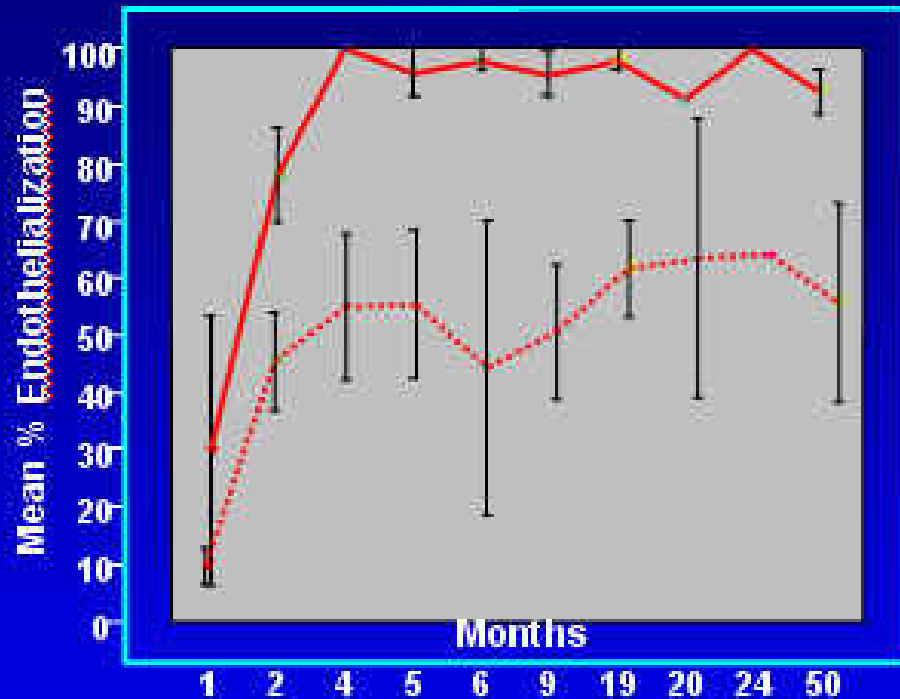


DES Pathobiology: Insights from Assessment of Endothelial Integrity and Function

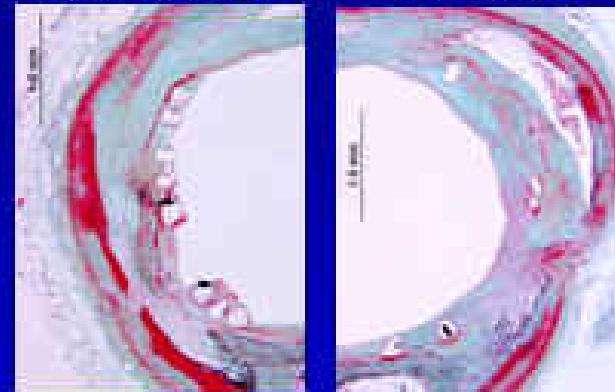
**Renu Virmani, MD
CVPath Institute Inc.
Gaithersburg, MD, USA.**

Delayed Arterial Healing in DES

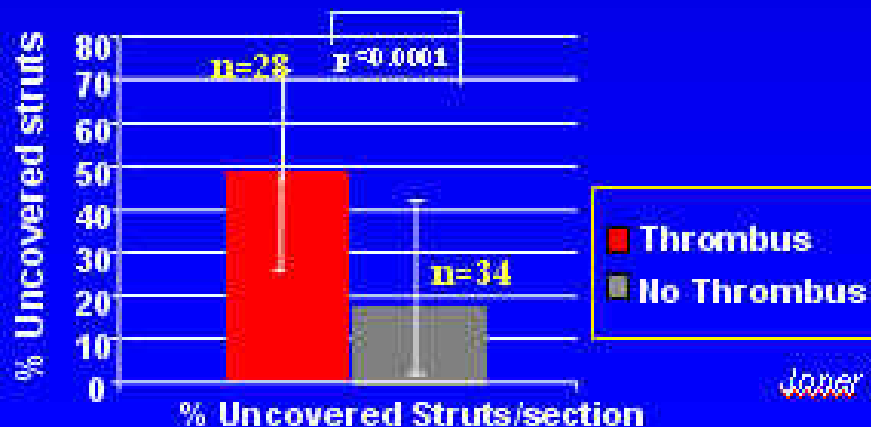
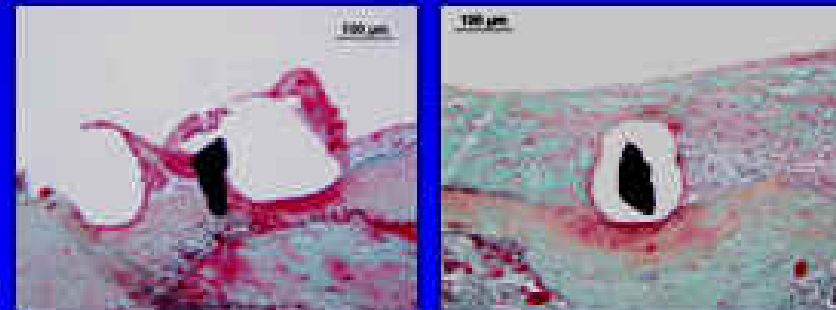
— BMS DES



> 30 days implants
uncovered covered



> 30 days implants
uncovered covered



Jaber M & Finn AV. *J Am Coll Cardiol.* 2006;48(1):193-202.

Finn AV, et al., *Circulation* 2007;115:2435-2441

Using a Measure of Endothelialization to Predict Stent Thrombosis

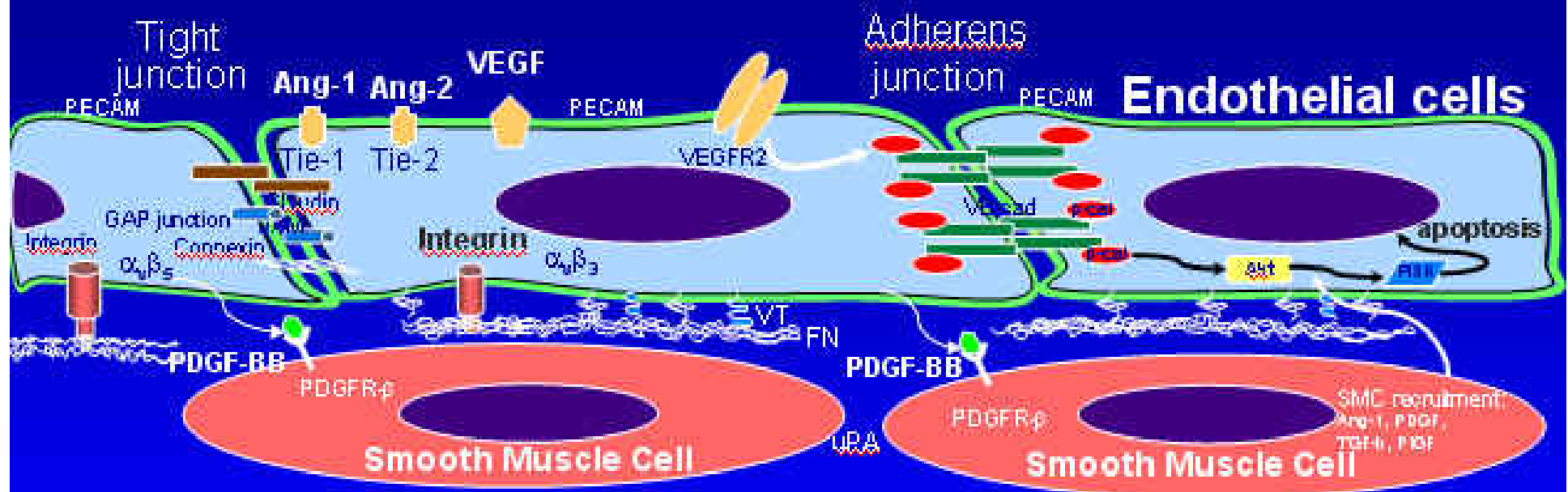
- Multiple Linear Regression Analysis to find significant correlations between endothelialization and morphometric parameters

	No. of uncovered struts/section	Ratio Uncovered/Total Struts	Interstrut Distance	Stent Length w/o Neointima	Fibrin Score
Multiple Linear regression	$r^2 = 0.64$	$r^2 = 0.86$	$r^2 = 0.15$	$r^2 = 0.20$	$r^2 = 0.32$
P Value	0.0001	0.0001	0.002	0.0005	0.0005

- The most powerful morphometric predictor of endothelialization was RUTSS (ratio of uncovered to total stent struts per section)
- Using RUTSS as a predictor in a logistic regression model, a lesion with RUTSS > 30% is 9 times more likely to have a stent thrombosis than lesions with RUTSS ≤ 30% [Odds Ratio = 9.0 (95%CI:3.5-22)]

Binn AV, et al., Circulation 2007;115:2435-2441

Endothelial Cell Intercellular Junctions and Antithrombotic actions



Tight Junctions:

Occludin
Claudin
JAM

GAP junctions

Connexins

Adherens Junctions:

VE-Cadherin

Antithrombotic Actions of Endothelial cells

- NO production through eNOS
- Prostacyclin (PGI_2)
- Heparin sulphate or chondroitine sulphate

- Thrombomodulin and PECAM-1
- Tissue Plasminogen activator
- Tissue factor pathway inhibitor

Endothelial Cell Assessment: Structure and Function

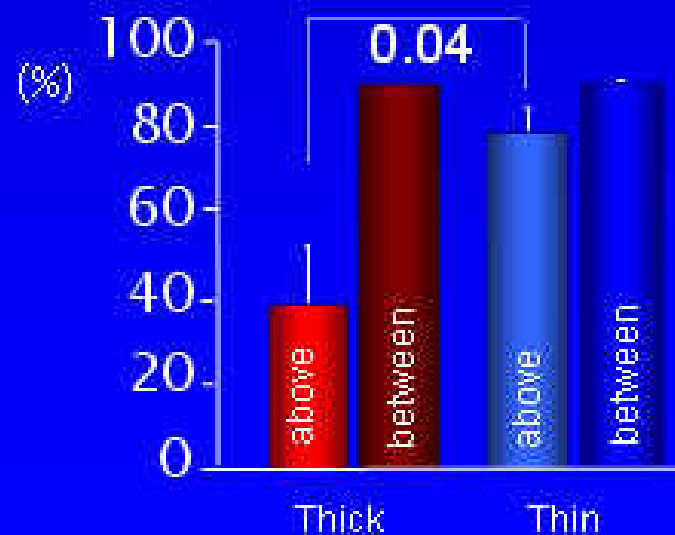
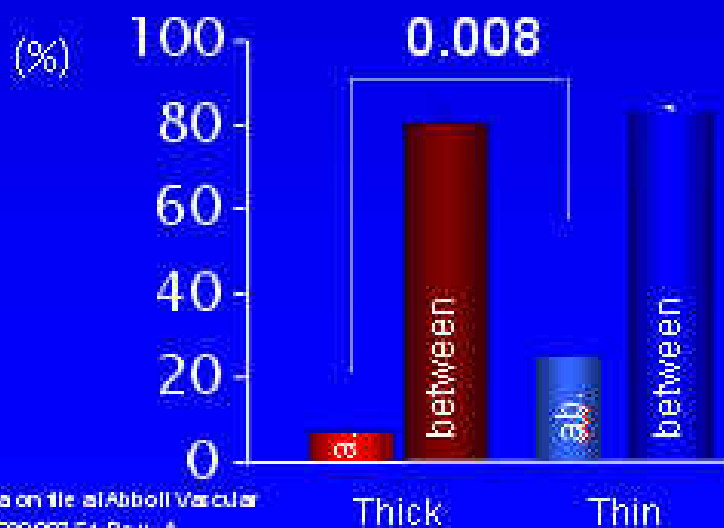
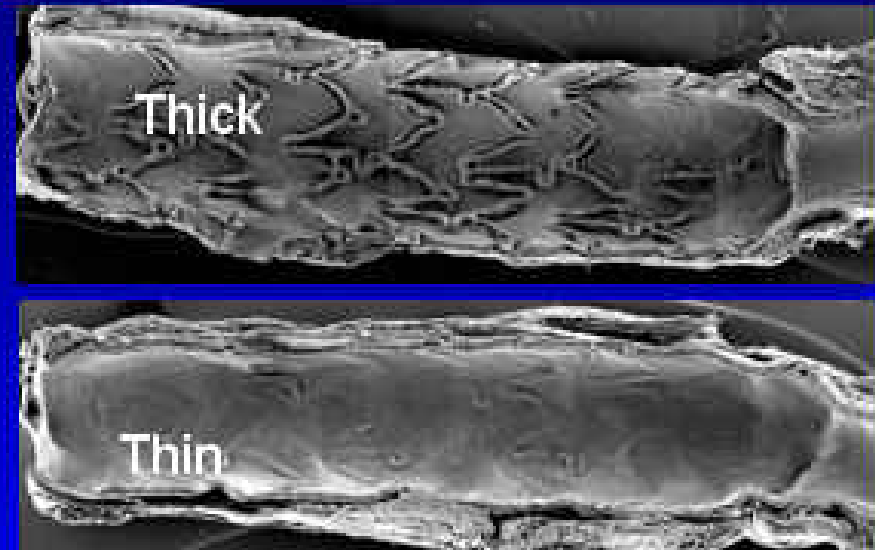
- SEM at 14 -, 28 -, and 42 – days
- PECAM-1- anticoagulant, inflammation, permeability and immune functions
- Thrombomodulin -anticoagulant
- VEGF-A - endothelial cell proliferation and permeability
- eNOS involved in enzymatic production NO

