The Value of Animal Models in Evaluating Pathobiologic Effects of Drug-Eluting Stents: *Insights from past successes and failures*

Angioplasty Summit 2004

April 29th, 2004

Renu Virmani, M.D.

Armed Forces Institute of Pathology

Washington, DC

Value of Animal Models in Evaluating Drug Eluting stent

- Why do we need animal models?
  - To determine safety of the product prior to human use.
- Can animal models help predict success or failure of drugs or devices in man?
  - Depends on the animal model and what we want the drug or device to treat:
    - Is it to treat atherosclerosis?
    - Or treating Acute Coronary Syndromes or Stable AP or Luminal Narrowing?
    - Or prevent restenosis?
- Animal models will only predict human disease if they have the disease we want to treat - unfortunately such models are either not available or are too expensive and therefore we compromise.

Conventional and Unconventional Models for the Evaluation of Coronary Stents

- Normal Vessels:
  - Pig coronary arteries
  - Rabbit iliac arteries
  - Rat carotid arteries
- Models of atherosclerosis:
  - Rabbit iliac arteries
  - Pig coronary arteries - presence or absence of diabetes - too expensive and time consuming
  - Primate models - peripheral vessels - too expensive and time consuming

Vessel Healing Following of Stent Placement in Man and Animals

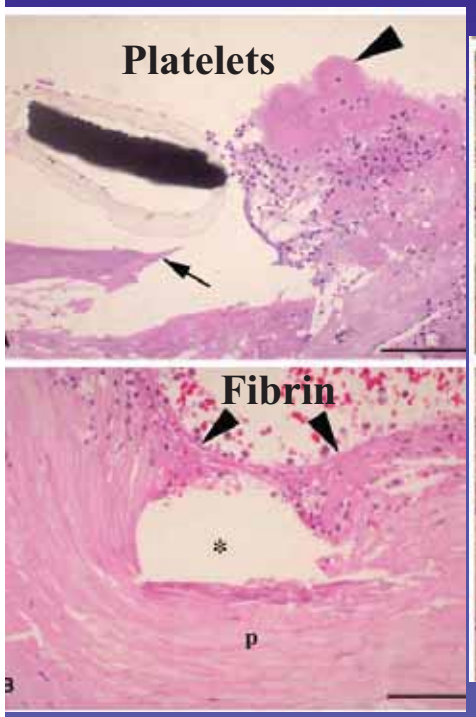
- Platelet/fibrin thrombus
- Inflammation-acute and chronic
- Smooth muscle cell proliferation and migration
- Matrix deposition-proteoglycans and collagen

Granulation tissue

- Reendothelialization
- Adventitial response

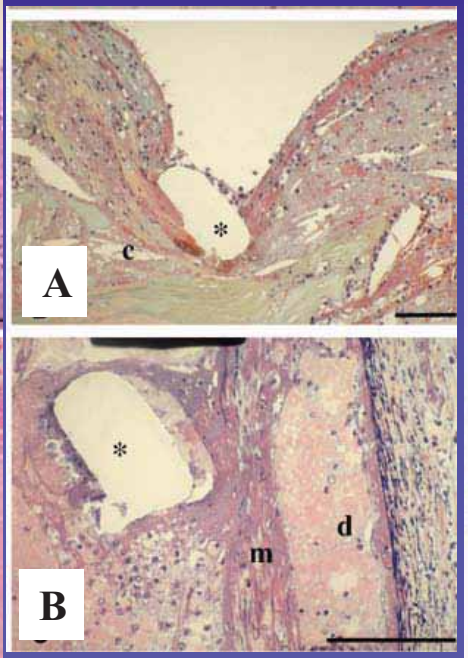
Balloon Expandable Stainless Steel Stent Healing in Man

Thrombus



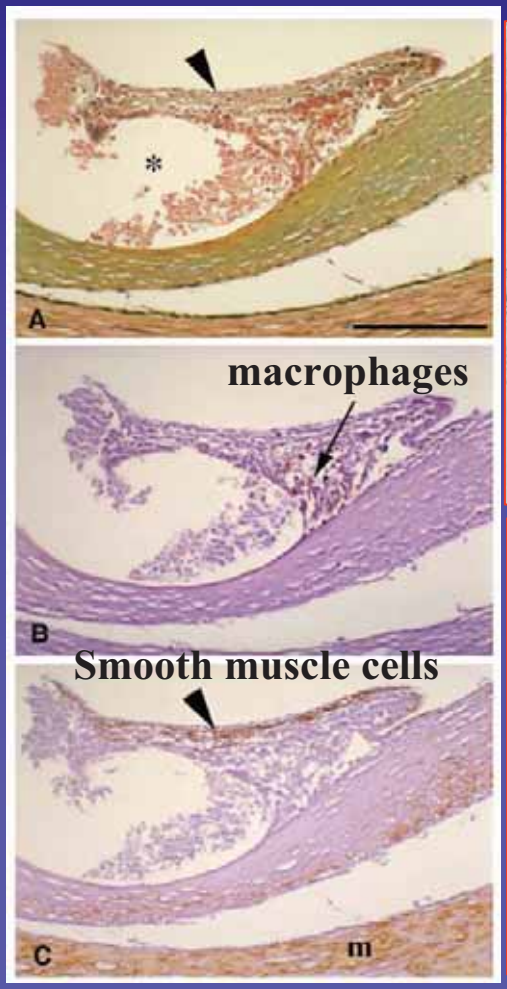
1-30 days

Inflammation



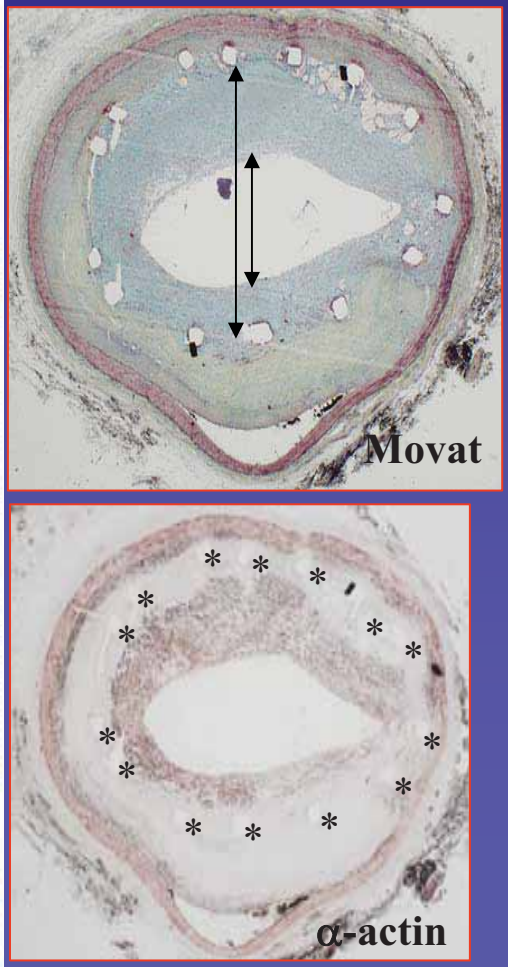
1-30 days

Granulation tissue



14 – 90 days

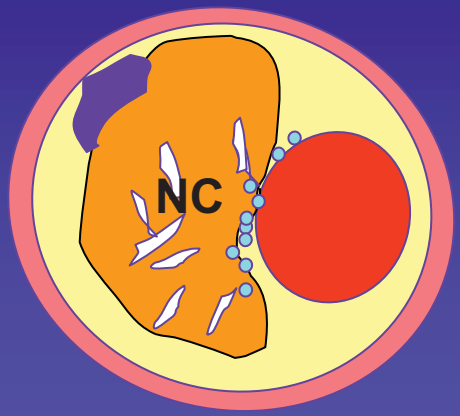
Smooth muscle cells and matrix



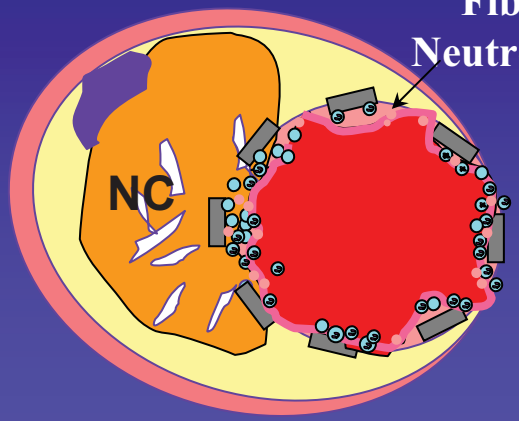
6 months

Diagram Illustrating Vascular Response to Inravascular Balloon Expandable Stainless Steel Stent Placement in Atherosclerotic Human Coronary Arteries

Virmani, R, et al. Heart 2003;89:133

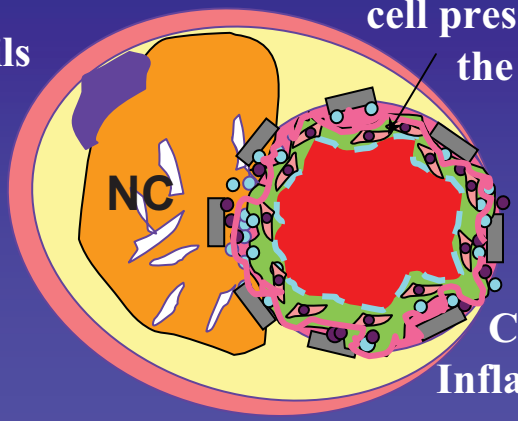


Atherosclerotic Coronary Artery
Prior to stent placement



Platelets/
Fibrin
Neutrophils

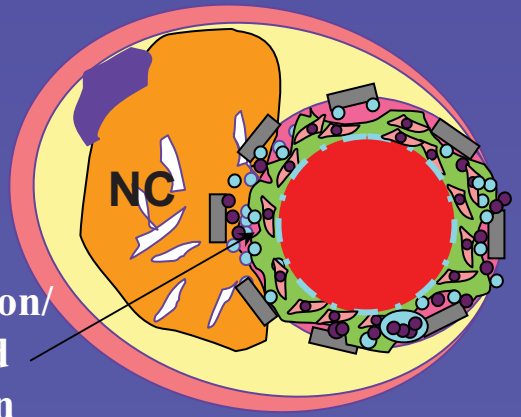
< 3 days



Smooth muscle
cell present inside
the stent

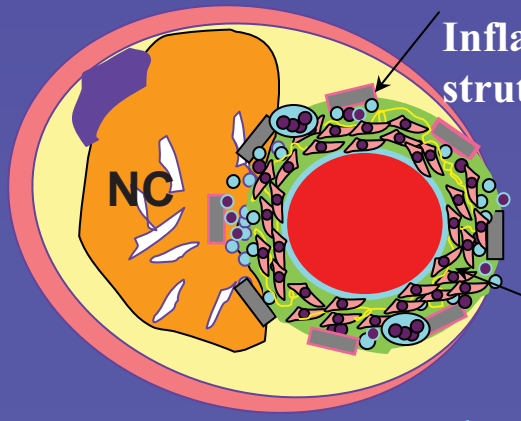
Chronic
Inflammation/
persistent
fibrin

14-30days



Chronic inflammation/
Persistent fibrin and
greater proteoglycan
matrix deposition

3 months



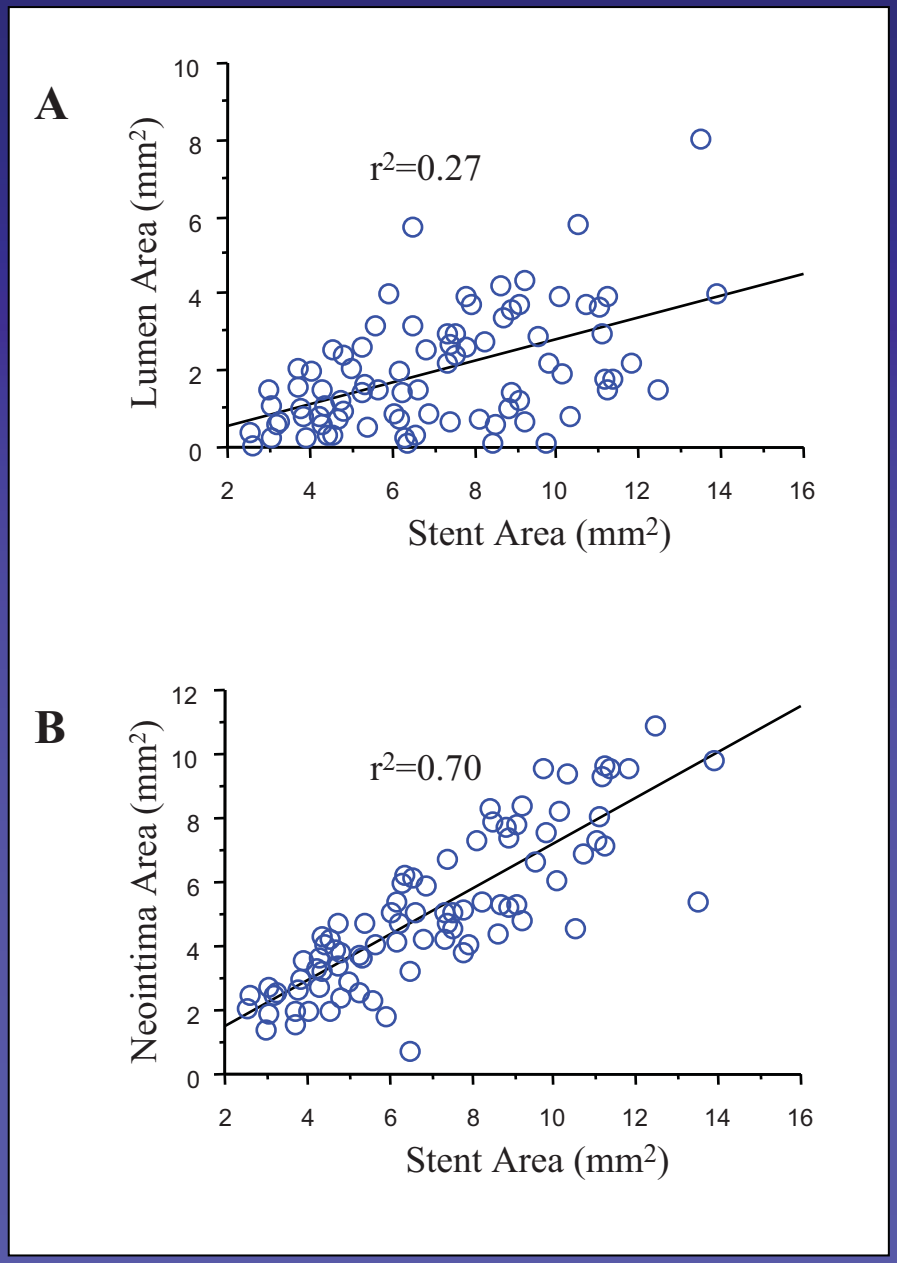
Persistent chronic
Inflammation close to
strut and endothelialization

Smooth muscle
cell rich neointima
with proteoglycan/
Collagen matrix

6 –12 months

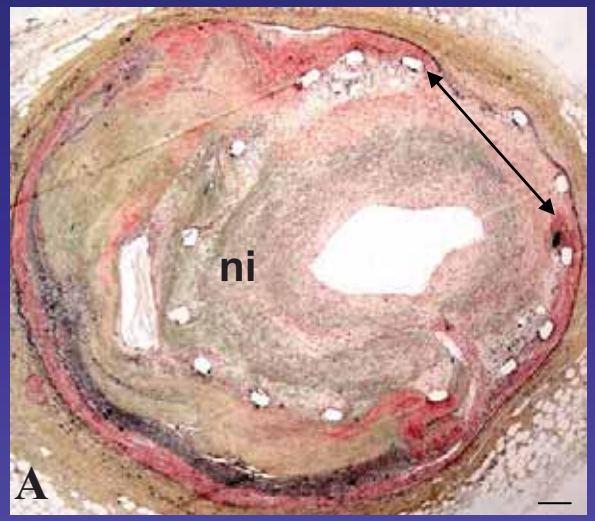
Factors that Influence the Extent of Neoinitmal Formation - *In-stent Restenosis*

