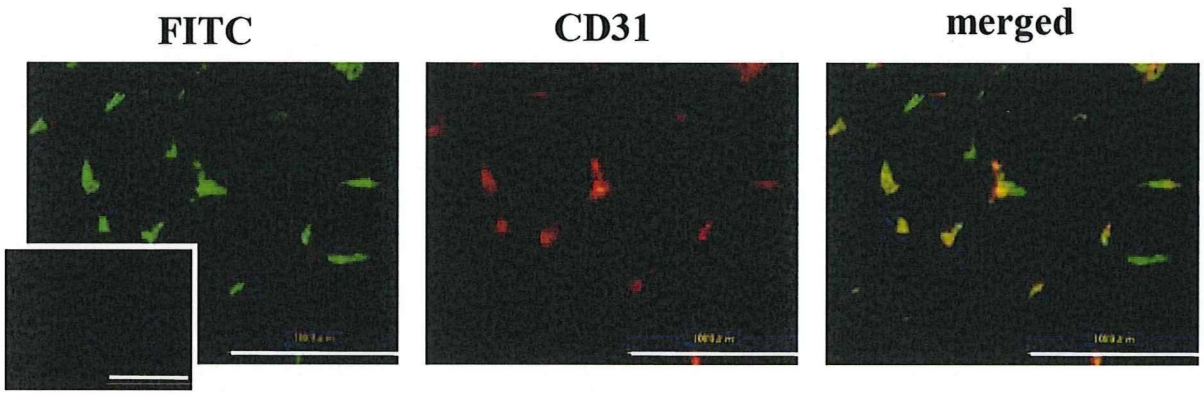
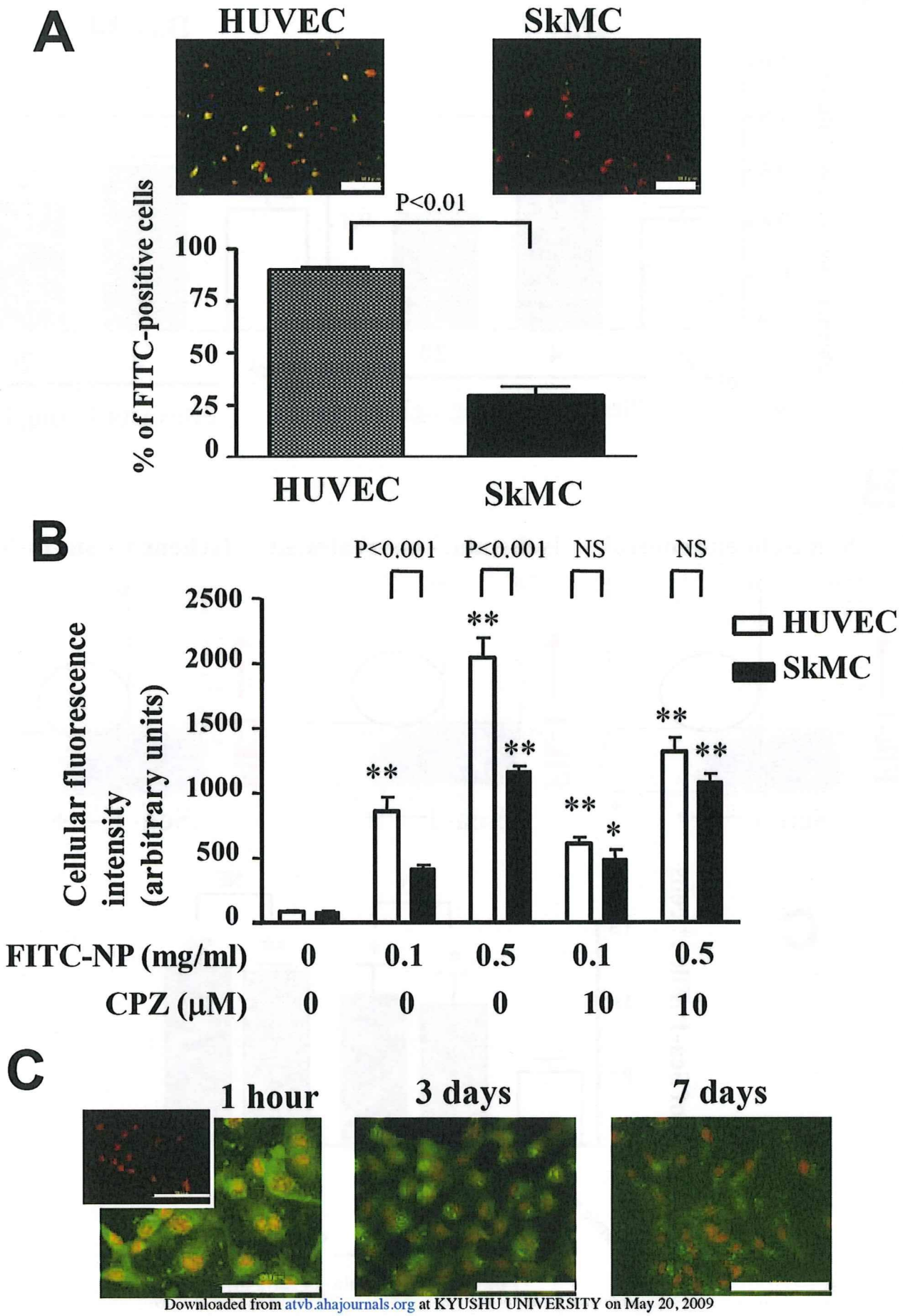
Supplementary Figure I *Kubo M et al.*