Endothelialization in the Rabbit Iliac Model: Comparison of thin and thick struts

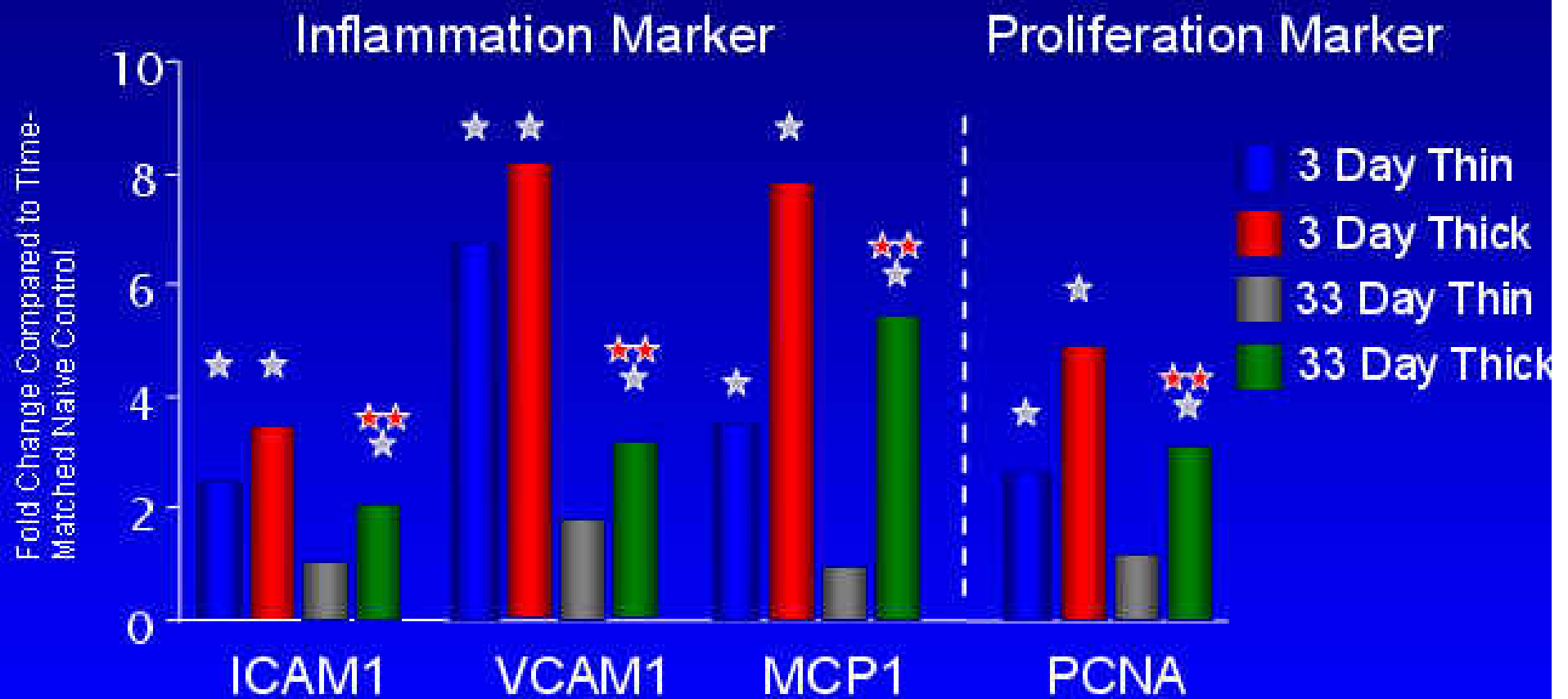
SEM of 7-Day Implants



SEM of 14-Day Implants

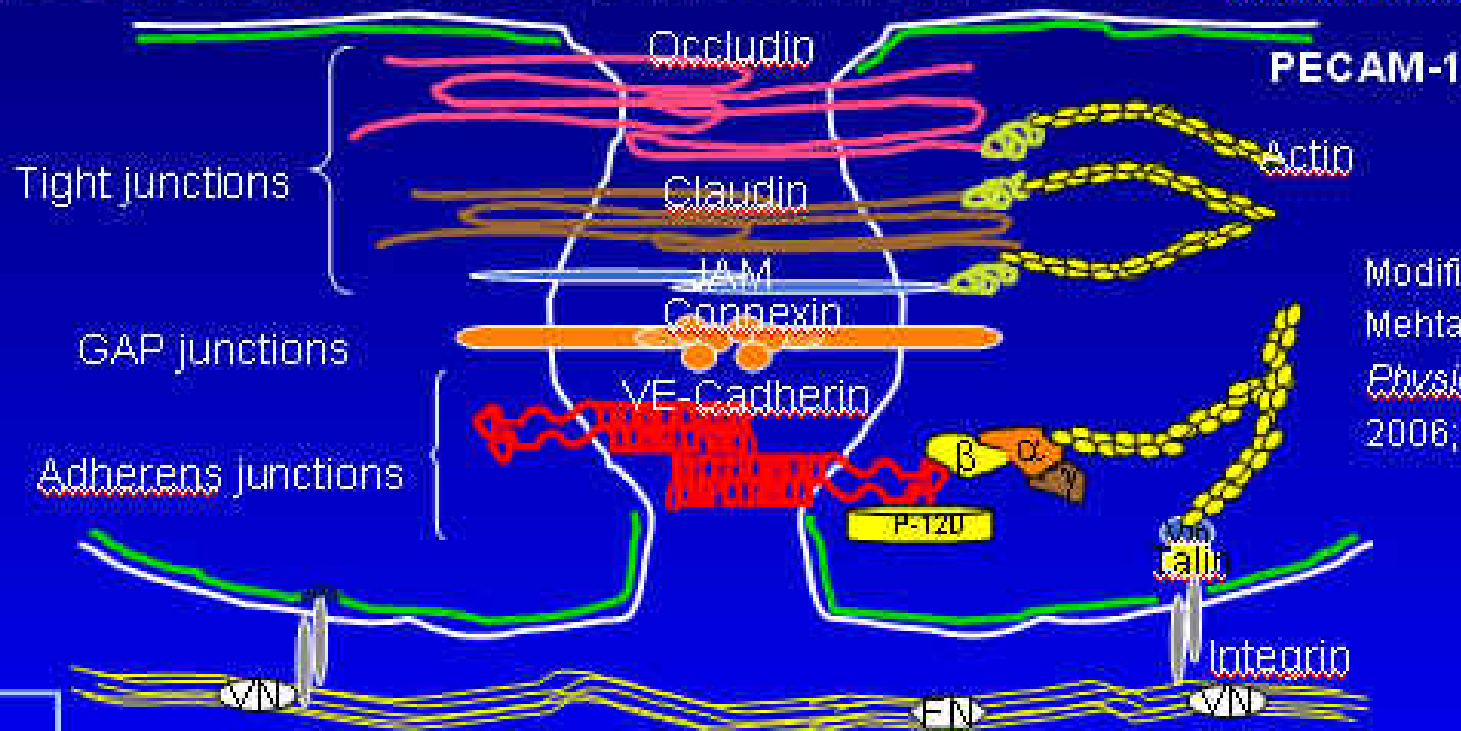


Thin Struts: Decreased Expression of Inflammatory Markers and Proliferation in Porcine Arteries



★ P < 0.05 from time matched unstented control ★★ p < 0.05 from time matched "thin" strut group

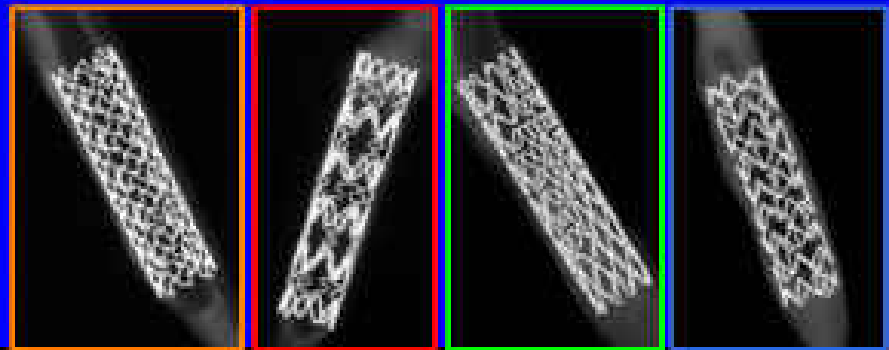
Structural Organization of Endothelial Cell Intercellular Junctions and Matrix Interaction



Modified from Dolly Mehta and AB Malik. *Physiol Rev* 2006;86:279

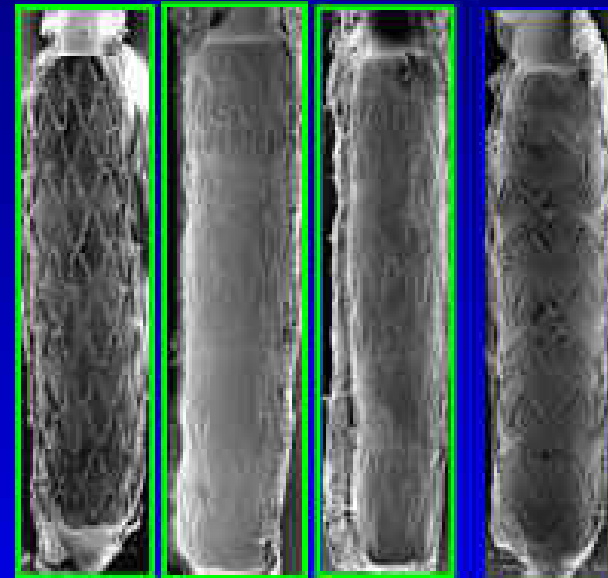
In Vivo

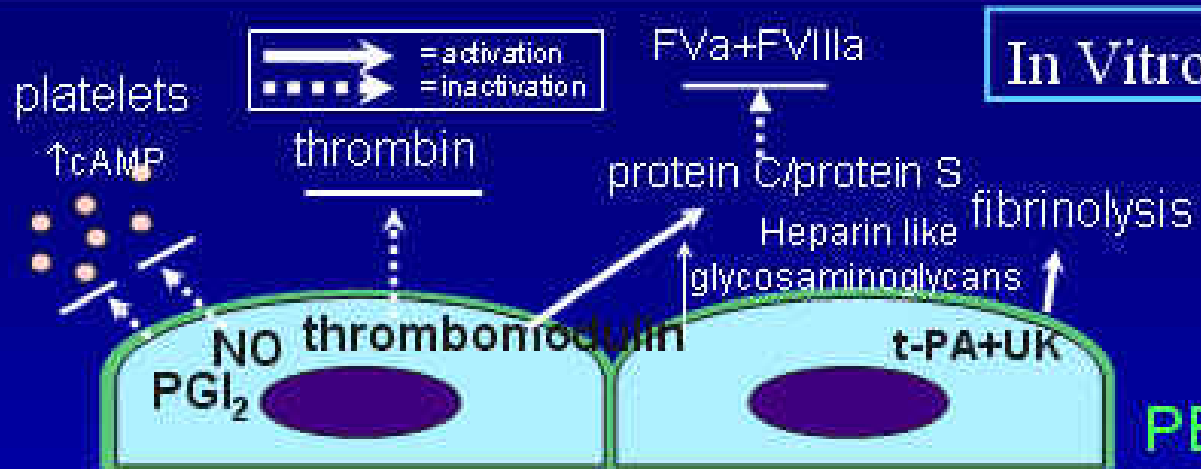
Taxus Cypher Endeavor Xience V



Comparison of Endothelialization following Implantation of Taxus, Cypher, Xience V and Endeavor Stents in Rabbit Iliac Arteries for 14- and 28- and 42-Days

Scanning Electron Microscopy at 14-, 28- and 42 Days





Thrombomodulin

CD141 is an integral membrane protein expressed on the surface of endothelial cells. It functions as a cofactor in the thrombin-induced activation of protein C in the anticoagulant pathway.

