly different between the two groups. However, the H/G ratios for the venous anastomosis site (paclitaxel 0.25 (0.17-0.38) *vs* sirolimus 0.38 (0.2-0.66), P = 0.4) and the arterial anastomosis site (paclitaxel 0.19 (0.08-0.39) *vs* sirolimus 0.41 (0.34-0.50), P = 0.1) did not differ significantly between the groups. In conclusion, there was no significant difference in the inhibition of neointimal hyperplasia by sirolimus- and paclitaxel-eluting AV grafts.

Key Words : Hemodialysis, Neointimal hyperplasia, Paclitaxel, Sirolimus

Introduction

Hemodialysis arteriovenous (AV) grafts are recommended as the initial primary vascular access in patients with poor vessels, such as elderly patients.[1,2] However, the unassisted patency of AV grafts has been poor because of stenoses that result from progressive neointimal hyperplasia.[3-5] Therefore, research into locally delivered treatments, such as the perivascular placement of drug-eluting wraps or drug-coated grafts, to inhibit the development of neointimal hyperplasia is ongoing.[3,6,7]

As antiproliferative agents, sirolimus blocks G1/S cell cycle progression by interacting with the mammalian target of rapamycin (mTOR), and paclitaxel blocks G2/M phase of the cell cycle by the depolymerization of actin microtubules. Therefore, they can be effective in preventing the neointimal hyperplasia at the anastomosis site of ePTFE vascular graft. Previously, we reported that expanded polytetrafluoroethylene (ePTFE) grafts coated with paclitaxel or sirolimus prevented neointimal hyperplasia and the stenosis of AV grafts, especially at the sites of graft–venous anastomoses, in animal models.[8,9] However, there have been few human studies of the inhibition of neointimal hyperplasia in AV grafts. A randomized controlled trial to assess the benefit of a paclitaxel-eluting mesh was terminated early because of an increased rate of infection. Recently, William *et al.* reported that the perivascular implantation of a sirolimus-eluting collagen membrane during graft surgery produced no technical failures or infections.[10] The 12- and 24-month primary unassisted patencies were 76% and 38%, respectively. That was the first human study to show the technical success and safety of a locally delivered treatment to inhibit neointimal hyperplasia.

Before planning a human study of drug-eluting ePTFE hemodialysis grafts, we compared the treatment efficacy of paclitaxel- and sirolimus-coated grafts with histological evaluation of neointimal hyperplasia in a porcine model. Terry *et al.* reported that the H/G ratio (the hyperplastic tissue area divided by the graft area) is a reproducible and quantitative method for assessing neointimal hyperplasia in an AV graft.[11] Therefore, using the method described above, we investigated the areas of hyperplastic tissue and graft in paclitaxel- and sirolimus-coated grafts and the corresponding H/G ratios.

Materials and Methods

Materials. Paclitaxel (Genexol) was purchased from Samyang Genex Inc., South Korea, and sirolimus was purchased from LC Laboratories, USA. The ePTFE vascular grafts (IMPRA, F4006C) were purchased from Bard Peripheral Vascular Inc., USA. High-performance liquid chromatography

[a]These authors contributed equally to this work.

(HPLC)-grade acetone and acetonitrile were obtained from Fisher Scientific, USA. Tween-20 was purchased from Hayashi Pure Chemical Industries Ltd, Japan. Phosphate-buffered saline (PBS) was obtained from Cambrex Corp., USA.

Preparation of Paclitaxel- and Sirolimus-Coated ePTFE Grafts. The drugs were applied with a single-dipping method. Briefly, paclitaxel or sirolimus was dissolved in acetone to a concentration of 2.0 mg/mL in a polypropylene tube. Each of the ePTFE vascular grafts (6 mm in diameter and 20 cm long) was dipped into one of these solutions and incubated for 30 min at 37 °C in a roller incubator (Combi-H; FinePCR, Korea). The drug-coated ePTFE grafts were then air-dried and kept under vacuum overnight to remove the solvent completely. These grafts were sterilized in ethylene oxide gas before use. The amount of paclitaxel or sirolimus coated onto each graft was 1.11 or 1.08 mg/mm^2, respectively.