Supplementary Figure II *Kubo M et al.*



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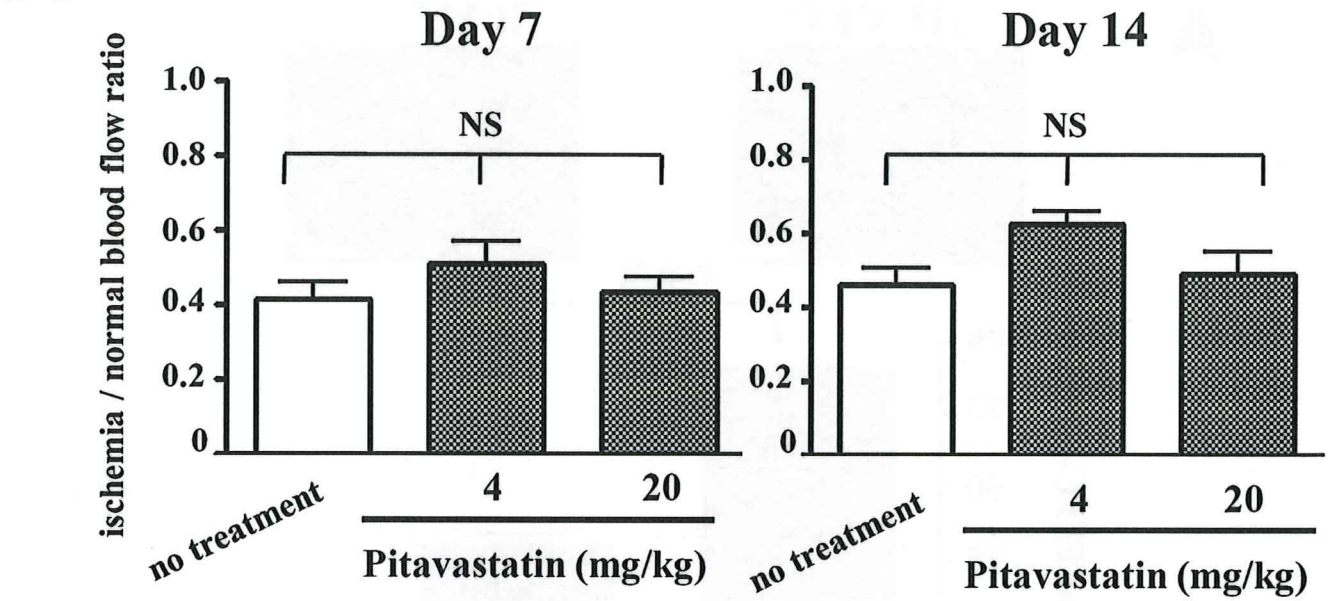
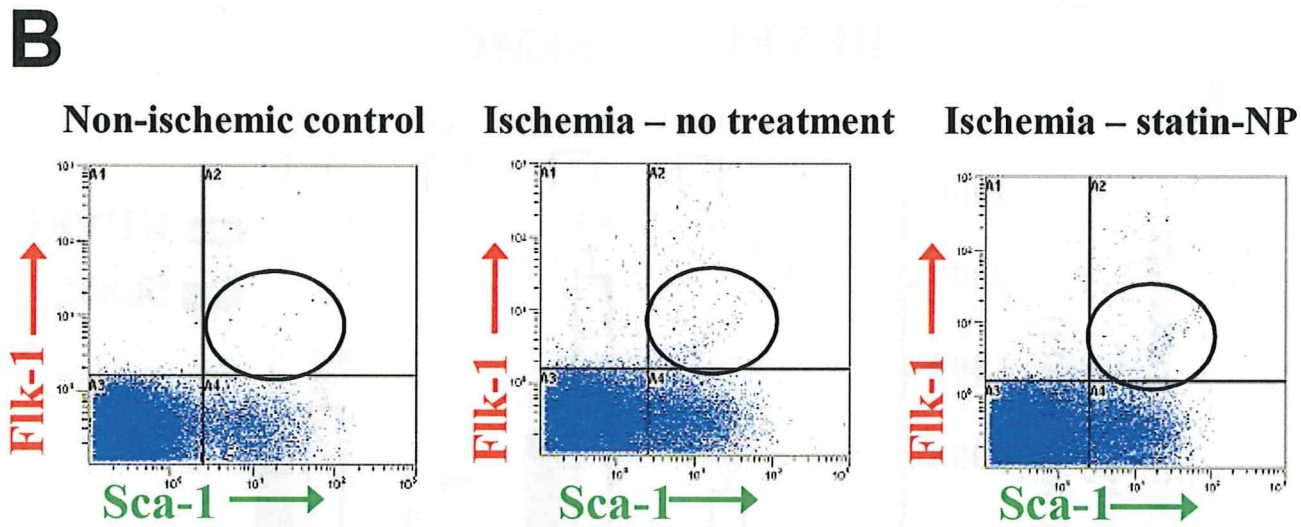
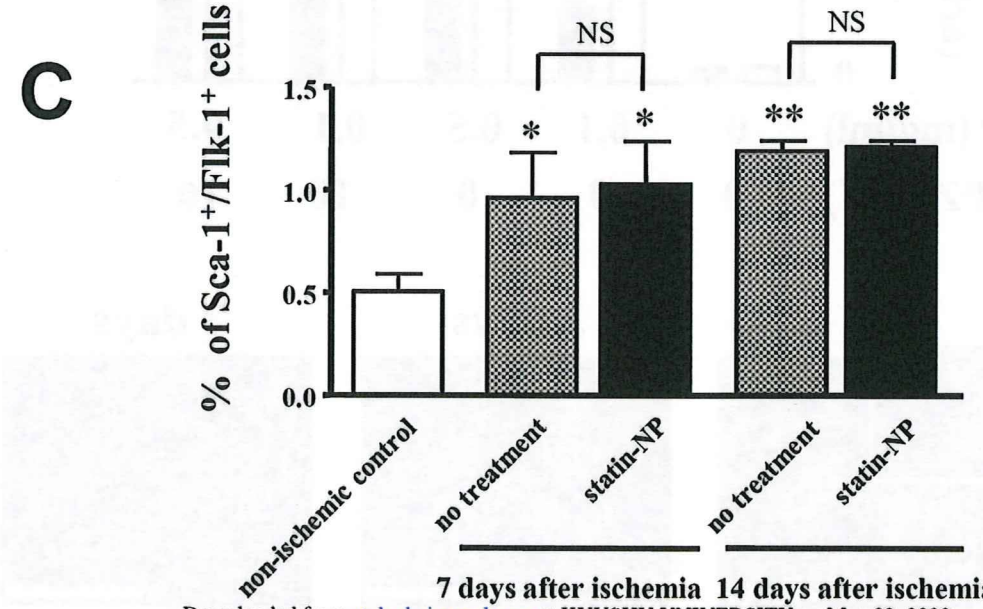
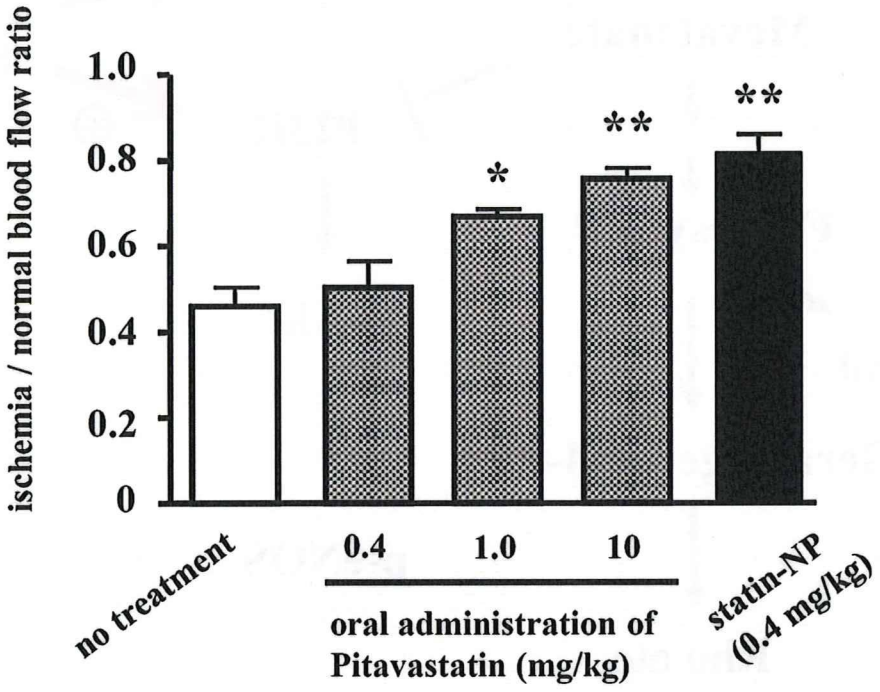