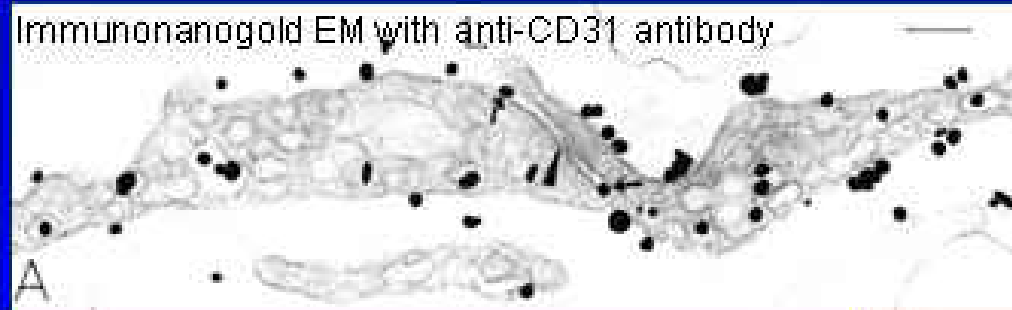
PECAM Thrombomodulin bound thrombin (TT) has no procoagulant effect. TT-complex stimulates fibrinolysis.

Inhibition of Platelet aggregation

Inhibition of coagulation

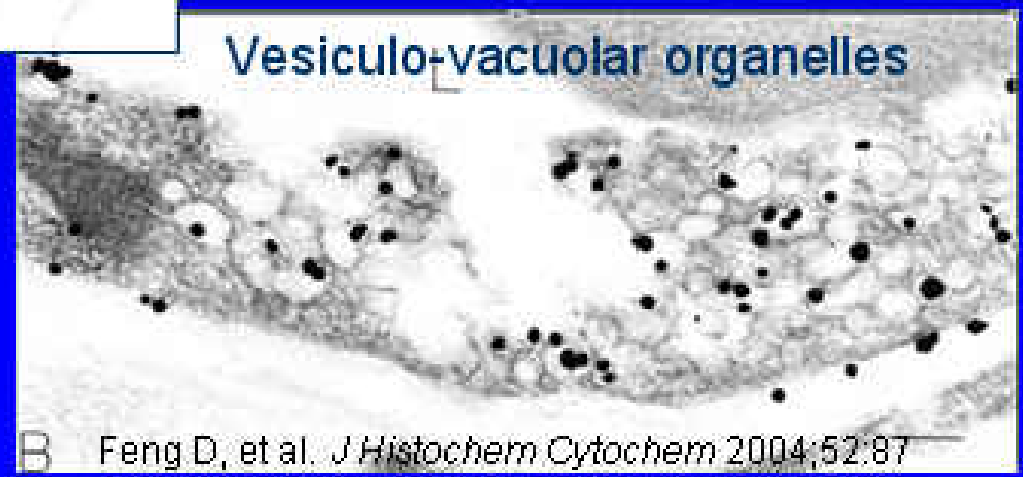
Activation of fibrinolysis

Platelet endothelial cell adhesion molecule (PECAM-1)

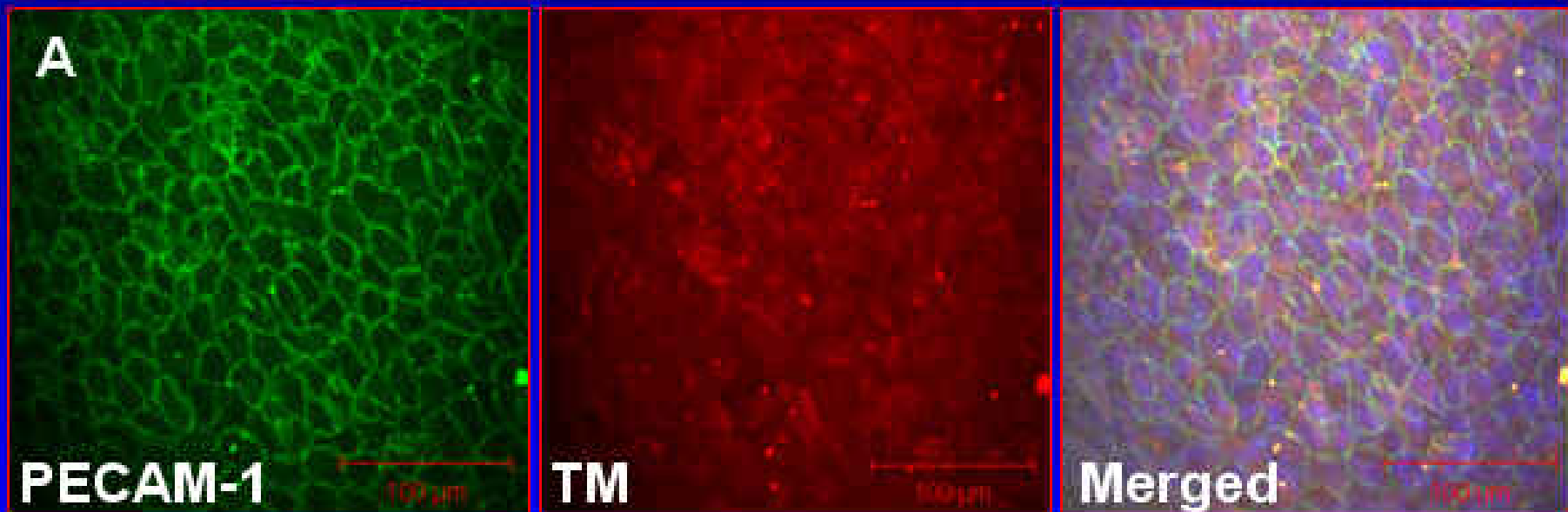


PECAM-1 is a membrane glycoprotein that is constitutively expressed by endothelial cells at cell borders and by platelets and leukocytes.

Its main functions are transendothelial leukocyte migration, vasculogenesis, apoptosis and hemostasis. In vitro and in vivo studies have demonstrated that PECAM-1 inhibits the aggregation of platelets and induces smaller thrombi which form slowly.
 (Falati S, et al., *Blood* 2006; 107:535)

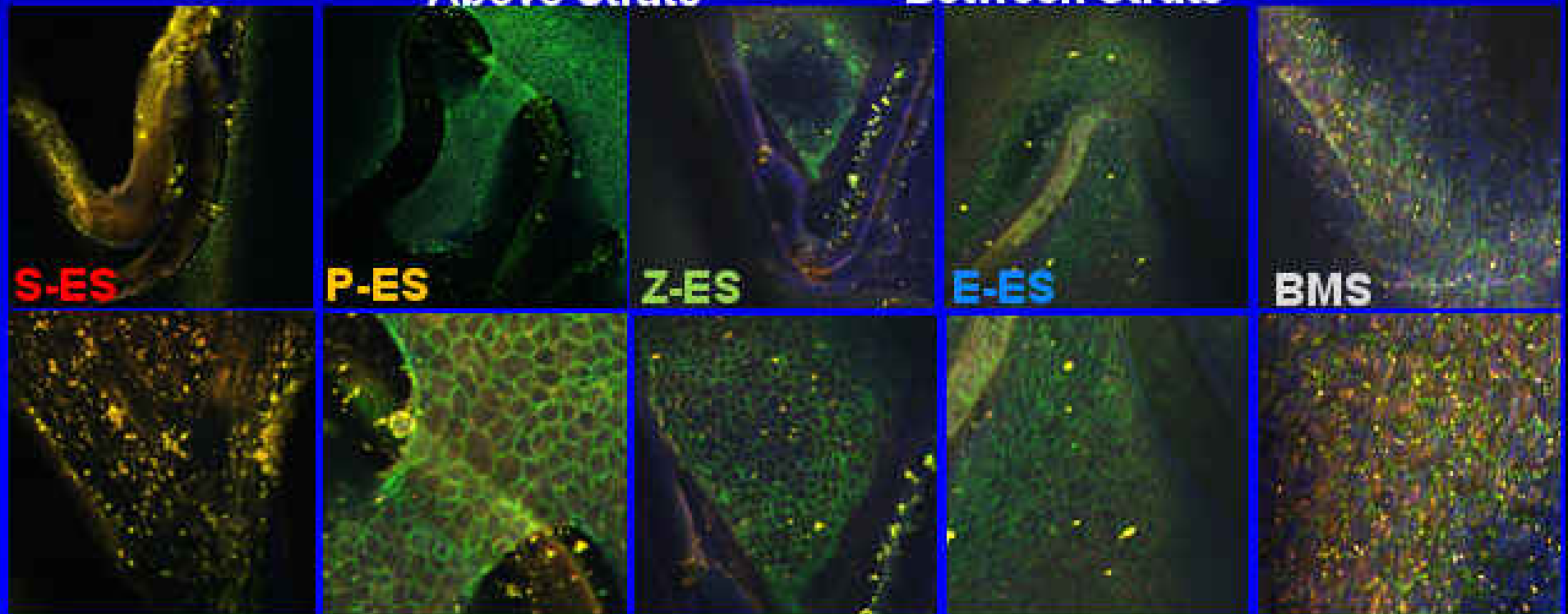
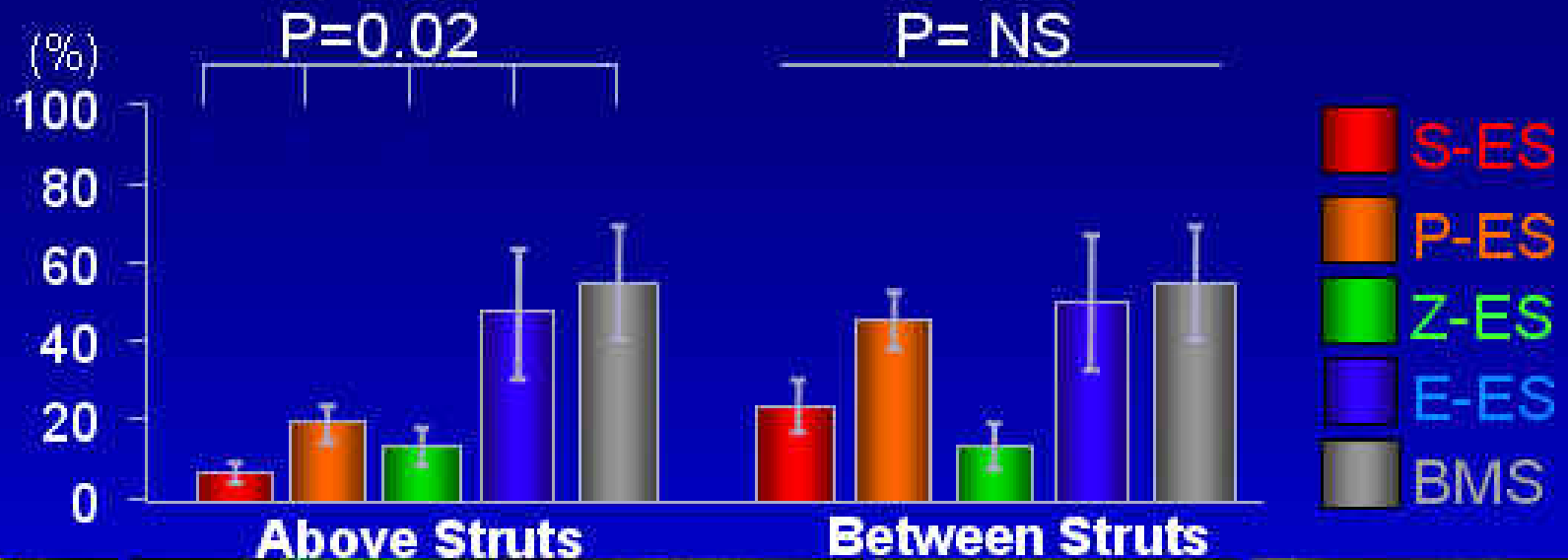


