4. 平成20年11月18日掲載、日本経済新聞「先端医療技術実用化促す　政府　スーパー特区24件決定」

5. 平成20年11月19日掲載、産経新聞「「先端医療開発特区」政府選定　スーパー特区24件　iPS応用、虫歯治療…」

6. 平成20年11月19日掲載、西日本新聞「初のスーパー特区に24件」

7. 平成20年11月28日掲載、科学新聞「先端医療開発特区の課題決定　iPSなど5分野24件　関係省庁一体で取り組み」

8. 平成20年12月17日掲載、西日本新聞「先進的医療　早期実現へ　九大など開発特区　「人工脳」実用化目指す」

9. 平成21年3月23日掲載、化学工業日報「九大-ホソカワ粉体技研　次世代DES、前臨床へ　ナノ粒子に薬剤封入　実用化でメーカーと交渉」

【研究成果の刊行物・別刷】

○をつけた論文の別刷あるいは資料を次のページ以降に添付します。

A Journal of the American College of Cardiology

April 2009
Volume 2, No. 4

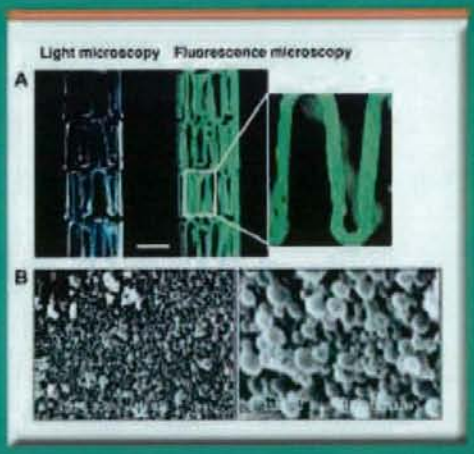
JACC cardiovascular Interventions

- Pulmonary Vein Stenosis Complicating Ablation for AF

MINI-FOCUS: Stent Technology

- Bioabsorbable Polymeric Nanoparticle-Eluting Stents
- Lowering the Dose of Sirolimus Reduces Delayed Healing
- Antirestenotic Efficacy in DES With and Without Polymer
- Biodegradable-Polymer Coated SES in "Real-World" Practice
- IVUS and Angiography Following Bioabsorbable Magnesium Stents

- Drug-Eluting and Bare-Metal Stents for Stable CAD
- Impact of DES vs. BMS on Mortality in Patients With Anemia
- Pre-Hospital ECGs and STEMI Receiving Center Networks
- Predictors and Outcomes of Ad Hoc vs. Non-Ad Hoc PCI
- Predictors of MACE and Optimal Post-Stent FFR After BMS



(A) Light and fluorescence stereomicroscopy of fluorescein-isothiocyanate–incorporated nanoparticle-eluting stent. (B) Scanning electron microscopy (low and high magnification). See page 279.

ELSEVIER

ISSN 1936-8798

www.jacc-interventions.org

JACC: CARDIOVASCULAR INTERVENTIONS
© 2009 BY THE AMERICAN COLLEGE OF CARDIOLOGY FOUNDATION
PUBLISHED BY ELSEVIER INC.

VOL. 2, NO. 4, 2009
ISSN 1936-8798/09/$36.00
DOI: 10.1016/j.jcin.2008.08.023

MINI-FOCUS: STENT TECHNOLOGY

Formulation of Nanoparticle-Eluting Stents by a Cationic Electrodeposition Coating Technology

Efficient Nano-Drug Delivery via Bioabsorbable Polymeric Nanoparticle-Eluting Stents in Porcine Coronary Arteries

Kaku Nakano, PHD,* Kensuke Egashira, MD, PHD,* Seigo Masuda, MD,* Kouta Funakoshi, MD,* Gang Zhao, MD, PHD,§ Satoshi Kimura, MD,† Tetsuya Matoba, MD, PHD,* Katsuo Sueishi, MD, PHD,‡ Yasuhisa Endo, PHD,¶ Yoshiaki Kawashima, PHD,‖ Kaori Hara, PHD,# Hiroyuki Tsujimoto, PHD,# Ryuji Tominaga, MD, PHD,† Kenji Sunagawa, MD, PHD*

Fukuoka, Aichi, Kyoto, and Osaka, Japan; and Shanghai, China

Objectives The objective of this study was to formulate a nanoparticle (NP)-eluting drug delivery stent system by a cationic electrodeposition coating technology.

Background Nanoparticle-mediated drug delivery systems (DDS) are poised to transform the development of innovative therapeutic devices. Therefore, we hypothesized that a bioabsorbable polymeric NP-eluting stent provides an efficient DDS that shows better and more prolonged delivery compared with dip-coating stent.

Methods We prepared cationic NP encapsulated with a fluorescence marker (FITC) by emulsion solvent diffusion method, succeeded to formulate an NP-eluting stent with a novel cation electrodeposition coating technology, and compared the in vitro and in vivo characteristics of the FITC-loaded NP-eluting stent with dip-coated FITC-eluting stent and bare metal stent.

Results The NP was taken up stably and efficiently by cultured vascular smooth muscle cells in vitro. In a porcine coronary artery model in vivo, substantial FITC fluorescence was observed in neointimal and medial layers of the stented segments that had received the FITC-NP-eluting stent until 4 weeks. In contrast, no substantial FITC fluorescence was observed in the segments from the polymer-based FITC-eluting stent or from bare metal stent. The magnitudes of stent-induced injury, inflammation, endothelial recovery, and neointima formation were comparable between bare metal stent and NP-eluting stent groups.

Conclusions Therefore, this NP-eluting stent is an efficient NP-mediated DDS that holds as an innovative platform for the delivery of less invasive nano-devices targeting cardiovascular disease. (J Am Coll Cardiol Intv 2009;2:277–83) © 2009 by the American College of Cardiology Foundation

From the Departments of *Cardiovascular Medicine, †Cardiovascular Surgery, and ‡Pathology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan; §Department of Cardiovascular Medicine, 6th People's Hospital, Shanghai Jiaotong University, Shanghai, China; ‖School of Pharmaceutical Science, Aichi Gakuin University, Aichi, Japan; ¶Division of Applied Biology, Kyoto Institute of Technology, Kyoto, Japan; and the #Hosokawa Powder Technology Research Institute, Osaka, Japan.
This study was supported by Grants-in-Aid for Scientific Research (19390216, 19650134) from the Ministry of Education, Science, and Culture, Tokyo, Japan; Health Science Research Grants (Research on Translational Research and Nano-medicine) from the Ministry of Health, Labor, and Welfare, Tokyo, Japan; and the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research, Tokyo, Japan. Dr. Egashira holds a patent on the results reported in this present study. Drs Nakano and Egashira contributed equally to this work.

Manuscript received July 29, 2008, accepted August 22, 2008.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 2, NO. 4, 2009
APRIL 2009:277–83

In the 3 years since they were introduced for clinical use, polymer-based drug-eluting stents (DES) that carry antiproliferative drugs such as sirolimus or paclitaxel, have been used extensively in percutaneous coronary interventions for the prevention of restenosis (1–3). Through the effects of these antiproliferation agents on vascular smooth muscle cells (VSMCs) and inflammatory cells, DES can reduce the rate of restenosis and target-vessel revascularization to below 10%. However, the increased risk of late in-stent thrombosis (resulting in acute myocardial infarction and death) associated with use of the first-generation DES devices has become a major issue (4). In particular, the off-label use of DES for complex coronary lesions (long and diffuse lesions, left main lesions, culprit lesion of acute coronary syndrome, and so forth) increases the risk of late in-stent thrombosis (5,6). These adverse effects are thought to result mainly from the antiproliferative effects of the drugs on the endothelial cells, leading to impaired arterial healing processes (impaired endothelial regeneration, excessive inflammation, proliferation, and fibrin deposition) and partly from the use of nonbiocompatible polymers in stent construction (7–9). Clearly, a novel DES system with fewer associated adverse effects is needed.