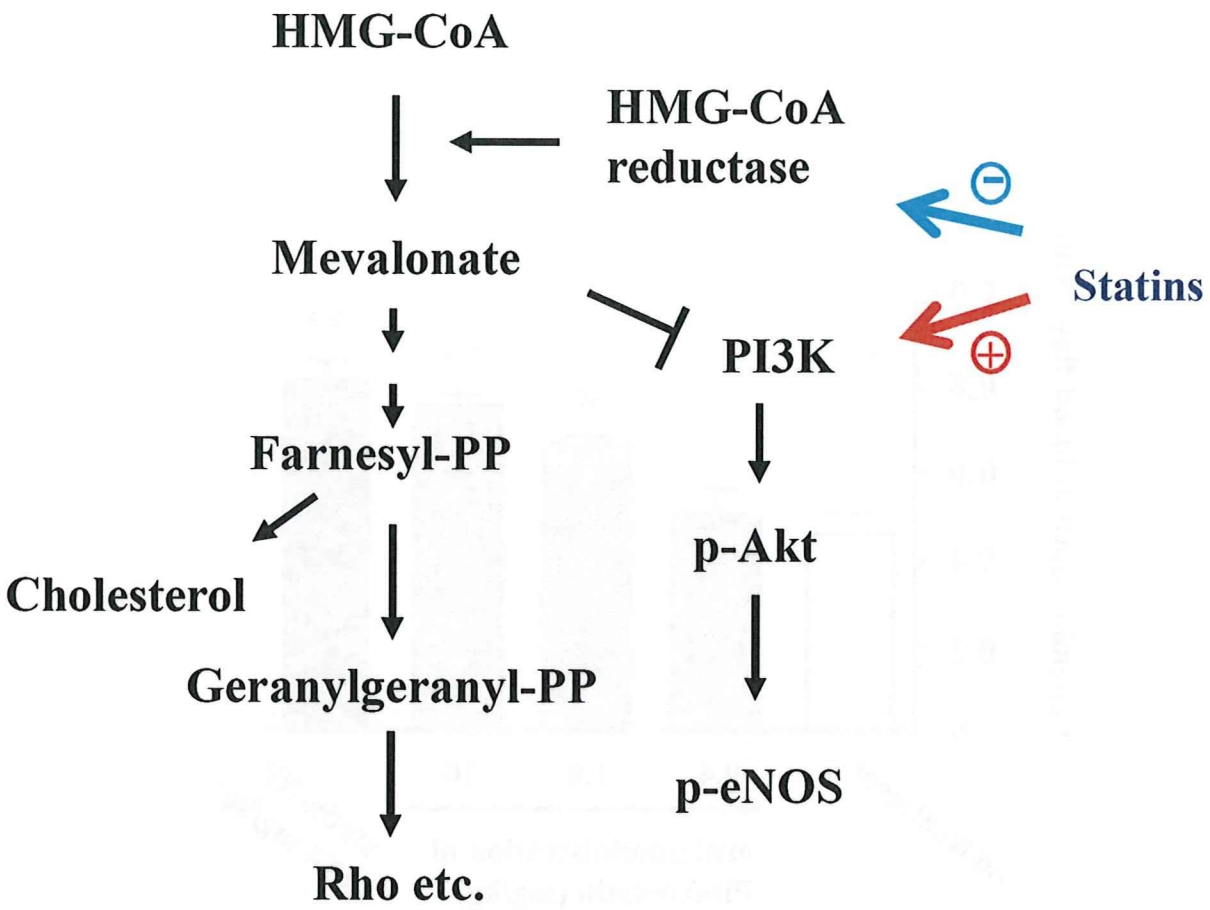




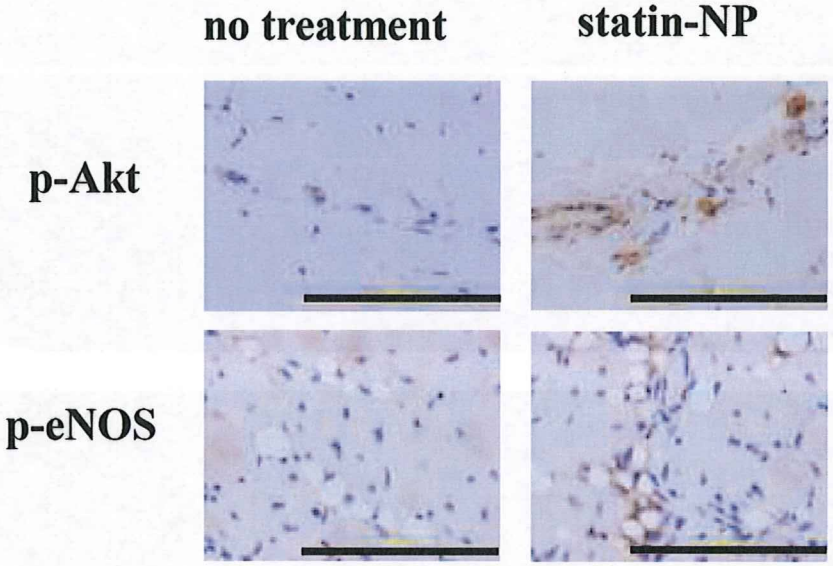
Supplementary Figure IV*Kubo M et al.*



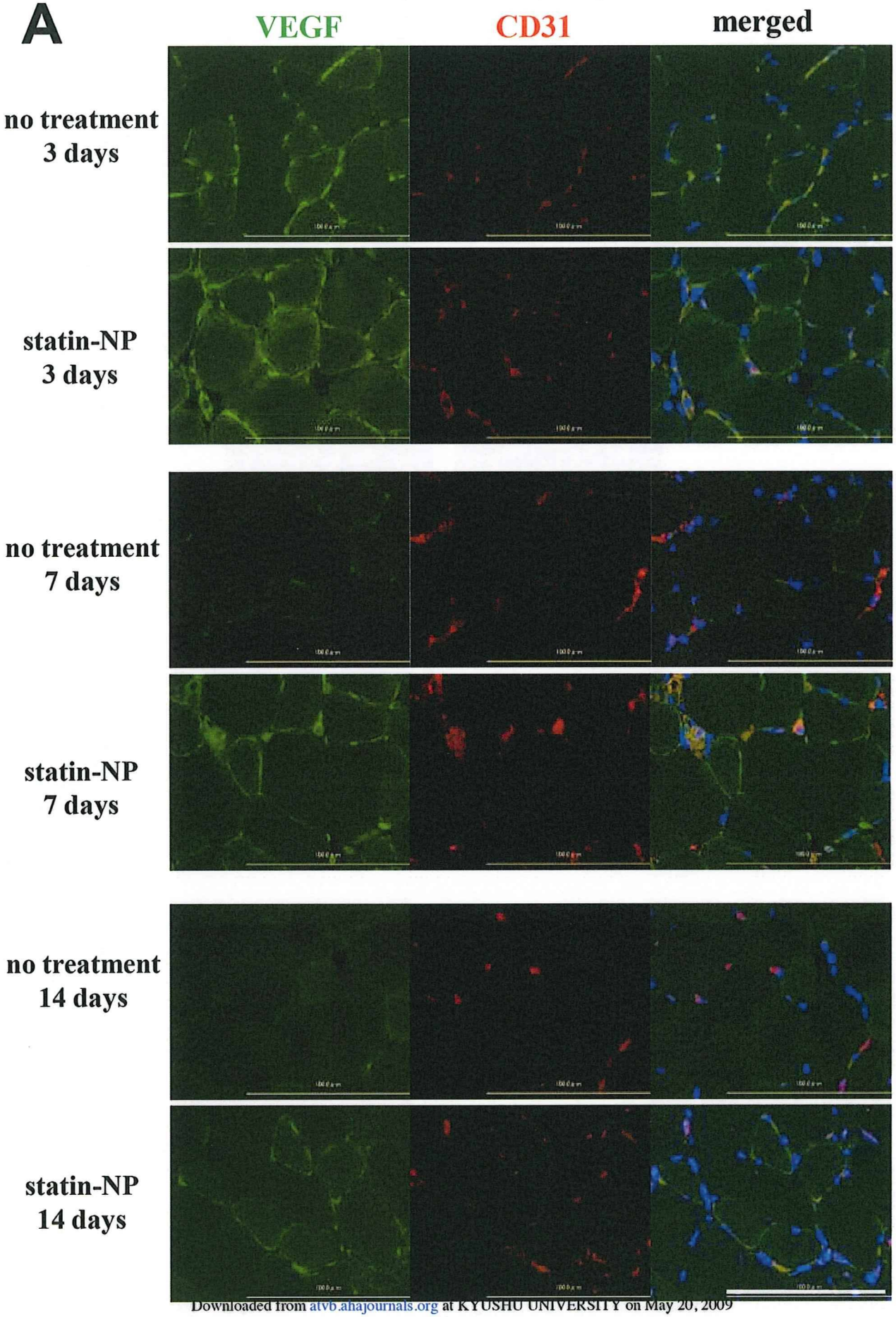
Supplementary Figure V *Kubo M et al.*



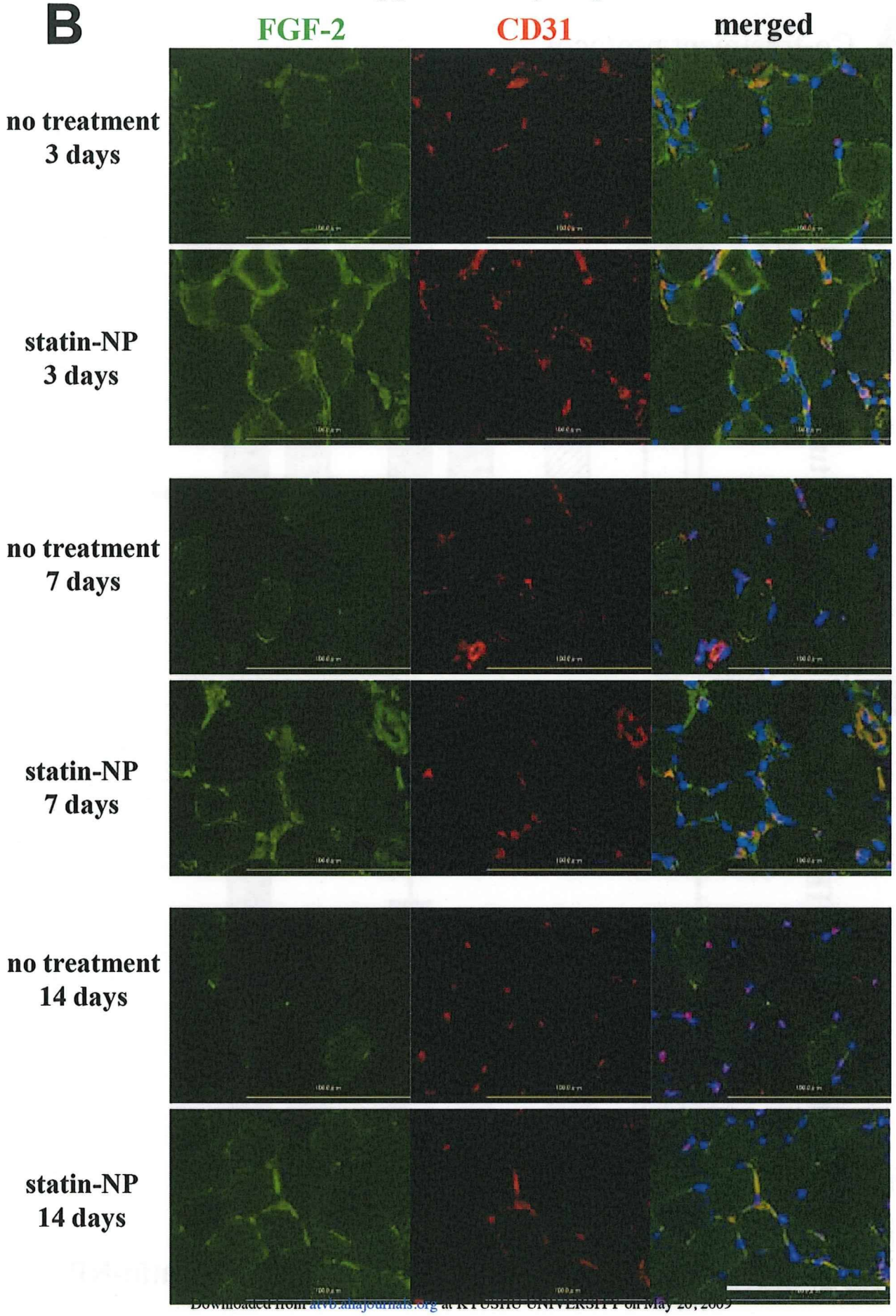
Supplementary Figure VI *Kubo M et al.*



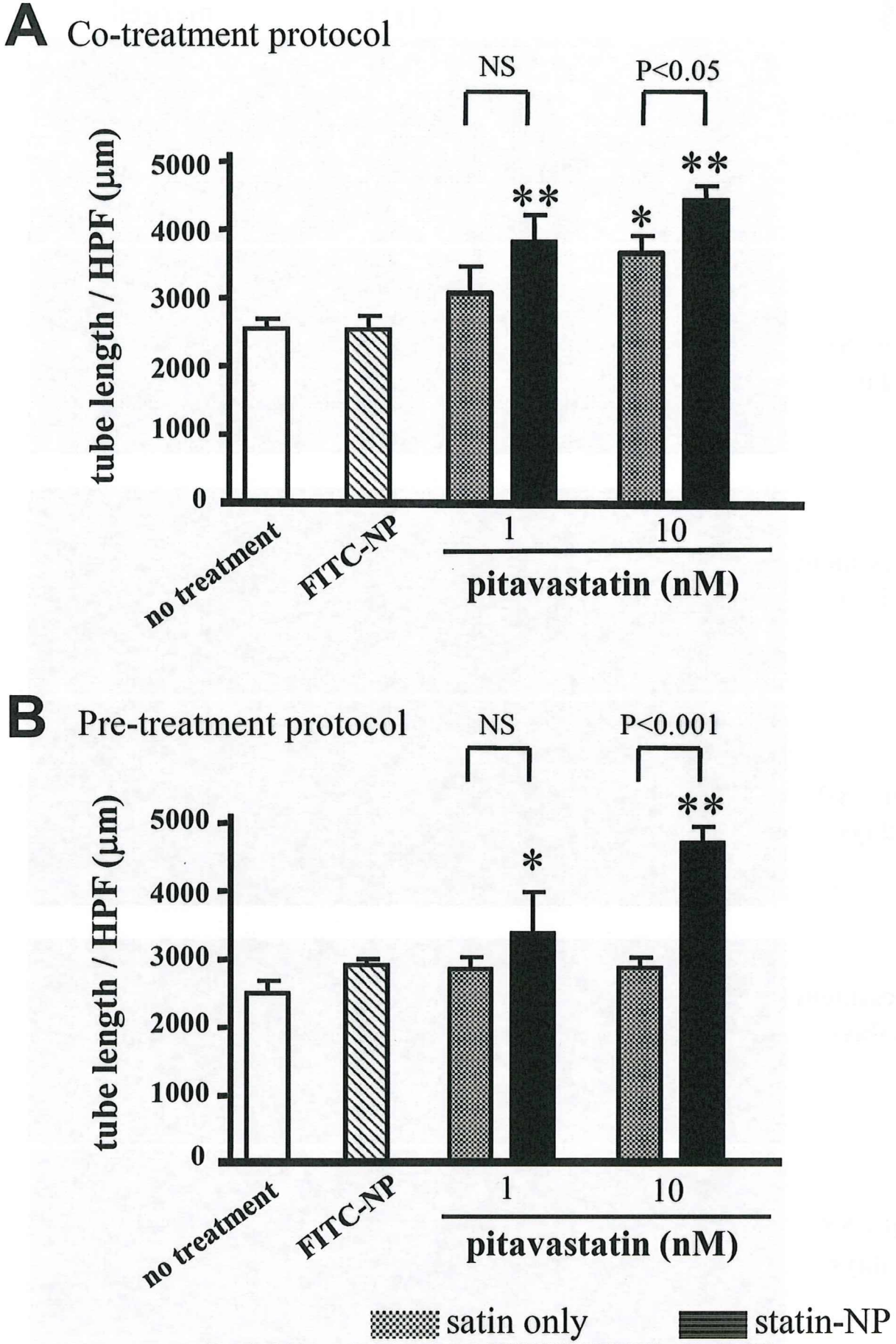
Supplementary Figure VII *Kubo M et al.*



Supplementary Figure VII *Kubo M et al.*



Supplementary Figure VIII *Kubo M et al.*



Supplement Material

Therapeutic Neovascularization by Nanotechnology-Mediated Cell-Selective Delivery of Pitavastatin into the Vascular Endothelium