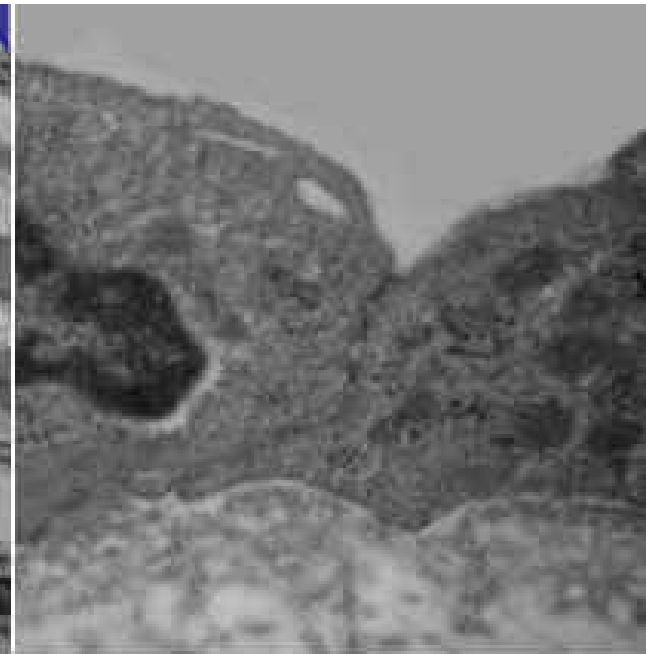
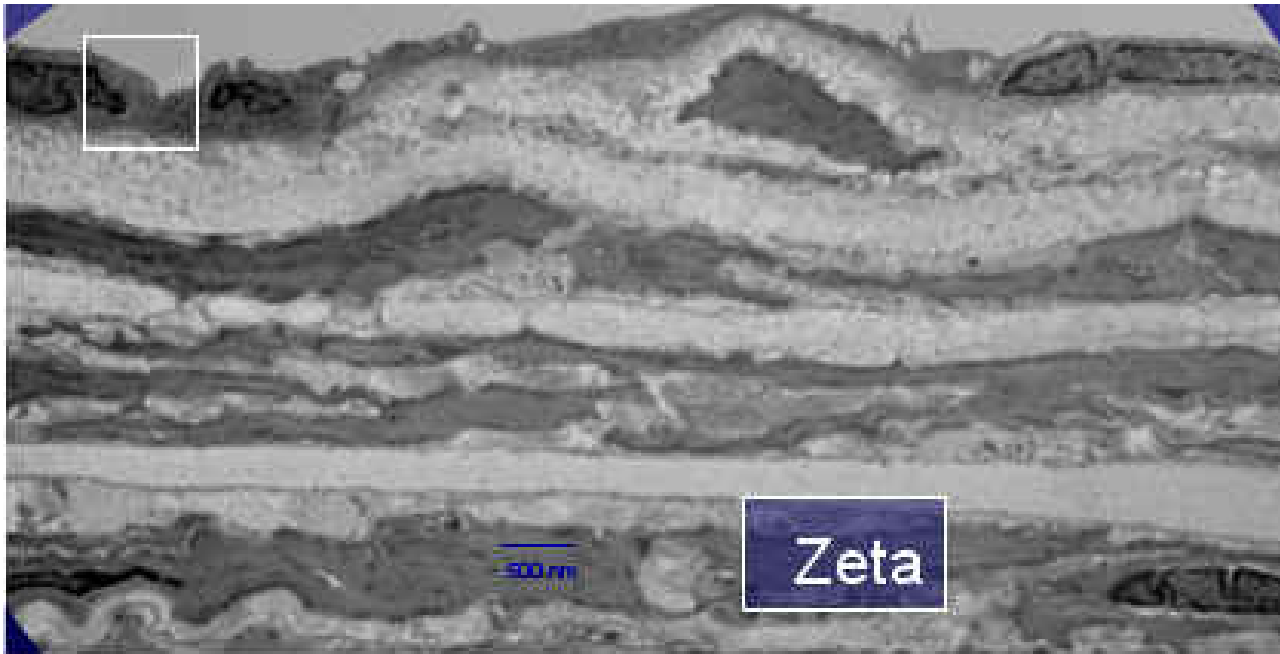
Confocal Microscopy in Normal Iliac Artery



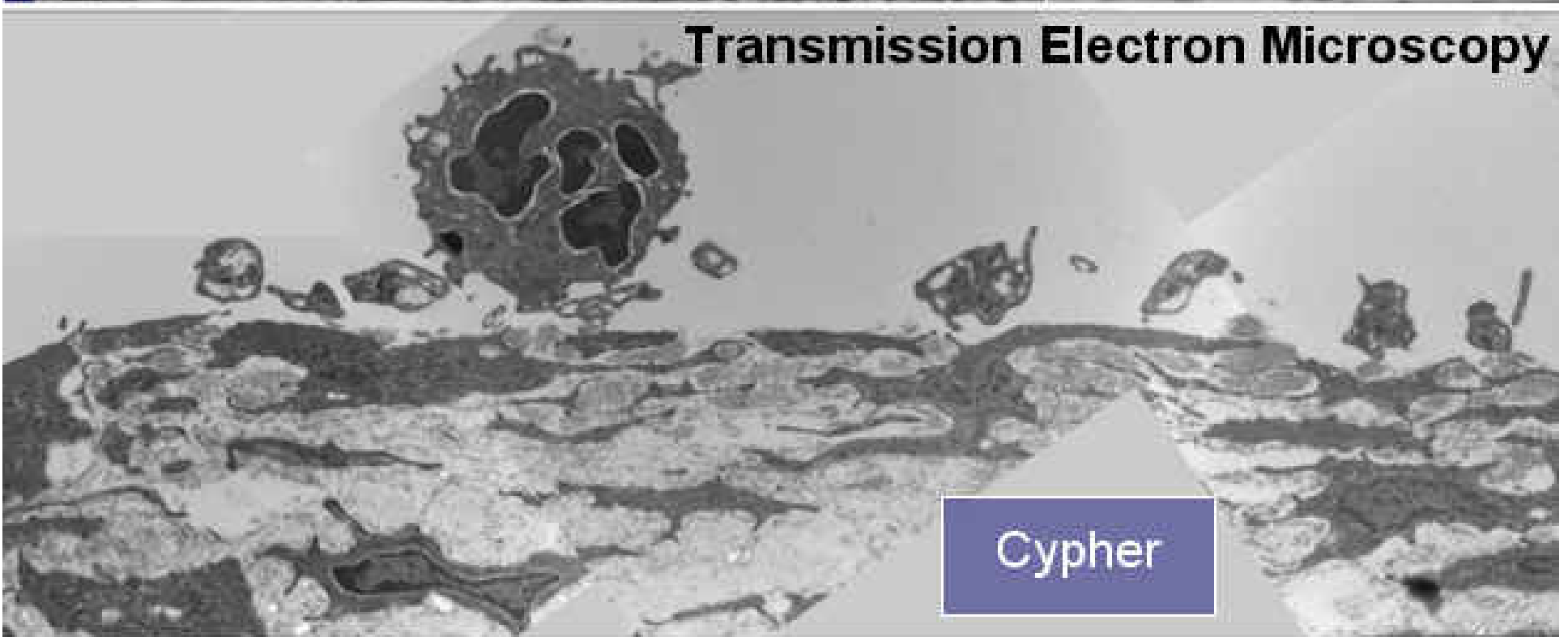
PECAM = platelet endothelial cell adhesion molecule
TM = thrombomodulin

En Face Confocal Analysis (PECAM-1) of Various Stents





Transmission Electron Microscopy

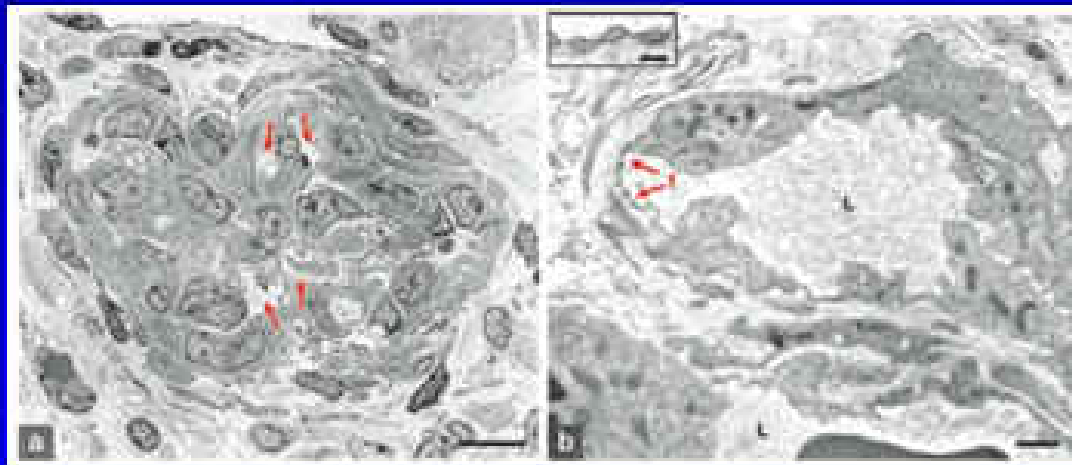


Endothelial Cell Specific Molecules Assessed

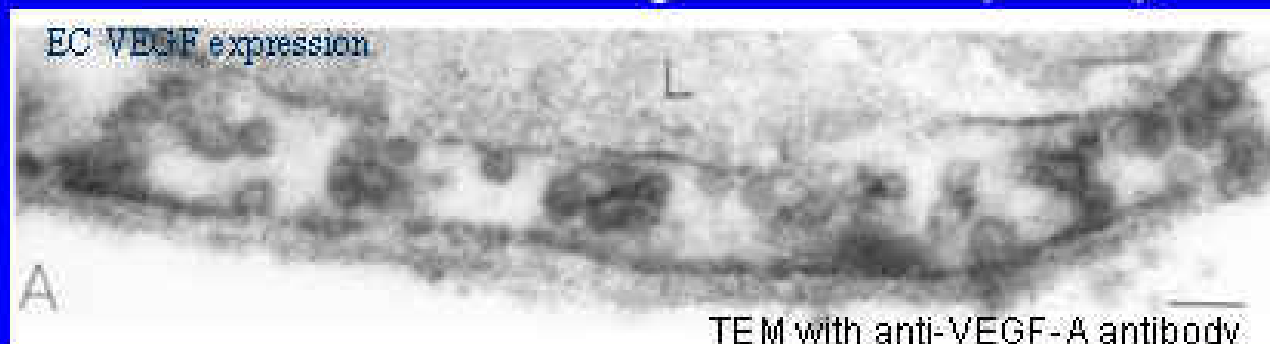
- PECAM-1- anticoagulant, inflammation, permeability and immune functions
- Thrombomodulin -anticoagulant
- VEGF - endothelial cell proliferation and permeability
- eNOS involved in enzymatic production NO release

Vascular Endothelial Growth Factor

VEGF a sub-family of growth factors are important signaling proteins involved in vasculogenesis (the *de novo* formation of embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature).