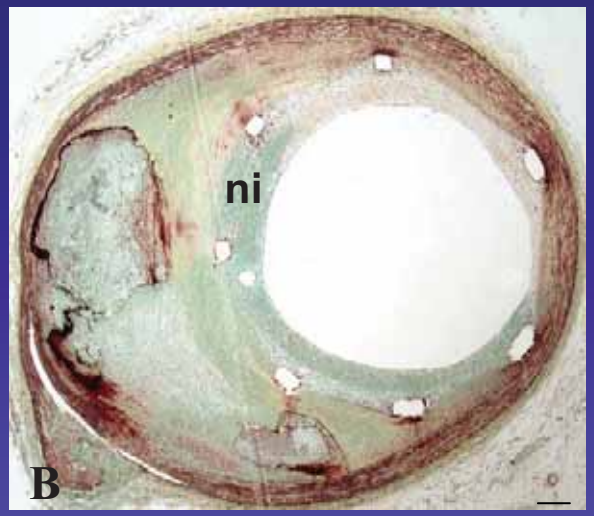
- Medial and arterial wall injury
- Extent of thrombosis
- Inflammation - acute and chronic
- Angiogenesis
- Proteoglycan/collagen matrix deposition
- Atherosclerosis vs. normal vessel
- Vessel size
- Length of diseased vessel

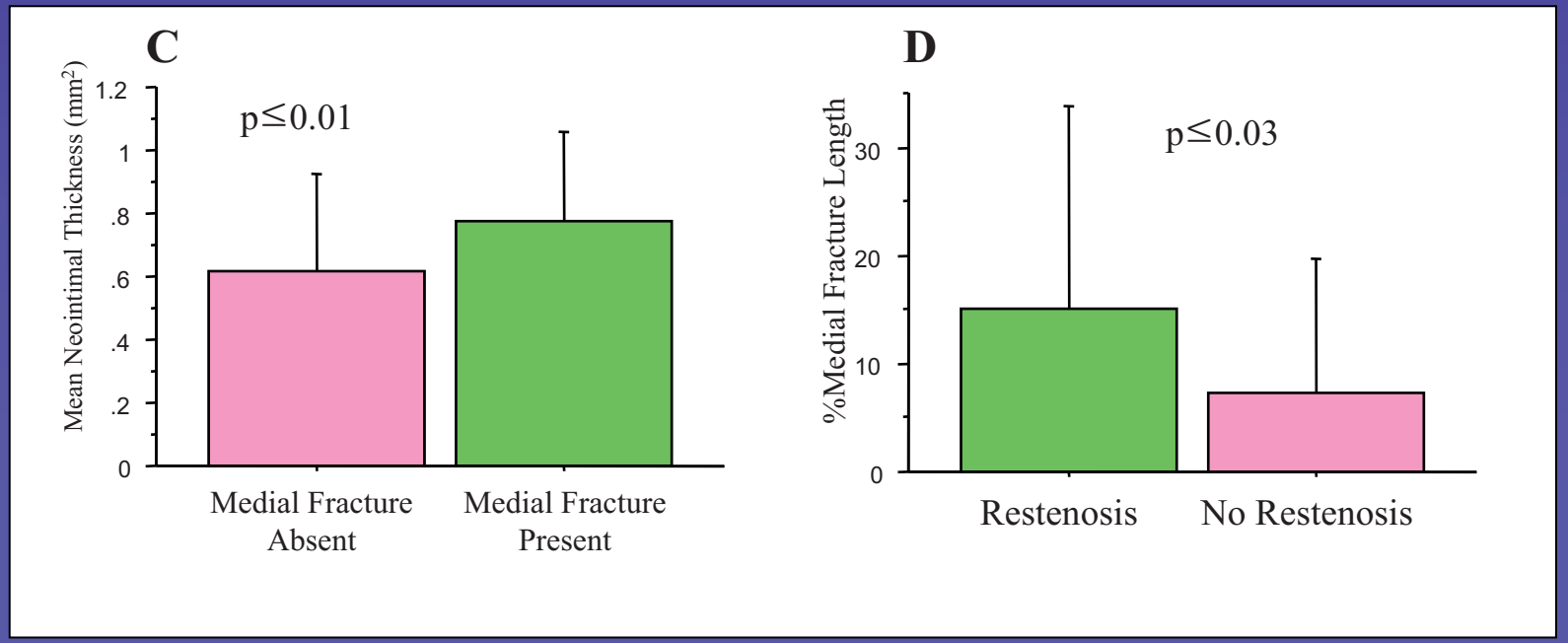


Regression plot of correlation of Stent Area With Lumen Area (A) (r^2=0.27) and Neointimal Area (r^2=0.70)

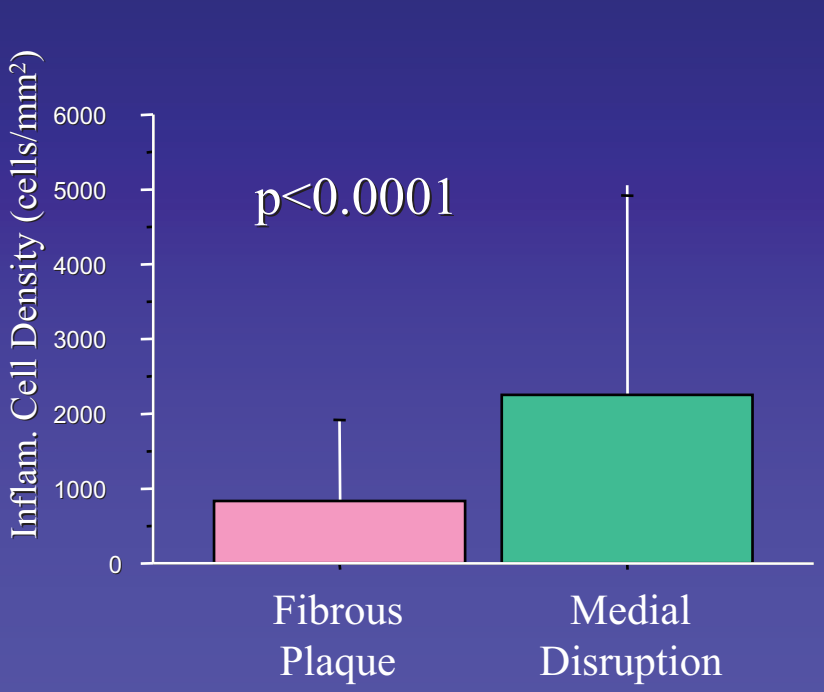
Influence of Medial Fracture on Neointimal Thickness and Restenosis