***In vitro* Release Test.** In the *in vitro* release test, PBS (pH 7.4) containing 0.05% (w/v) Tween-20 was used as the drug-release medium. Paclitaxel- or sirolimus-coated vascular grafts with a length of 5 cm were soaked in polypropylene tubes with 8 mL of medium and shaken at 37 °C and 20 rpm in a roller incubator. At this moment, the mechanical sinkers were used to fully immerse the ePTFE grafts in release media. At the designated time points over a period of 28 days, the release medium was removed completely from the tubes and analyzed by HPLC using a 4.6 × 150 mm C18 reverse-phase column. UV detection set at 227 nm for paclitaxel and 278 nm for sirolimus. The HPLC analysis was performed using a mobile phase of water: acetonitrile (50:50 v/v) at a flow rate of 0.8 mL/min. Under these conditions, the peaks for paclitaxel and sirolimus were eluted at 9.5 min and 18 min, respectively.

Experimental Animals and Operative Technique. Twelve female Landrace pigs in good health, weighing 50 ± 7 kg on average, received a single paclitaxel- or sirolimus-coated ePTFE graft between the common carotid artery and the external jugular vein.

The pigs were anesthetized with intramuscular ketamine HCl (20 mg/kg) and xylazine HCl (2 mg/kg). They were then intubated and ventilated with a mixture of O_2 and air (1:2) containing enflurane (2%) to maintain the anesthesia. Vecuronium bromide (0.1 mg/kg) was administered continuously through an ear vein.

As in previous studies, we adopted the animal experiment model proposed by Rotmans *et al.*[12] After standard surgical cleansing, a longitudinal incision was made in the right lateral side of the neck, along the sternocleidomastoid muscle. The common carotid artery and the external jugular vein were exposed and heparin was given intravenously at 100 IU/kg before vessel manipulation. The common carotid artery was clamped using vessel loops and an 8-mm arteriotomy was made. An end-to-side anastomosis was created at 45° using a 6-0 polypropylene suture. Venous anastomoses were created in a similar manner. The animals were maintained in standard animal-care facilities at Samsung Biomedical Research Institute. All operating procedures conformed to the Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

The animals were sacrificed six weeks after surgery, and the implanted ePTFE grafts were excised together with the adjacent vessels and immersion fixed in 10% neutral-buffered formalin (NBF) for at least 24 h. All the pigs were administered aspirin (100 mg/day) and clopidogrel (PLAVIX®, Sanofi Aventis, France; 75 mg/day) from day 0 to the day of euthanasia.

Tissue Preparation and Morphometry. The artery, vein, and graft were explanted en bloc and the excised specimens were fixed in 10% NBF and embedded in paraffin. The graft and vessels were cut into five 5-mm blocks in the longitudinal direction to yield lumen cross-sections around the center of the graft–venous anastomosis.

All sections were stained with hematoxylin and eosin to roughly confirm the presence of inflammation around the implanted ePTFE graft. Fresh thrombi, when present, were distinguishable from hyperplasia by their distinctive red coloration and the absence of smooth muscle cells. The areas of hyperplastic tissue and graft were traced manually on the captured images using Aperio ImageScope software (Aperio). The H/G ratio was obtained by drawing a straight line across the 'mouth' of the graft, then measuring the cross-sectional area of the hyperplastic tissue growing within the lumen of the graft (Figure 1). The cross-sectional area of the graft was measured in the same section and used as a normalization factor. The area of hyperplastic tissue delimited by the line and graft wall was divided by the graft area. The intima and media layers within the lumen of the graft are not measured

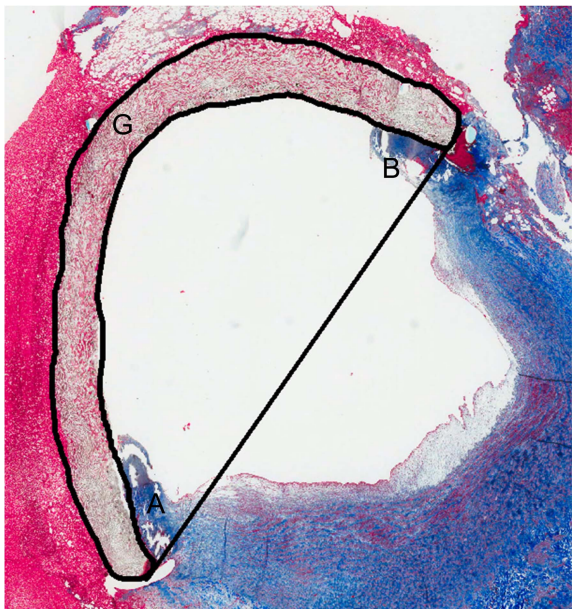

Figure 1. Diagram of the H/G method. Using Aperio ImageScope software, a line was drawn across the mouth of the graft, and the area of hyperplastic tissue growing within the lumen of the graft (A and B) and the area of the graft (G) were measured. The area of hyperplastic tissue (A+B) is divided by graft area to obtain the H/G ratio.

separately with this method. The H/G ratio was thus defined in this method as hyperplasia the developed within the lumen of the graft divided by the graft area. We compared the median values for the cross-sectional areas of the hyperplastic tissue growing within the lumen of the graft and H/G ratios between the paclitaxel- and sirolimus-coated groups.