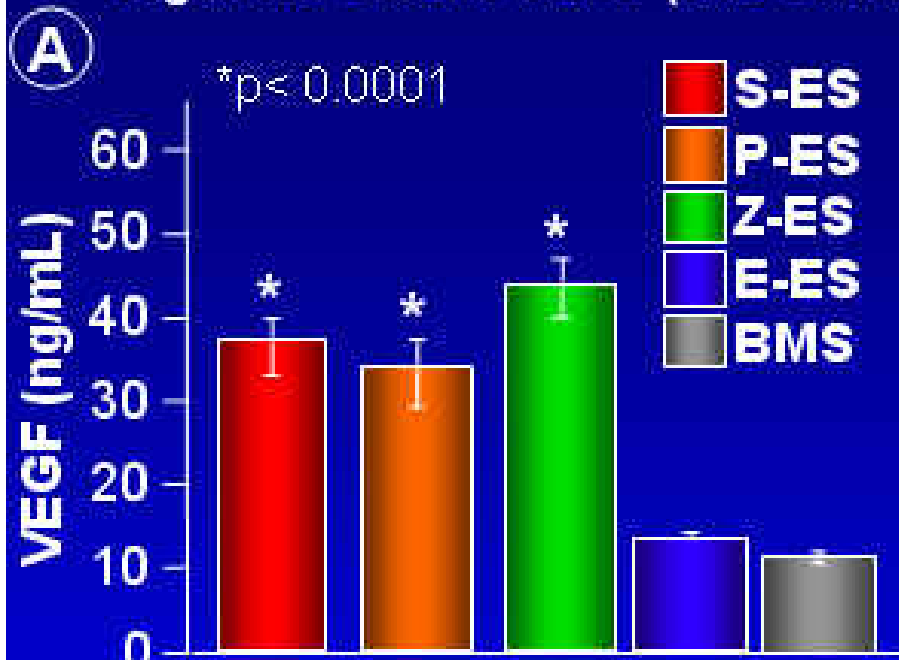
Vascular endothelial growth factor (VEGF)



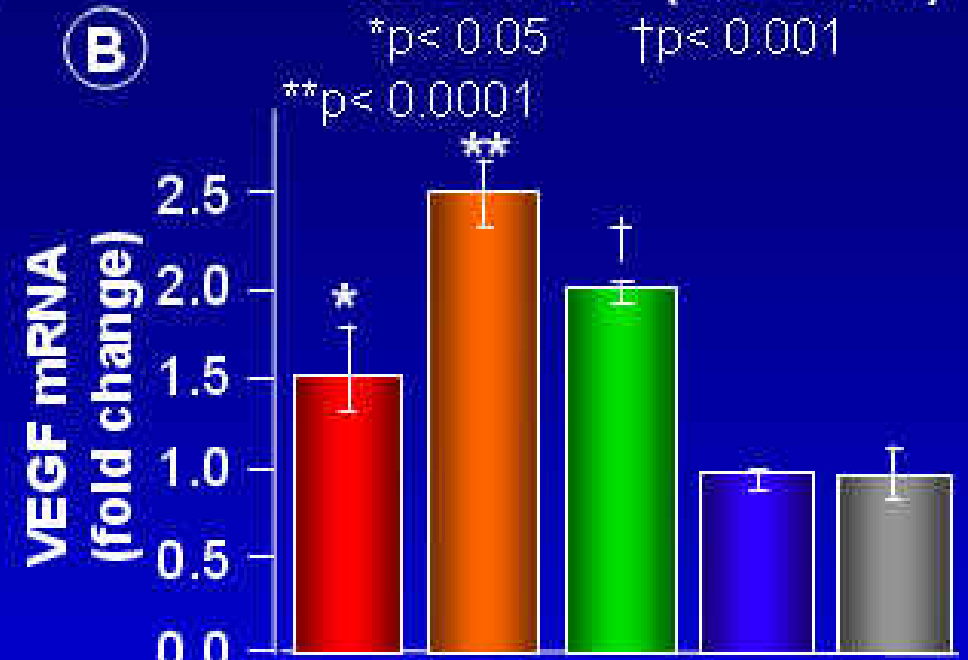
Tumor with vascular expression of VEGF

VEGF has endothelial cell-specific mitogenic activity and is believed to be an important regulator of normal and pathologic angiogenesis and vascular permeability. VEGF protein and mRNA levels are significantly upregulated in tissues with ongoing angiogenesis while there is down regulation following complete vascularization. VEGF-A is responsible for induction of pathologic angiogenesis as well as maintenance of normal vascular endothelium

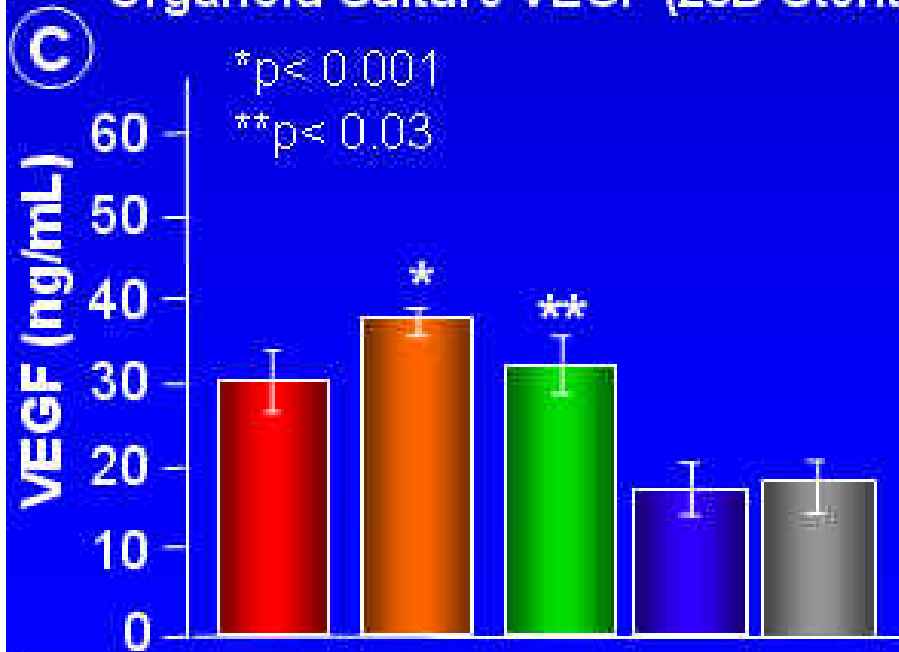
Organoid Culture VEGF (14D Stents)



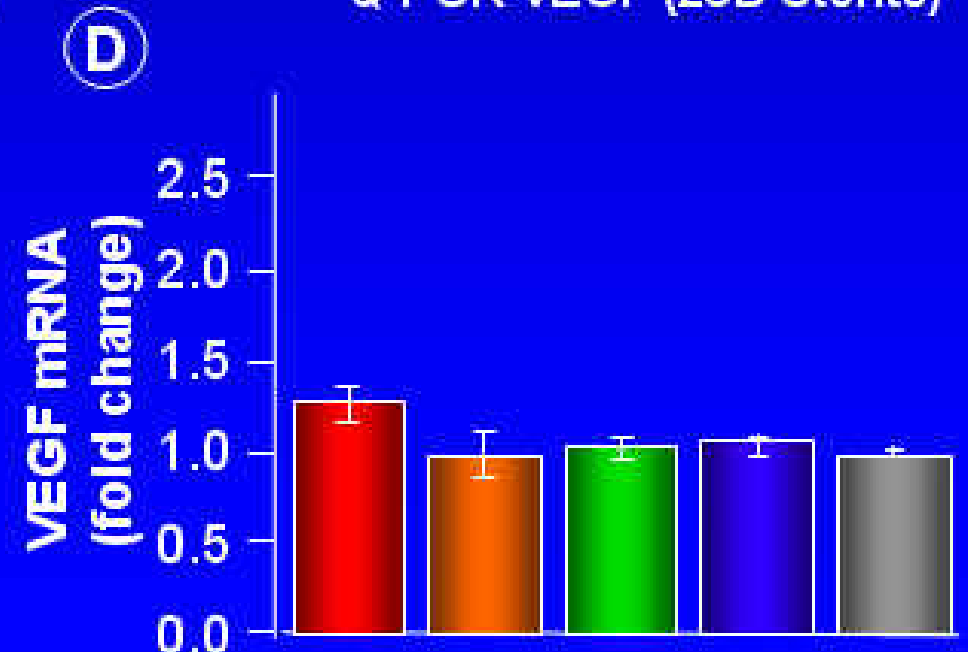
Q-PCR VEGF (14D Stents)



Organoid Culture VEGF (28D Stents)



Q-PCR VEGF (28D Stents)