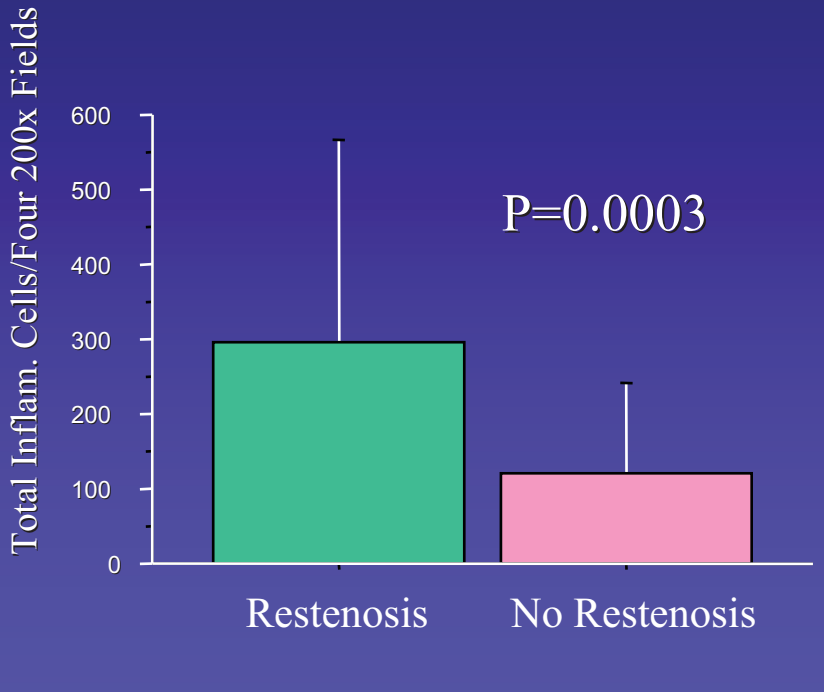


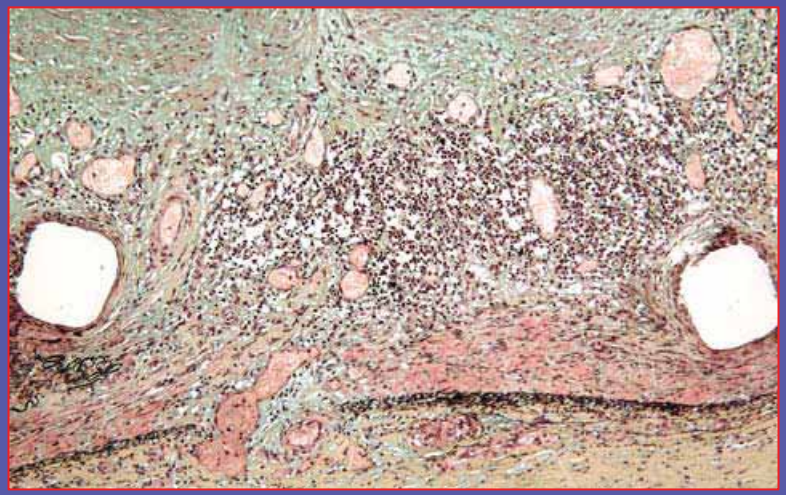


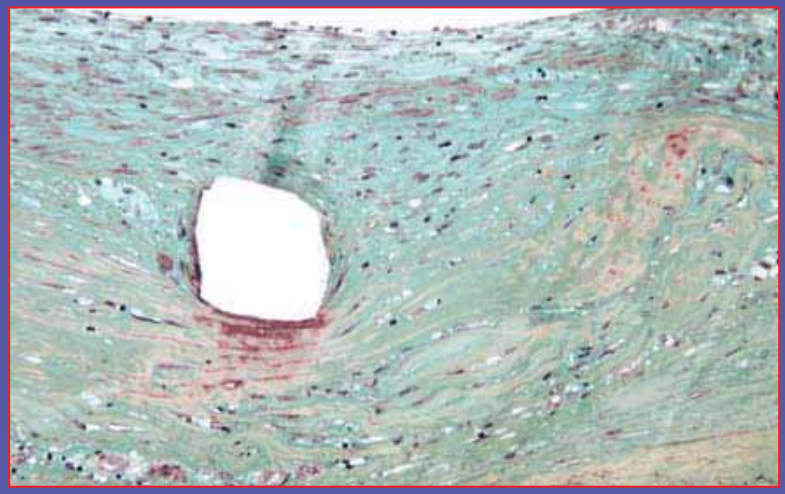


Morphologic Predictors of Restenosis
Arterial Injury & Inflammation

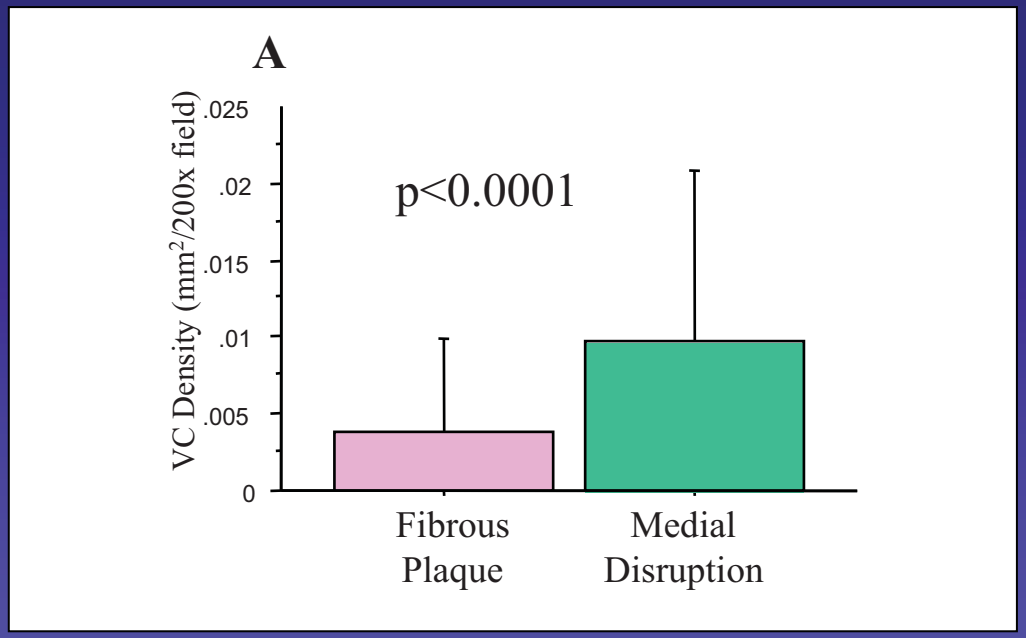


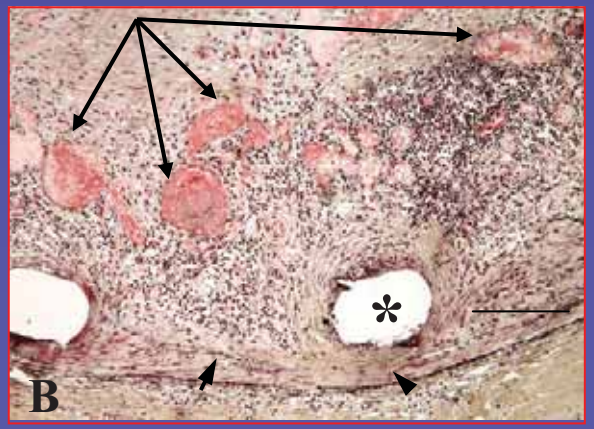


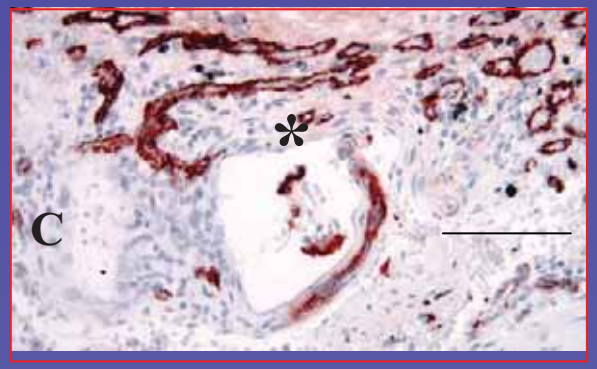




Increased neointimal neoangiogenesis is associated with medial injury







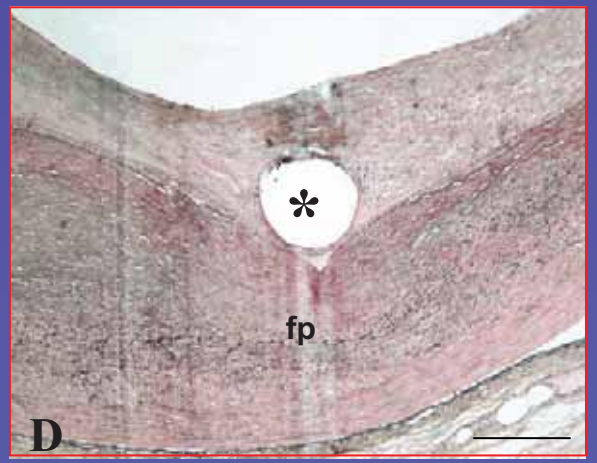
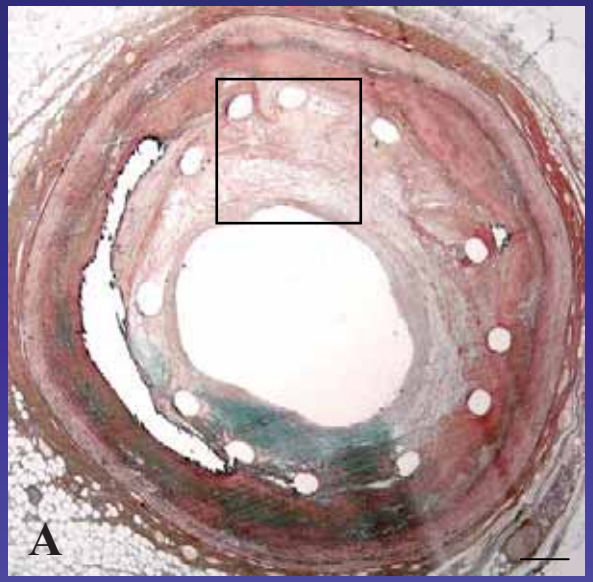
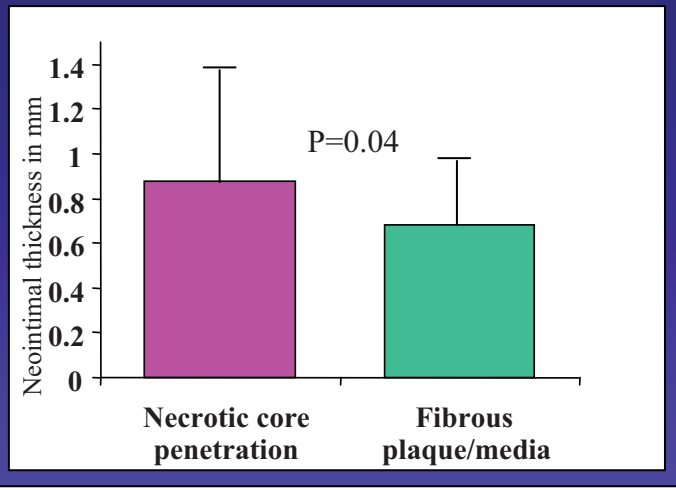
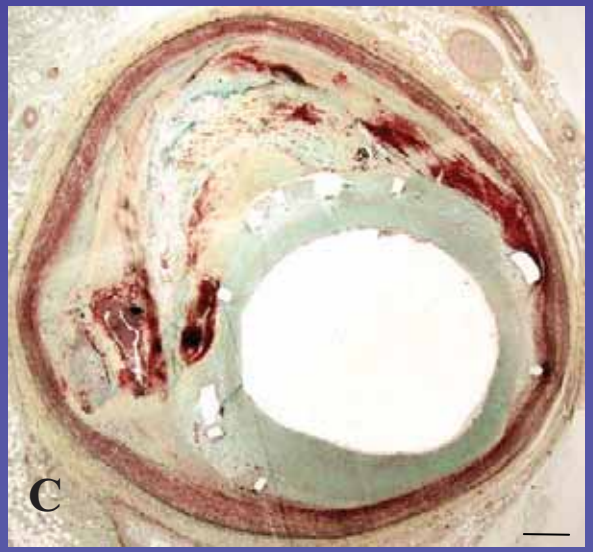


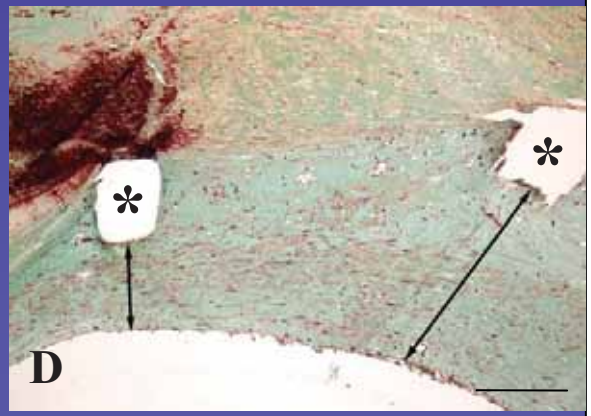
Figure 6

Influence of Necrotic core Penetration by Stent Strut









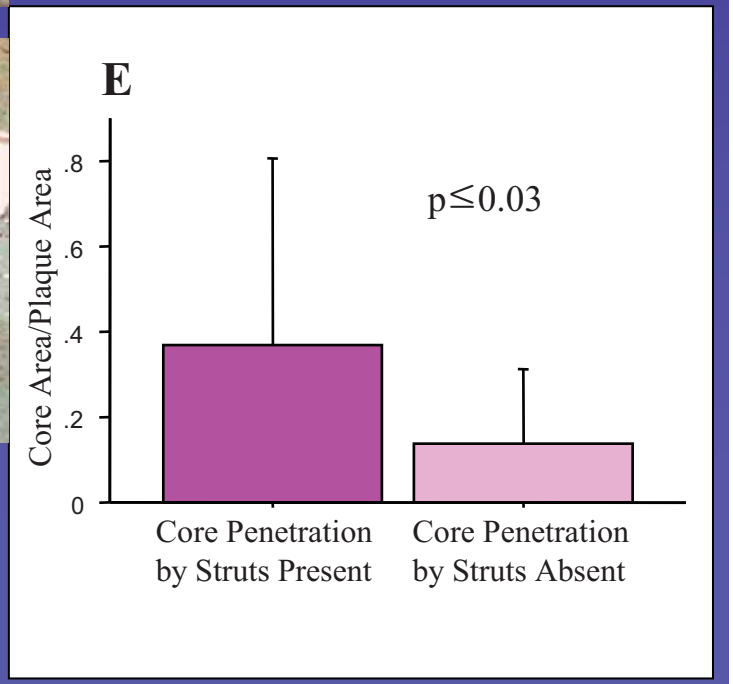
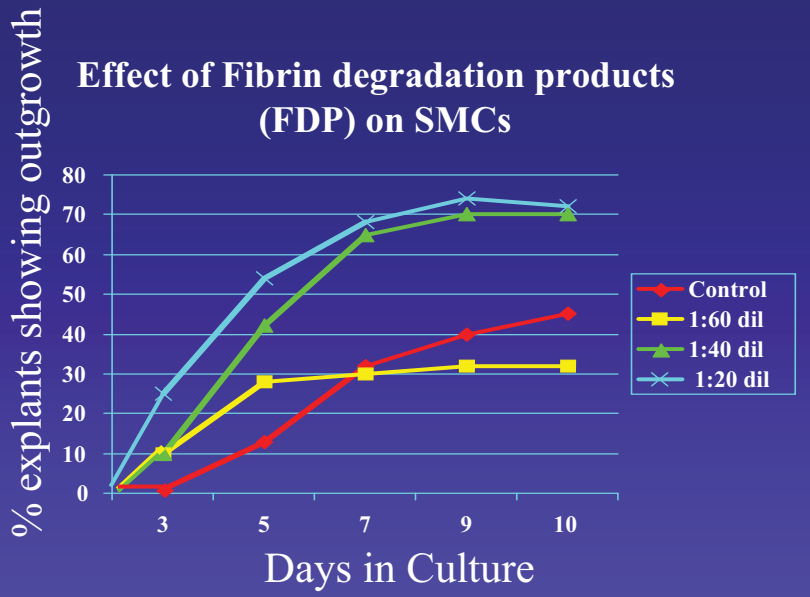
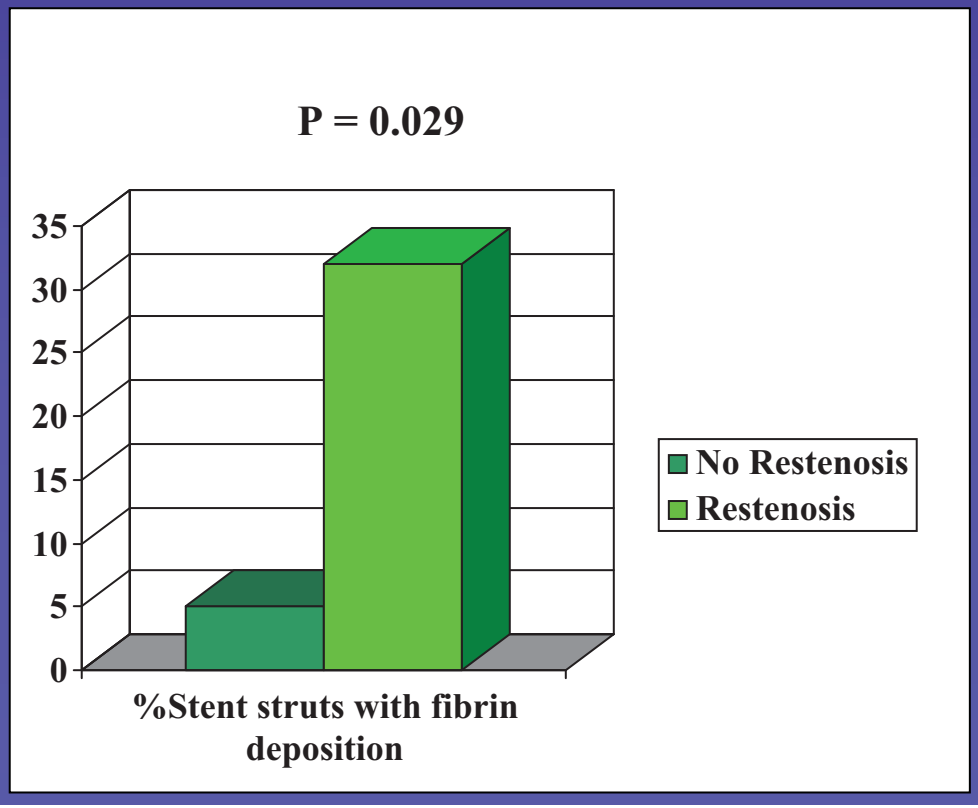


Figure 7

Non-DES Human Coronary Stents >90 Days

Neointimal Fibrin Deposition





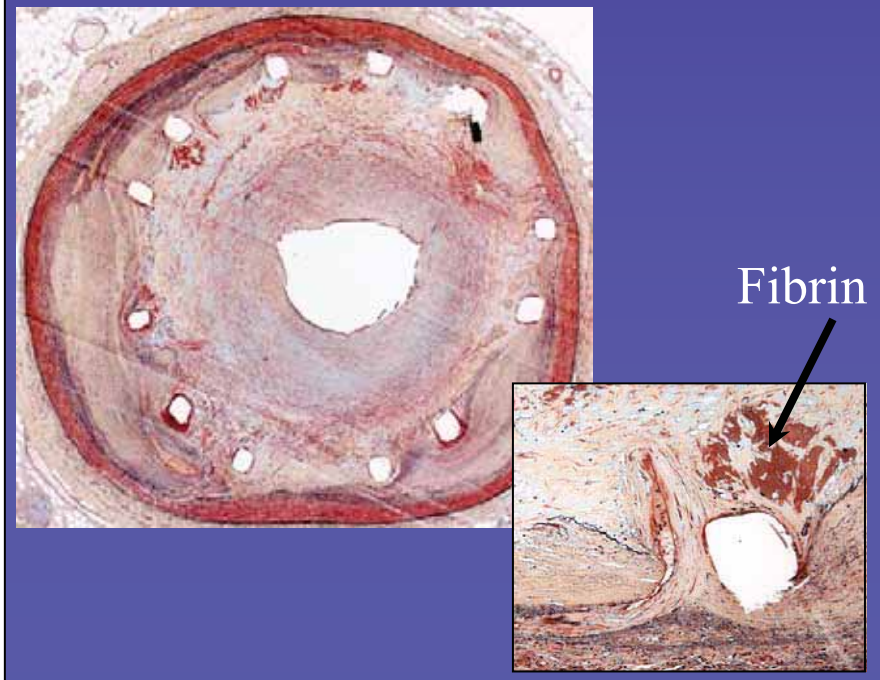
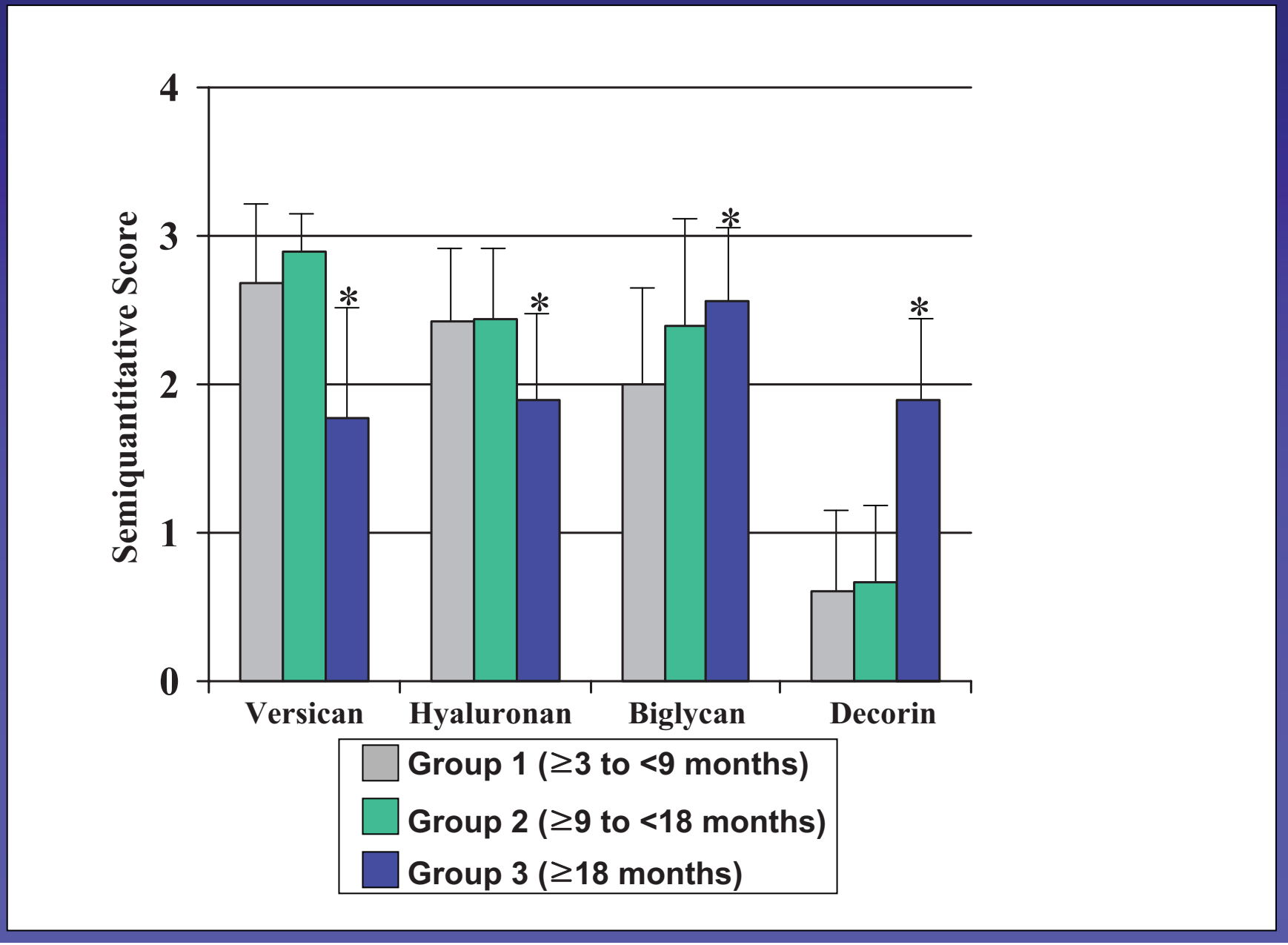
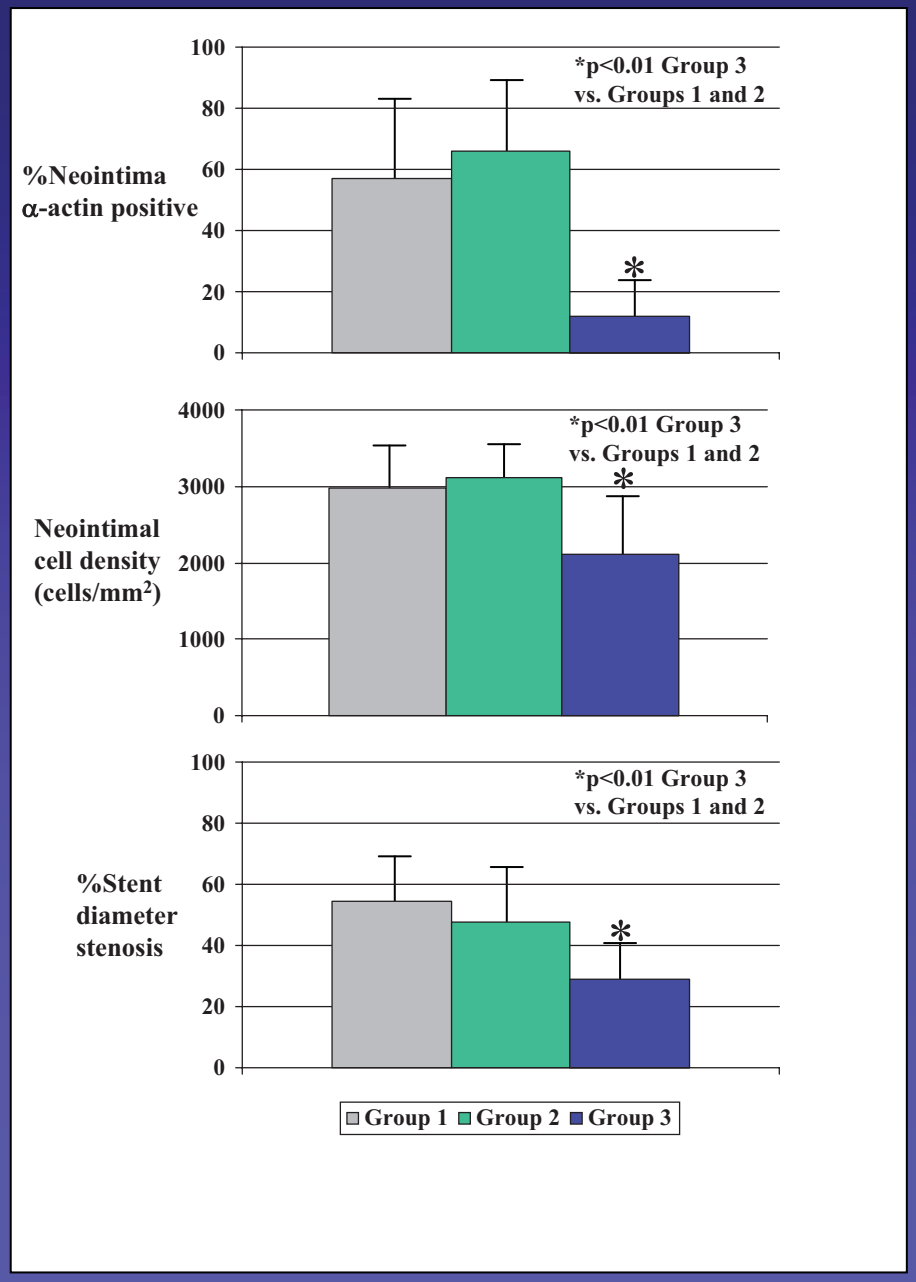