Abbreviations and Acronyms

DDS = drug delivery system
DES = drug-eluting stent(s)
FITC = fluorescein isothiocyanate
NP = nanoparticle(s)
PLGA = poly (DL-lactide-co-glycolide)
VSMC = vascular smooth muscle cell

Current DES polymer-coating technology uses dip- and/or spray-coating methodology. These methods are useful for coating stents with strongly lipophilic drugs such as sirolimus but not for water-soluble drugs or deoxyribonucleic acid (DNA). Recently, we and others reported the formulation of plasmid DNA- or oligonucleotide-coated stents with a water-soluble polymer, which showed limited delivery efficacy and non optimal therapeutic effects for clinical application (10–13). The application of nanotechnology-based drug delivery system (DDS) is expected to have a major impact on the development of efficient and safe DDS.(14) Previously, we reported the development of such a DDS, a polymeric nanoparticle (NP) formulated from the biodegradable polymer poly (DL-lactide-*co*-glycolide) (PLGA) (15,16) that can entrap hydrophilic agents (protein, oligonucleotide, DNA, and the like), penetrate cellular membrane via endocytosis, and deliver the encapsulated therapeutic agents into the cellular cytoplasm. The PLGA hydrolyzes slowly, is metabolized, and is eliminated from the body. The PLGA NP offers the advantages of efficient intracellular delivery of different classes of therapeutic agents and the capacity for sustained intracytoplasmic release (17). Until now, no existing technology could produce an active coating of NP on the surface of metallic stents.

We have formulated a bioabsorbable polymeric NP-eluting stent with a novel cation electrodeposition coating technology. We hypothesized that this NP-eluting stent system would be an efficient innovative platform for in vivo drug delivery. The aims of this study were to: 1) formulate a bioabsorbable polymeric NP-eluting metallic stent with cation electrodeposition NP coating technology; 2) characterize the in vitro kinetics of drug release from NP and the cellular uptake and localization of NP; and 3) evaluate the feasibility of using NP-eluting stents in vivo in porcine coronary arteries.

Methods

Preparation of cationic PLGA NP with chitosan-mediated surface modification. Cationic PLGA NP encapsulated with fluorescein-isothiocyanate (FITC) were prepared by a previously reported emulsion solvent diffusion method in purified water (15,16). Additional details are provided in the Online Appendix.

Preparation of NP-eluting stents by cationic electrodeposition coating technology. Cationic electrodeposition coating was prepared in NP solution at a concentration of 50 mg/ml in distilled water and deposited on cathodic, 15-mm stainless-steel, balloon-expandable stents (Multilink, Guidant, Indianapolis, Indiana) with current maintained at 2.0 mA by a direct current power supply (Nippon Stabilizer Co., Tokyo, Japan) for different periods under sterile conditions (Online Fig. III). This electrodeposition coating procedure produced a coating of approximately 654.0 ± 167.5 μg (n = 12) of NP/stent and 31 ± 2 μg of FITC/stent (n = 12). Additional details are provided in the Online Appendix.

Measurement of in vitro NP release kinetics. To measure FITC release kinetics, FITC-NP (n = 8) was immersed in Tris-EDTA buffer, and the released FITC from NP was measured. The FITC-NP–eluting stents (n = 8) were also immersed in Tris-EDTA buffer, and the eluted FITC-containing NP was measured.

Cellular uptake and intracellular distribution of NP. Human coronary artery smooth muscle cells (SMCs) were used to perform this study. Additional details are provided in the Online Appendix.

Cytotoxicity assay. The cytotoxicity of PLGA NP on human coronary artery SMCs was determined with an MTS assay (Promega, Dojin, Japan). Additional details are provided in the Online Appendix.

Animal preparation, stent implantation, and coronary angiography. Domestic male pigs (weighing 25 to 30 kg) were anesthetized and divided into 3 groups that received noncoated bare metal stents (1 week, n = 3; 2 week, n = 3; 4 week, n = 8; 6 week, n = 3), FITC-incorporated NP-eluting stents (2 week, n = 3; 2 week, n = 3; 4 week, n = 8; 6 week, n = 3), or dip-coated stents with thin layers of PLGA polymer containing FITC (1 week; n = 3, 2 week; n = 3, 4 week; n = 3), to either the left anterior descending

or the left circumflex coronary artery. Animals were killed after 1, 2, 4, or 6 weeks, and the stented arterial sites and contralateral non stented sites were excised for biochemical, immunohistochemical, and morphometric analyses. Left coronary angiography was performed before, immediately after, and 4 weeks after stent implantation. Additional details are provided in the Online Appendix.

Histopathological studies. The stented arterial segment at 4 weeks after stent implantation was divided into 2 parts at the center of the stent, and the proximal part was embedded in resinoid. The distal part of the stent was used for either fluorescence or pathological analysis after the stent struts were gently removed with micro-forceps. Additional details are provided in the Online Appendix.

Statistical analysis. Data are expressed as mean ± SEM. The statistical analysis of differences between 2 groups was performed with the unpaired *t* test. The analysis of differences among 3 groups was compared by 1-way analysis of variance. Values of $p < 0.05$ were considered to be statistically significant.

Results

Fabrication of NP-eluting stent and NP release kinetics in vitro. The cationic electrodeposit coating formed a thin and uniform layer of NP on the surface of stents without webbing between the struts (Fig. 1A). Interestingly, amount of coating of NP on the stent surface increased with period of electricity (Online Fig. III).

Light and fluorescence microscopy analysis revealed that the polymer stretched after balloon expansion, but no fragmentation was observed (Fig. 1A). Scanning electron microscopy analysis revealed that NP was structurally intact and cohesive (Fig. 1B). An analysis of the in vitro FITC release kinetics from FITC-NP showed an early burst of FITC release such that approximately 40% of the total amount ultimately released was present on day 1, followed by sustained release of the remaining FITC over the next 30 days (Fig. 1C). The in vitro FITC release kinetics from NP-eluting stents also showed a similar release pattern (Fig. 1D).

In vitro cell uptake and intracellular distribution of NP. Incubation of human coronary artery SMCs with FITC-loaded NP (0.1 mg/ml PLGA) showed highly efficient and stable intracellular delivery of NP (Fig. 2A). In contrast, no fluorescence was detected when the SMCs were incubated with blank NP or FITC only. Fluorescence confocal microscopy revealed that: 1) the SMCs took up the NP rapidly, and NP remained stable in the cell for at least 24 h of incubation; and 2) NP was seen in both the nuclei and the cytoplasm (Figs. 2B and 2C). Transmission electron microscopy of the cellular cross-sections revealed the intracellular localization of NP at 24 h of incubation (Fig. 2D). Furthermore, NP eluted from the FITC-NP-eluting stent was added to human coronary artery SMCs and incubated

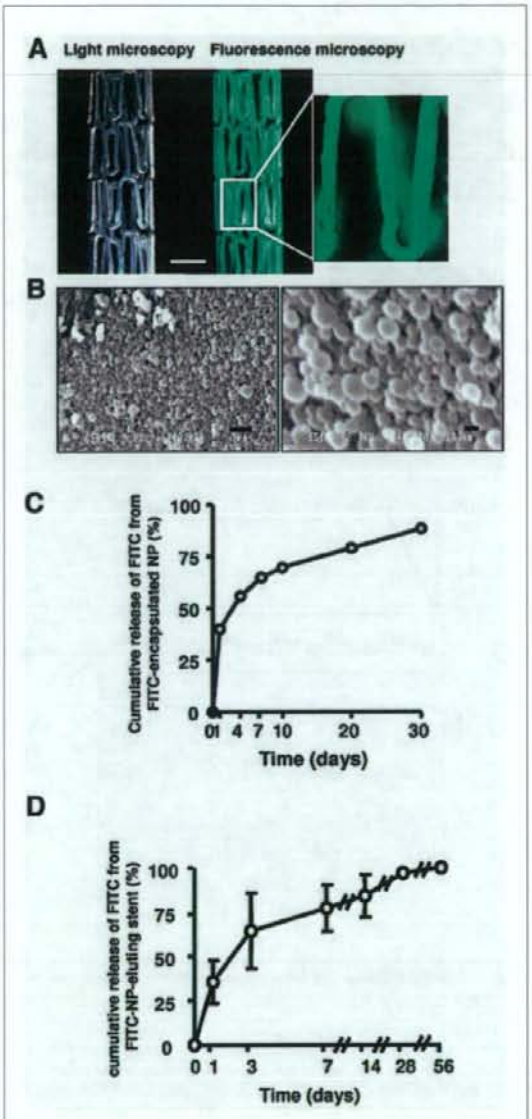


Figure 1. Formulation of FITC-Encapsulated NP-Eluting Stents by a Cationic Electrodeposition Coating Technology