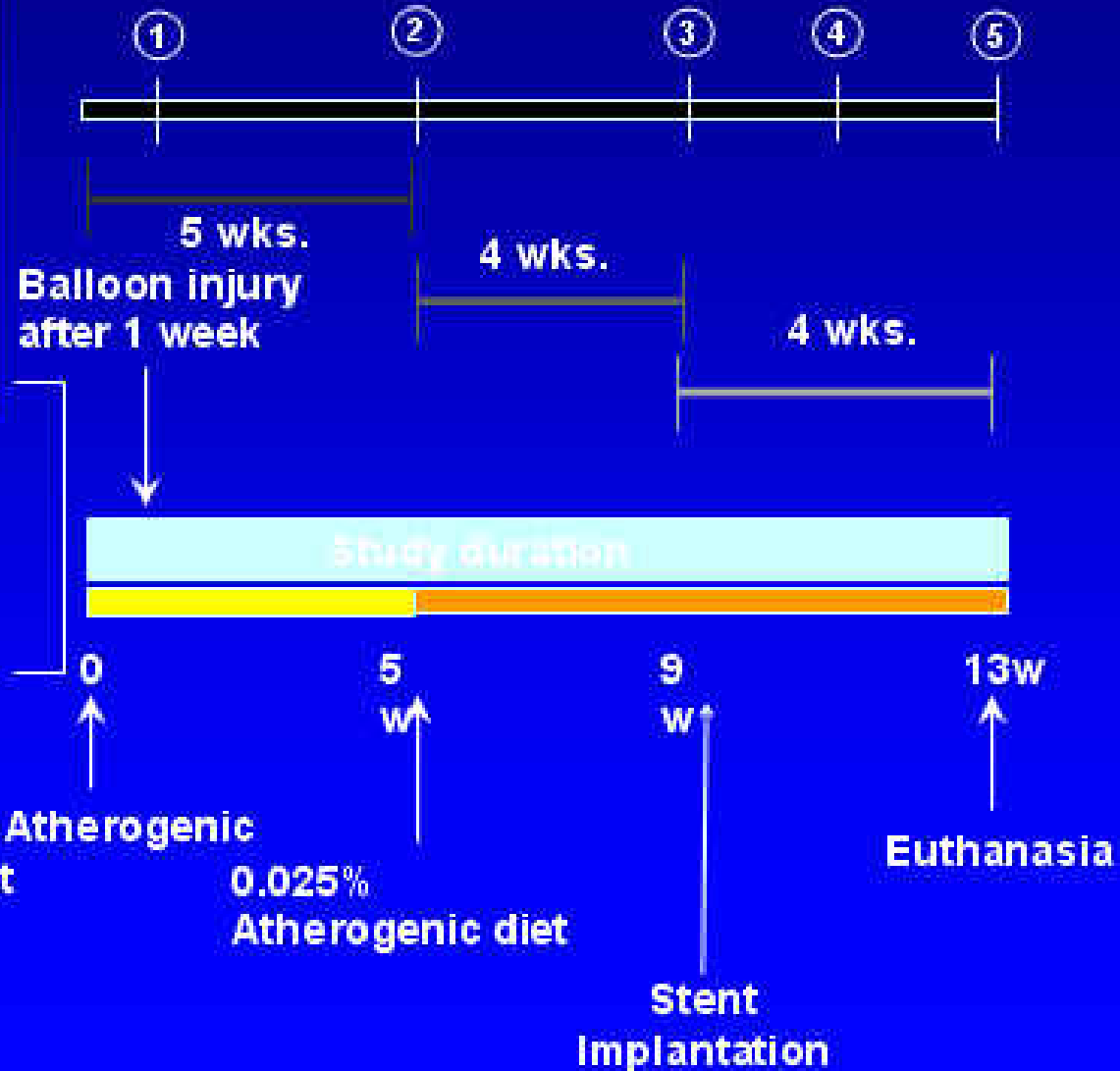
DES must be studied in Atherosclerosis



New Zealand White Rabbits

Groups:
Diver
Endeavor
Xience
Cypher

Blood Collection for Cholesterol measurements

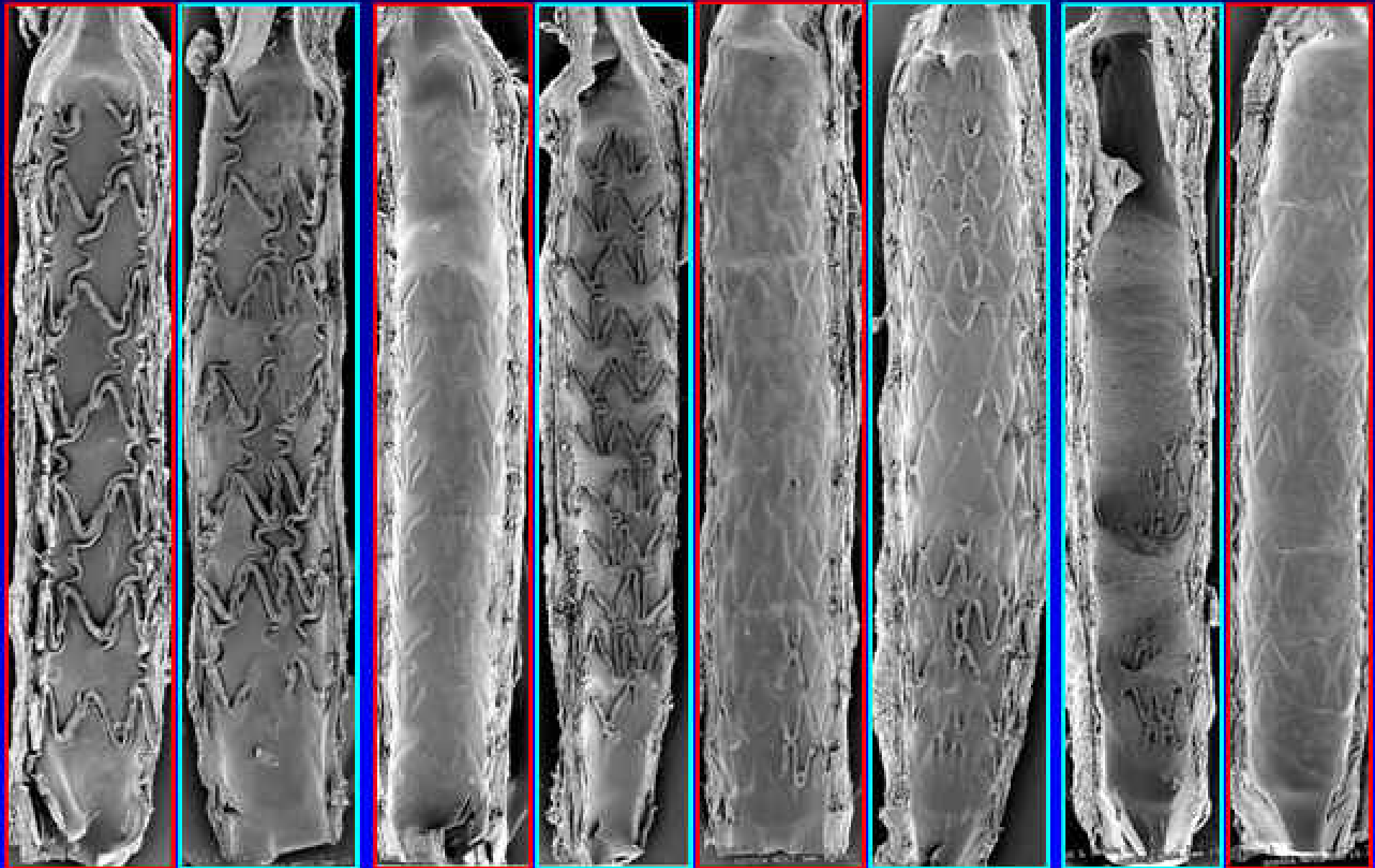


Cypher

Xience V

Endeavor

Driver



TC=593,

2307

824

2337

1313

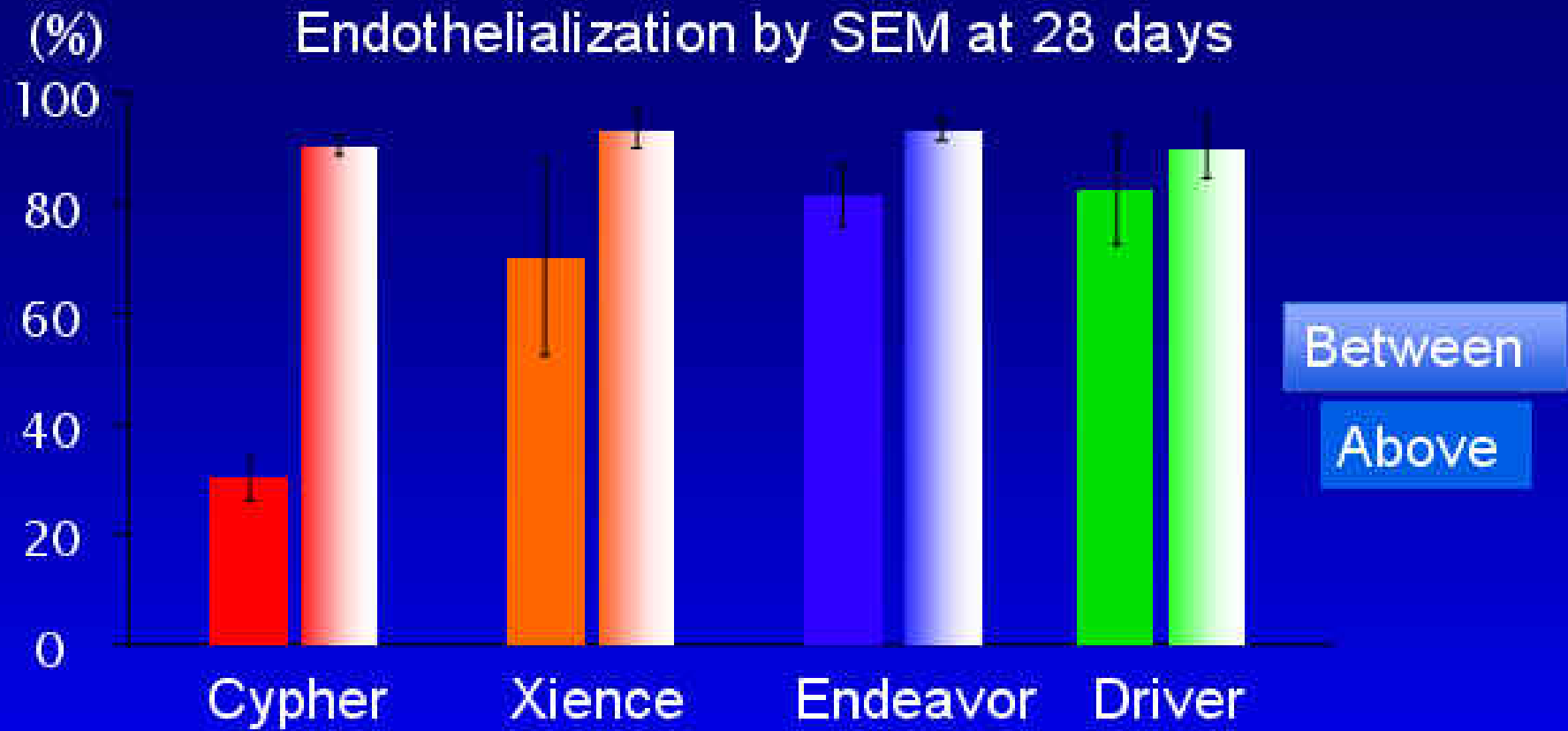
392

1358

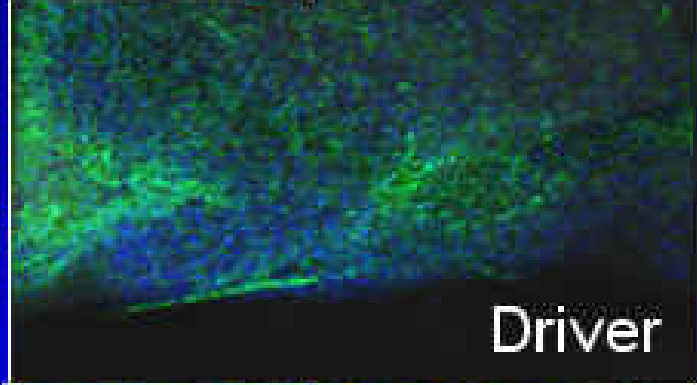
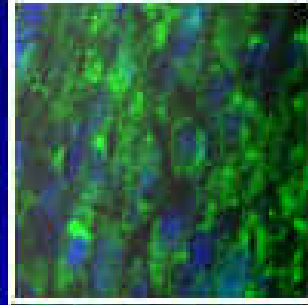
1305mg/dl

Rabbit model of atherosclerosis at 28-days

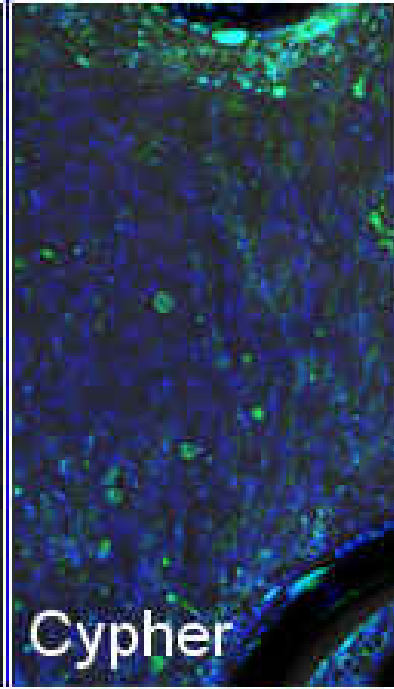
Endothelialization by SEM at 28 days



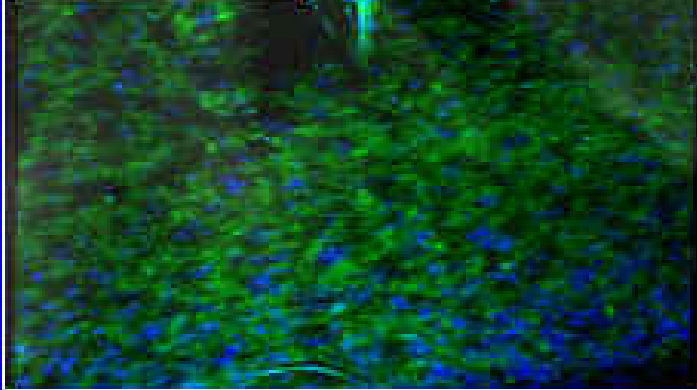
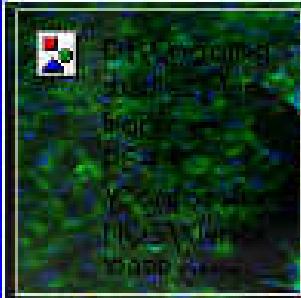
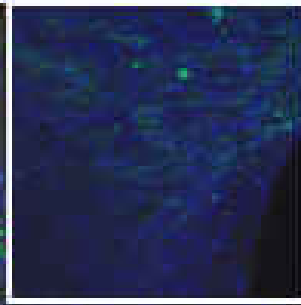
Confocal Microscopy (eNOS)