Statistics. All data are presented as medians and quartiles. The significance of differences in the areas and H/G ratios of the paclitaxel- and sirolimus-coated groups was evaluated using the Mann–Whitney test. Repeated measure analysis of variance (ANOVA) was used to compare the *in vitro* drug release profiles of the two kinds of drug-eluting vascular grafts. P values < 0.05 were considered statistically significant. We used PSS 18.0 software (SPSS Inc.) for all statistical analyses.

Results and Discussion

***In vitro* Release Test.** The *in vitro* drug-release profiles for 28 days were constructed to compare the release patterns of the drugs coated onto the ePTFE grafts, as shown in Figure 2. The burst release of paclitaxel or sirolimus was followed by a slower sustained release. The paclitaxel-coated grafts showed faster cumulative drug release than the sirolimus-coated grafts, but the release profiles of drugs were not statistically different (P = 0.998 by repeated measure ANOVA).

Animal Experiments and Morphometric Analysis. We implanted six paclitaxel-coated grafts and six sirolimus-coated grafts in Landrace pigs. In the paclitaxel-coated group, one pig was sacrificed before five weeks after surgery because of hemorrhage around the graft–venous anastomosis, and another pig showed an incomplete graft–venous junction, which was later occluded by fibrotic tissue. These two pigs were excluded from the paclitaxel-coated group, and

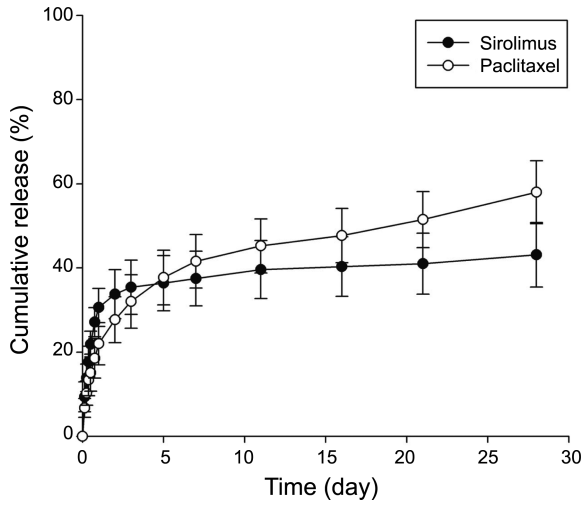

Figure 2. Cumulative *in vitro* release profiles for paclitaxel and sirolimus from ePTFE grafts containing 1.11 μg/mm^2 paclitaxel and 1.08 μg/mm^2 sirolimus, respectively (P = 0.998 by repeated measure ANOVA). Data are the means of four experiments, and the bars represent standard deviations.

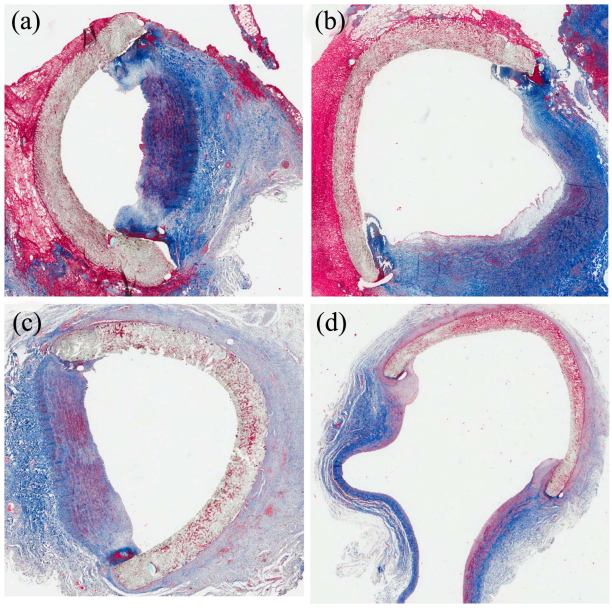

Figure 3. Representative samples of graft–arterial and graft–venous anastomosis tissue sections. (a) arterial anastomosis tissue in the paclitaxel group. (b) venous anastomosis tissue in the paclitaxel group. (c) arterial anastomosis tissue in the sirolimus group. (d) venous anastomosis tissue in the sirolimus group.

histomorphological analyses were performed for four paclitaxel-coated grafts and six sirolimus-coated grafts.

