Int J Clin Exp Med 2014;7(12):5317-5323
www.ijcem.com /ISSN:1940-5901/IJCEM0003972

Original Article

Poly-l-lactic acid/amorphous calcium phosphate bioabsorbable stent causes less inflammation than poly-l-lactic acid stent in coronary arteries

Lingting Kong[1*], Wenbo Liu[2*], Ge Yan[3], Qiang Li[2], Haiyan Yang[1], Fengxia Yu[1], Haoming Song[4]

[1]Department of Emergency, Yantaishan Hospital, Yantai 264000, China; [2]Department of Cardiology, Yantaishan Hospital, Yantai 264000, China; [3]Department of Medical Administration, Yantaishan Hospital, Yantai 264000, China; [4]Department of Cardiology, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China. [*]Equal contributors.

Received November 19, 2014; Accepted November 20, 2014; Epub December 15, 2014; Published December 30, 2014

Abstract: Aim: Poly-l-lactic acid (PLLA) based bioabsorbable stents with or without amorphous calcium phosphate (ACP) were implanted and compared the inflammation in coronary arteries. Methods: 6 PLLA and 6 PLLA/ACP based paclitaxel-eluting stents were randomly implanted into the coronary arteries of 12 healthy mini-pigs. The segments with stent were used to evaluate inflammation score and endothelialization score by hematoxylin-eosin staining. Results: At the 28th day after stent implantation, no in-stent restenosis or stent thrombosis was found in both PLLA and PLLA/ACP group. Histological analysis indicated that the inflammation score in PLLA/ACP group was less than that of in PLLA group (2.20±0.42 vs. 2.80±0.48, $P<0.05$). Consist with that, the expression of NF-κB was lower in PLLA/ACP group. The results from immunohistochemistry showed that the expressions of endothelial nitric oxide synthase (eNOS) and CD 31 in PLLA/ACP group were dramatically higher than those in PLLA group respectively. The serum levels of vascular endothelial growth factor (VEGF) and nitric oxide (NO) in PLLA/ACP group were significantly higher than those in PLLA group respectively (509.86±49.37 pg/ml vs. 322.04±35.16 pg/ml and 139.46±7.52 μmol/L vs. 29.55±16.55 μmol/L, $P<0.05$). Conclusion: The application of ACP helps to reduce the inflammation caused by PLLA, and is also helpful in endothelial formation and function for PLLA-based bioabsorbable stent.

Keywords: Poly-l-lactic acid, amorphous calcium phosphate, bioabsorbable stent, inflammation

Introduction

Since Sigwart successfully implanted the first coronary stent after operation in 1987, stenting has been widely used in treatment of coronary heart disease clinically. With the update of technology innovations, coronary stents are currently used mainly in metal bracket for the platform without degradation or absorption. As for platform, side-effects like persistent mechanical guyed or foreign body inflammatory reaction would cause a series of problems to the stent-planted coronary segments, such as endothelium injury, hyperplasia of endometrial and smooth muscle, in-stent restenosis, and so on. Drug-eluting stent (DES) can inhibit neointima hyperplasia with antiproliferative function by its polymeric coating, but concerning of the late stent thrombosis (ST) or very late stent thrombosis caused by the DES, which may increase the incidence of myocardial infarction or even mortality on the basis of the BASKET-LATE study published in 2006 [1]. In addition, drug-eluting stents carry anti-proliferative drugs at the local quick-release, which not only inhibit the proliferation of smooth muscle cells, but also delay the endothelialization without inhibiting thrombosis, which leads to the contradiction between anti-smooth muscle cell proliferation and promotion of endothelial repair. Furthermore, delayed re-endothelialization also plays an important role in the late stent thrombosis [2, 3], and the sequent problems such as long-term endothelial dysfunction [4, 5], allergic reaction [6], chronic artery inflammation, caused by current DES were limitations of the application in DES in clinical.