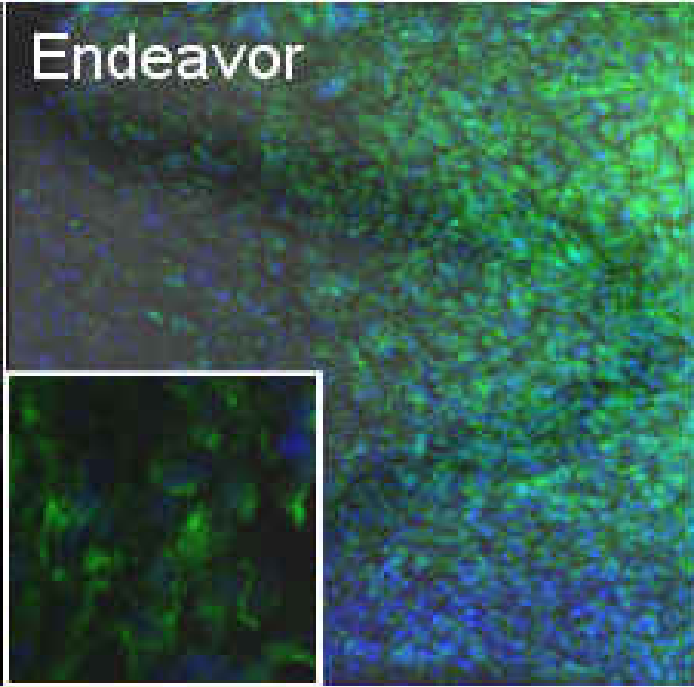
Driver



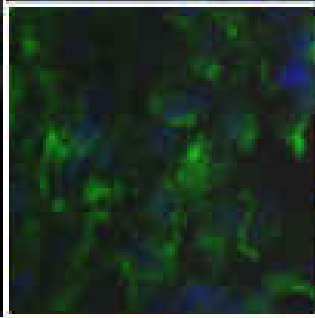
Cypher



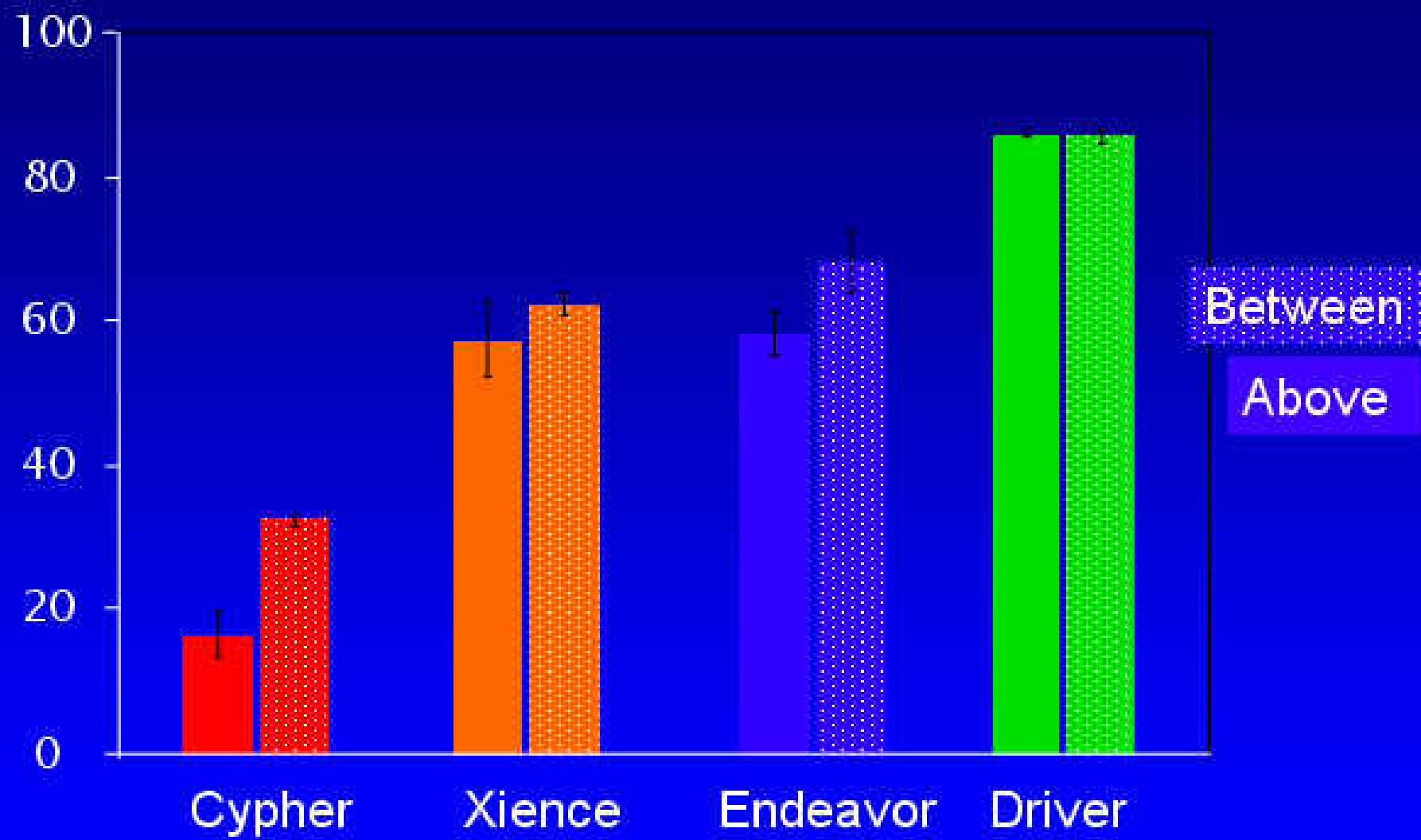
Xience



Endeavor



(%) Endothelialization by Confocal (eNOS) at 28 days



Can Endothelialization be Improved in DES?

- Possibilities:
- Abluminal delivery of drug
 - Coating with Anti-CD34
 - RGD Peptide

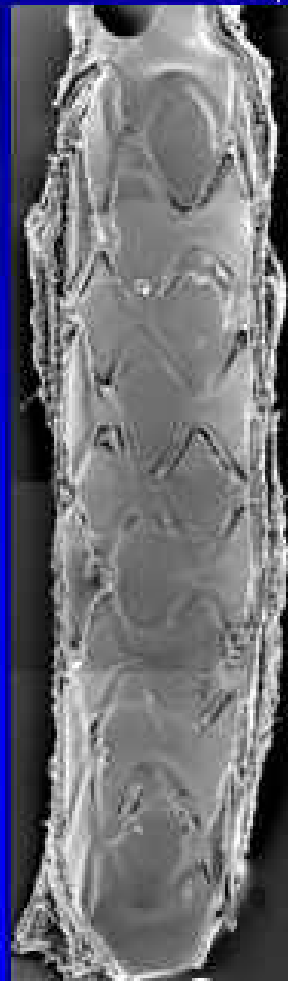
Endothelialization at 14 days (SEM; Rabbit iliac model)

BMS

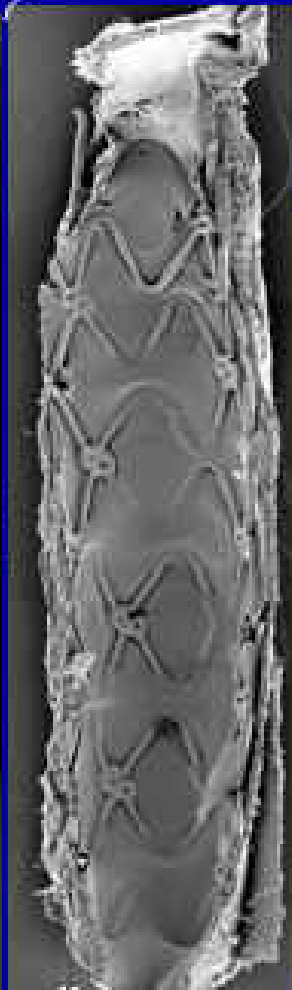
Polymer-free DES



$89.9 \pm 9.9\%$



Overall
Coverage



$78.4 \pm 10.7\%$



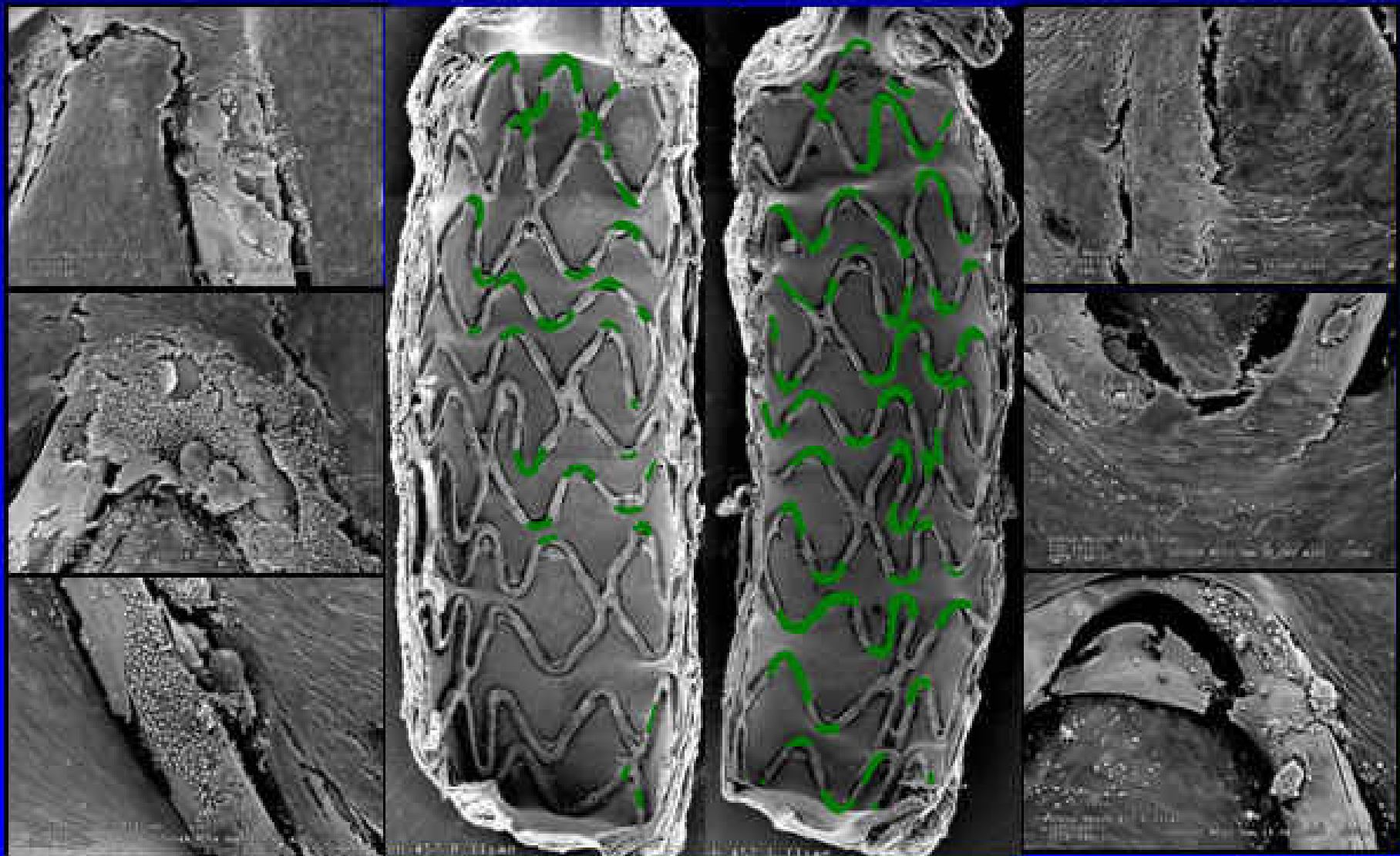
BioRx 61500
R112-26

Early endothelialization of Endothelial Progenitor Cell capture stents (Genous) In the rabbit iliac model

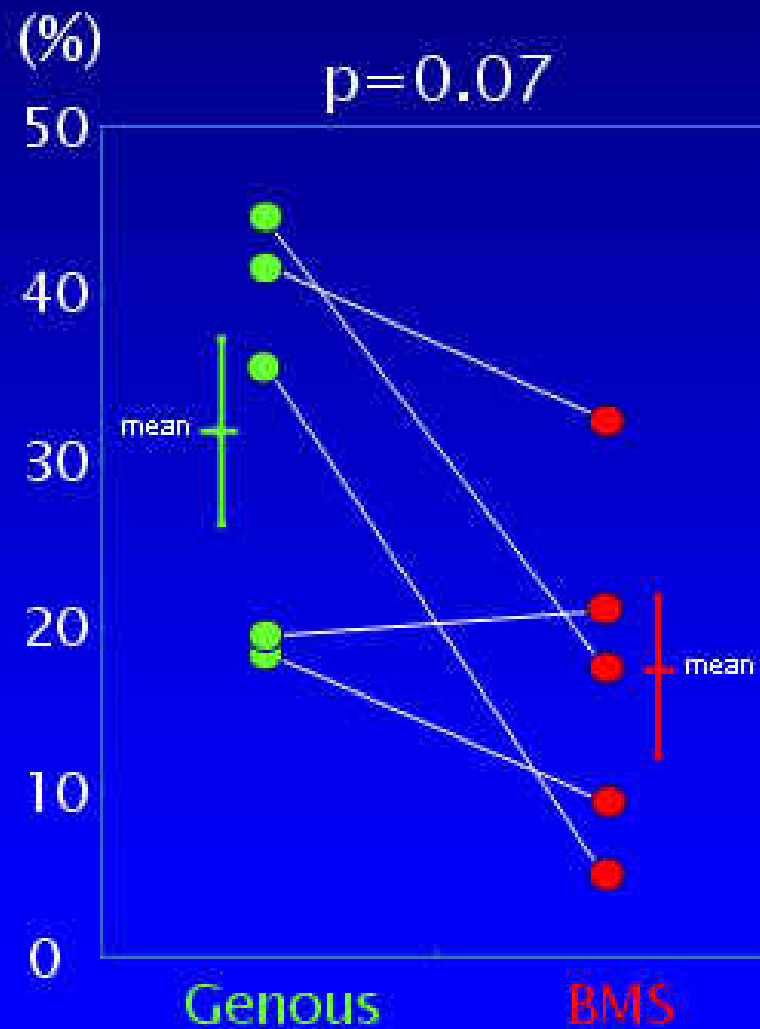
BMS

7-day

Genous

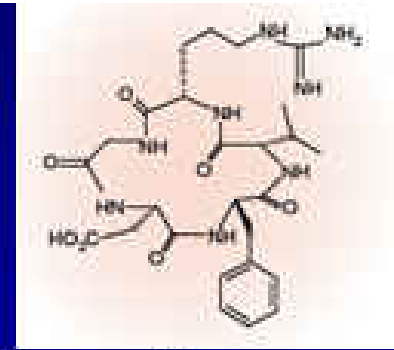


Stent strut coverage – 7 day



In rabbit iliac model

RGD (Arg-Gly-Asp) peptide

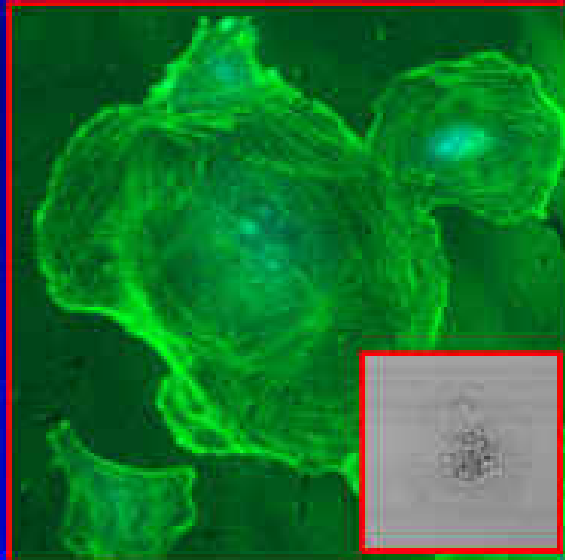


- Integrins mediate adhesion between cells and the extracellular matrix (ECM) by binding to ligands with an exposed arginine-glycine-aspartate (RGD) sequence
- Focal adhesion kinase (FAK) is activated by tyrosine phosphorylation in response to integrin activation and regulates cell adhesion and contraction which are closely connected to permeability