Representative cross-sections of the anastomotic tissues from the paclitaxel- and sirolimus-coated grafts six weeks after surgery are shown in Figure 3. The grafts of both groups retained their patency at six weeks. Compared with the control grafts in a previous study,[9] all of the grafts were patent and neointimal hyperplasia was markedly suppressed inside the graft wall.

A reproducible and quantitative method is required to assess the efficacy in antihyperplasia treatments. Several studies have used the intima-to-media (I/M) ratio to evaluate neointimal hyperplasia.[13,14] However, the I/M ratio technique requires the delineation of the intimal and medial layers, which are easily discernible in arteries but poorly defined in veins on routine histology. In our previous studies, we used the percentage luminal stenosis, defined as the area of intima divided by the total luminal area inside the graft and vascular tissues, to evaluate the intimal hyperplasia on an AV graft.[8,9] However, that method also requires the delineation of the intimal and medial layers. Terry *et al.* reported that the I/M ratio had greater interobserver variability than the H/G method, the results of which correlated positively with the visual scores and I/M ratios.[11] In the H/G method, only hyperplasia that grows within the lumen of the graft is measured and it is normalized to the graft area within the same histological section. They proposed that the H/G method is preferable for assessing hyperplasia because it is quantitative, less variable, and does not require the delineation of the elastic lamina.

Based on these reports, we used the H/G method to compare the degree of intimal hyperplasia in our two experi-

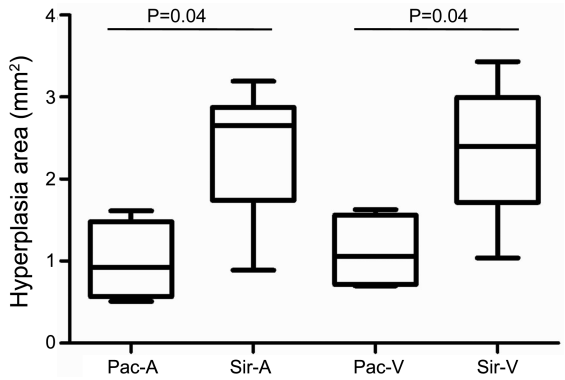

Figure 4. Comparison of the areas of intimal hyperplasia. Abbreviations: Pac-A, arterial anastomosis tissue in the paclitaxel group (n=4); Pac-V, venous anastomosis tissue in the paclitaxel group; Sir-A, arterial anastomosis tissue in the sirolimus group (n=6); Sir-V, venous anastomosis tissue in the sirolimus group.

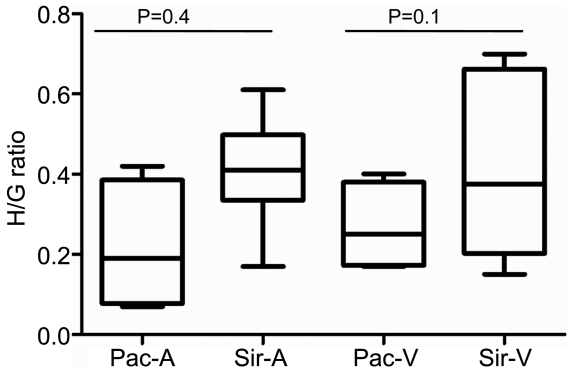

Figure 5. Comparison of the H/G ratios. Abbreviations: Pac-A, arterial anastomosis tissue in the paclitaxel group (n=4); Pac-V, venous anastomosis tissue in the paclitaxel group; Sir-A, arterial anastomosis tissue in the sirolimus group (n=6); Sir-V, venous anastomosis tissue in the sirolimus group.

mental groups. Figure 4 and Figure 5 show the median values (25^{th}–75^{th} percentiles) for the areas of intimal hyperplasia and the H/G ratios. The areas of intimal hyperplasia at the venous anastomosis sites (paclitaxel 1.06 [0.72-1.56] *vs* sirolimus 2.40 [1.72-3.0] mm^2, P = 0.04) and arterial anastomosis sites (paclitaxel 0.93 [0.57-1.48] *vs* sirolimus 2.40 [1.72-3.0] mm^2, P = 0.04) differed significantly between the two groups. However, the H/G ratios at the venous anastomosis sites (paclitaxel 0.25 [0.17-0.38] *vs* sirolimus 0.38 [0.2-0.66], P = 0.4) and arterial anastomosis sites (paclitaxel 0.19 [0.08-0.39] *vs* sirolimus 0.41 [0.34-0.50], P = 0.1) did not differ significantly between the two groups.