Figure 1

Extracellular Matrix in Stent Human Coronary Arteries





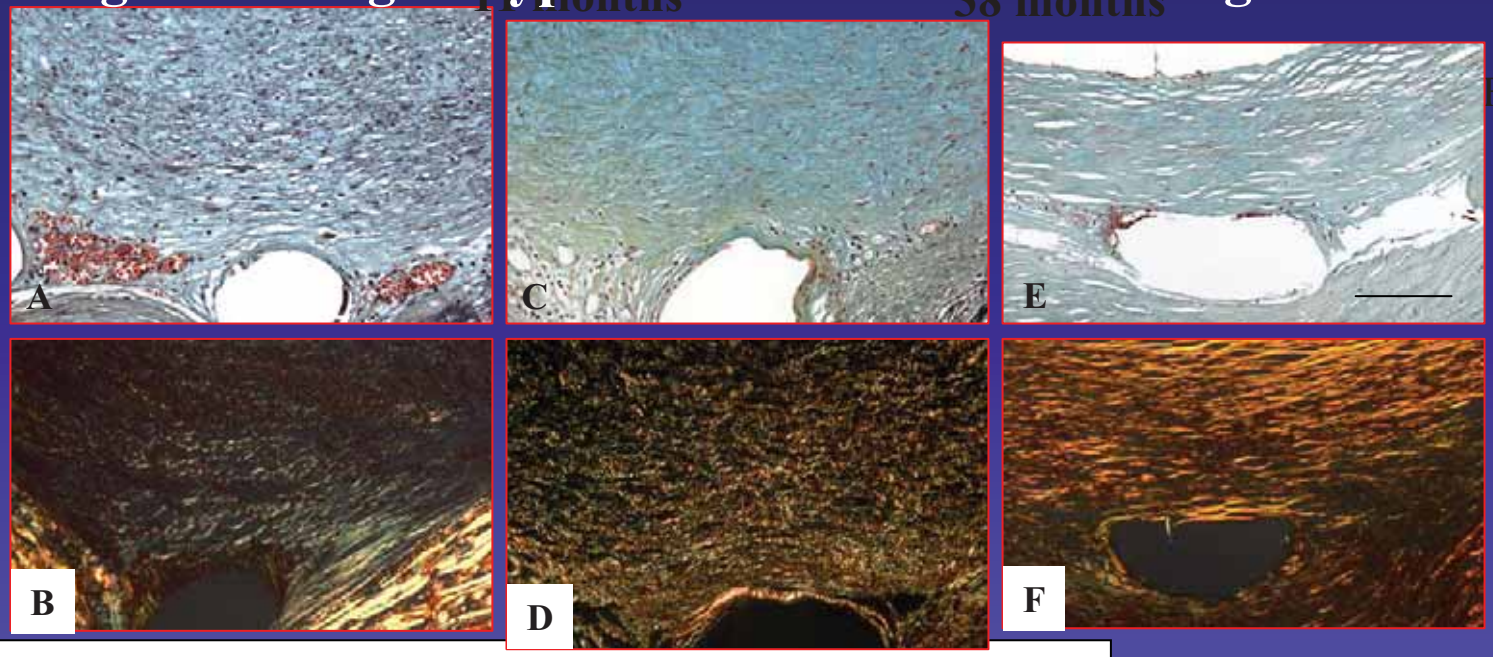
Percent Neointimal α-actin, Neointimal Cellularity, and % Diameter Stentosis at Various time intervals after Stenting

Group 1 (≥3 to <9 months)

Group 2 (≥9 to <18 months)

Group 3 (≥18 months)

Change in Collagen Type with Duration of Stenting in Man



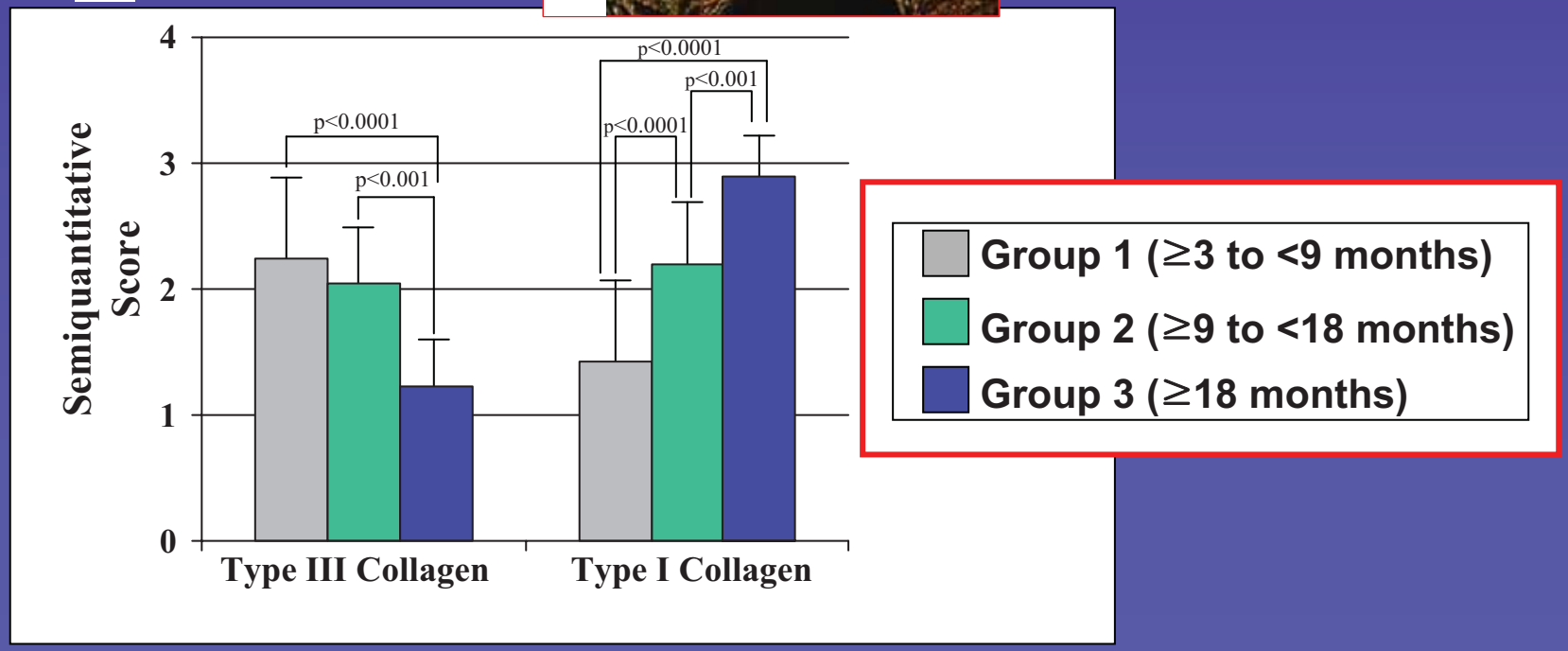


Figure 5

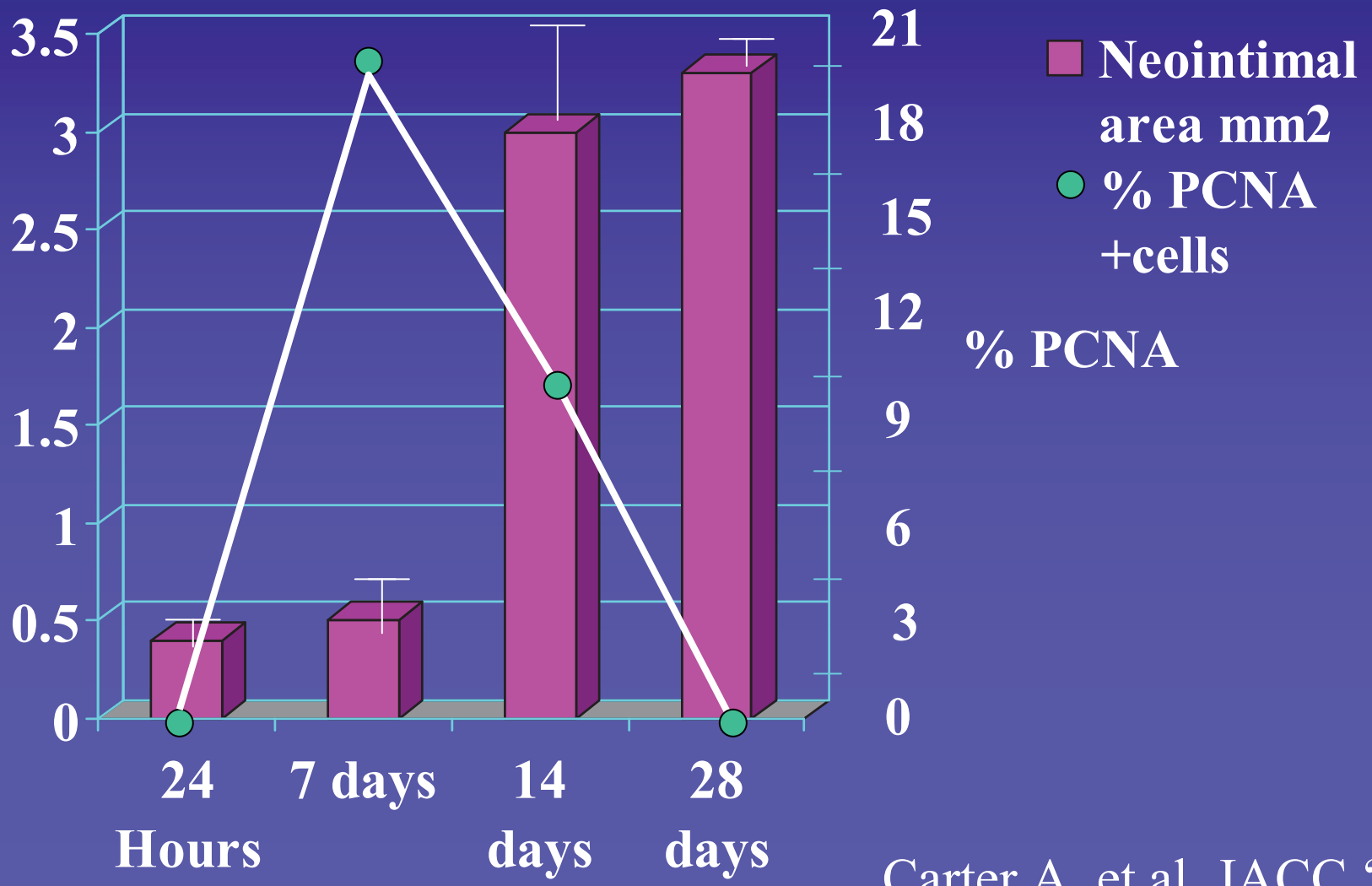
Animal Models used to study In-stent Restenosis

Pig (Dog)

Rabbit

Rat

Time Course of Intimal Thickness and Cell Proliferation After Balloon Expandable Stent Placement in Normal Porcine Coronary Arteries