Nowadays, fully biodegradable stent platform are under investigation (e.g., Igaki-Tamai device,

Igaki Medical Planning Company, Japan; BVS, Abbott Vascular, CA) [7, 8], and the emerging of biodegradable drug-eluting stent (BDS) has solved the problem of permanent residue due to metal stent implantation. Poly-l-lactic acid (PLLA), acted as a semi-crystalline polymer with sufficient mechanical strength, is favored in the application of biodegradable stent platformsearch now [9, 10], and constitute as the main constructing stent material, has obvious advantages; however, poor biocompatibility and the contradictions between antiproliferative and endothelialization are still the unresolved problems of biodegradable stent. What's more, the vessel inflammatory response caused by the acidic degraded products released by PLLA can limit its applications [11, 12]. According to international research progress, high biocom\patibility of biomaterial and efficient drug delivery coating system are becoming the focus issues in the field of BDS research.

Calcium phosphates have been widely used in tissue engineering scaffolds and composite material [13]. And amorphous calcium phosphate (ACP) has been proved to have better bioabsorbable and biocompatible characteristic compare to other tricalcium phosphates owe to its high solubility in aqueous solution. Moreover, the ions released from ACP are considered to aggravate the biocompatibilities of PLLA by counteracting the acidic products released during PLLA degradation, hence ultimately decrease the inflammation response [14], and the ACP also can form the uniform microporous structure which can facilitate cell adhesion and hold the differentiated cell function, thus accelerate endothelialization of coronary artery and make greater biocompatibility [15, 16]. The stents used in this study are of fully bioabsorbable drug-eluting stent (NFBS), which are primarily constituted by poly-l-lactic acid and a special nanomaterials-amorphous calcium phosphate (ACP). Besides, paclitaxel drugs are evenly added to the stent body by using a special manufacturing process for the first time. This experiment aims to investigate the effects of the new stent on vascular inflammatory reaction to observe the endothelialization of stent segments. It will promote the new development of the BDS, which provide experimental foundation and theoretical basis for the future research.

Materials and methods

Stent preparation

In this experiment, the body material of BDS is PLLA and the body material of NFBS is PLLA+ACP. ACP was uniformly dispersed into PLLA with a ratio of PLLA/ACP of 98/2 (w/w), using a speed mixer under class 100 clean room conditions. 2% paclitaxel (w/w) was incorporated into both PLLA and PLLA/ACP composites prior to extrusion by a single screw extruder. The extruded tubes were laser-automated according to design specifications (3.0 mm diameter ×13 mm length ×150 μm width). Two radiopaquemetal markers were incorporated, one on each end. All stents were crimped on 3.0 mm×15 mm balloon catheter and sterilized with gamma radiation for 60 minutes prior to implantation.

Animal preparation and stent implantation

Twelve healthy experimental mini-pigs (16 to 18 months old, weighing 30-40 kg) were randomly divided into PLLA group and PLLA/ACP group. Aspirin 100 mg and clopidogrel 50 mg were fed to the pigs orally for three days before the procedure and continued throughout the observation period. Animal study protocol was approved by Institutional Animal Care Committee at Renmin Hospital of Wuhan University. Pigs utilized in our study were taken care of according to the policies and principles established by the Animal Welfare Act.

Ketamine 15 mg/kg, midazolam Lun 0.1 mg/kg and scopolamine 0.01 mg/kg were intramuscularly injected into the back ear muscle of each mini-pig. Mini-pigs were fixed and transported to the cardiac catheterization laboratory after basal anesthesia. Surgical skin preparation was performed while establishing a venous channel, Ketamine and propofol anesthesia were used for surgery. A 6F guiding catheter was inserted into the coronary arteries through left femoral artery percutaneously. After angiograms were taken from three directions/views, one stent per animal was placed at a selected site (stent to artery ratio =1.1:1) and expanded at 8-12 ATM pressure. Once the stent was deployed, a final coronary arterial angiogram was taken out of the site. Recovered from anesthesia, animals were sent back to the animal care facility where they received continuous dual anti-platelet therapies throughout the study period.