(A) Light and fluorescence stereomicroscopy photograph of balloon-expanded fluorescein-isothiocyanate (FITC)-incorporated nanoparticle (NP)-eluting stent. **Scale bar** = 1 mm. **(B)** Scanning electron microscopy photograph (**left**: low magnification, **scale bar** = 1 μm; **right**: high magnification; **scale bar** = 100 nm) of balloon-expanded NP-eluting stent. **(C, D)** In vitro time course of cumulative FITC release from the FITC-incorporated NP and FITC-incorporated NP release from the NP-eluting stents (n = 8 each). The percentage of incremental quantities of released FITC was plotted against time.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 2, NO. 4, 2009
APRIL 2009:277–83

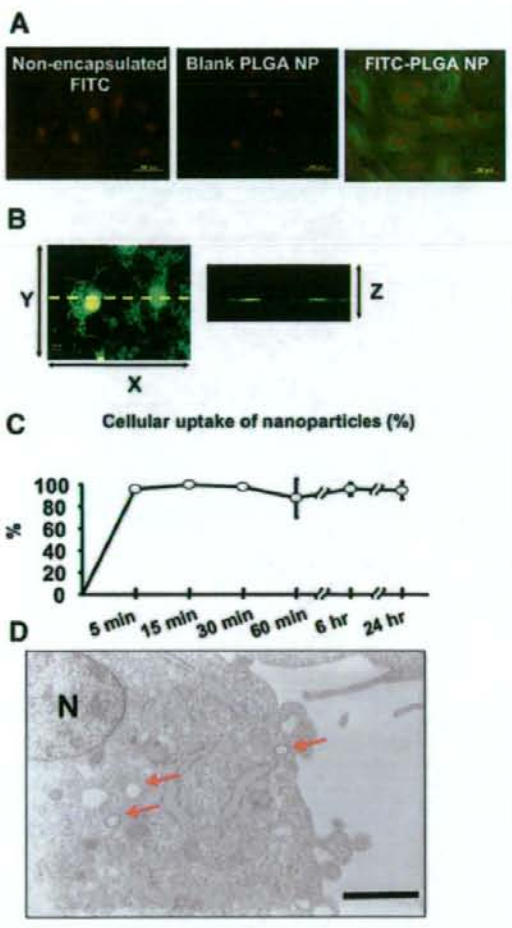


Figure 2. Cellular Uptake and Intracellular Distribution of NP in Human Coronary Artery SMCs in Vitro

(A) Fluorescence microscopy photographs of human coronary artery smooth muscle cells (SMCs) incubated with FITC only, blank NP, or FITC-NP (0.1 mg/ml) for 1 h. **(B)** Confocal fluorescence microscopy photographs (**left**: an XY-axis image; **right**: a Z-axis image of cross-sections from **yellow dashed line** displayed on an XY-axis image) of human coronary artery SMCs incubated with FITC-NP at 0.1 mg/ml. The FITC fluorescence is **green**; nuclei are **red**. **Scale bar** = 10 μm. **(C)** In vitro time course of the percentage of cellular uptake of FITC-incorporated NP by human coronary artery SMCs (n = 6 to 8 at each time point). **(D)** Transmission electron microscopy photograph of a cross-section of human coronary artery smooth muscle incubated with NP for 60 min. **Arrows** indicate NP in the cellular cytoplasm. **Scale bar** = 500 nm. N = nucleus; PLGA = poly (DL-lactide-*co*-glycolide); other abbreviations as in Figure 1.

for 1 h, resulting in prominent cellular FITC positivity (Online Fig. IV A). In contrast, scarce FITC fluorescence was observed in the SMCs 1 h after addition of FITC-PLGA matrix eluted from PLGA polymer-based FITC-eluting stent (Online Fig. IV B).

Results of a cytotoxicity assay showed that human coronary artery SMCs incubated with PLGA NP for 48 h at concentrations of 0.1, 0.3, and 1 mg/ml remained 100% viable relative to control (data not shown).

Deployment of FITC-NP–eluting stent in porcine coronary arteries in vivo. After 1 week of stent deployment, a thin layer of platelets and fibrin deposition formed around the stent strut. Strong FITC fluorescence was detected in the stented coronary arterial segments that had received the FITC-NP–eluting stent (Fig. 3).

After 2 weeks, when a thin neointima associated with monocyte infiltration had formed mainly around stent struts, intense FITC fluorescence was detected in the stented coronary arterial segments from the NP-eluting stented groups (Fig. 4).

No substantial FITC fluorescence was observed in coronary segments from the non-NP polymer-based FITC stent site (Figs. 3 and 4) or from the bare metal stent sites (data not shown) 1 and 2 weeks after stenting.

After 4 weeks, when a significant in-stent neointima formed in stent sites and the neointima consisted mainly of VSMCs, FITC fluorescence was noted only in cross-sections from the NP-eluting stent site but not from bare metal stent sites (Fig. 5A). No substantial FITC fluorescence was observed in cross-

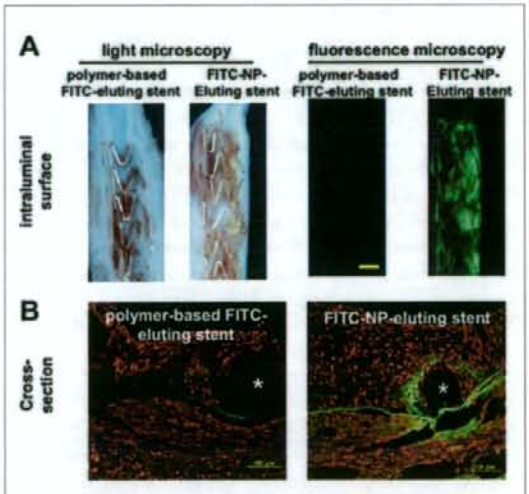


Figure 3. Localization of FITC Fluorescence After Deployment of FITC-NP–Eluting Stent in Porcine Coronary Arteries 1 Week After Stenting

(A) En-face light and fluorescence stereomicroscopic pictures of the intraluminal surface of an isolated stented segment of coronary artery taken from the FITC-NP–eluting and the polymer-based FITC-eluting stent sites. **Scale b** = 1 mm. **(B)** Fluorescence microscopic pictures of cross-sections from the FITC-NP–eluting stent and polymer-based FITC-eluting stent sites. Microscopic settings (exposure condition, filter, intensity of excitation light, and so forth) are the same between 2 pictures. **Scale b** = 100 μm. Abbreviations as in Figure 1.

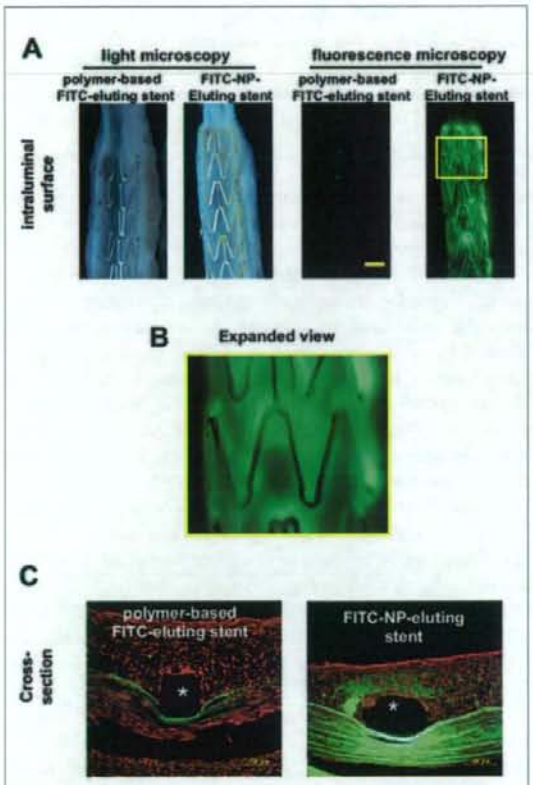


Figure 4. Localization of FITC Fluorescence After Deployment of FITC-NP–Eluting Stent in Porcine Coronary Arteries 2 Weeks After Stenting

(A) En-face light and fluorescence stereomicroscopic pictures of the intraluminal surface of an isolated stented segment of coronary artery taken from the FITC-NP–eluting and the dip-coated FITC stent sites. **Scale b** = 1 mm. **(B)** Expanded images of **boxed area in A**. Expanded images reveal that a number of discrete patterns of fluorescence can be seen, indicating local retension of FITC in the form of NP. **(C)** Fluorescence microscopic pictures of cross-sections from the FITC-NP–eluting stent and polymer-based FITC-eluting stent sites. Microscopic settings (exposure condition, filter, intensity of excitation light, and so forth) are the same between 2 pictures. **Scale b** = 100 μm. Abbreviations as in Figure 1.