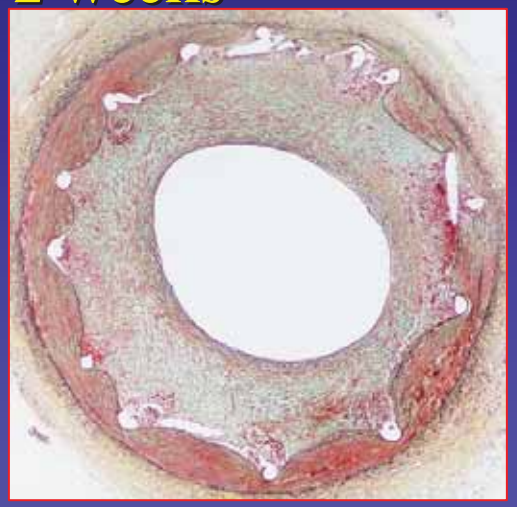


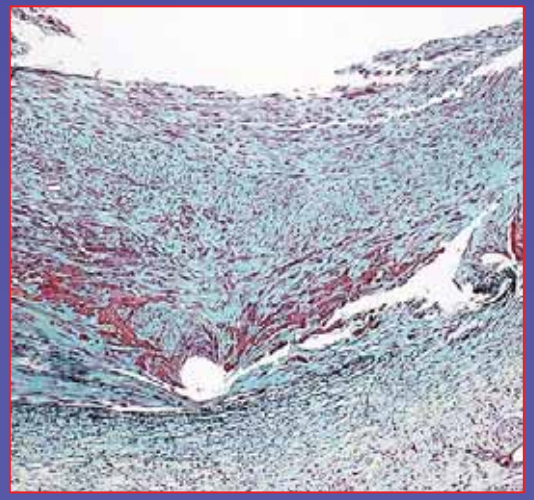
Carter A, et al. JACC '97

Balloon Expanded Stents in Pig Coronary Arteries at 2 and 4 Weeks

2 weeks

2 weeks

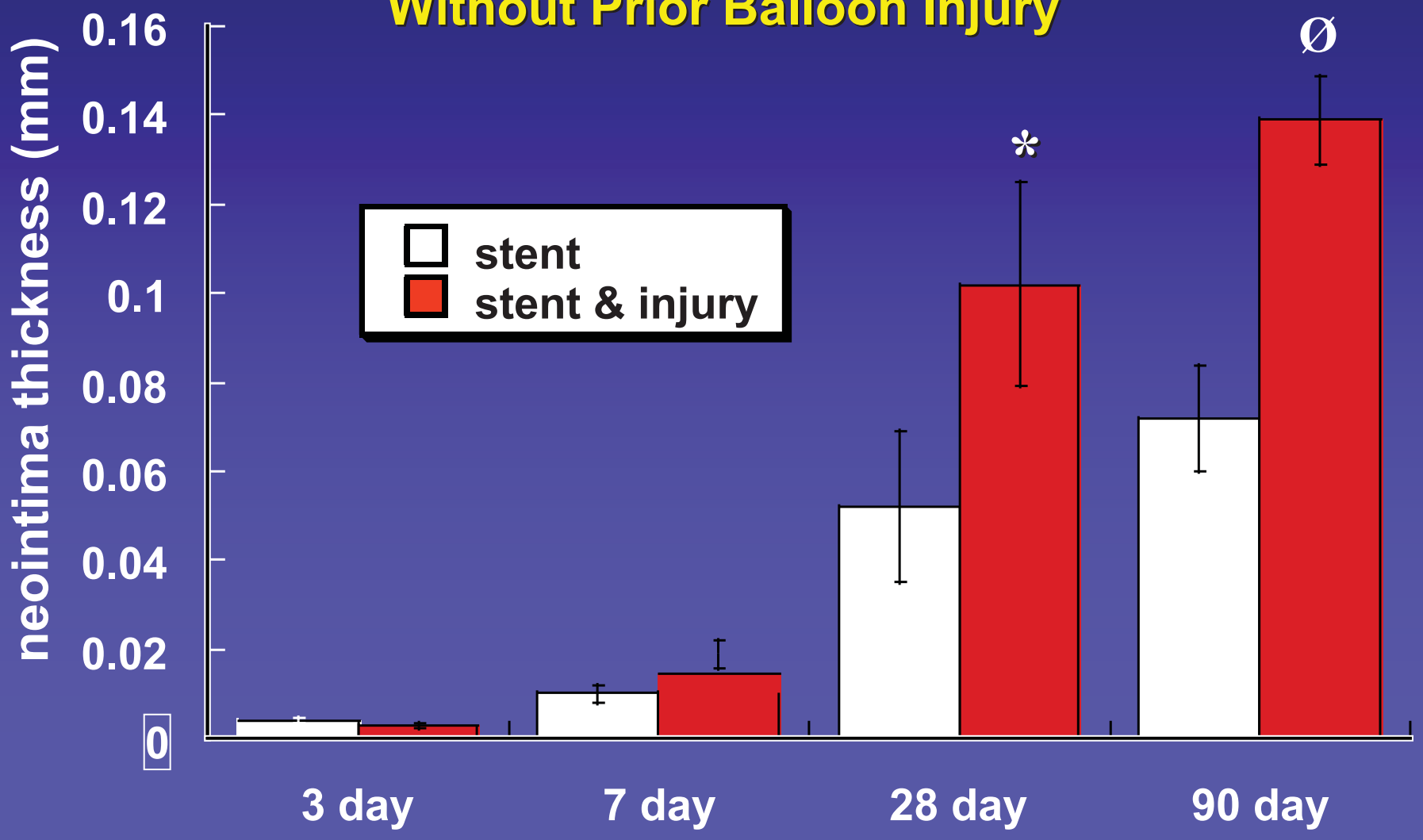




2 weeks

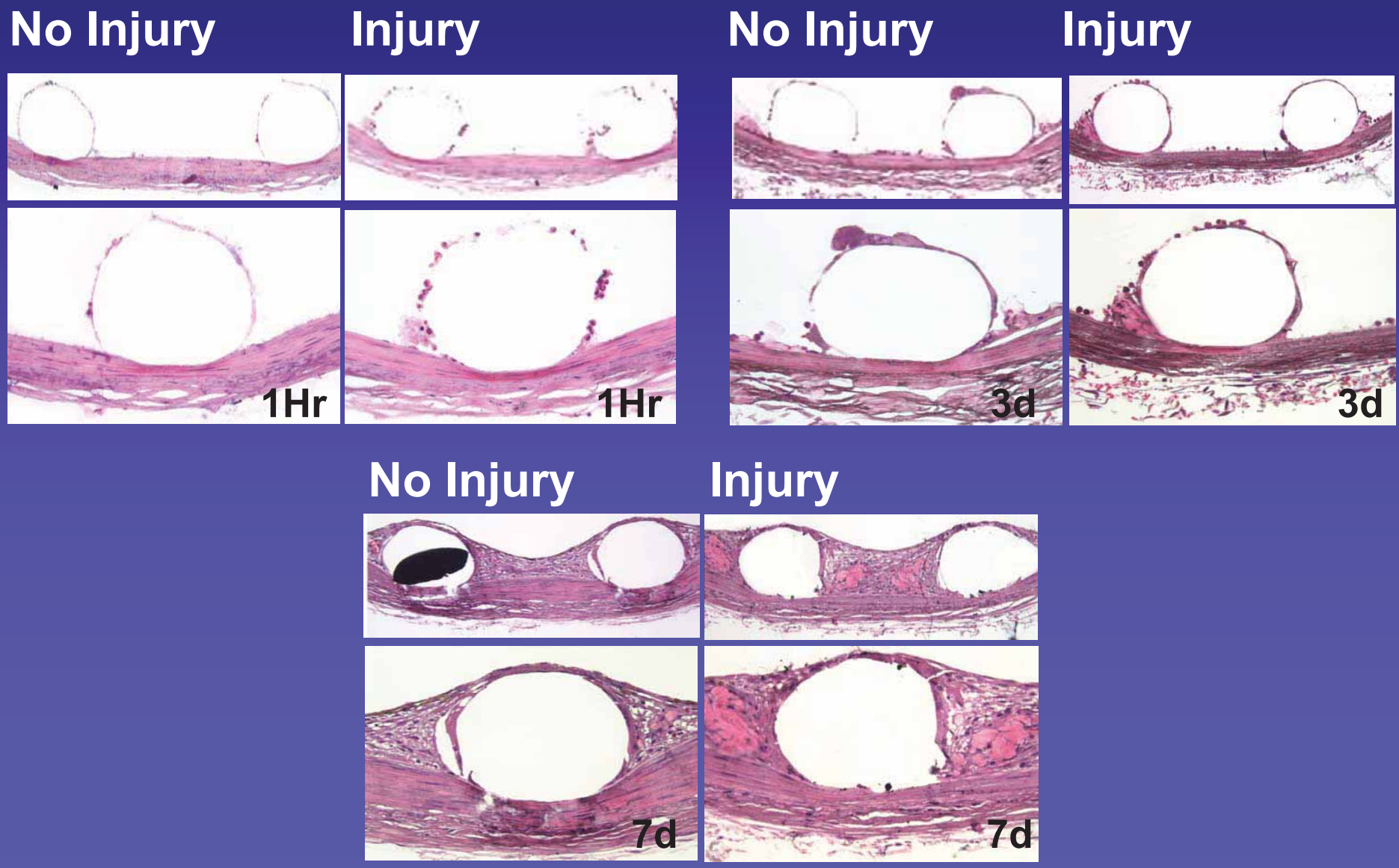
4 weeks

Morphometric Analysis of Neointimal Thickness at Various Time Points after Stenting in the Rabbit Iliac Model With and Without Prior Balloon Injury

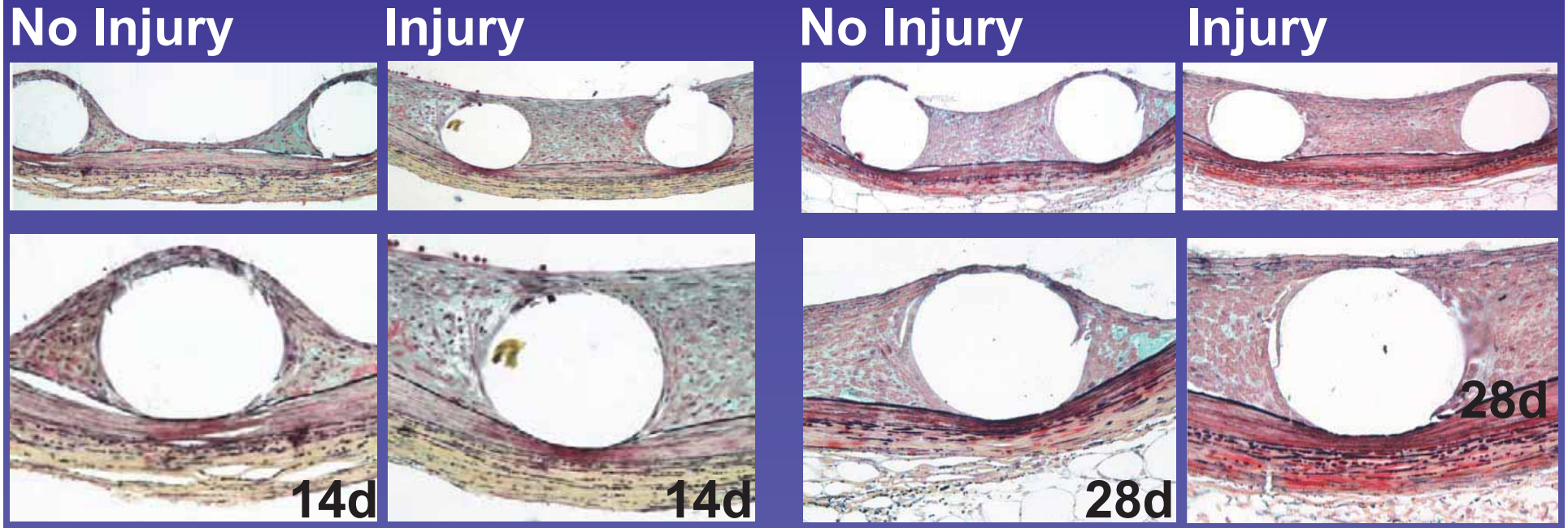


* P=0.05, Ø p<0.006 vs. stent alone

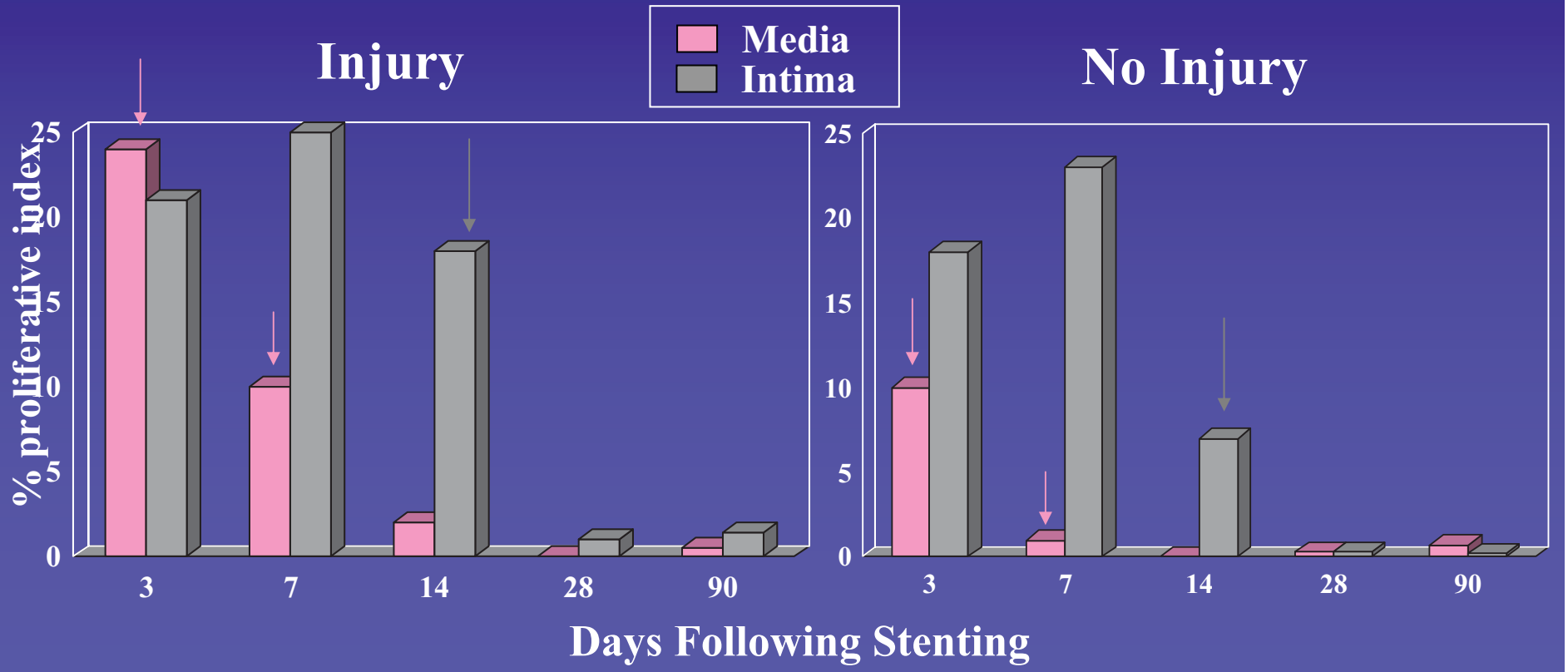
Rabbit Iliac Artery Stent Implants at Various Time Intervals With or Without Previous Balloon Injury



Rabbit Iliac Artery Stent Implants at Various Time Intervals With or Without Previous Balloon Injury



Proliferative Index (BrdU) following Stent Placement in the Presence and Absence of Balloon injury



Medial proliferation is significantly (p=0.05) greater at 3 and 7 days and intimal proliferation is greater at 14 days in injured arteries compared to vessel without injury.

