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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

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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

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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 nonoptimal 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 for 1 h, resulting in prominent cellular FITC positivity

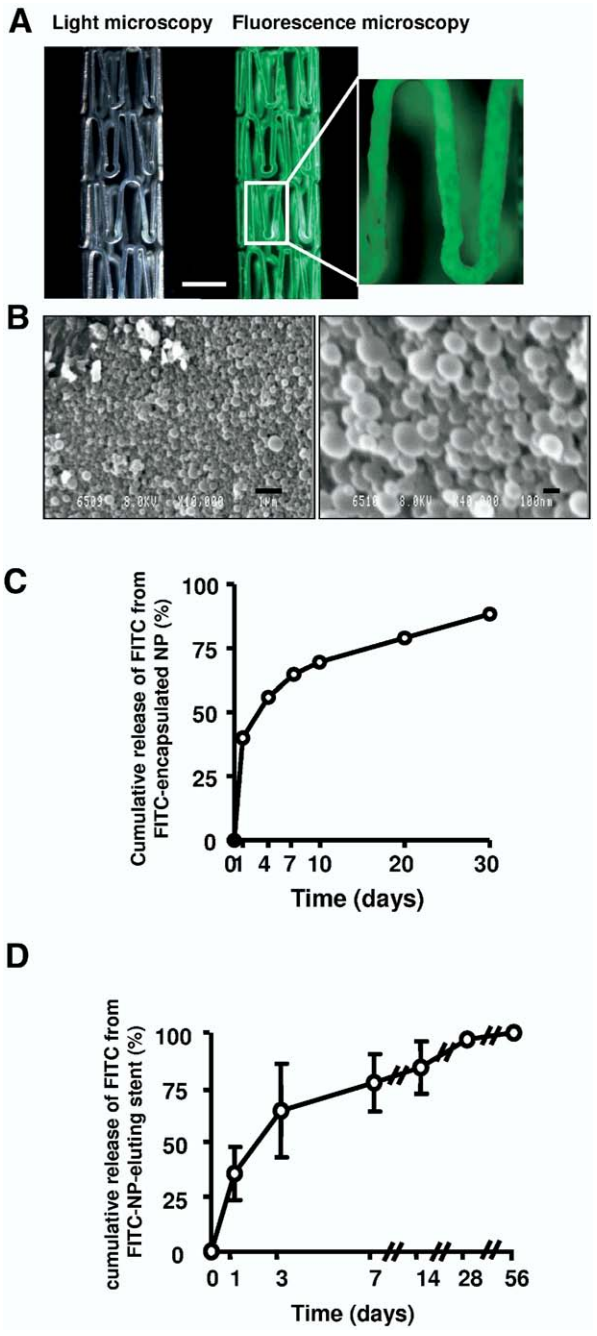

Figure 1. Formulation of FITC-Encapsulated NP-Eluting Stents by a Cationic Electrodeposition Coating Technology

(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.

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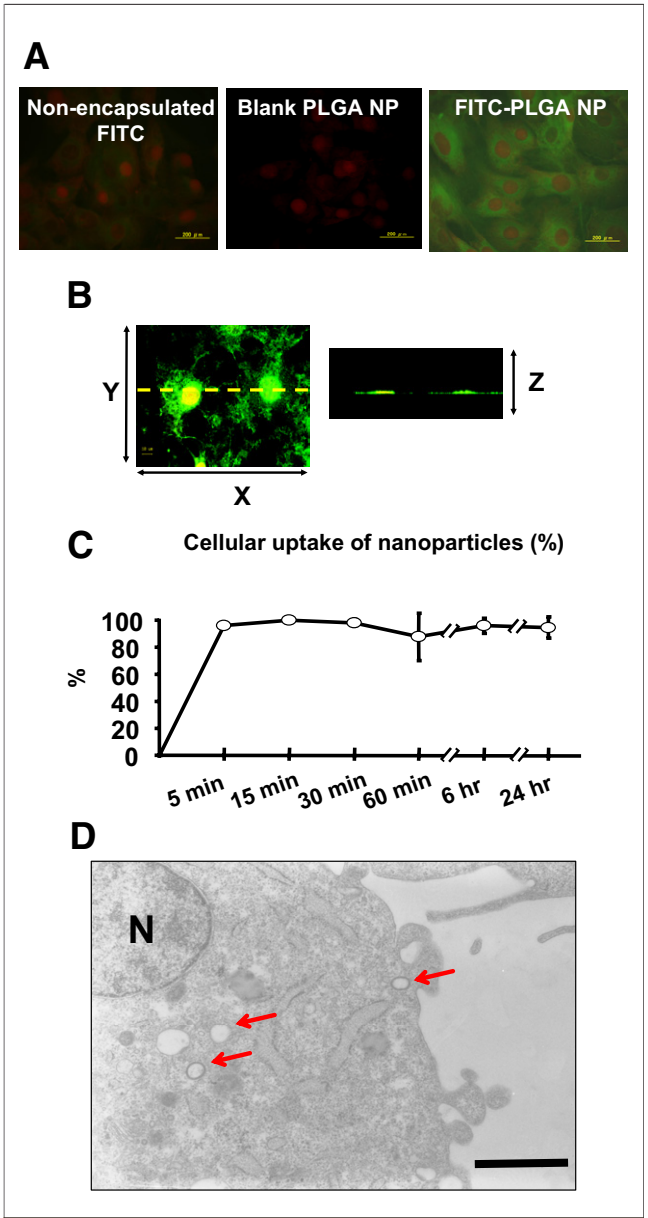