sections from the non-NP polymer-based FITC stent site (data not shown). Autofluorescence of the internal and external elastic laminas made assessing the FITC distribution in the neointima and media possible. The neointima and media around the stent strut expressed intense fluorescence, whereas a discrete and circular-shaped fluorescence was noted in cells of either layer distal to the stent strut (Fig. 5B). Fluorescence-positive cell counts revealed that cellular FITC positivity was 90 ± 12% and 76 ± 10% (n = 8), in the neointima and the media, respectively. Adventitial delivery was more difficult to quantify, due to the presence of autofluorescence. After 6

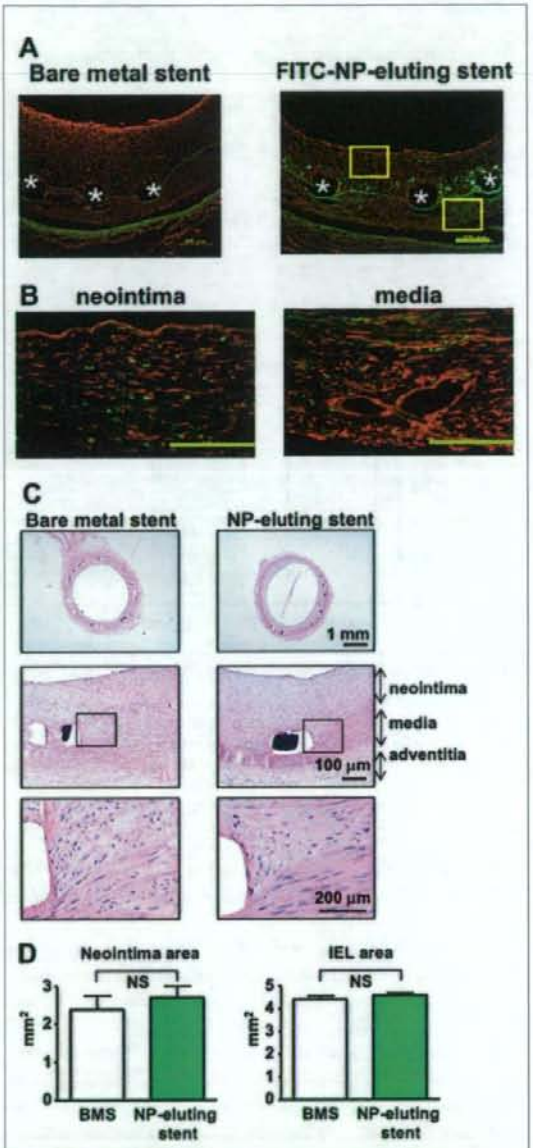


Figure 5. Localization of FITC Fluorescence and Histopathology in Porcine Coronary Artery 4 Weeks After Stenting

(A) Fluorescence microscopy photographs of cross-sections from bare-metal stent (BMS) and NP-eluting stent sites. **Scale b** = 100 μm. **(B)** Expanded images of neointima and media from boxed areas in **(A)**. **(C)** Representative low- **(upper panels)**, middle- **(middle panels)**, and high-magnification **(lower panels)** photomicrographs of hematoxylin-eosin stained sections of coronary arteries after 4 weeks from coronary artery segments that received BMS and the NP-eluting stents. **(D)** The neointimal area and the area within the internal elastic lamina (IEL) at the BMS and the NP-eluting stent sites (n = 6 each). *site of stent strut. Abbreviations as in Figure 1.

JACC: CARDIOVASCULAR INTERVENTIONS, VOL. 2, NO. 4, 2009
APRIL 2009:277–83

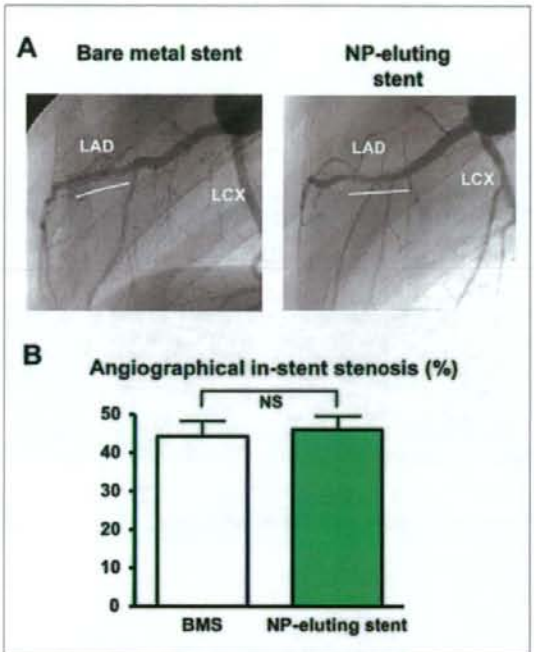


Figure 6. Coronary Arteriography and In-Stent Stenosis

(A) Representative coronary arteriographic images 4 weeks after nanoparticle (NP)-eluting stent and bare-metal stent (BMS) implantation. **White bars** in the images denote stent segment sites. **(B)** Angiographic in-stent stenosis 4 weeks after NP-eluting stent site (n = 8) and BMS site (n = 8). LAD = the left anterior descending coronary artery; LCX = left circumflex coronary artery.

weeks, cellular FITC signal declined spontaneously (cellular FITC positivity was <10% in the neointima and media, data not shown).

In hematoxylin-eosin stained sections, there were no significant differences in the degrees of inflammation among the 3 groups after 4 weeks (Fig. 5C, Online Fig. V, and Online Table). A significant in-stent neointima was formed in the non-coated bare metal stent and NP-eluting stent sites, and the neointima consisted mainly of VSMC. Quantitative analysis of the neointima after 4 weeks revealed no significant differences in neointima formation, stent area, or medial area between the 2 groups (Fig. 5D). In addition, angiographically examined in-stent stenosis was comparable between the 2 groups (Fig. 6).

Discussion

We present the first NP-eluting stent formulated with a newly invented cation electrodeposition coating technology as an excellent drug delivery platform. Importantly, this NP-mediated DDS is able to carry hydrophilic agents such as FITC (15,17), which offers advantages over the current stent-coating technology (see also introductory text). The PLGA is a bioabsorbable polymer with a long history of safe use in medical applications. For clinical use, PLGA-NP can be manufactured in pyrogen-free form under the good manufacture practice guidelines.

Characteristics of NP-eluting stent. To create a cationic electrodeposition coating, NP surface was rendered cationic with chitosan-mediated surface-modification (16). This cationization offers several advantages. Firstly, compared with anionic NP, cationic NP increases the intracellular incorporation rate of NP. Secondly, it accelerates the escape of entrapped NP from the endosomal compartment to cytoplasmic compartment. This escape is important, because therapeutic agents (drug, protein, DNA, and the like) are prone to destabilize in the endosomal compartment. Thirdly, NP retained in the cytoplasm or extracellular spaces release encapsulated drug slowly in conjunction with the hydrolysis of PLGA-NP and diffusion from NP. The slow intracellular release might result in sustained intracellular drug delivery. It is likely that these advantages contribute to the highly efficient delivery of NP eluted from the NP-eluting stent into the neointima and media in vitro (Fig. 2, Online Fig. IV) and in vivo (Figs. 3 to 5). Because FITC is hydrophilic and free FITC released from FITC-encapsulated NP must be washed out rapidly, a considerable part of small circular shaped fluorescence might come from NP still containing FITC within the cell or in extracellular spaces. The FITC release kinetics from the NP-eluting stent supports this notion. In contrast, prior reports showed that the intracellular drug/gene delivery capacity of stents coated with polymer and water-soluble drug/gene is limited (10,11); drug/gene delivery persisted for up to 7 days, and the percentage of positive cells ranged from 1% to 2%. Therefore, our present NP-eluting stent system is a more efficient DDS than those created with non-NP polymeric DES coating technology. Recently, we found that NP-eluting stents could deliver other water-soluble agents such as oligonucleotide or plasmid DNA into porcine coronary arteries (Drs. Nakano K and Egashira K, unpublished observations, May 2007). Therefore, our NP-eluting stent system provides an innovative platform for delivering therapeutic agents in the future treatment of cardiovascular diseases.