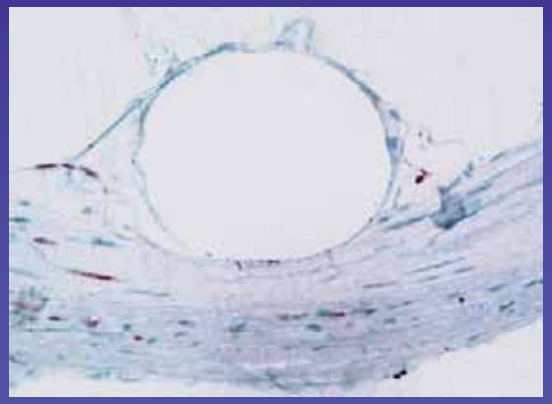
Cell Proliferation(BrdU) in Rabbit Iliac Artery Stent Implants With or Without Previous Balloon Injury

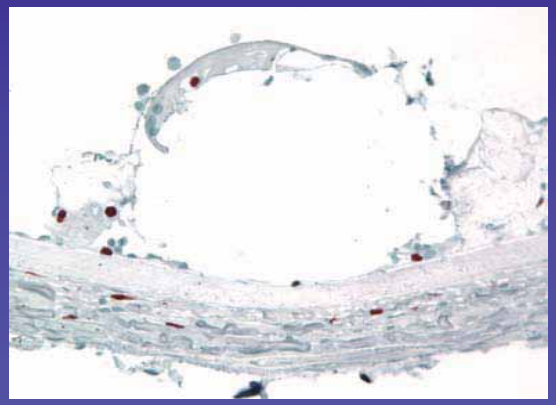
3 days —

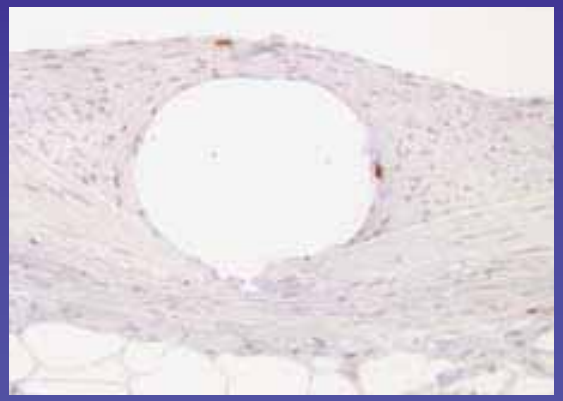
7 days —

14 days —

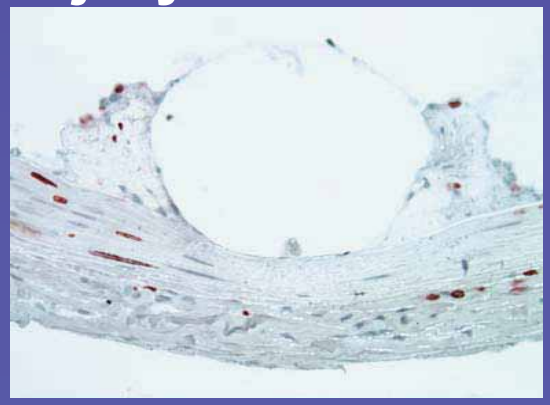
No Injury

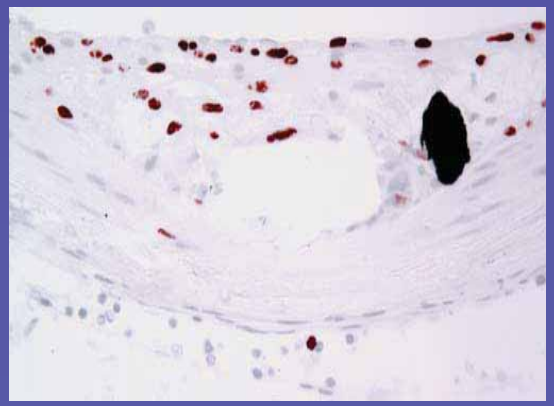


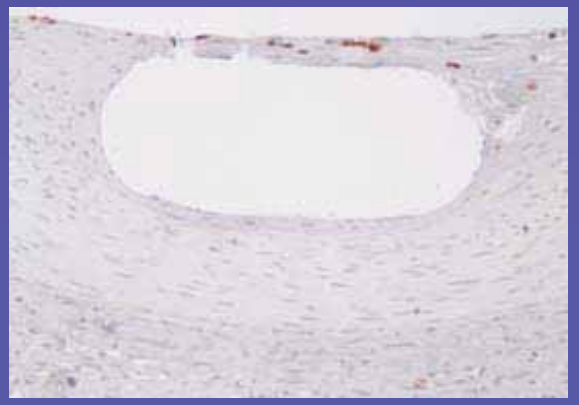




Injury

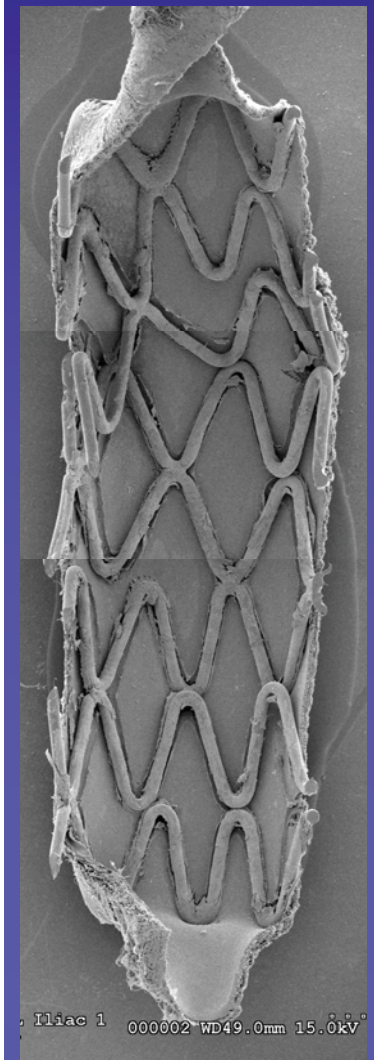


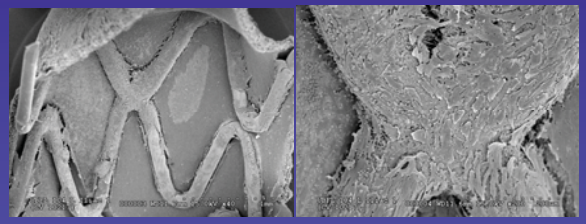


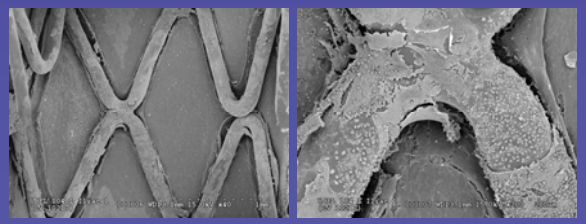


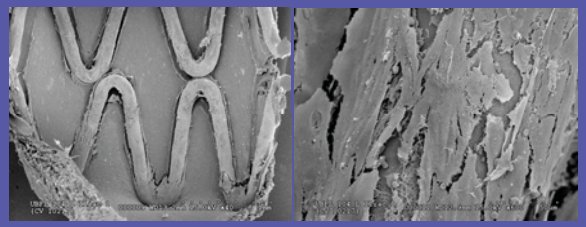
En Face Analysis of Surface Endothelialization in 3 Day Rabbit Iliac Artery Stent Implants With or Without Previous Balloon Injury

No Injury

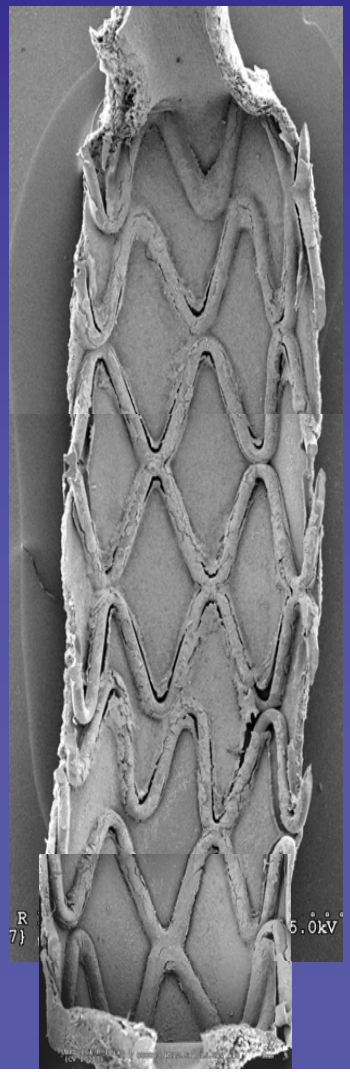


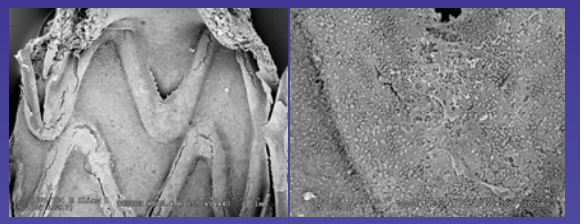


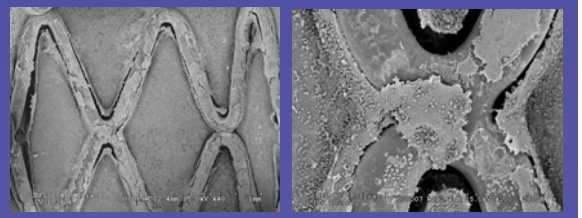


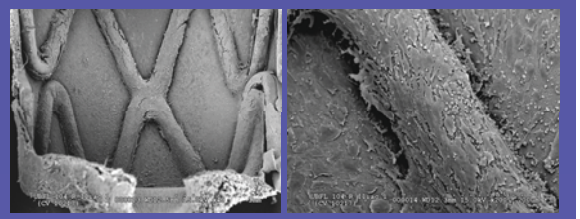


Injury



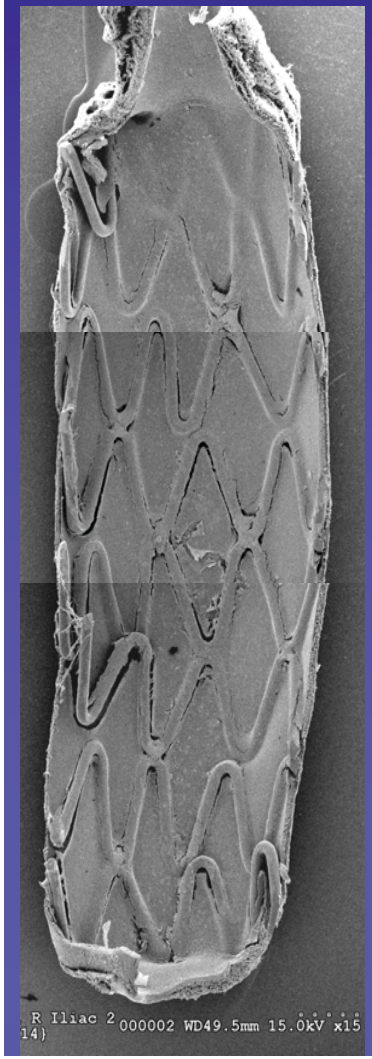


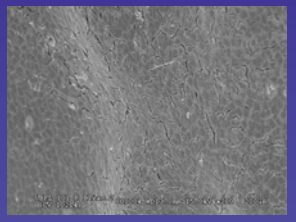


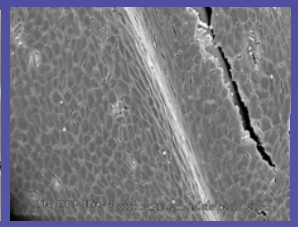


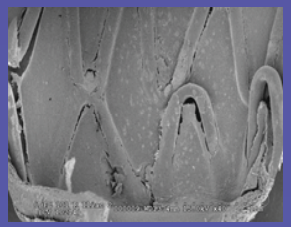
En Face Analysis of Surface Endothelialization in 7 Day Rabbit Iliac Artery Stent Implants With or Without Previous Balloon Injury