Mitsuki Kubo, MD; Kensuke Egashira, MD PhD; Takahiro Inoue, MD; Jun-ichiro Koga, MD; Shinichiro Oda, MD; Ling Chen, MD; Kaku Nakano, PhD; Tetsuya Matoba, MD PhD; Yoshiaki Kawashima, PhD; Kaori Hara, PhD; Hiroyuki Tsujimoto, PhD; Katsuo Sueishi, MD PhD; Ryuji Tominaga MD PhD; Kenji Sunagawa, MD PhD

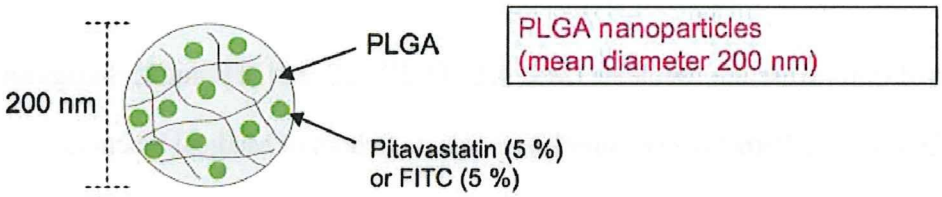
Department of Cardiovascular Medicine (MK, KE, TI, JK, LC, KN, TK and K Sunagawa), Surgery (SO, RT), and Pathology (K Sueishi), Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, School of Pharmaceutical Science (YK), Aichi Gakuin University, Aichi, Japan, and Hosokawa Powder Technology Research Institute (KH, HT), Osaka, Japan.