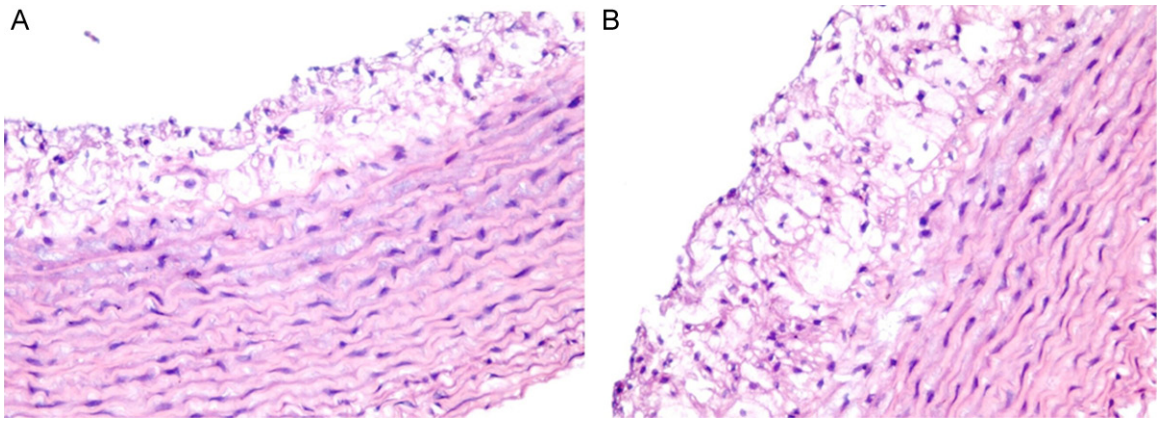

Figure 1. Histological analysis indicated that no severe inflammation occurred in both groups, but PLLA/ACP group have less inflammation score. A. PLLA/ACP group; B. PLLA group.

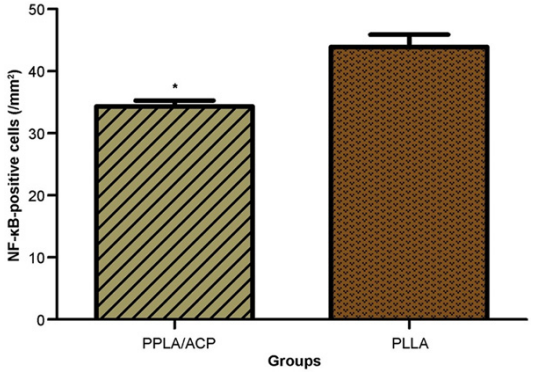

Figure 2. The difference of the expression of NF-κB between the PLLA/ACP and PLLA groups. *P<0.05 vs. PLLA.

Angiography follow-up

Twenty-eight days after stent implantation, animals were anesthetized and subjected to angiography to assess the patency of coronary artery, thrombosis and vascular restenosis at stent segments. Each treated vessel underwent follow up angiography and quantitative coronary angiography (QCA) analysis, which was performed using representative single digital images selected from the digital video sequences. The following standard parameters were assessed: 1) minimal lumen diameter (MLD), 2) percent diameter stenosis.

Blood examination

Blood samples were taken from porcine arteries pre-operation and 28 days post implantation. According to the kit of nitric oxide (NO) and the enzyme linked immunosorbent assay kit (ELISA) kit of vascular endothelial growth factor (VEGF) (RB Company, America), we detected the concentrations of NO (μmol/L) and VEGF (pg/ml) in the serum of arterial blood.

Histology and immunohistochemistry

After the end of the observation period, the animals were sedated, anesthetized deeply, and given euthanasia. The hearts were harvested, and the coronary arteries were perfusion-fixed with 4% paraformaldehyde at 100 mmHg. The vascular stent segments were 4% paraformaldehyde-fixed. After fixation, each stented specimen was sectioned into proximal, central, and distal segments. Each segment was cut into 5-μm-thick slices, which were stained with hematoxylin-eosin (HE) for histological analysis.