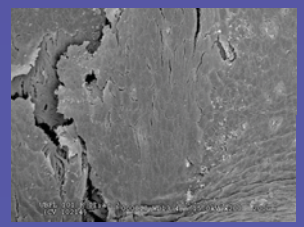
No Injury



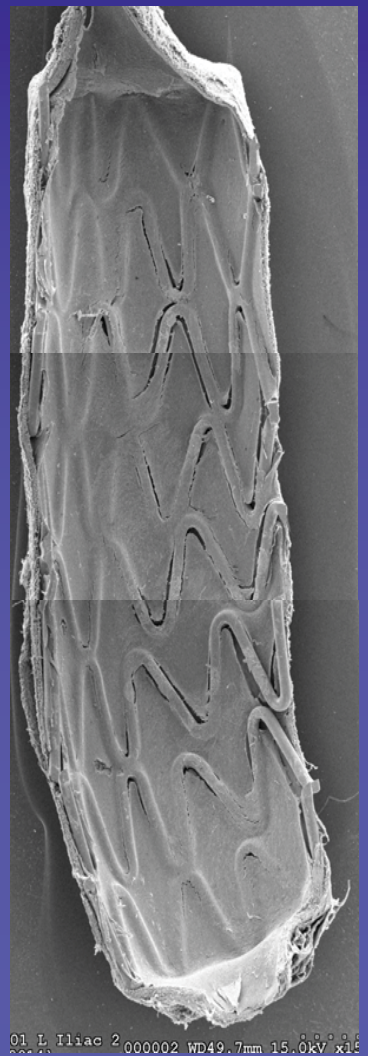


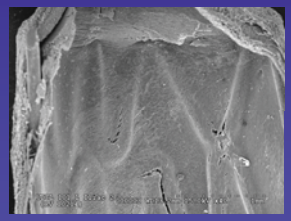


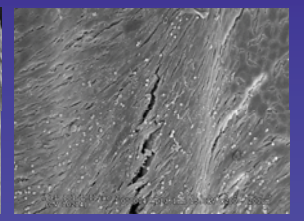


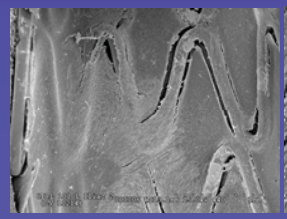


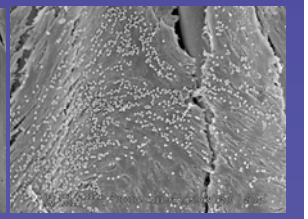
Injury

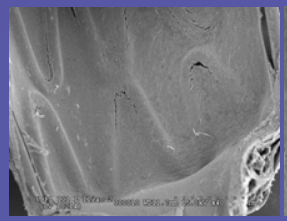


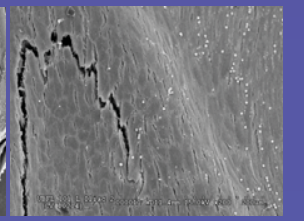








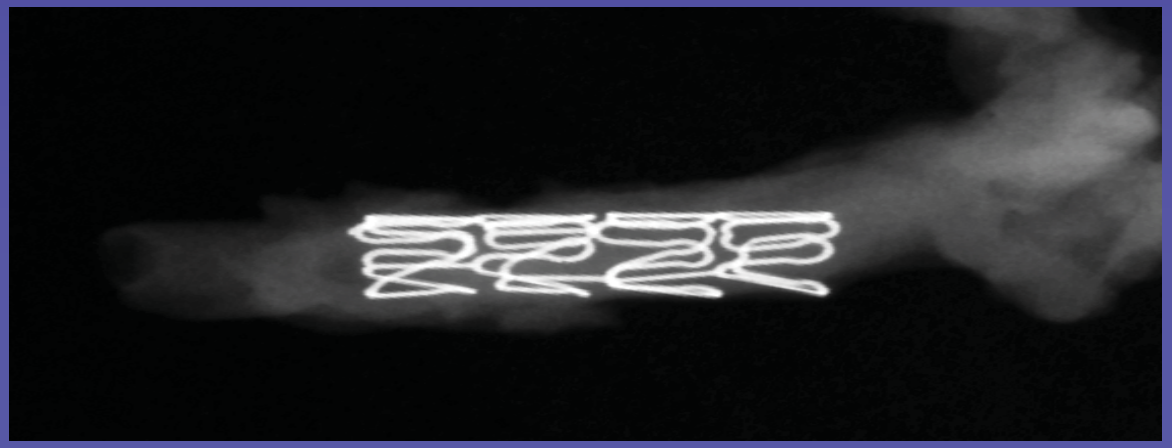




Gross Appearance and X-Ray of the Stented Rat Carotid Artery

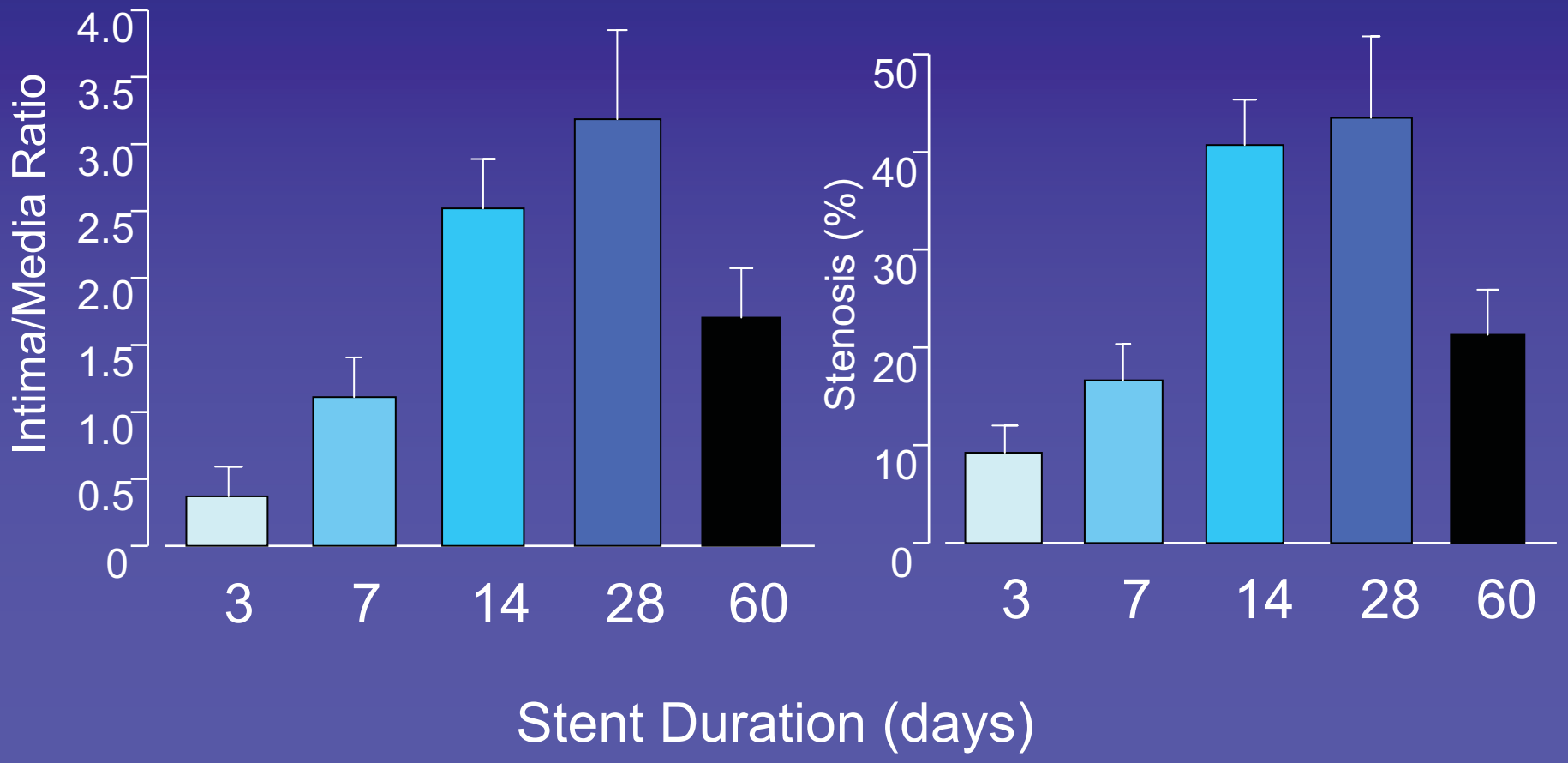
A

B

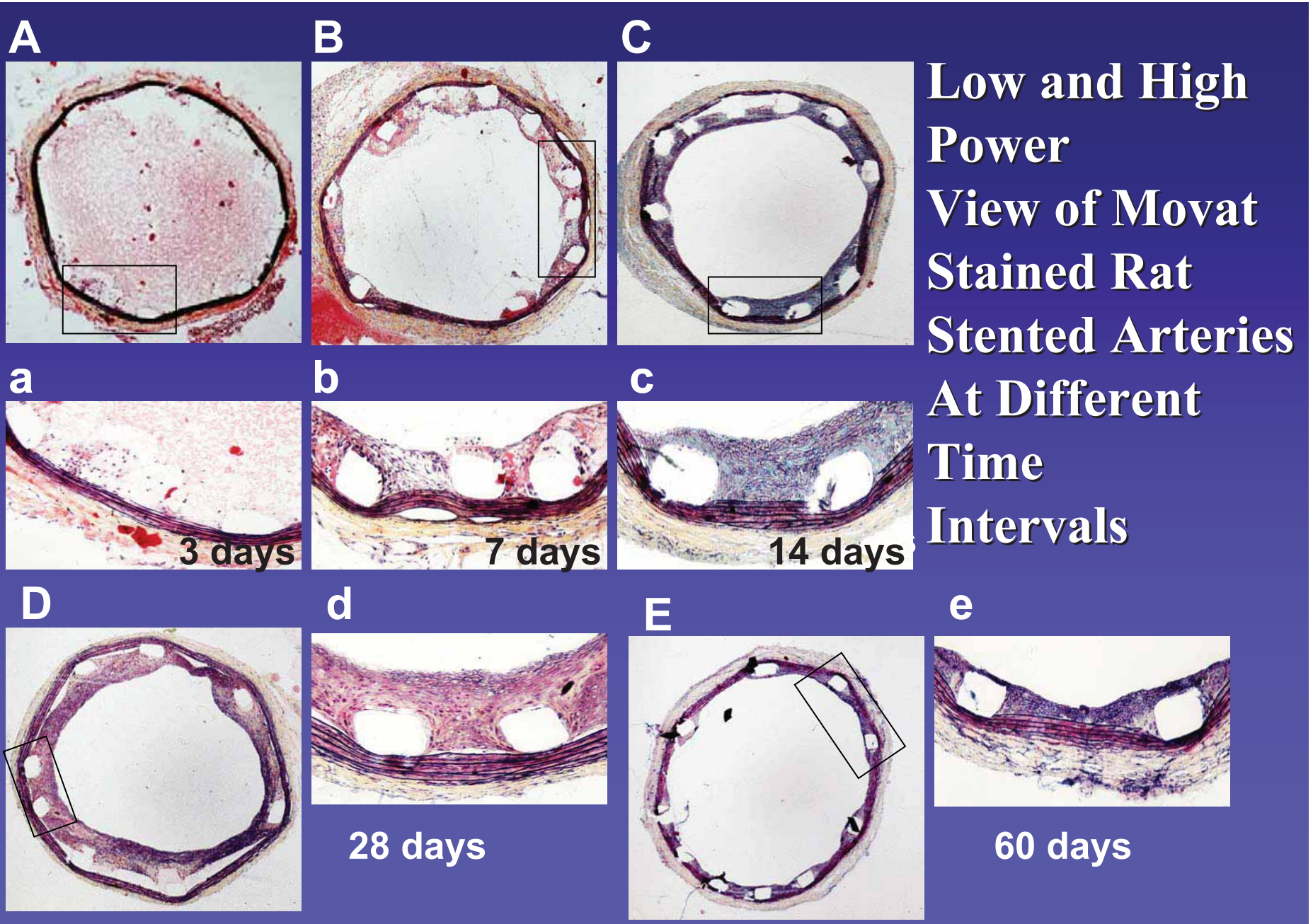


Finn AV, et al. J Vasc Res 2002

Intimal/Medial Ratio and % Stenosis in Rat Cartoid Stented Arteries Harvested At Different Time Intervals

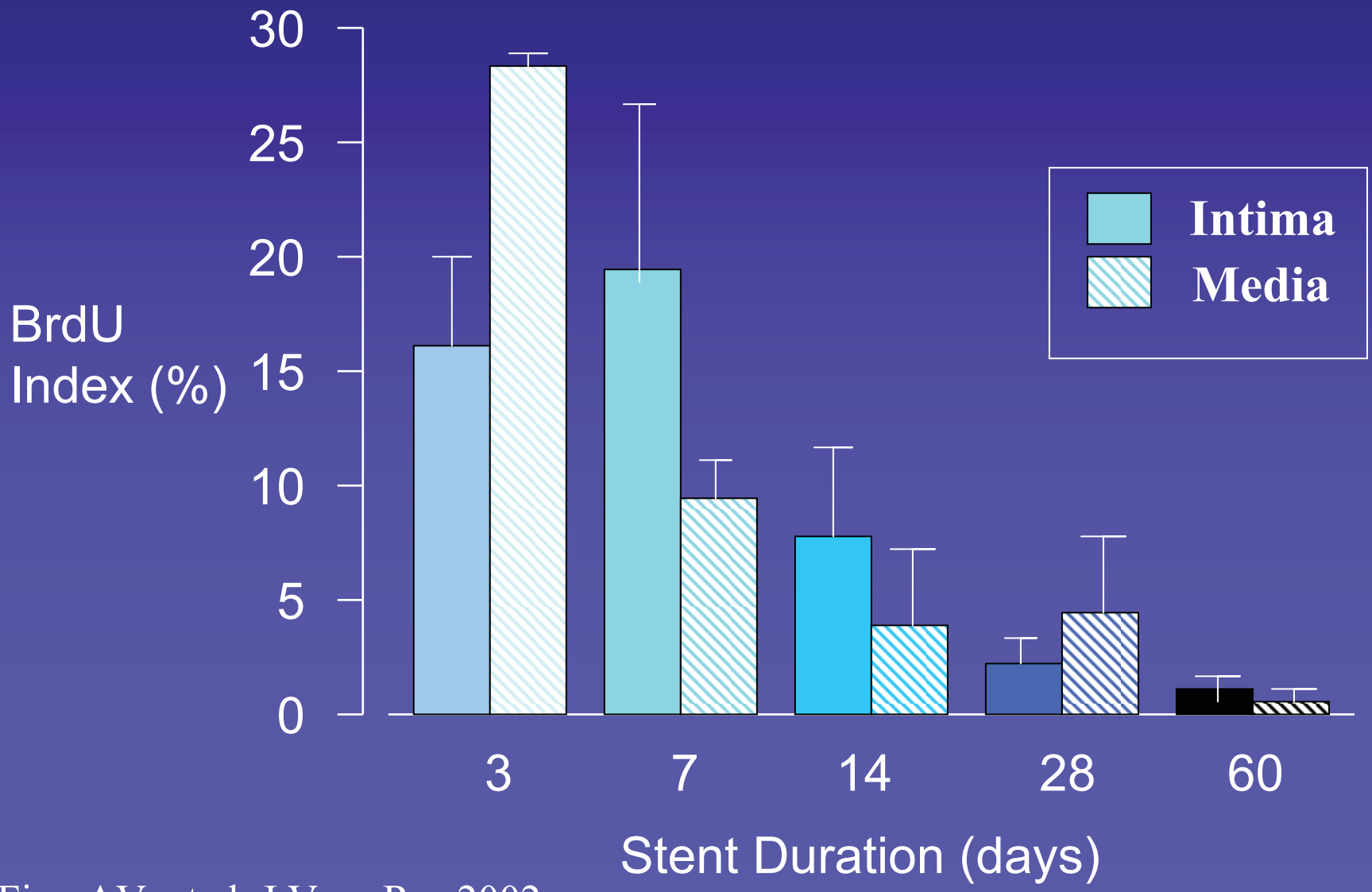


Finn AV, et al. J Vasc Res 2002



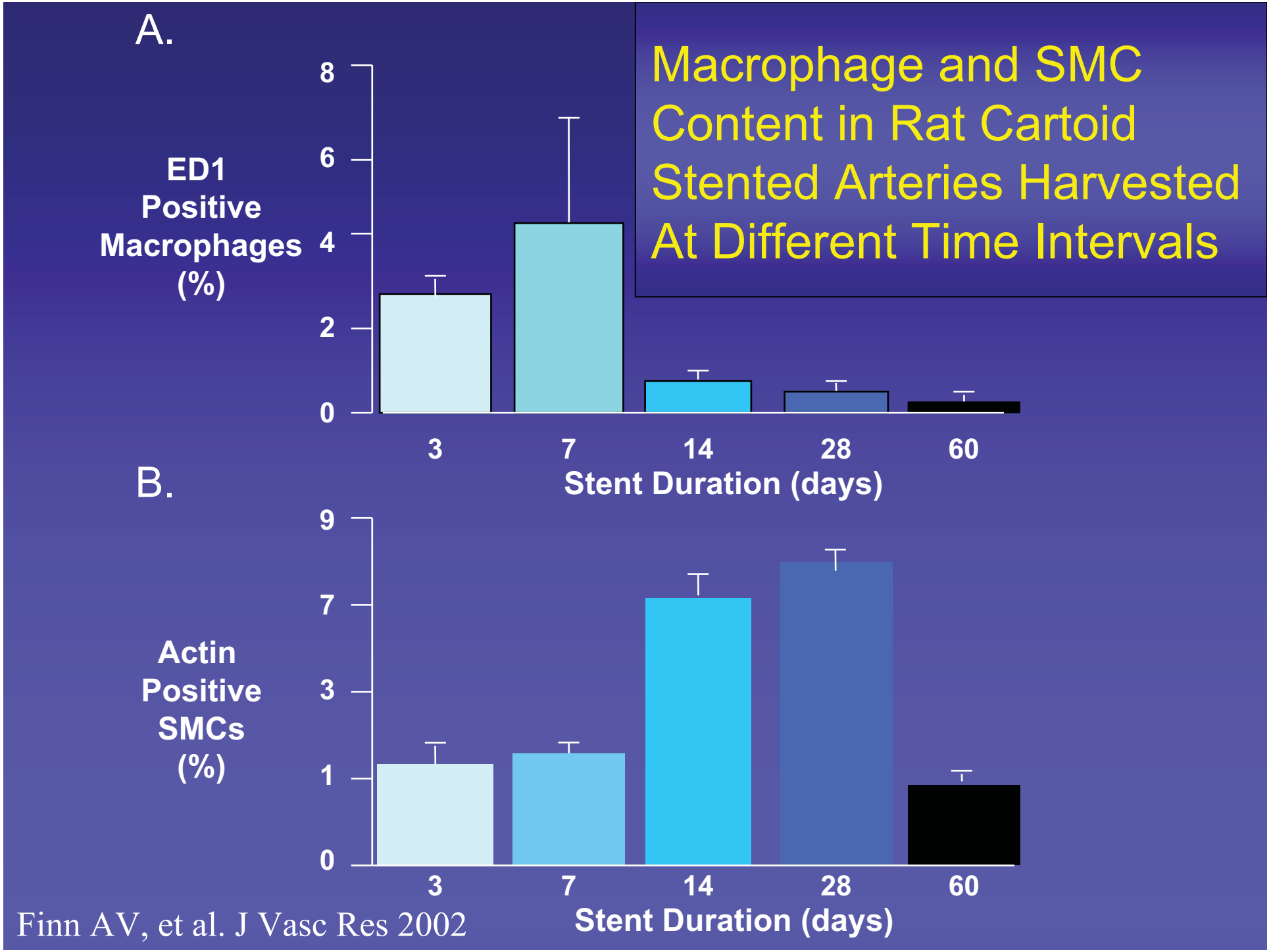
Finn AV, et al. J Vasc Res 2002

Cell Proliferation Index in Rat Cartoid Stented Arteries Harvested at Different Time Intervals