Clinical implication. Evidence of impaired arterial healing process is a major histopathological feature in the arteries of experimental animals (18,19) and humans (8,9) exposed to DES in current use. Therefore, it is important to consider potential toxicities associated with the delivery of PLGA NP from an NP-eluting stent system. Of a number of polymer matrix materials evaluated for stent coating, it has been shown that PLGA do not increase the incidence of thrombosis in a porcine coronary artery model (18). Our NP-eluting stents had no apparent effect on stent-induced injury, inflammation, or endothelial regeneration in vivo, suggesting that PLGA NP transfer to the arterial wall does not impair the arterial healing

process in this model. Overall, these data of vascular compatibility support the notion that this bioabsorbable PLGA NP-eluting stent system could be applied to human subjects without vascular toxicity.

Efficacy studies in animals are needed to determine the therapeutic potential of this NP-eluting stent system. Potential therapeutic strategies for this stent-based platform include the delivery of proteins or genes that inhibit inflammation, SMC proliferation, and thrombosis. We plan to examine the effects of antimonocyte chemoattractant protein-1 (13,20–22) or nuclear factor κ-B (12) delivered via the NP-eluting stent. Furthermore, it would be interesting to deliver multiple agents with different time courses from 1 NP-eluting stent. The bioabsorbtion time of PLGA in living body is controlled by molecular make-up of PLGA (the ratio of D,L-lactic acid and glycolic acid) (23), allowing us to modulate the time course of intracellular drug delivery. Finally, cell-specific delivery of NP into VSMC to suppress neointima formation or into endothelial cells to accelerate endothelial regeneration might be attractive strategies. Future studies will test the feasibility of each of these innovative approaches.

Conclusions

These data suggest that this bioabsorbable polymeric NP-eluting stent system has unique aspects in novel electrodeposition coating technology, vascular compatibility, and an efficient DDS (better and more prolonged delivery of FITC into the stented coronary artery), compared with dip-coated polymer-eluting stent. This system might hold promise as an innovative platform for the delivery of less invasive nano-devices targeting cardiovascular disease. Further efficacy and good laboratory practice-compliant safety studies are needed to prove this notion.

Acknowledgments

The authors would like to thank E. Iwata and M. Miyagawa for their excellent technical assistance.

Reprint requests and correspondence: Dr. Kensuke Egashira, Department of Cardiovascular Medicine, Graduate School of Medical Science, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: egashira@cardiol.med.kyushu-u.ac.jp.

REFERENCES

1. Babapulle MN, Eisenberg MJ. Coated stents for the prevention of restenosis: Part I. Circulation 2002;106:2734–40.
2. Babapulle MN, Eisenberg MJ. Coated stents for the prevention of restenosis: Part II. Circulation 2002;106:2859–66.
3. Serruys PW, Kutryk MJ, Ong AT. Coronary-artery stents. N Engl J Med 2006;354:483–95.
4. Laskey WK, Yancy CW, Maisel WH. Thrombosis in coronary drug-eluting stents: report from the meeting of the Circulatory System Medical Devices Advisory Panel of the Food and Drug Administration Center for Devices and Radiologic Health, December 7–8, 2006. Circulation 2007;115:2352–7.
5. Shuchman M. Debating the risks of drug-eluting stents. N Engl J Med 2007;356:325–8.
6. Shuchman M. Trading restenosis for thrombosis? New questions about drug-eluting stents. N Engl J Med 2006;355:1949–52.
7. Luscher TF, Steffel J, Eberli FR, et al. Drug-eluting stent and coronary thrombosis: biological mechanisms and clinical implications. Circulation 2007;115:1051–8.
8. Finn AV, Nakazawa G, Joner M, et al. Vascular responses to drug eluting stents. Importance of delayed healing. Arterioscler Thromb Vasc Biol 2007;27:1500–10.
9. Finn AV, Joner M, Nakazawa G, et al. Pathological correlates of late drug-eluting stent thrombosis: strut coverage as a marker of endothelialization. Circulation 2007;115:2435–41.
10. Klugherz BD, Jones PL, Cui X, et al. Gene delivery from a DNA controlled-release stent in porcine coronary arteries. Nat Biotechnol 2000;18:1181–4.
11. Walter DH, Cejna M, Diaz-Sandoval L, et al. Local gene transfer of phVEGF-2 plasmid by gene-eluting stents: an alternative strategy for inhibition of restenosis. Circulation 2004;110:36–45.
12. Ohtani K, Egashira K, Nakano K, et al. Stent-based local delivery of nuclear factor-kappaB decoy attenuates in-stent restenosis in hypercholesterolemic rabbits. Circulation 2006;114:2773–9.
13. Egashira K, Nakano K, Ohtani K, et al. Local delivery of anti-monocyte chemoattractant protein-1 by gene-eluting stents attenuates in-stent stenosis in rabbits and monkeys. Arterioscler Thromb Vasc Biol 2007;27:2563–8.
14. Wickline SA, Neubauer AM, Winter P, Caruthers S, Lanza G. Applications of nanotechnology to atherosclerosis, thrombosis, and vascular biology. Arterioscler Thromb Vasc Biol 2006;26:435–41.
15. Niwa T, Takeuchi H, Hino T, Kunou N, Kawashima Y. In vitro drug release behavior of D,L-lactide/glycolide copolymer (PLGA) nanospheres with nafarelin acetate prepared by a novel spontaneous emulsification solvent diffusion method. J Pharm Sci 1994;83:727–32.
16. Yamamoto H, Kuno Y, Sugimoto S, Takeuchi H, Kawashima Y. Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions. J Control Release 2005;102:373–81.
17. Panyam J, Zhou WZ, Prabha S, Sahoo SK, Labhasetwar V. Rapid endo-lysosomal escape of poly(DL-lactide-co-glycolide) nanoparticles: implications for drug and gene delivery. Faseb J 2002;16:1217–26.
18. van der Giessen WJ, Lincoff AM, Schwartz RS, et al. Marked inflammatory sequelae to implantation of biodegradable and nonbiodegradable polymers in porcine coronary arteries. Circulation 1996;94:1690–7.
19. Lincoff AM, Furst JG, Ellis SG, Tuch RJ, Topol EJ. Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. J Am Coll Cardiol 1997;29:808–16.
20. Ohtani K, Usui M, Nakano K, et al. Antimonocyte chemoattractant protein-1 gene therapy reduces experimental in-stent restenosis in hypercholesterolemic rabbits and monkeys. Gene Ther 2004;11:1273–82.
21. Egashira K, Zhao Q, Kataoka C, et al. Importance of monocyte chemoattractant protein-1 pathway in neointimal hyperplasia after periarterial injury in mice and monkeys. Circ Res 2002;90:1167–72.
22. Egashira K. Molecular mechanisms mediating inflammation in vascular disease: special reference to monocyte chemoattractant protein-1. Hypertension 2003;41:834–41.
23. Miller RA, Brady JM, Cutright DE. Degradation rates of oral resorbable implants (polylactates and polyglycolates): rate modification with changes in PLA/PGA copolymer ratios. J Biomed Mater Res 1977;11:711–9.

Key Words: drug delivery system ■ nanotechnology ■ restenosis ■ smooth muscle cells ■ stents.

APPENDIX

For a supplementary Methods section and supplementary figures, please see the online version of this article.

Journal Scan

Kim A. Eagle, M.D., M.A.C.C., Associate Editor

Interventional Cardiology

Talk Back

Title: Formulation of Nanoparticle-Eluting Stents by a Cationic Electrodeposition Coating Technology: Efficient Nano-Drug Delivery via Bioabsorbable Polymeric Nanoparticle-Eluting Stents in Porcine Coronary Arteries
Topic: Interventional Cardiology
Date Posted: 4/20/2009 5:00:00 PM
Author(s): Nakano K, Egashira K, Masuda S, et al.
Citation: *J Am Coll Cardiol Intv* 2009;2:277-283.
Clinical Trial: No

Study Question: Can stents be designed to efficiently deliver nanoparticles (NPs)?

Related Resources

JACC Intv: Formulation of Nanoparticle-Eluting Stents by a Cationic Electrodeposition Coating Technology: Efficient Nano-Drug Delivery via Bioabsorbable Polymeric Nanoparticle-Eluting Stents in Porcine Coronary Arteries

Methods: Cationic NPs encapsulated with a fluorescence marker (FITC) were prepared and coated on a stent with cation electrodeposition technology. The in vitro and in vivo characteristics of the FITC-loaded NP-eluting stents were compared with dip-coated FITC-eluting stents and bare-metal stents.