Materials and Methods

Preparation of PLGA NP

A lactide/glycolide copolymer (PLGA) with an average molecular weight of 20,000 and a copolymer ratio of lactide to glycolide of 75:25 (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] Polyvinylalcohol (PVA-403; Kuraray, Osaka, Japan) was used as a dispersing agent. Fluorescein-isothiocyanate (FITC; Dojin Chemical, Tokyo, Japan) was used as a fluorescent marker of the NP.

We prepared bioabsorbable poly-lactide-glycolide copolymer (PLGA) nanoparticles (NP) by emulsion solvent diffusion method. The encapsulated agents are entrapped into the polymer matrix as shown below.



Advantages of PLGA NP-based drug delivery system (DDS) include:

- Matrix polymer (PLGA) is bioabsorbable.
- NP can incorporate water-soluble drugs/oligonucleotides/DNAs.
- NP can cross cell membrane via endocytosis (efficiency of cellular uptake: 90 % or more), and deliver the encapsulated agents into the cytoplasm.
- Incorporated drugs are slowly released from NP with hydrolysis of PLGA, which works intracellular DDS after intracellular uptake.

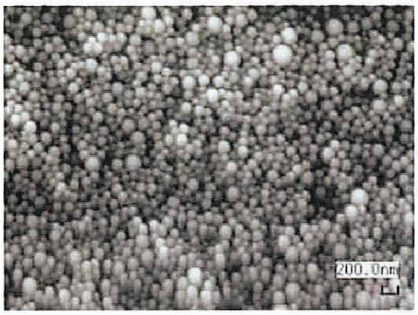
PLGA NP incorporated with FITC or pitavastatin (Kowa Pharmaceutical Co. Ltd., Tokyo, Japan) were prepared by a previously reported emulsion solvent diffusion method in purified water[2,3]. PLGA were dissolved in a mixture of acetone and methanol. Then, FITC or pitavastatin were added into this solution. The resultant polymer-FITC or polymer-statin solution was emulsified in PVA solution under stirring at 400 rpm using the propeller-type agitator with three blades (Heidon 600G; Shinto Scientific, Japan). After agitating the system for 2 h under reduced pressure at 40 °C, the entire suspension was centrifuged (20,000×*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. The FITC- and pitavastatin-loaded PLGA NP contained 5 % (w/v) FITC and 5 % (w/v) pitavastatin, respectively.

Particle size and surface charge measurements

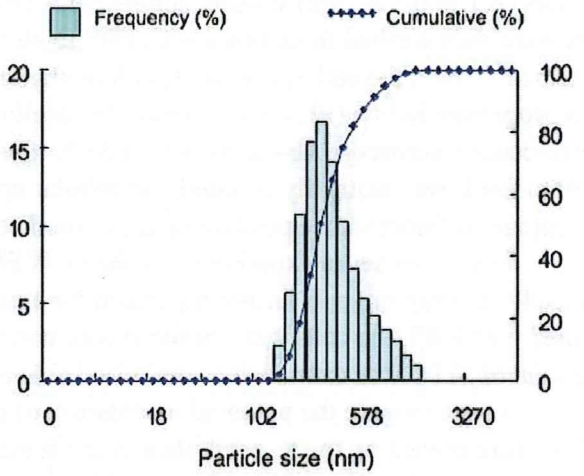
Scanning electron microscopy picture of the PLGA NP indicates that the NP is prepared in the form of powder. The mean particle size was analyzed by light scattering

method (Microtrack UPA150; Nikkiso, Tokyo, Japnan). A sample of nanoparticulate suspension in distilled water was used for particle size analysis. The diameter of PLGA NP was 196 ± 29 nm. Surface charge (zeta potential) was also analyzed by Zetasizer Nano (Sysmex, Hyogo, Japan) and was anionic charge (-15±10 mV at pH 4.4).

Scanning electron microscopy (SEM) image



Particle size distribution of FITC-incorporated PLGA nanoparticles in water

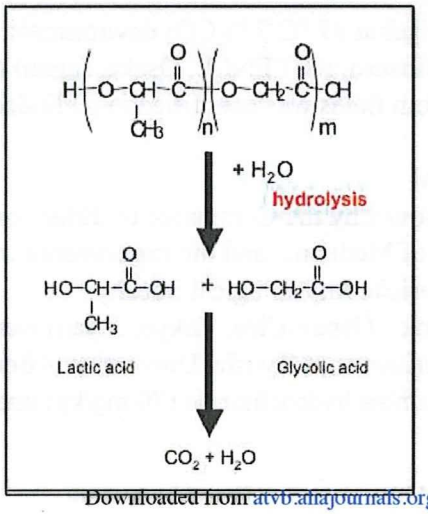


Bioabsorption process of PLGA NP

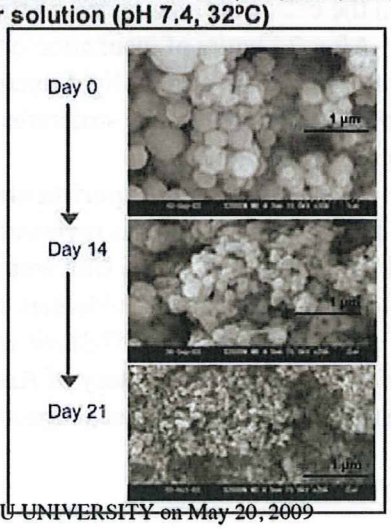
The chemical structure of PLGA and its bioabsorption process (hydrolysis) are indicated below. Scanning electron microscopic examination of time course of biodegradation in phosthe phate buffer solution shows slow degradation of NP with time.

Bioabsorption Process (hydrolysis) of PLGA

Chemical structure of PLGA and its hydrolysis process in living body



Biodegradation of PLGA nanoparticles (a mean diameter: 200 nm) in phosphate buffer solution (pH 7.4, 32°C)



Intracellular uptake and intracellular distribution of NPs

Human umbilical vein endothelial cells (HUVEC) were obtained from Cambrex BioScience Walkersville, Inc., cultured in EGM-2 (Lonza, Charles City, IA) with supplements (Lonza), and used between passages 4 to 8. Human skeletal muscle cells (SkMC) were also obtained from Cambrex BioScience Walkersville, Inc. and cultured in SkGM (Lonza).

The HUVEC and SkMC were seeded on the 8-well-chamber slide to an initial concentration of 1.5×10^4 cells per well and incubated at 37 °C/5 % CO_2 environment until cells were subconfluent. The growth medium was replaced with the FITC-NP suspension medium (0.1 to 0.5 mg/ml) without supplements and then further incubated for 1 hour. The cells were then washed three times with PBS to eliminate extracellular NP as previously described[4]. Then, the cells were fixed with methyl-alcohol and nuclei were counterstained with propidium iodide (PI; vector shield). Intracellular uptake of FITC-NP was evaluated by fluorescence microscopy (Biozero; KEYENCE, Osaka, Japan). The number of cells in 5 random fields was manually counted and cellular uptake percentage was assessed by the percentage of fluorescence positive cells per total cells.

In another set of experiments using HUVEC, the growth medium was replaced with FITC-NP (0.5 mg/ml) and further incubated for 1 hour. After excess extracellular NP were washed with PBS, the cells were cultured with normal growth medium and intracellular retention of FITC was examined as described above at days 3 and 7.

To investigate the potential mechanism of cellular uptake of NP, HUVEC and SkMC were seeded on the 96-well plate in the presence or absence of an inhibitor of clathrin-mediated endocytosis pathway[5], chlorpromazine (CPZ; Sigma) at 10 or 30 μM for 30 min at 37 °C in the culture medium without supplements, and then incubated with FITC-NP suspension medium (0.1 to 0.5 mg/ml) for further 30 min. After incubation, cells were washed and lysed with triton X and NaOH and then the amount of fluorescence in each wells were analyzed with fluorescence-plate reader (Mithras LB940; BERTHOLD BIOTECHNOLOGY, Germany).