Sirolimus, an antiproliferative agent, is reported to inhibit vascular smooth muscle cell proliferation after balloon angioplasty.[15,16] The local administration of paclitaxel also prevented neointimal hyperplasia in a rabbit model of carotid artery injury.[17] The efficacy of sirolimus- and paclitaxel-eluting stents in the prevention of neointimal hyperplasia following coronary artery angioplasty has been established.[18-20] Studies that have compared the clinical outcomes of sirolimus- and paclitaxel-eluting stents showed no significant differences in rates of target lesion revascularization, cardiac death and stent thrombosis.[21,22] In the present study, we have demonstrated that the area of intimal hyperplasia was larger in the sirolimus-treated group. However, when this area was divided by the graft area in the same section, as a normalization factor, the H/G ratios of the two groups were not significantly different. There are few data with which to compare the outcomes of sirolimus- and paclitaxel-eluting grafts. In our previous studies, some degree of neointimal hyperplasia was observed with high-dose sirolimus-coated grafts, whereas little neointimal hyperplasia was observed with paclitaxel-coated grafts.[8,9] Different mechanisms underlying late stent stenosis and the vascular healing response, involving either hypersensitivity or excessive fibrin, were identified for sirolimus- and paclitaxel-eluting coronary stents.[23] Further experimental analyses may be required to assess the degree of neointimal hyperplasia depending on the dose of each drug and to determine the mechanisms involved in their inhibition of neointimal hyperplasia. In addition, the long-term influences of safety factors such as hematoma and edema should be considered as well as efficacy of drug-eluting hemodialysis grafts.

Conclusion

In this study, we have shown that the inhibition of neointimal hyperplasia by sirolimus- and paclitaxel-coated AV grafts is not significantly different. However, the area of neointimal hyperplasia was larger in the sirolimus-treated group. Further experiments are required to evaluate the efficacy and mechanisms of both drug-coated grafts. The successful suppression of neointimal hyperplasia in a small human study of sirolimus-eluting collagen membranes[10] and the similar degree of this suppression by paclitaxel- and sirolimus-coated grafts in this study encourage further human studies.

Acknowledgments. This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A092099).

References

1. Staramos, D. N.; Lazarides, M. K.; Tzilalis, V. D.; Ekonomou, C. S.; Simopoulos, C. E.; Dayantas, J. N. *Eur. J. Surg.* **2000**, *166*, 777.
2. Rooijens, P. P.; Burgmans, J. P.; Yo, T. I.; Hop, W. C.; de Smet, A. A.; van den Dorpel, M. A.; Fritschy, W. M.; de Groot, H. G.; Burger, H.; Tordoir, J. H. *J. Vasc. Surg.* **2005**, *42*, 481.
3. Rotmans, J. I.; Pattynama, P. M.; Verhagen, H. J.; Hino, I.; Velema, E.; Pasterkamp, G.; Stroes, E. S. *Circulation* **2005**, *111*, 1537.
4. Cinat, M. E.; Hopkins, J.; Wilson, S. E. *Ann. Vasc. Surg.* **1999**, *13*, 191.
5. Gibson, K. D.; Gillen, D. L.; Caps, M. T.; Kohler, T. R.; Sherrard, D. J.; Stehman-Breen, C. O. *J. Vasc. Surg.* **2001**, *34*, 694.
6. Masaki, T.; Rathi, R.; Zentner, G.; Leypoldt, J. K.; Mohammad, S. F.; Burns, G. L.; Li, L.; Zhuplatov, S.; Chirananthavat, T.; Kim, S. J.; Kern, S.; Holman, J.; Kim, S. W.; Cheung, A. K. *Kidney Int.* **2004**, *66*, 2061.
7. Nugent, H. M.; Groothuis, A.; Seifert, P.; Guerraro, J. L.; Nedelman,