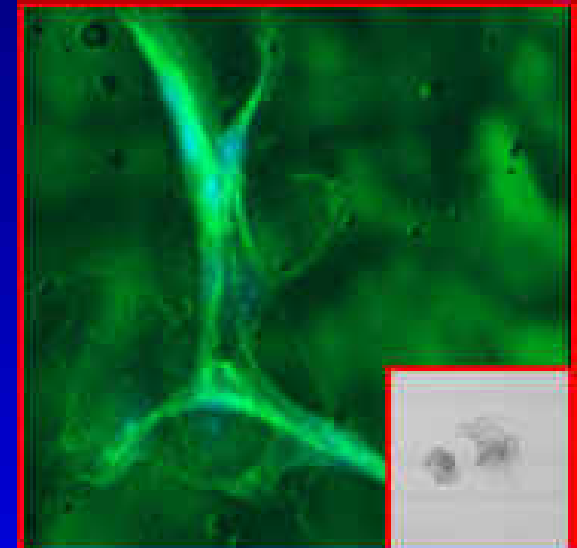
The Impact of RGD-coating Nitinol on Endothelial Cell Growth

coated

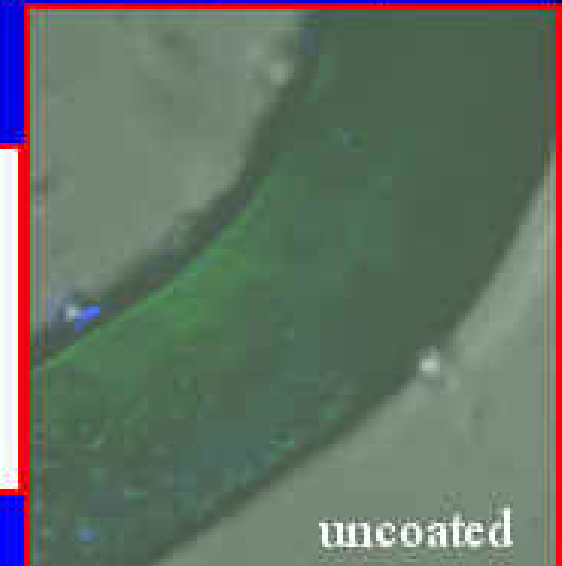
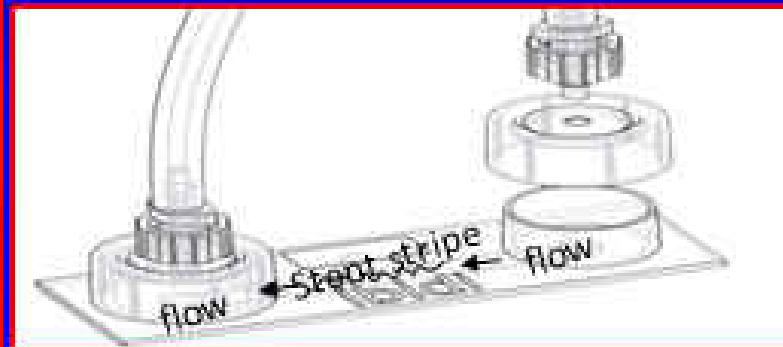
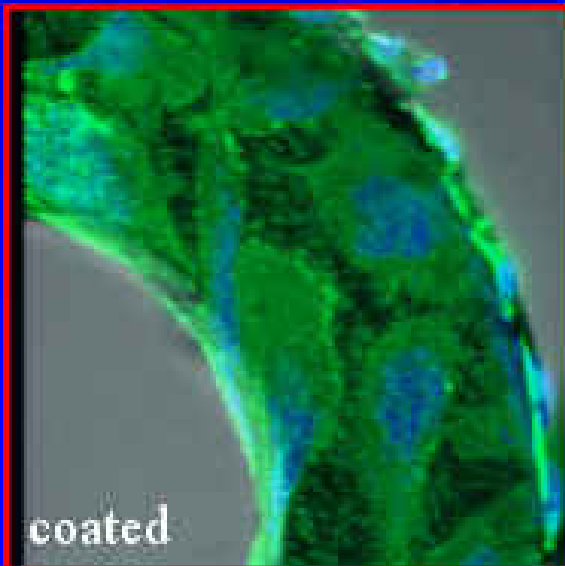
uncoated



**Coated and Non-coated
Nitinol Coupons**

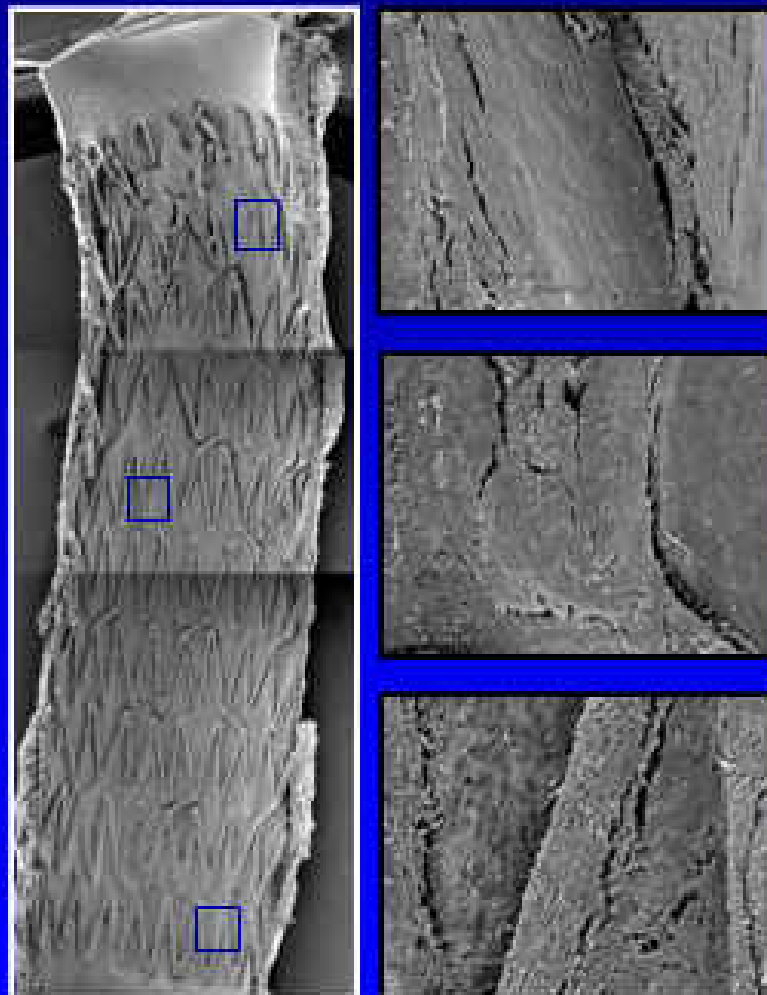


**Coated and Non-coated
Nitinol Stent under flow**

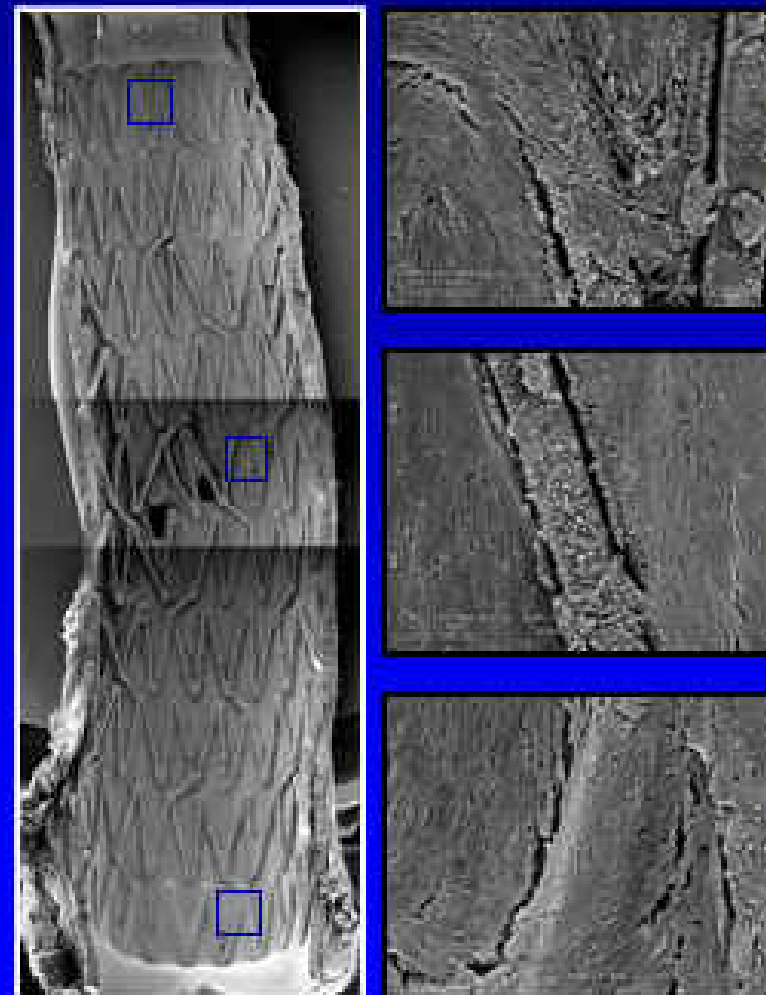


SEM for cRGD coated and uncoated Nitinol stents were implanted into the abdominal aorta of New Zealand White Rabbit for 7 days

cRGD coated



uncoated



In Vitro and in Vivo Assessment of Endothelialization

SUMMARY

- FDA approved CYPHER and TAXUS stents are associated with late stent thrombosis and is likely related to the extent of endothelial stent strut coverage.
- We need to utilize appropriate animal models of endothelialization to better assess DES. Not only for extent of endothelial coverage but importantly also its integrity and functionality.
- In vivo and in vitro organoid cultures show that VEGF continues to be released only in those arteries with incomplete endothelialization in the rabbit model
- In vivo studies in atherosclerotic model for extent of endothelialization and presence of eNOS show that the 2nd generation of DES are better than the 1st generation (CYPHER and TAXUS).
- 3rd Generation of DES are likely to be better than the 1st generation because enhancing endothelialization is less likely to result in late stent thrombosis

Acknowledgments

- Frank Kolodgie, Ph.D.
- Gaku Nakazawa, M.D.
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- Andrew Farb, M.D.
- Ed Campado, D.V.M.
- Robert Kutz, M.S.
- You-hui Liang, M.D.
- Hedwig Avallone
- Lila Adams
- Russ Jones
- Rosellin Mathew



Madagascar, 2003.
Photograph by R. Virmani

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