Figure 2. Cellular Uptake and Intracellular Distribution of NP in Human Coronary Artery SMCs In Vitro

(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.

(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

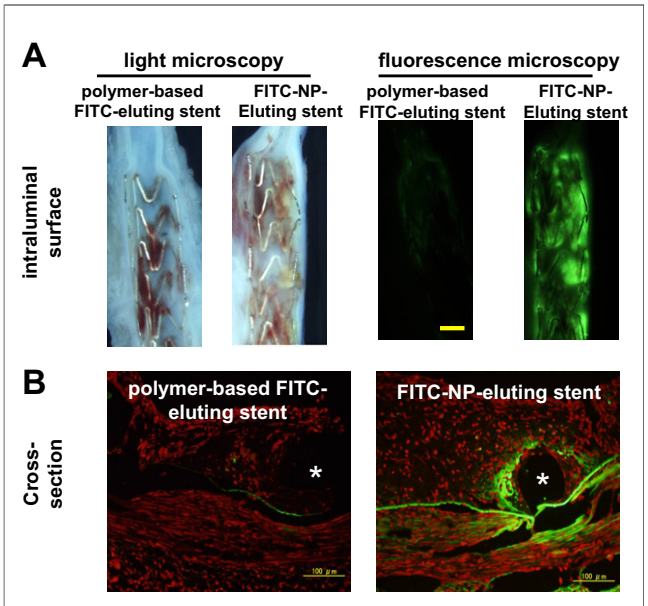

Figure 3. Localization of FITC Fluorescence After Deployment of FITC-NP–Eluting Stent in Porcine Coronary Arteries 1 Week After Stenting

(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 bar** = 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 bar** = 100 μm. *Site of stent strut. Abbreviations as in Figure 1.

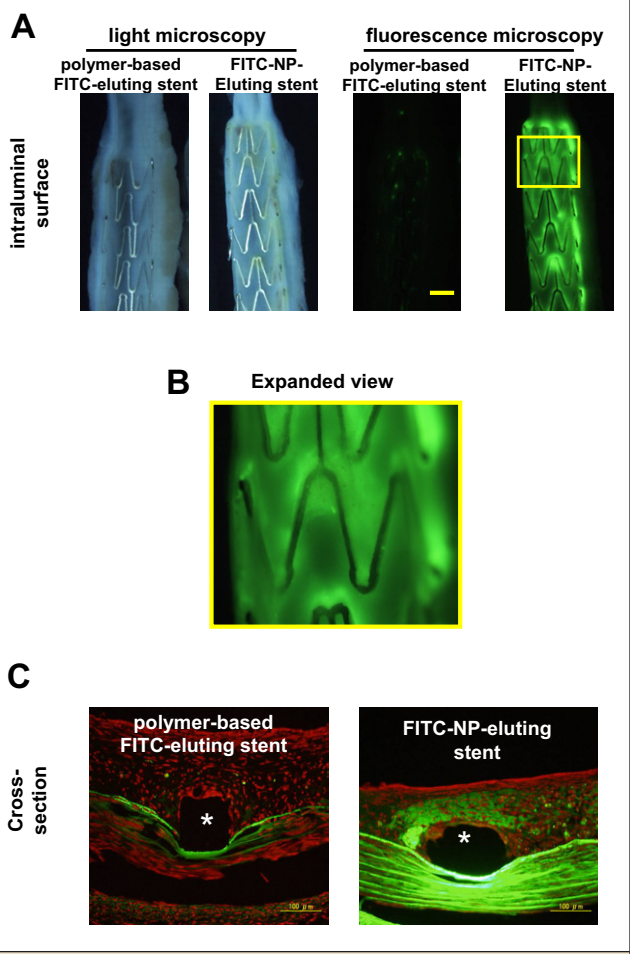

Figure 4. Localization of FITC Fluorescence After Deployment of FITC-NP–Eluting Stent in Porcine Coronary Arteries 2 Weeks After Stenting

(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 bar** = 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 bar** = 100 μm. Abbreviations as in Figure 1.