Angiogenesis Assay of Human Endothelial Cell

Angiogenesis assay of human endothelial cells was tested by 2-dimensional Matrigel assay as previously described.[6] HUVECs (2×10^4) were suspended on the 8-well-chamber slide pre-coated with 200 μl Matrigel (BD Bioscience) in 500 μl EBM-2 medium with supplements (Lonza) in the presence or absence of pitavastatin or NP incorporated with pitavastatin at 1 and 10 nM. In another set of experiments, HUVEC were pre-treated with pitavastatin or NP incorporated with pitavastatin at 1 and 10 nM for 24 hours and washed, and then the cells were suspended on the Matrigel.

After 24 hours of incubation on the Matrigel at 37 °C/5 % CO_2 environment, tube formation were quantified by light microscopy (Biozero; KEYENCE, Osaka, Japan) and the length of completed tube-like structures in 5 random fields was quantified in a blinded fashion in each expreiment.

Animal Preparation and Experimental Protocol

The study protocol was reviewed and approved by the Committee on Ethics on Animal Experiments, Kyushu University Faculty of Medicine, and the experiments were conducted according to the Guidelines of the American Physiological Society.

Male 8-weeks-old C57BL/6J wild-type mice (Japan-Clea, Tokyo, Japan) were bred and maintained in the Laboratory of Animal Experiments at Kyushu University. After anesthesia with an intraperitoneal injection of ketamine hydrochloride (70 mg/kg) and

xyladine hydrochloride (3 mg/kg), we induced unilateral hindlimb ischemia to mice as previously described.[7, 8] Briefly, the proximal portion of the left femoral artery and vein including the superficial and deep branch as well as the distal portion of saphenous artery and vein were ligated and resected after all side branches were dissected free. Immediately after induction of ischemia, animals were randomly divided into 4 groups; a control no treatment group and others received intramuscular injections of FITC-NP (PLGA at 0.18 mg/100 μl) (NP group), intramuscular injections of pitavastatin at 0.01 mg/100 μl (0.4 mg/kg)(Pitava only group), or intramuscular injections of pitavastatin-NP [PLGA at 0.18 mg/100 μl containing 0.01 mg (0.4 mg/kg) of pitavastatin] (Pitava-NP group) into the left femoral and thigh muscles with a 27-gauge needle. This dose of pitavastatin NP was selected because we examined effects of pitavastatin-NP containing pitavastatin at 0.1, 0.4, 1.0 and 1.5 mg/kg 1n preliminary studies and confirmed that pitavastatin NP containing 0.4 mg/kg pitavastatin was an optimal dose in our experiments. Biochemical parameters listed in supplemental Table 1 were measured 3, 7, and 14 days after treatment.

In another set of experiments, effects of intramuscular injections of pitavastatin-NP were examined in $eNOS^{-/-}$ mice and wild-type mice chronically treated with *N*ω-nitro-L-arginine methyl ester (L-NAME; Sigma), an NO synthase inhibitor, in drinking water (2 mg/kg) from 7 days before operation to sacrifice[9]. Two other groups received intramuscular injections of non-nanoparticulated soluble pitavastatin at high doses at 4 and 20 mg/kg. Furthermore, three other groups received systemic daily oral administration of pitavastatin at doses of 0.4, 1.0 and 10 mg/kg, solved in 0.5 % carboxymethyl cellulose by gavage from the day of surgery until the mice were sacrifice on day 14.

Histological and immunohistochemical analyses

Histological evaluation was performed in 5-μm paraffin embedded sections from gastrocnemius muscle 14 days after hindlimb ischemia. Capillary and arteriolar density in ischemic muscle were determined by immunohistochemical staining with anti-mouse platelet endothelial cell adhesion molecule (PECAM)-1 antibody (CD31; Santa Cruz Biotechnology) and α-smooth muscle actin (α-SMA; DAKO), respectively. Digital images of 5 microscopic fields from 4 different sections from each animal were stored. Capillary density was expressed as the number of CD31 positive cells per mm^2 and arteriolar density was expressed as the number of circumvented brown signals of α-SMA per mm^2 as previously described[10]. To determine intracellular molecular signals for angiogensis, cross sections were stained with anti-phosphorylated-Akt antibody (Cell Signaling) or anti-phosphorylated-eNOS antibody (Cell Signaling)[9]. To determine cellular localization of angiogenic growth factors 3, 7 and 14 days after ischemia, cross sections were stained with anti-VEGF or anti-FGF-2 antibody with anti-PECAM-1 (CD31) antibody, as a primary antibody (all from Santa Cruz Biotechnology), and anti-mouse IgG (Alexa 488; Molecular Probes) or anti-rabbit IgG (FITC; Santa Cruz Biotechnology) with anti-goat IgG antibody (Alexa 555; Molecular Probes), as a secondary antibody, respectively. Nuclei were counterstained with 4', 6-diamidino-2-phenylindole (DAPI; Vector Shield).

Distribution of nanoparticles *in vivo*

Three, 7 and 14 days after hindlimb ischemia and intramuscular injection of FITC-NP, gastrocnemius muscle was isolated from ischemic and non-ischemic limbs, and FITC signals were examined under a fluorescent stereomicroscope. Frozen cross sections of those muscles were then prepared and examined under a fluorescent microscope (Biozero, KEYENCE, Osaka, Japan). Nuclei were counterstained with propidium iodide (PI; Vector Shield). Another sections were stained with anti-mouse PECAM-1 antibody (CD31; Santa

Cruz Biotechnology), as a primary anti-body, and anti-goat IgG (Alexa 555; Molecular Probes), as a secondary anti-body. Frozen cross sections of liver, spleen and kidney were also examined.

Western blotting

Homogenates of muscle tissues were analyzed for immunoblotting 7 days after induction of hindlimb ischemia. Proteins were separated in 7.5 % or 15 % SDS-polyacrylamide gels and then blotted onto a membrane. Membrane was incubated with antibodies against phosphorylated-Akt, phosphorylated-eNOS, Akt (1:1000, Cell Signaling), eNOS (1:1000, Affinity BioReagents), VEGF, FGF-2 and MCP-1 (1:200, Santa Cruz Biotechnology) and then the blots were reprobed with GAPDH (1:1000, Santa Cruz Biotechnology).

Flow Cytometric Analyses of EPC Mobilization

Peripheral blood was obtained from mice 7 and 14 days after hindlimb ischemia. EPC are thought to derive from mononuclear leukocytes that are positive for both Sca-1 and Flk-1 (vascular endothelial cell growth factor receptor-2)[11, 12]. The percentage of mononuclear cells that were positive for both the Sca-1-FITC and Flk-1-PE antibodies (Pharmingen) was then analyzed with a FACS Caliber flow cytometer (Becton Dickinson).

Measurements of statin concentration in serum and muscle tissue

Statin concentration in serum and muscle were measured at predetermined time points by using column-switching high performance liquid chromatography (HPLC) system as previously reported[13]. Briefly, the column-switching HPLC system consists of two LC-10AD pumps, an SIL-10A auto-sampler, a CTO-10A column oven, a six-port column-switching valve and an SPD-10A UV-detector (all from Shimadzu, Kyoto, Japan). The column temperature was maintained at 40 °C. Preprepared serum or tissue homozynates sample solutions were injected from auto-sampler into HPLC system and the detection of statin in sample solutions was carried out at 250 nm with a UV-detector. The detected peak-area was measured with Lcsolution software (Shimadzu, Kyoto, Japan).