Results: The NP was taken up by cultured vascular smooth muscle cells in vitro. In a porcine coronary artery model, substantial FITC fluorescence was observed in the neointimal and medial layers of the stented segments that had received the FITC-NP-eluting stent for 4 weeks. In contrast, no substantial FITC fluorescence was observed in the segments from the polymer-based FITC-eluting stent or from the bare-metal stent. The magnitude of stent-induced injury, inflammation, endothelial recovery, and neointima formation were comparable between the bare-metal stent and NP-eluting stent groups.

Conclusions: This NP-eluting stent is an efficient NP-mediated drug delivery system that may serve as an innovative platform for the delivery of less invasive nano-devices targeting cardiovascular disease.

Perspective: Drug-eluting stents (DES) have been highly successful in reducing in-stent restenosis; however, current DES are associated with increased late stent thrombosis, an often catastrophic complication. Late stent thrombosis is likely due to long-term antiproliferative effects of the current drugs used to coat stents, which impair healing of the vessel wall. Polymers used in the construction of DES may also impair healing. The current methods used to construct DES involve dip or spray techniques that are useful for strongly lipophilic drugs. Stent drug delivery platforms with biodegradable polymers capable of releasing water-soluble drugs, including oligonucleotides, may be highly advantageous. The current study shows promising preliminary data that such a stent platform is feasible. This system should allow targeting of numerous molecular pathways that may prevent restenosis without thrombosis risk. Daniel T. Eitzman, M.D., F.A.C.C.

Talk Back

Source

Content provided by the American College of Cardiology Foundation

Cardiosource
American College of Cardiology

Welcome Kaku Nakano | Logout
My Account | My CME/CE
Sign Up Now

Search [Go] • MEDLINE

Institutional Access • Feedback

Home | Library & Images | News & Views | Guidelines & Trials | Recertification (MOC) | Annual Meeting/i2 | Practice Tools | Registry Resources | Products | CME/CE

Cardiosource en Español

Clinical Collections
- Arrhythmias
- Cardiovascular Surgery
- Congenital Heart Disease
- General Cardiology
- Heart Failure/Transplant
- Interventional Cardiology
- Noninvasive Cardiology
- Prevention/Vascular

Cardiosource Quick Links
- ACC.org
- JACC Journals
- ACCEL Audio
- CardioSmart for Your Patients NEW!
- Braunwald's Heart Disease
- Cardiac Care Associates/CE
- Fellows-in-Training
- Case Studies
- CME/CE for Health Care Providers
- Conversations with Experts
- CVN - Cardiosource Video Network
- Guidelines
- Heart Songs
- Performance Improvement CME/CE
- RSS Feed
- Talk Back

Highlights & Features

Editor's Choice
April 23, 2009


Chris Cannon, MD, FACC Editor-in-Chief

From *EHJ*: Infarct size and myocardial salvage after primary angioplasty in early presenters vs. late presenters.

ACC supports clinical and cost-effectiveness research.

From *JAMA*:
- Association of physician certification and outcomes among patients receiving an ICD — analysis of data from the NCDR.
- Screening for asymptomatic CAD in patients with type 2 diabetes (DIAD)

From *JACC*:
- CMR in myocarditis: a JACC White Paper.
- Efficacy of ranolazine in patients with chronic angina: MERLIN-TIMI 36 Trial.

From *JACC Interventions*: Formulation of nanoparticle-eluting stents.

From *Arch Intern Med*:
- Impact of guideline adherence intervention on cholesterol management in primary care practices.
- Link between dietary factors and CHD.

CV News Digest – What Your Patients Are Reading

Apr 23, 2009 | Research suggests drinks sweetened with fructose may lead to higher levels of LDL cholesterol, triglycerides. (*Time*)

Apr 23, 2009 | FDA approves new version of Abiomed heart pump. (*AP*)

Apr 23, 2009 | Managed care providers experiencing enrollment losses due to recession. (*Wall Street Journal*)

- More News

ADVERTISEMENT

A 54 y.o. man on aspirin presents with chest pain, elevated troponin, and ST depression; a cath is planned for the following day. Would you treat with:

- A. Unfractionated heparin (UFH) only
- B. Enoxaparin only
- C. UFH and clopidogrel
- D. Enoxaparin and clopidogrel
- E. Bivalirudin and clopidogrel
- F. UFH and GP IIb/IIIa inhibitor
- G. UFH, GP IIb/IIIa inhibitor, and clopidogrel

View Results

ADVERTISEMENT

Co-sponsored CME

Journal Scan *(updated daily)*
Apr 24, 2009

A Comparison of Abciximab and Small Molecule Glycoprotein IIb/IIIa Inhibitors in Patients Undergoing Primary Percutaneous Coronary Intervention: A Meta-Analysis of Contemporary Randomized Controlled Trials (*Circ Cardiovasc Intervent* 2009;Apr 21:[Epub ahead of print].)

Syncope and Risk of Sudden Death in Hypertrophic Cardiomyopathy (*Circulation* 2009;119:1703-1710.)

Cardiometabolic Abnormalities in Current National Football League Players (*Am J Cardiol* 2009;103:969-971.)

Amiodarone for the Prevention of Sudden Cardiac Death: A Meta-Analysis of Randomized Controlled Trials (*Eur Heart J* 2009;Mar 31:[Epub ahead of print].)

Cardiac Outcomes After Screening for Asymptomatic Coronary Artery Disease in Patients With Type 2 Diabetes: The DIAD Study: A Randomized Controlled Trial (*JAMA* 2009;301:1547-1555.)

- More Journal Scan

JACC Online
Apr 20, 2009

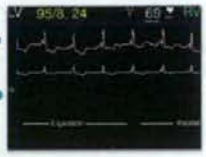
Cardiovascular Magnetic Resonance in Myocarditis: A JACC White Paper

Five-Year Follow-Up After Sirolimus-Eluting Stent Implantation: Results of the SIRIUS (Sirolimus-Eluting Stent in De-Novo Native Coronary Lesions) Trial

- More JACC

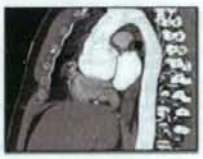
Expert Opinions
Apr 10, 2009

Analyze This Image
Apr 9, 2009



Analyze This Image: Simultaneous Intracardiac Pressure Tracing From a 52-Year-Old Woman With Worsening Dyspnea CME CE

Case Studies
Apr 22, 2009



A 47-Year-Old Male Presents to the ER With Persistent, Sharp Chest Pain CME

Echo of the Month: A 45-Year-Old Woman Is Referred for a Second Opinion Regarding Treatment of a Valve Condition CME

- More Case Studies
- Guideline-Based Case Studies

Clinical Trials
Apr 24, 2009

Heart Failure–A randomized Controlled Trial Investigating Outcomes of exercise traiNing (HF-ACTION)

Warfarin and Antiplatelet Trial in Chronic

Journal Scan

Kim A. Eagle, M.D., M.A.C.C., Associate Editor

Editor of Journal Scan selects must-read journal articles and summarizes them with incisive commentary. Most recent updates listed below.

Interventional Cardiology

Result Pages: [1] 2 3 4 5 6 7 8 9 10 > >> Viewing 1-20 of 691

Apr 27, 2009
Benefits From Small Molecule Administration as Compared With Abciximab Among Patients With ST-Segment Elevation Myocardial Infarction Treated With Primary Angioplasty: A Meta-Analysis
De Luca G, Usel G, Cassetti E, Marino P.
J Am Coll Cardiol 2009;53:1668-1673.

Apr 24, 2009
A Comparison of Abciximab and Small Molecule Glycoprotein IIb/IIIa Inhibitors in Patients Undergoing Primary Percutaneous Coronary Intervention: A Meta-Analysis of Contemporary Randomized Controlled Trials
Gurm HS, Tamhane U, Meier P, Grossman PM, Chetcuti SJ, Bates ER.
Circ Cardiovasc Interv 2009;Apr 21:[Epub ahead of print].

Apr 23, 2009
Infarct Size and Myocardial Salvage After Primary Angioplasty in Patients Presenting With Symptoms for <12 h vs. 12-72 h
Busk M, Kaltoft A, Nielsen SS, et al.
Eur Heart J 2009;Apr 8:[Epub ahead of print].