Inflammation was graded as 0, none; 1, scattered inflammatory cells; 2, inflammatory cells encompassing 50% of a strut in at least 25% to 50% of the circumference of the artery; 3, inflammatory cells surrounding a strut in at least 50% of the circumference of the artery. *Endothelial scores* were defined as the extent of the circumference of the arterial lumen covered by the endothelial cells and were graded from 0 to 3 (0≤25%, 1=25-50%, 2=50-75%, 3≥75%).

For immunohistochemical analysis, selected arteries were immunohistochemically stained for detection of NF-Kb, endothelial nitric oxide synthase (eNOS) and CD31 by SP method. The mean value of three parts (the proximal, middle and distal) was calculated as the final measure-

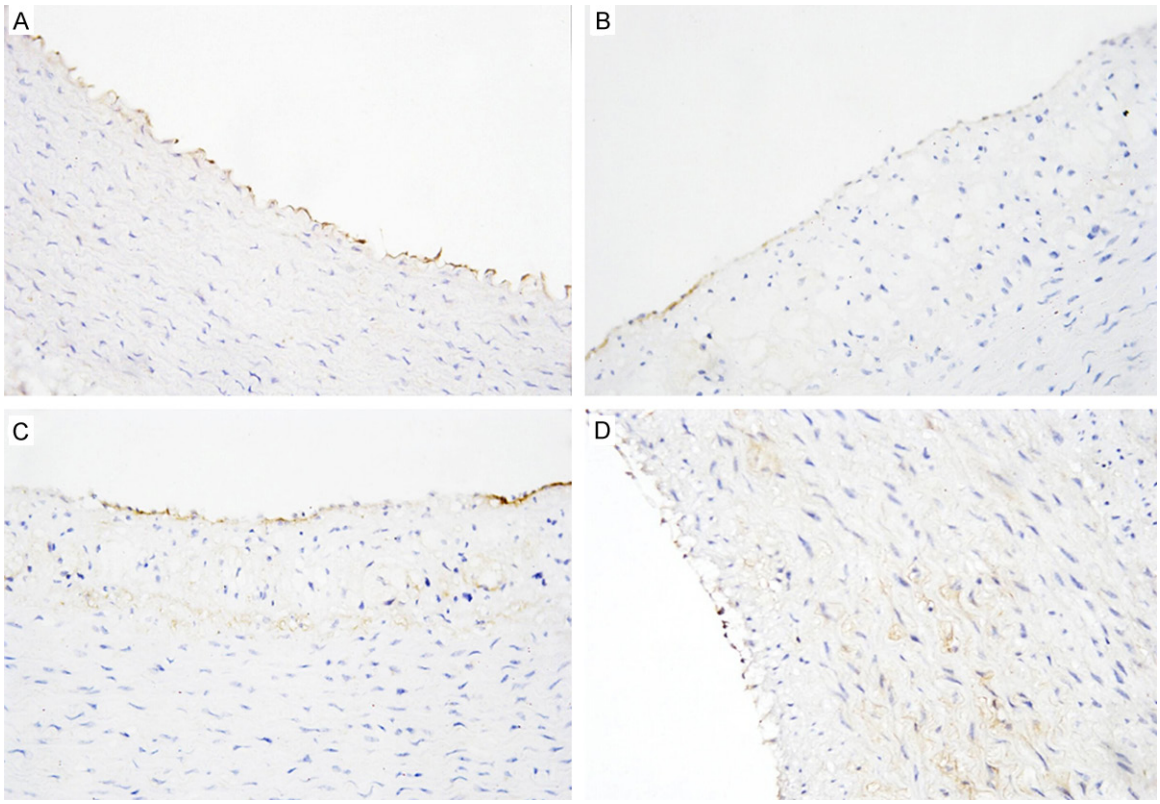

Figure 3. Positive staining of eNOS and CD31 in PLLA/ACP and PLLA group. A. eNOS in PLLA/ACP group; B. eNOS in PLLA group; C. CD31 in PLLA/ACP group; D. CD31 in PLLA group.

ment for each specimen. Each slice was taken five visions, percentage of positive cell and average optical density were analyzed by Image-Pro Plus 6.0 analysis software (Media Cybernetics, Inc). The positive expression of the indicator index will be defined as: percentage of positive cell × average optical density ×100.