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;8:787-791.
2. Kawashima Y, Yamamoto H, Takeuchi H, Hino T, Niwa T. Properties of a peptide containing DL-lactide/glycolide copolymer nanospheres prepared by novel emulsion solvent diffusion methods. *Eur J Pharm Biopharm.* 1998;45:41-48.
3. Kawashima Y, Yamamoto H, Takeuchi H, Fujioka S, Hino T. Pulmonary delivery of insulin with nebulized DL-lactide/glycolide copolymer (PLGA) nanospheres to prolong hypoglycemic effect. *J Control Release.* 1999;62:279-287.
4. Kimura S, Egashira K, Nakano K, Iwata E, Miyagawa M, Tsujimoto H, Hara K, Kawashima Y, Tominaga R, Sunagawa K. Local delivery of imatinib mesylate (STI571)-incorporated nanoparticle ex vivo suppresses vein graft neointima formation. *Circulation.* 2008;118:S65-70.
5. Kanatani I, Ikai T, Okazaki A, Jo J, Yamamoto M, Imamura M, Kanematsu A, Yamamoto S, Ito N, Ogawa O, Tabata Y. Efficient gene transfer by pullulan-spermine occurs through both clathrin- and raft/caveolae-dependent mechanisms. *J Control Release.* 2006;116:75-82.
6. Weis M, Heeschen C, Glassford AJ, Cooke JP. Statins have biphasic effects on

angiogenesis. *Circulation.* 2002;105:739-745.

7. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med.* 2003;9:1370-1376.
8. Urbich C, Heeschen C, Aicher A, Dernbach E, Zeiher AM, Dimmeler S. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation.* 2003;108:2511-2516.
9. Hiasa K, Ishibashi M, Ohtani K, Inoue S, Zhao Q, Kitamoto S, Sata M, Ichiki T, Takeshita A, Egashira K. Gene transfer of stromal cell-derived factor-1alpha enhances ischemic vasculogenesis and angiogenesis via vascular endothelial growth factor/endothelial nitric oxide synthase-related pathway: next-generation chemokine therapy for therapeutic neovascularization. *Circulation.* 2004;109:2454-2461.
10. Masaki I, Yonemitsu Y, Yamashita A, Sata S, Tanii M, Komori K, Nakagawa K, Hou X, Nagai Y, Hasegawa M, Sugimachi K, Sueishi K. Angiogenic gene therapy for experimental critical limb ischemia: acceleration of limb loss by overexpression of vascular endothelial growth factor 165 but not of fibroblast growth factor-2. *Circ Res.* 2002;90:966-973.
11. Landmesser U, Engberding N, Bahlmann FH, Schaefer A, Wiencke A, Heineke A, Spiekermann S, Hilfiker-Kleiner D, Templin C, Kotlarz D, Mueller M, Fuchs M, Hornig B, Haller H, Drexler H. Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. *Circulation.* 2004;110:1933-1939.
12. Iwakura A, Shastry S, Luedemann C, Hamada H, Kawamoto A, Kishore R, Zhu Y, Qin G, Silver M, Thorne T, Eaton L, Masuda H, Asahara T, Losordo DW. Estradiol enhances recovery after myocardial infarction by augmenting incorporation of bone marrow-derived endothelial progenitor cells into sites of ischemia-induced neovascularization via endothelial nitric oxide synthase-mediated activation of matrix metalloproteinase-9. *Circulation.* 2006;113:1605-1614.
13. Kojima J, Fujino H, Yosimura M, Morikawa H, Kimata H. Simultaneous determination of NK-104 and its lactone in biological samples by column-switching high-performance liquid chromatography with ultraviolet detection. *J Chromatogr B Biomed Sci Appl.* 1999;724:173-180.

Overview

衝撃：DESは誰の心を痛くするのだろう?
―患者，医師，それとも企業

九州大学大学院医学研究院循環器内科学

江頭健輔
Kensuke Egashira

連載企画として「薬剤溶出ステントと遅発性血栓症 ～DESの陰から光を探る～」を企画致しました．DESは，いわゆるbreakthroughテクノロジーとして登場し，初期臨床試験において格段の有効性が報告されたことから，予想以上に膨大な数が使用され，少なくとも数年間に600万人以上がDESの治療を受けたとされています．一般に，承認後に市場で爆発的に使用された場合，予想外の成果や副作用がまれながら認められることがあります．今回のDESにおける遅発性ステント内血栓症の発生がそれに当たると考えられます．

2006年9月，バルセロナで開催された欧州心臓学会議(ESC)において衝撃が走りました．DESが遅発性ステント内血栓を増加させ，その結果，急性心筋梗塞や心臓死が増加するという衝撃的報告が複数の施設から報告されました．私は現場で，該当するいくつかの発表を聞きましたが，「本当なら患者の死をもたらす重篤な副作用であり，現場の医療を変える重大な発表だ」，「現行のDESはもはや最終ゴールではなくなった」，「DESは反省期を迎える」と感じたことを鮮明に覚えています．DESを製造販売している医療機器会社の市販後調査の結果ではなく(医療機器会社はこの状況を把握していなかったと発表)，実際に患者を診療し，遅発性血栓症を実感した医師グループからの臨床研究であったことも不思議に思いました．医薬品業界では，このようなことはきわめて稀です．

これらの発表を受けて“Trading restenosis for thrombosis?”というeditorialがN Engl J Med誌(**図1**)に掲載され[1]，The Wall Street Journal誌に“Coated stents deliver heart burn”という記事(Johnson A：The Wall Street Journal. November 11, 2006)が掲載されるに至り，DES使用後の遅発性血栓症は世界的問題となりました．DESは誰に心の痛みをもたらすのでしょうか？ DESを植え込まれた患者でしょうか？ むしろ，DESを患者に植え込んだ医師やDES製造会社の心を痛くするのでしょうか？ 同年12月，FDAが急遽，諮問委員会を開催したことは，その重要性を如実に表しています[2)3)]．さらに，N Engl J Med誌の2007年3月8日号に7つの論文が一挙に掲載されました．その詳細を連載の初めに取り上げます．

ご存知のように，DESに用いられている薬剤はパクリタキセル(抗癌剤)やシロリムス(免疫抑制薬)です．より優れた血管保護作用を有する多数の薬剤・遺伝子が知られていた

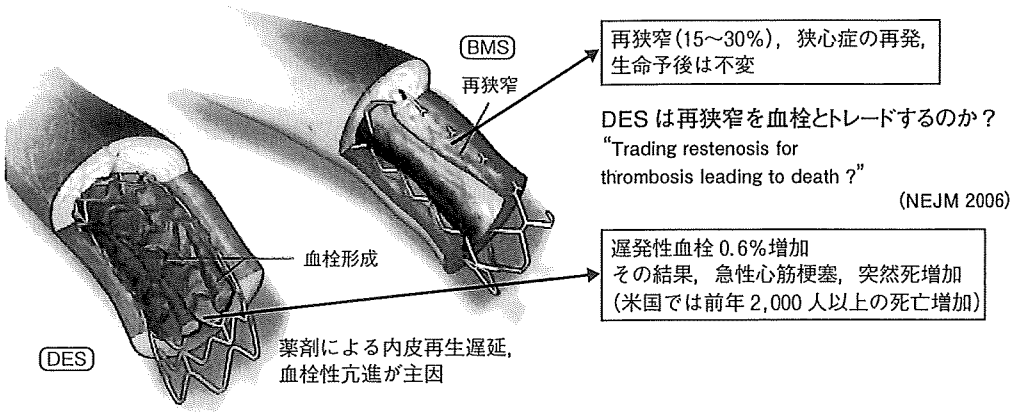


図1．薬剤溶出ステント(DES)の重篤な副作用(遅発性血栓→急性心筋梗塞・心臓死)は世界的問題
薬剤がコーティングされていない通常のベアメタルステント(BMS)では，再狭窄(新生内膜形成)によって狭窄・閉塞が生じる．一方，DES では，再狭窄は著明に減少するが，内皮細胞の再生が遅延しステントが血管内に露出することが多く，薬による組織因子の発現亢進と組み合わさって遅発性血栓が生じる．(文献1より改変して引用)