M.; Mohanakumar, T.; Edelman, E. R. *J. Vasc. Res.* **2002**, *39*, 524.
8. Baek, I.; Bai, C. Z.; Hwang, J.; Park, J.; Park, J. S.; Kim, D. J. *Nephrol. Dial. Transpl.* **2012**, *27*, 1997.
9. Lee, B. H.; Nam, H. Y.; Kwon, T.; Kim, S. J.; Kwon, G. Y.; Jeon, H. J.; Lim, H. J.; Lee, W. K.; Park, J. S.; Ko, J. Y.; Kim, D. J. *Nephrol. Dial. Transpl.* **2006**, *21*, 2432.
10. Paulson, W. D.; Kipshidze, N.; Kipiani, K.; Beridze, N.; DeVita, M. V.; Shenoy, S.; Iyer, S. S. *Nephrol. Dial. Transpl.* **2012**, *27*, 1219.
11. Terry, C. M.; Blumenthal, D. K.; Sikharam, S.; Li, L.; Kuji, T.; Kern, S. E.; Cheung, A. K. *Nephrol. Dial. Transplant.* **2006**, *21*, 3172.
12. Rotmans, J. I.; Velema, E.; Verhagen, H. J.; Blankensteijn, J. D.; Kastelein, J. J.; de Kleijn, D. P.; Yo, M.; Pasterkamp, G.; Stroes, E. S. *J. Surg. Res.* **2003**, *113*, 161.
13. Kiechl, S.; Willeit, J. *Arterioscler Thromb. Vasc. Biol.* **1999**, *19*, 1491.
14. Ferns, G. A.; Forster, L.; Stewart-Lee, A.; Konneh, M.; Nourooz-Zadeh, J.; Anggard, E. E. *Proc. Natl. Acad. Sci. U S A* **1992**, *89*, 11312.
15. Braun-Dullaeus, R. C.; Mann, M. J.; Seay, U.; Zhang, L.; von Der Leyen, H. E.; Morris, R. E.; Dzau, V. J. *Arterioscler Thromb. Vasc. Biol.* **2001**, *21*, 1152.
16. Gallo, R.; Padurean, A.; Jayaraman, T.; Marx, S.; Roque, M.; Adelman, S.; Chesebro, J.; Fallon, J.; Fuster, V.; Marks, A.; Badimon, J. J. *Circulation* **1999**, *99*, 2164.
17. Axel, D. I.; Kunert, W.; Goggelmann, C.; Oberhoff, M.; Herdeg, C.; Kuttner, A.; Wild, D. H.; Brehm, B. R.; Riessen, R.; Koveker, G.; Karsch, K. R. *Circulation* **1997**, *96*, 636.
18. Moses, J. W.; Leon, M. B.; Popma, J. J.; Fitzgerald, P. J.; Holmes, D. R.; O'Shaughnessy, C.; Caputo, R. P.; Kereiakes, D. J.; Williams, D. O.; Teirstein, P. S.; Jaeger, J. L.; Kuntz, R. E. *N. Engl. J. Med.* **2003**, *349*, 1315.
19. Sabate, M.; Jimenez-Quevedo, P.; Angiolillo, D. J.; Gomez-Hospital, J. A.; Alfonso, F.; Hernandez-Antolin, R.; Goicolea, J.; Banuelos, C.; Escaned, J.; Moreno, R.; Fernandez, C.; Fernandez-Aviles, F.; Macaya, C. *Circulation* **2005**, *112*, 2175.
20. Silber, S.; Colombo, A.; Banning, A. P.; Hauptmann, K.; Drzewiecki, J.; Grube, E.; Dudek, D.; Baim, D. S. *Circulation* **2009**, *120*, 1498.
21. Naito, R.; Sakakura, K.; Wada, H.; Funayama, H.; Sugawara, Y.; Kubo, N.; Ako, J.; Momomura, S. *Int. Heart. J.* **2012**, *53*, 149.
22. Nasu, K.; Oikawa, Y.; Yoshikawa, R.; Kadotani, M.; Takeda, Y.; Ota, H.; Kamiya, H.; Muto, M.; Okamura, A.; Yamaki, M.; Usui, S.; Tohara, S.; Yamashita, J.; Suzuki, M.; Kawaguchi, R.; Kawajiri, K.; Nakatsu, Y.; Uchida, Y.; Kashima, Y.; Kawashima, N.; Ozaki, T.; Ogawa, T.; Aizawa, T.; Suzuki, T. *Int. J. Cardiol.* 2011.
23. Nakazawa, G.; Finn, A. V.; Vorpahl, M.; Ladich, E. R.; Kolodgie, F. D.; Virmani, R. *J. Am. Coll. Cardiol.* **2011**, *57*, 390.