Statistical analysis

Statistical analyses wer e performed with SPSS software version 17.0. All data were expressed as mean ± standard deviation (SD), categorical variables as counts (%). t-test was performed to detect the difference between groups and a value of $P<0.05$ was considered statistically significant.

Results

General condition

All stents were implanted successfully. All animals survived the scheduled follow-up period without any major adverse cardiac event. No influence and differences on animal diet, activity and other general conditions of the two groups.

Results of coronary angiography

A total of 12 stents were successfully implanted, and reached a predetermined diameter after balloon dilation. Follow-up angiography revealed that all stented vessels and the distal branches were opened without any sign of peripheral embolization or thrombosis. Gross examination of the hearts showed no sign of coronary abnormalities, epicardial hemorrhage, myocardial infarction and aneurysms. No stents collapsed or broke up. There was no difference between experimental group and control group.

Quantitative coronary angiography revealed comparable baseline characteristics between groups. Furthermore, the degree of balloon/vessel oversized of two groups was similar. At 28-day follow-up, MLD andpercent diameter stenosis were not significantly different between the two groups.

Inflammatory response

At necropsy, stented coronary arteries segments were dissected to reveal presence of inflammation. Extensive inflammation was evident in 1 of 6 PLLA-stented coronary samples. However, the tissue inflammation can't be found in the PLLA/ACP group. Histological analysis indicated that no severe inflammation occurred in both groups, but only some monocyte around the stent, and compared with the control group, PLLA/ACP group have less inflammation score (2.20±0.42 vs. 2.80±0.48, $P<0.05$) (**Figure 1**).

Immunohistochemical result revealed that positive staining of NF-κB viewed as tan particles mainly distributed in the nuclei of vascular endothelium and endangium. The expression quantity of PLLA/ACP group was much less than PLLA group (27.02±2.18 vs. 54.34±4.21, $P<0.05$) (**Figure 2**). Positive staining of eNOS and CD31 viewed as yellow particles mainly distributed in the nuclei of vascular endothelium and endangium. The expression quantity of eNOS in PLLA/ACP group was higher than PLLA group ($P<0.05$). The expression quantity of CD31 in PLLA/ACP group was higher than that in PLLA group (58.53±4.25 vs. 26.53±3.55, $P<0.05$) (**Figure 3**).

Serum concentrations of NO and VEGF

The serum concentrations of NO in both the two groups at 28 days were all significantly lower than before stenting. But on the 28th day, the VEGF and NO level in PLLA/ACP group were higher than that of PLLA group (509.86±49.37 pg/ml vs. 322.04±35.16 pg/ml and 139.46±7.52 μmol/L vs. 29.55±16.55 μmol/L $P<0.05$).

Discussion

Coronary stenting has been viewed to be a giant achievement in the history of percutaneous coronary interventions (PCIs) since Sigwart successfully implanted the first coronary stent after operation in 1987. Moreover, drug-eluting stent (DES) can inhibit neointima hyperplasia with antiproliferative function by its polymeric coating. From this point of view, DES reduces the risk of in-stent restenosis and improves prognosis contrast with bare metal stent (BMS). However, concerning of the late stent thrombosis (ST) and inflammatory caused by the metal platform, the bioabsorbable stent are preferentially used in Drug-Eluting Stents. Currently, the fully biodegradable stent are under investigation, and the fully biodegradable Drug-Eluting Stents may become a tendency for clinical applications. Poly-l-lactic acid (PLLA), act as a semi-crystalline polymer with sufficient mechanical strength, is favored in the applications of biodegradable stent platform search now. The stents used in our study are of fully bioabsorbable drug-eluting stent (NFBS), which are primarily constituted by poly-l-lactic acid and a special nanomaterials-amorphous calcium phosphate (ACP). In addition, paclitaxel drugs are evenly added to the stent body by using a special manufacturing process for the first time.