にもかかわらず，何故この2剤が選ばれたのでしょうか？ 私から見ると不思議です．その理由のひとつは，脂溶性がきわめて強く，ステントコーティングに用いるポリマーとの相性が良いという点であったようです[4]．再狭窄の主因が「血管平滑筋の増殖」であるという立場から，その抑制作用が強力であることも理由でしょう[4]．しかし，血管壁は平滑筋細胞だけで構成されているわけではないこと，他の構成細胞である内皮細胞が傷害後の再生修復反応に重要であること，は忘れられていたのでしょうか．動脈硬化性プラークにおいては，平滑筋の機能抑制はプラークの不安定化を促進することがあることも知られています．繰り返しになりますが，血管生物学の立場からは，パクリタキセルやシロリムスより優れた血管保護(再狭窄抑制，動脈硬化抑制)作用を有する化合物や遺伝子はたくさんあります．再狭窄(平滑筋増殖抑制)だけを注視して，臓器としての血管壁全体を見なかったのではないかと考えてしまいます．たとえば，血管平滑筋の増殖抑制なら，PDGF シグナル抑制が良かったかもしれません(PDGF 抑制は内皮の再生にはほとんど影響しませんし，線維芽細胞の増殖も抑えます)．また，血管内皮の再生促進や抗炎症も強力な血管保護をもたらすことから，これらをターゲットにしても良かったかもしれません．

また，生体残存性ポリマーが使われている点も科学的ではありません．生体残存性ポリマーが冠動脈血栓症につながる血管傷害を惹起する可能性は，すでに1996年に Circulation 誌に掲載されていました[5]．DES による冠動脈の遷延性炎症，内皮再生遅延などの副作用の懸念は，2000年以降，病理学者からも剖検所見に基づいて発表されていました(図2)[6)-9)]．私見ですが，DES は「血管生物医学の基礎研究成果を基盤にして開発された医療機器」なのかどうかは疑問です．

DES のステント内血栓は，今回初めて取り上げられたわけではありません．2004年，FDA は，稀ではあるが，ステント内血栓症が生じるリスクがあることを発表し，適切な

適応のもとにDESを使用することを提唱していました[10]．しかし，このような基礎研究者やFDAから発信された心配は杞憂であるとして，インターベンションに関わる臨床医や企業からは顧みられませんでした．DESは，その劇的再狭窄防止効果を有することから，米国では冠動脈インターベンションの90％近くに使用され，承認後600万人以上の患者に過剰使用されてきました．安定狭心症の予後と医療費の調査から，冠動脈インターベンションの効果は内科的治療と同程度であることがN Engl J Med誌などに発表され，注目されています[11]．

現在，DESの遅発性ステント内血栓症を議論する場合，少なくとも3つの点を考える必要があると思います．

第一に，すでにDESを植え込まれた患者については最善の策を配慮しなくてはなりません．患者にどこまで通知するかは難しい点ですが，少なくとも安易に抗血小板薬を中止しないよう指導することが重要でしょう．

第二に，今後，冠動脈インターベンションを受ける患者において，DESはどのように位置づけられるのでしょうか？ 冠動脈インターベンションを受ける患者が治療後，歯科治療や外傷，手術などによって，抗血小板薬を中止せざるを得ない状況になる確率は少なくとも50％以上はあると思います．抗血小板薬の中止は，ステント内血栓症誘発のリスクであることは明白です．急性心筋梗塞症や心臓死のリスクがあるのであれば，「DESは慎重に使用すべきである」というのは自明です．議論の余地は少ないと思います．少な

急性心筋梗塞か突然心臓死で死亡した23症例

Group	Inflammation score	Eosinophils /strut	Fibrin score	% Struts with fibrin	% Struts endothelialized
DES(n=32)	1.7±1.5	5.6±11.1	2.3±1.1	49.3±30.8	55.8±26.5
BMS(n=36)	1.3±0.8	0.6±2.3	0.9±0.8	22.3±17.8	89.8±20.9
p value	ns	0.01	0.0001>	0.0005	0.0001

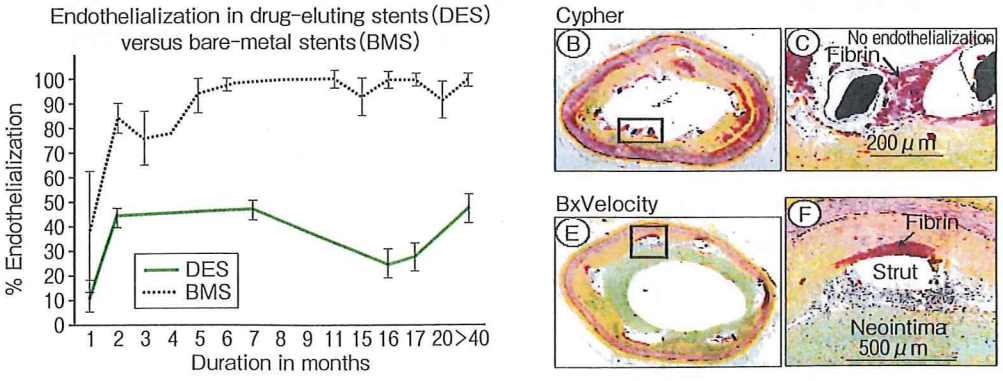


図2．ヒト病理組織は語る：DES使用症例では血管内皮細胞再生不全による遅発性血栓のリスクが増加する
（文献9より改変して引用）

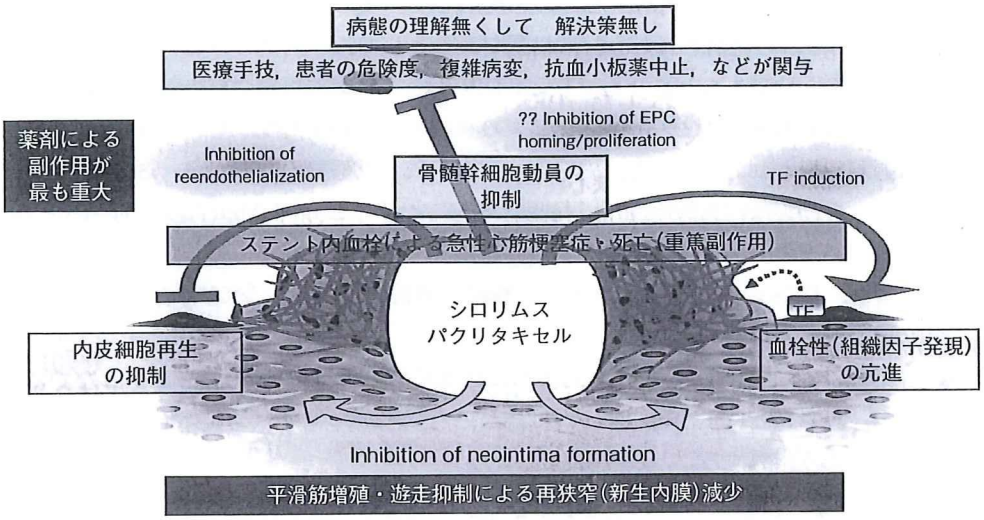


図3．薬剤溶出ステント（DES）による遅発性ステント内血栓症の病態

くとも企業と冠動脈インターベンションを行う医師は，この点を患者に知らせるべきでしょう．

第三に，早急に遅発性ステント内血栓症の分子機序を理解し，それを基盤に次世代デバイスの開発を進めなければなりません．**図3**に示すように，DESにコーティングされた薬とポリマーによる内皮再生の遅延，骨髄内皮前駆細胞の増員抑制，組織因子の発現亢進が，遅発性ステント内血栓症の最も重要な病態と考えられています．したがって，次世代ステントは細胞選択的に増殖や炎症を抑制，内皮細胞の再生を促進，抗血栓性の促進，などをもたらす新しいコンセプトに基づいた対策が必要と考えます．細胞特異的ドラッグデリバリーシステム（DDS），あるいは細胞内シグナル応答性DDSなどの日本が得意とする新技術の導入はイノベーションをもたらすかもしれません．今まさに，血管生物医学の知識を基盤にした，より効果的で，かつ安全な血管内医療システムの開発が期待されています．現在，臨床治験が進められているDESはすべてシロリムスのアナログであり，根本的な解決になるかどうか疑問です．

最後に，今回取り上げる「薬剤溶出ステントと遅発性血栓症 ～DESの陰から光を探る～」は平成19年11月29，30日に開催される第15回日本血管生物医学会学術大会（於 九州大学医学部内 九州大学医学部100年講堂）のプレナリーセッションとして取り上げます．学会に国内外の多くの研究者に参加していただき，活発な交流や討論を通じて光を探るきっかけになれば幸いです．

どうぞよろしくお願い申し上げます．