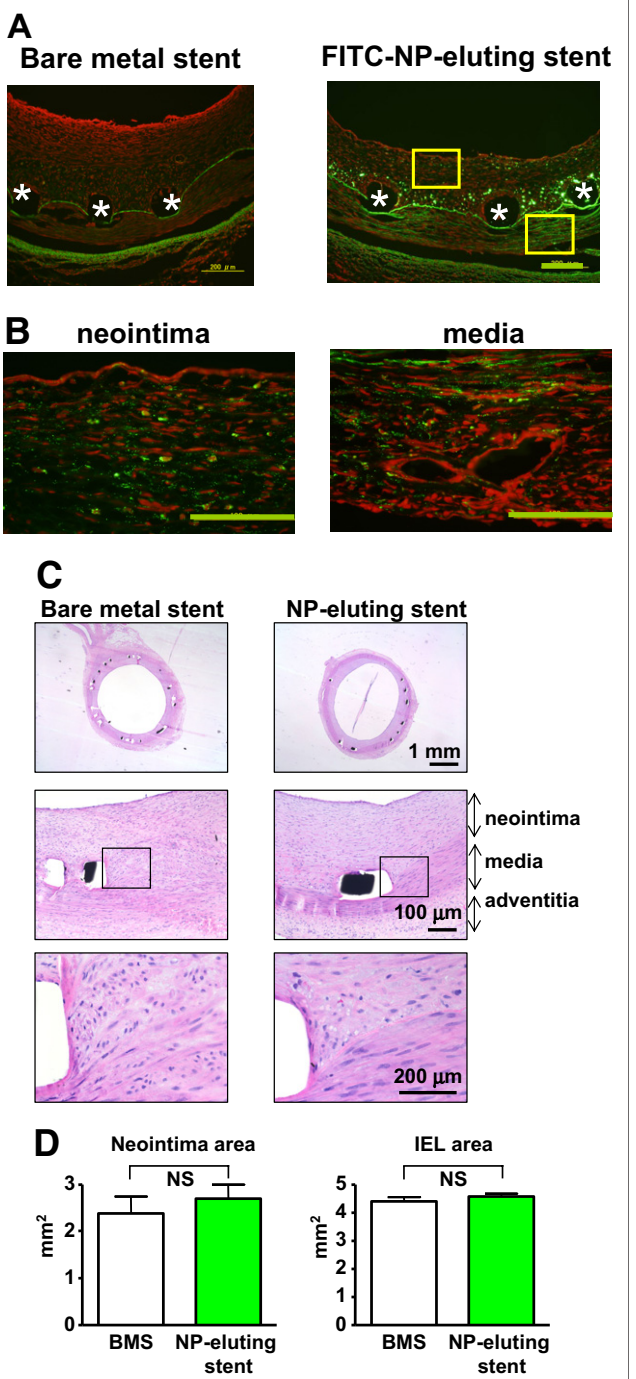

Figure 5. Localization of FITC Fluorescence and Histopathology in Porcine Coronary Artery 4 Weeks After Stenting

(A) Fluorescence microscopy photographs of cross-sections from bare-metal stent (BMS) and NP-eluting stent sites. **Scale bar** = 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.

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-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 =

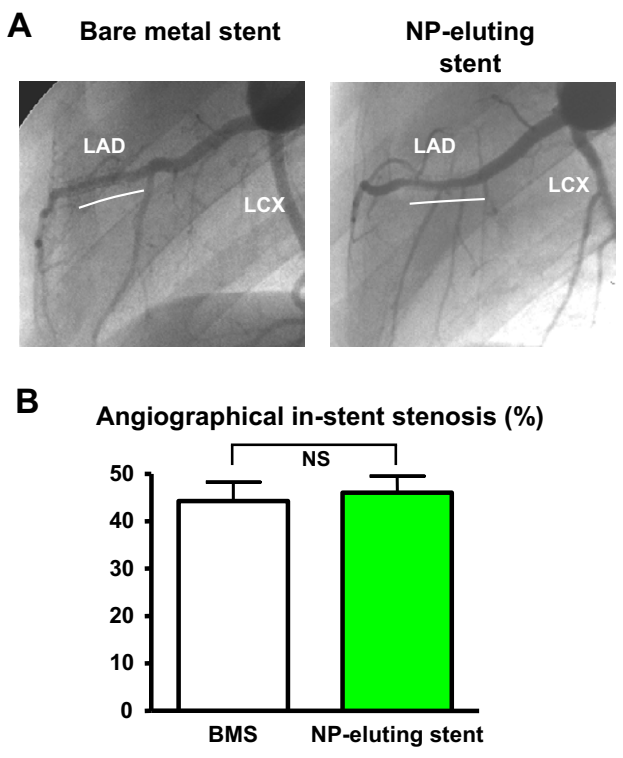

Figure 6. Coronary Arteriography and In-Stent Stenosis

(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.

8), in the neointima and the media, respectively. Adventitial delivery was more difficult to quantify, due to the presence of autofluorescence. After 6 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 released 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. K. Nakano and K. Egashira, 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, suggest-

ing 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.

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

APPENDIX

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