The principal findings of our study are as follow: 1) NFBS which is primarily constituted PLLA and ACP and PLLA based bioabsorbable stents without ACP were both non-obstructed, with no sign of peripheral embolization or thrombosis by coronary angiography. 2) The inflammatory response of PLLA/ACP Bioabsorbable Stent was similar to that of PLLA stent, and no severe inflammation can be found in both groups. 3) above 90% stent surface endothelialization were shown in the PLLA/ACP group, while the BDS was partially covered by a thin layer of neointima; and this result can be explained by that the ACP can generate uniform microporous structure which can facilitate cell adhesion and hold the differentiated cell function, thus accelerate endothelialization of coronary artery and make greater biocompatibility. Hence, using biodegradable PLLA that incorporated with ACP to modify the stent is practical for clinic. 4) The expression quantity of NF-κB positive cells of PLLA/ACP Bioabsorbable Stent group was much less than that of PLLA stent group. NF-κB, viewed as a marker of inflammation and can mediate the expression of numerous inflammatory cytokines [17], and as the literature reports, it includes the monocyte chemoattractant protein (MCP-1) and the interleukin-6 (IL-6). One explanation for the anti-inflammatory effect of the PLLA/ACP Bioabsorbable Stent is the co-formulation of ACP into PLLA. Amorphous calcium phosphate (ACP) has been proved to have better bioabsorbable and biocompatible characteristic compare to other tricalcium phosphates owe to its high solubility in aqueous solution. Moreover, the ions released from

ACP are considered to aggravate the biocompatibilities of PLLA by counteracting the acidic products release [17].

Conclusions

The result of PLLA polymers blended with ACP shows significantly less inflammatory response than the group of the PLLA without amorphous calcium phosphate suggesting that the incorporation of ACP with PLLA improves the stent's biocompatibility. This preclinical study was projected to promote the new development of the BDS, which provide initiatory data for future search.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Haoming Song, Department of Cardiology, Tongji Hospital, Tongji University School of Medicine, 389 Zhidan Road, Shanghai 200065, China. E-mail: tjsonghaoming@163.com

References

[1] Pfisterer M, Brunner-La Rocca HP, Buser PT, Rickenbacher P, Hunziker P, Mueller C, Jeger R, Bader F, Osswald S, Kaiser C; BASKET-LATE Investigators. Late clinical events after clopidogrel discontinuation may limit the benefit of drug-eluting stents: an observational study of drug-eluting versus bare-metal stents. J Am Coll Cardiol 2006; 48: 2584-2591.

[2] Lang NN and Newby DE. Emerging thrombotic effects of drug eluting stents. Arterioscler Thromb Vasc Biol 2007; 27: 261-262.

[3] Joner M, Finn AV, Farb A, Mont EK, Kolodgie FD, Ladich E, Kutys R, Skorija K, Gold HK and Virmani R. Pathology of drug-eluting stents in humans: delayed healing and late thrombotic risk. J Am Coll Cardiol 2006; 48: 193-202.

[4] Hofma SH, van der Giessen WJ, van Dalen BM, Lemos PA, McFadden EP, Sianos G, Ligthart JM, van Essen D, de Feyter PJ and Serruys PW. Indication of long-term endothelial dysfunction after sirolimus-eluting stent implantation. Eur Heart J 2006; 27: 166-170.

[5] Togni M, Windecker S, Cocchia R, Wenaweser P, Cook S, Billinger M, Meier B and Hess OM. Sirolimus-eluting stents associated with paradoxic coronary vasoconstriction. J Am Coll Cardiol 2005; 46: 231-236.