Apr 20, 2009
Formulation of Nanoparticle-Eluting Stents by a Cationic Electrodeposition Coating Technology: Efficient Nano-Drug Delivery via Bioabsorbable Polymeric Nanoparticle-Eluting Stents in Porcine Coronary Arteries
Nakano K, Egashira K, Masuda S, et al.
J Am Coll Cardiol Intv 2009;2:277-283.

Apr 15, 2009
Prognostic Significance of Small Troponin I Rise After a Successful Elective Percutaneous Coronary Intervention of a Native Artery
De Labriolle A, Lemesle G, Bonello L, et al.
Am J Cardiol 2009;103:639-645.

Apr 14, 2009
A Randomized Controlled Trial of a Paclitaxel-Eluting Stent Versus a Similar Bare-Metal Stent in Saphenous Vein Graft Lesions: The SOS (Stenting Of Saphenous Vein Grafts) Trial
Briakis ES, Lichtenwalter C, de Lemos JA, et al.
J Am Coll Cardiol 2009;53:919-928.

Apr 9, 2009
Catheter-Based Renal Sympathetic Denervation for Resistant Hypertension: A Multicentre Safety and Proof-of-Principle Cohort Study
Krum H, Schlaich M, Whitbourn R, et al.
Lancet 2009;373:1275-1281.

Apr 3, 2009
The Uncertain Value of Renal Artery Interventions: Where Are We Now?
Textor SC, Lerman L, McKusick M.
J Am Coll Cardiol Intv 2009;2:175-182.

Apr 2, 2009
Mini-Crush Versus T-Provisional Techniques in Bifurcation Lesions: Clinical and Angiographic Long-Term Outcome After Implantation of Drug-Eluting Stents
Galassi AR, Tomasello SD, Capodanno D, Barrano G, Ussia GP, Tamburino C.
J Am Coll Cardiol Intv 2009;2:185-194.

Apr 1, 2009
Integration of Multislice Computed Tomography With Magnetic Navigation Facilitates Percutaneous Coronary Interventions Without Additional Contrast Agents
Ramchandar S, Pugliese F, Schultz C, et al.
J Am Coll Cardiol 2009;53:741-746.

Mar 26, 2009
A Bioabsorbable Everolimus-Eluting Coronary Stent System (ABSORB): 2-Year Outcomes and Results From Multiple Imaging Methods
Serruys PW, Ormiston JA, Onuma Y, et al.
Lancet 2009;373:897-910.

Mar 25, 2009
Quality of Life After Late Invasive Therapy for Occluded Arteries
Mark DB, Pan W, Clapp-Channing NE, et al., on behalf of the Occluded Artery Trial Investigators.
N Engl J Med 2009;360:774-783.

Mar 23, 2009
Clinical and Angiographic Outcomes With Sirolimus Eluting Stents in Total Coronary Occlusions: The ACROSS/TOSCA-4 (Approaches to Chronic Occlusions With Sirolimus-Eluting Stents/Total Occlusion Study of Coronary Arteries-4) Trial
Kandzari DE, Rao SV, Moses JW, et al., on behalf of the ACROSS/TOSCA-4 Investigators.
J Am Coll Cardiol Intv 2009;2:97-106.

Mar 20, 2009
A Prospective Feasibility Trial Investigating the Use of the Impella 2.5 System in Patients Undergoing High-Risk Percutaneous Coronary Intervention (The PROTECT I Trial): Initial U.S. Experience
Dixon SR, Henriques J, Mauri L, et al.
J Am Coll Cardiol Intv 2009;2:91-96.

Mar 19, 2009
2-Year Clinical Outcomes After Implantation of Sirolimus-Eluting, Paclitaxel-Eluting, and Bare-Metal Coronary Stents: Results From the WDHR (Western Denmark Heart Registry)
Kaltoft A, Jensen LO, Maeng M, et al.
J Am Coll Cardiol 2009;53:658-664.

Mar 18, 2009
Antiplatelet Therapy and Stent Thrombosis After Sirolimus-Eluting Stent Implantation
Kimura T, Morimoto T, Nakagawa Y, et al., on behalf of the j-Cypher Registry Investigators.
Circulation 2009;119:987-995.

Mar 16, 2009
Nine-Month Clinical, Angiographic, and Intravascular Ultrasound Results of a Prospective Evaluation of the Axxess Self-Expanding Biolimus A9-Eluting Stent in Coronary Bifurcation Lesions: The DIVERGE (Drug-Eluting Stent Intervention for Treating Side Branches Effectively) Study
Verheye S, Agostoni P, Dubois CL, et al.
J Am Coll Cardiol 2009;53:1031-1039.

Mar 9, 2009

Prasugrel Compared With Clopidogrel in Patients Undergoing Percutaneous Coronary Intervention for ST-Elevation Myocardial Infarction (TRITON-TIMI 38): Double-Blind, Randomised Controlled Trial
Montalescot G, Wiviott SD, Braunwald E, et al., on behalf of the TRITON-TIMI 38 investigators.
Lancet 2009;373:723-731.

Mar 9, 2009
Intracoronary Electrocardiogram Recording With a Bare-Wire System: Perioperative ST-Segment Elevation in the Intracoronary Electrocardiogram Is Associated With Myocardial Injury After Elective Coronary Stent Implantation
Ueiani T, Amano T, Kumagai S, et al.
J Am Coll Cardiol Intv 2009;2:127-135.

Mar 6, 2009
Percutaneous Transcatheter Closure of Prosthetic Mitral Paravalvular Leaks: Are We There Yet?
Kim MS, Casserly IP, Garcia JA, Klein AJ, Salcedo EE, Carroll JD.
J Am Coll Cardiol Intv 2009;2:81-90.

Result Pages: [1] 2 3 4 5 6 7 8 9 10 > >> **Viewing 1-20 of 691**

Online Data Supplements

Methods

Preparation of cationic PLGA NP with surface modification with chitosan

A lactide/glycolide copolymer (PLGA) with an average molecular weight of 20,000 and a copolymer ratio of lactide to glycolide of 75:25 (PLGA7520; Wako Pure Chemical Industries, Osaka, Japan) was used as a wall material for the NP. According to manufacturer's instruction, a bioabsorption half-life of this product is 2 weeks in rat tissue.[1] Chitosan (Mw. 50,000; deacetylation degree 80%; Katakurachikkarin, Tokyo, Japan) was used to coat the surface of PLGA NP. Polyvinylalcohol (PVA-403; Kuraray, Osaka, Japan) was used as a dispersing agent. Fluorescein-isothiocyanate (FITC; Dojindo laboratories, Kumamoto, Japan), a water-soluble reagent, was used as a fluorescent marker of the NP. PLGA NP encapsulated with FITC were prepared by a previously reported emulsion solvent diffusion method in purified water. PLGA (2 g) were dissolved in a mixture of acetone (40 ml), ethanol (20 ml). Then, FITC (100 mg) was added into this solution. The resultant polymer-FITC solution was emulsified in PVA and chitosan solution under stirring at 400 rpm using the propeller-type agitator with four blades. After agitating the system for about 1 h under reduced pressure at 40 °C, the entire suspension was centrifuged (41,000 x *g* for 20 min at -20 °C). After removing the supernatant, purified water was added to mix with the sediment. The wet mixture was then centrifuged again to remove the excess PVA and the unencapsulated reagent that could not adsorb on the surfaces of NP. After repeating this process, the resultant dispersion was freeze-dried under the same conditions (See online Figure IA).

The mean particle size was analyzed by light scattering method (Microtrack

UPA150; Nikkiso, Tokyo, Japan). A sample of nanoparticulate suspension in distilled water was used for particle size analysis. An average particles size of 241.6 nm (D10 = 147.2 nm, D90 = 284.1 nm) with a narrow size distribution (see online Figure IB). The FITC-encapsulated PLGA NP contained 5 % FITC. The zeta potential of the NP as measured by Zetasizer Nano Z (Malverm, America) was +6.7 mV.