[6] Kim JW, Suh SY, Choi CU, Na JO, Kim EJ, Rha SW, Park CG, Seo HS and Oh DJ. Six-month comparison of coronary endothelial dysfunction associated with sirolimus-eluting stent versus Paclitaxel-eluting stent. JACC Cardiovasc Interv 2008; 1: 65-71.

[7] Serruys PW, Ormiston JA, Onuma Y, Regar E, Gonzalo N, Garcia-Garcia HM, Nieman K, Bruining N, Dorange C, Miquel-Hebert K, Veldhof S, Webster M, Thuesen L and Dudek D. A bioabsorbable everolimus-eluting coronary stent system (ABSORB): 2-year outcomes and results from multiple imaging methods. Lancet 2009; 373: 897-910.

[8] Serruys PW, Onuma Y, Dudek D, Smits PC, Koolen J, Chevalier B, de Bruyne B, Thuesen L, McClean D, van Geuns RJ, Windecker S, Whitbourn R, Meredith I, Dorange C, Veldhof S, Hebert KM, Sudhir K, Garcia-Garcia HM and Ormiston JA. Evaluation of the second generation of a bioresorbable everolimus-eluting vascular scaffold for the treatment of de novo coronary artery stenosis: 12-month clinical and imaging outcomes. J Am Coll Cardiol 2011; 58: 1578-1588.

[9] Laskey WK and Osborne L. Comment on: overestimation of aortic valve gradient measured by Doppler echocardiography in patients with aortic stenosis. Catheter Cardiovasc Interv 2005; 65: 176-9. Catheter Cardiovasc Interv 2005; 66: 598.

[10] Nishio S, Kosuga K, Igaki K, Okada M, Kyo E, Tsuji T, Takeuchi E, Inuzuka Y, Takeda S, Hata T, Takeuchi Y, Kawada Y, Harita T, Seki J, Akamatsu S, Hasegawa S, Bruining N, Brugaletta S, de Winter S, Muramatsu T, Onuma Y, Serruys PW and Ikeguchi S. Long-Term (>10 Years) clinical outcomes of first-in-human biodegradable poly-l-lactic acid coronary stents: Igaki-Tamai stents. Circulation 2012; 125: 2343-2353.

[11] van der Giessen WJ, Lincoff AM, Schwartz RS, van Beusekom HM, Serruys PW, Holmes DR Jr, Ellis SG and Topol EJ. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. Circulation 1996; 94: 1690-1697.

[12] Onuma Y and Serruys PW. Bioresorbable scaffold: the advent of a new era in percutaneous coronary and peripheral revascularization? Circulation 2011; 123: 779-797.

[13] Combes C and Rey C. Amorphous calcium phosphates: synthesis, properties and uses in biomaterials. Acta Biomater 2010; 6: 3362-3378.

[14] Stamboulis A, Hench LL and Boccaccini AR. Mechanical properties of biodegradable polymer sutures coated with bioactive glass. J Mater Sci Mater Med 2002; 13: 843-848.

[15] Shen Y, Wang G, Chen L, Li H, Yu P, Bai M, Zhang Q, Lee J and Yu Q. Investigation of surface endothelialization on biomedical nitinol

(NiTi) alloy: Effects of surface micropatterning combined with plasma nanocoatings. Acta Biomater 2009; 5: 3593-3604.

[16] Gao Y, Weng W, Cheng K, Du P, Shen G, Han G, Guan B and Yan W. Preparation, characterization and cytocompatibility of porous ACP/PLLA composites. J Biomed Mater Res A 2006; 79: 193-200.

[17] Gogo PB Jr, Schneider DJ, Watkins MW, Terrien EF, Sobel BE and Dauerman HL. Systemic inflammation after drug-eluting stent placement. J Thromb Thrombolysis 2005; 19: 87-92.