Preparation of NP-eluting stent by a cationic electrodeposition coating technology

A 15-mm-long stainless-steel, balloon-expandable stents (Multilink, Guidant) were ultrasonically cleaned by acetone, ethanol (70%), and demineralized water. Cationic electrodeposition coating was prepared on cathodic stents in PLGA NP solution at a concentrations of 50 mg/mL in distilled water with current maintained at 2.0 mA by a direct current power supply (DC power supply, Nippon Stabilizer Co, Tokyo, Japan) for different period under sterile conditions (illustrative explanation in next page). The coated stents were-dried vacuously overnight. This electrodeposition coating procedure produced a coating of approximately 654 ± 168 μg of NP per stent and 31 ± 2 μg of FITC per stent (n= 12). Surface of some NP-coating stents were observed with scanning electron microscopy (JXM8600, JEOL, Tokyo, Japan).

As control, dip-coated stents with thin layers of PLGA polymer, containing about 600 μg of PLGA polymer and 30 μg of FITC, were prepared as we previously described [2]. Prior to experimental use, non-coated bare metal and NP-coated stents were mounted over the 3-mm balloon for implantion in the coronary artery. These balloon mounted stent sets were sterilized using ethylene oxide gas.

Schematic illustration of the electrodeposition coating system (chamber, electrodes, and DC power supply) for a cation nanoparticle coating technology. Three steps of electrodeposit coating procedure are shown in online Figure II.

Measurement of *in vitro* release kinetics To measure NP release kinetics *in vitro*, the FITC-NP-coated stents (n=8) were immersed in Tris-EDTA buffer, and the FITC containing NP that was subsequently eluted into the buffer was measured using a fluorescence plate reader (Mithras LB 940-AS, Berthold Technologies, Bad Wildbad, Germany) at excitation and emission wavelengths of 480 and 525 nm, respectively. To measure FITC release kinetics, FITC-NP ($n = 8$) were immersed in Tris-EDTA buffer, and the released FITC from NP was measured.

Cellular uptake and intracellular distribution of nanoparticles Human coronary artery smooth muscle cells (SMCs) were obtained from Cambrex Bio Science Walkersville, Inc. and cultured in SmGM-2 (Cambrex Bioscience, Charles City, IA). Each cells were used between passages 4 to 8.

The SMCs were seeded on 48-well culture plate to an initial concentration of 1×10^5 cells per well and incubated at 37 °C/5 % CO_2 environment until cells were subconfluent. The growth medium was replaced with the FITC-loaded PLGA nanoparticles suspension medium (0.1 to 0.5 mg/mL) and then further incubated for 1 hour. The cells were then washed three times with PBS to eliminate extracellular nanoparticles which were not incorporated into the cells. Then, the cells were fixed with 1 % formaldehyde/PBS buffer and nuclear was counterstained with propidium iodide. Cellular uptake of FITC-loaded PLGA NP was evaluated by confocal fluorescence

microscopy. The images were digitized and analyzed with Adobe Photoshop and Scion Image Software. The total number of fluorescence positive cells in each field and the number of total cells was counted. Cellular uptake percentage was assessed by the percentage of fluorescence positive cells per total cells in each field. In part of experiment, the internal properties of NP were examined by transmission electron microscopy (H-7000E, Hitachi, Tokyo, Japan).

Cytotoxicity assay The cytotoxicity of PLGA nanoparticles on human coronary artery SMCs was determined using a MTS assay (Cell Counting Kit-8, Dojindo Inc., #343-07626). The cells were grown in 96-well microtiter plates for 24 hours, and then they were treated with different concentrations of FITC-loaded PLGA nanoparticles suspension medium with 10 % FBS. The plates were incubated for 48 hours and then the medium was replaced with 200 μl of fresh medium. Next, 2 mg/ml MTS solution was added and the plates were incubated again for 4 hours at 37°C/5%CO_2. Finally, absorbance was measured at 490 nM using an enzyme-linked immunosorbent assay reader. Cell viability was expressed as the ratio between the amount of formazan determined for cells treated with PLGA nanoparticles and for control non-treated cells.

Animal Preparation and Stent implantation The animal-model experiments were reviewed and approved by the Committee on Ethics on Animal Experiments, Kyushu University Faculty of Medicine, and were performed according to the guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. Domestic male pigs (Kyudo, Tosu, Japan; aged 2 to 3 months and weighing 25 to 30 kg) were used.

Animals were anesthetized and divided into 3 groups, which underwent deployment of either a non-coated bare metal stents (1 week, n = 3; 2 week, n=3; 4 week, n = 8; 6 week, n = 3), FITC-incorporated NP-eluting stents (2 week, n = 3; 2 week, n=3; 4 week, n= 8; 6 week, n = 3), or dip-coated stents with thin layers of PLGA polymer containing FITC (1 week; n=3, 2 week; n=3, 4 week; n=3), to either the left anterior descending (LAD) or the left circumflex coronary (LCx) arteries. After arterial blood samples were taken, animals were euthanized with a lethal dose of anesthesia after 1, 4, or 6 weeks, and the stented arterial sites and contralateral non-stented sites were excised for biochemical, immunohistochemical, and morphometric analyses.

A segment with a mean coronary diameter of 2.5 mm was selected by using quantitative coronary angiography with a stent-to-artery ratio of approximately 1.1 to 1.2. A balloon catheter mounted with a stent was then advanced to the pre-selected coronary segments for deployment over a standard guide wire. The balloon catheter was inflated at 15 atm for 60 seconds once and was then slowly withdrawn, leaving the stent in place. All animals received aspirin (330 mg/day) and ticlopidine (200 mg/day) until euthanasia from 3 days before stent implantation procedure.

Coronary Angiography Left coronary angiography was performed before, immediately after, and 4 weeks after the stent implantation. A preshaped Judkins catheter was inserted into the right or left carotid artery, and coronary angiography in a left anterior oblique view was performed. Arterial pressure, heart rate, and ECG were continuously monitored and recorded on a recorder (Nihon Kohden, Tokyo, Japan).

Histopathological study The left coronary artery was perfused with 10 % buffered formalin at 120 mm Hg and fixed for 24 hours. The stented artery segments were isolated and processed as described previously[2,3]: The segment was divided into two

parts at the center of the stent, and the proximal part was embedded in methyl methacrylate mixed with *n*-butyl methacrylate to allow for sectioning through the metal stent struts. Serial sections were stained with elastica van Gieson or with hematoxylin-eosin (HE). The neointimal area, the area within the internal elastic lamina (IEL), and the lumen area were measured by computerized morphometry, which was carried out by a single observer who was blinded to the experimental protocol. All images were captured by an Olympus microscope equipped with a digital camera (HC-2500) and were analyzed using Adobe Photoshop 6.0 and Scion Image 1.62 Software. The injury and inflammatory scores were determined at each strut site, and mean values were calculated for each stented segment (Supplementary Table online). The extent of re-endothelialization was semi-quantitatively expressed as the percentage of circumference covered by the endothelium, as described previously. [4] The 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 (1=25%; 2=25% to 75%; 3=>75%).

The distal part of the stent was used for either fluorescence or pathological analysis after the stent struts were gently removed with micro-forceps. For fluorescence microscopic analysis, the tissue was frozen and subjected to cryosection (5-μm) and counterstain with propidium iodide and examined using fluorescence microscopy. At least 5 high-power field pictures from 2 to 3 sections were taken, and cells positive with nuclear or peri-nuclear FITC among cells with nuclear propidium iodide positivity.

References

1. Okada H, Inoue Y, Heya T, Ueno H, Ogawa Y, Toguchi H. Pharmacokinetics of once-a-month injectable microspheres of leuprolide acetate. *Pharm Res.*

1991;8:787-91.

2. Ohtani K, Egashira K, Nakano K, Zhao G, Funakoshi K, Ihara Y, Kimura S, Tominaga R, Morishita R, Sunagawa K. Stent-based local delivery of nuclear factor-kappaB decoy attenuates in-stent restenosis in hypercholesterolemic rabbits. *Circulation*. 2006;114:2773-9.
3. Ohtani K, Usui M, Nakano K, Kohjimoto Y, Kitajima S, Hirouchi Y, Li XH, Kitamoto S, Takeshita A, Egashira K. Antimonocyte chemoattractant protein-1 gene therapy reduces experimental in-stent restenosis in hypercholesterolemic rabbits and monkeys. *Gene Ther*. 2004;11:1273-82.
4. Suzuki T, Kopia G, Hayashi S, Bailey LR, Llanos G, Wilensky R, Klugherz BD, Papandreou G, Narayan P, Leon MB, Yeung AC, Tio F, Tsao PS, Falotico R, Carter AJ. Stent-based delivery of sirolimus reduces neointimal formation in a porcine coronary model. *Circulation*. 2001;104:1188-93.