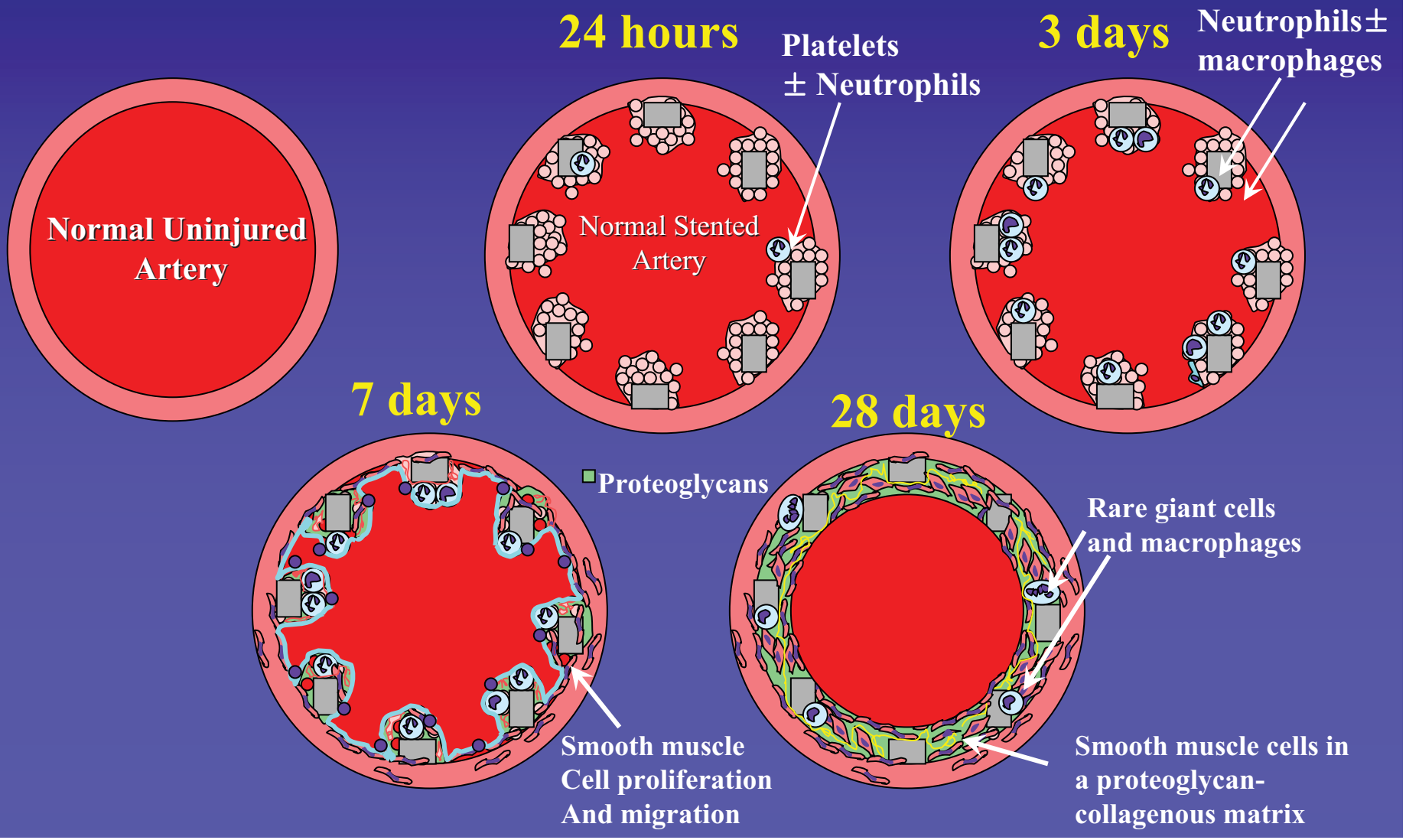
Finn AV, et al. J Vasc Res 2002

Macrophage and SMC Content in Rat Cartoid Stented Arteries Harvested At Different Time Intervals



Finn AV, et al. J Vasc Res 2002

Diagram Illustrating Vascular Response to Intravascular Stent Placement

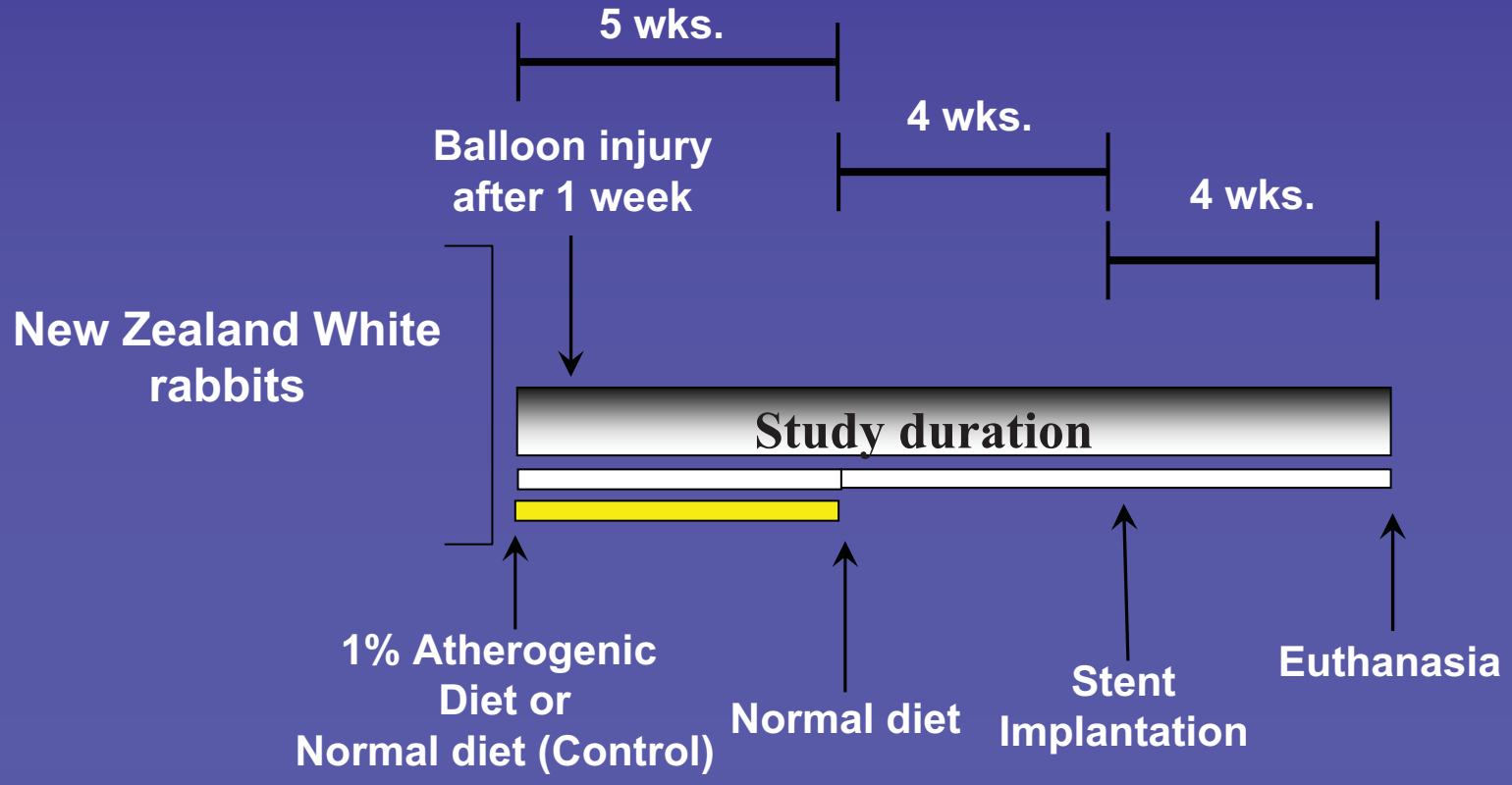


Influence of Underlying Atherosclerosis in Animal Model

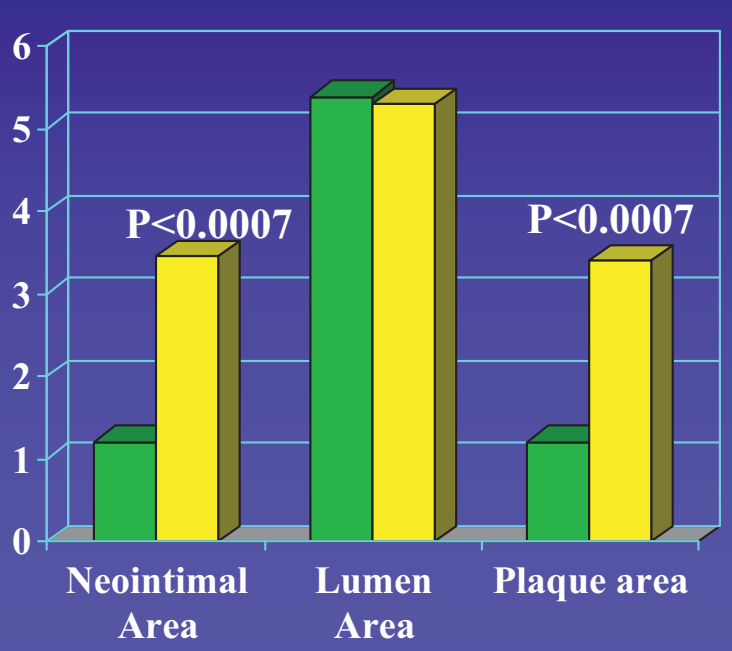
- Rabbit
- Pig
- Primate

Rabbit Iliac Artery Model of Stenting in the Presence and Absence of Underlying Atherosclerosis

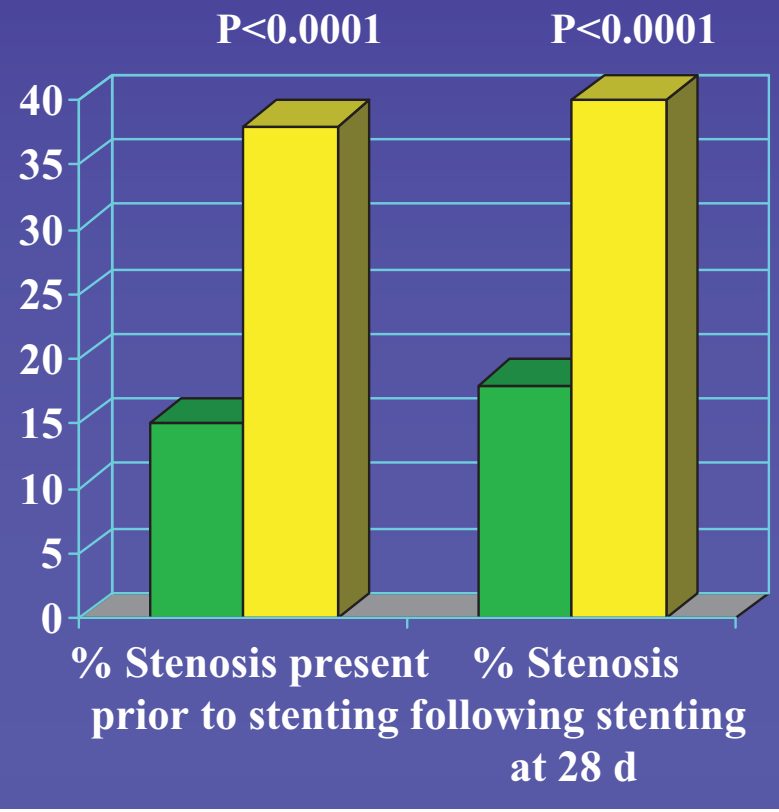
Does atherosclerotic base make a difference in neointimal formation and proliferation?



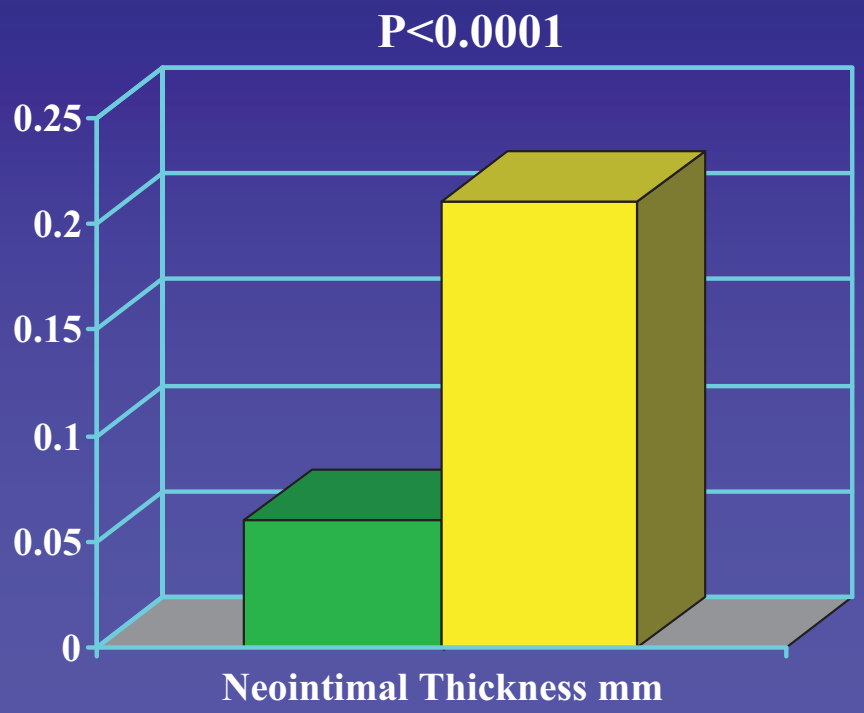
Comparison of Stenting in the Presence or Absence of Atherosclerosis (balloon injury with or without Cholesterol) in Rabbit Iliac Model at 28 days



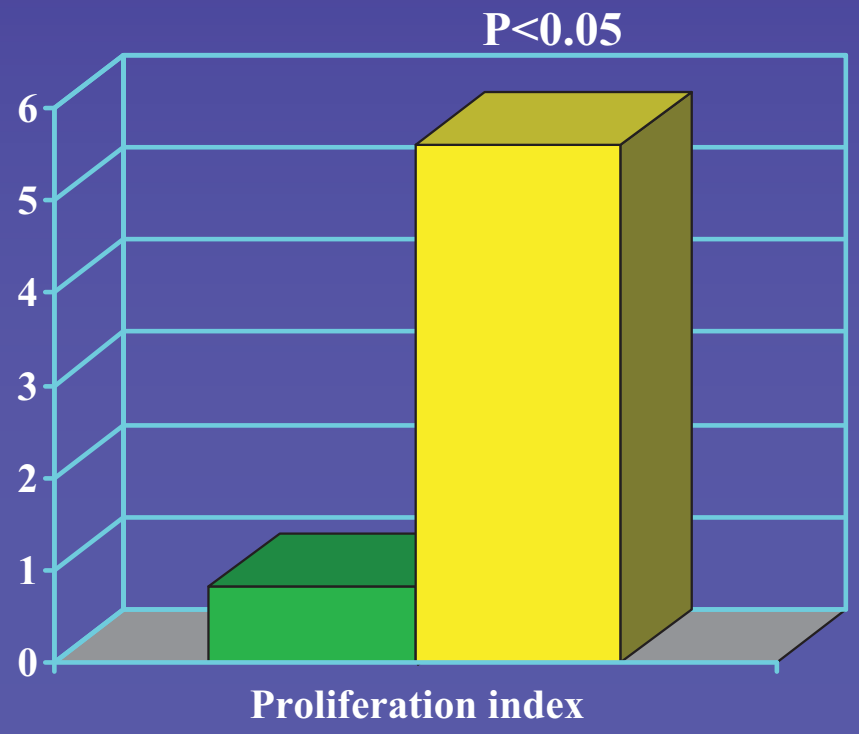
Balloon injury - cholesterol
Balloon injury + cholesterol



Comparison of Neointimal Thickness and Proliferation Index Following Stenting in the Presence or Absence of Underlying Atherosclerosis in Rabbit Iliac Model

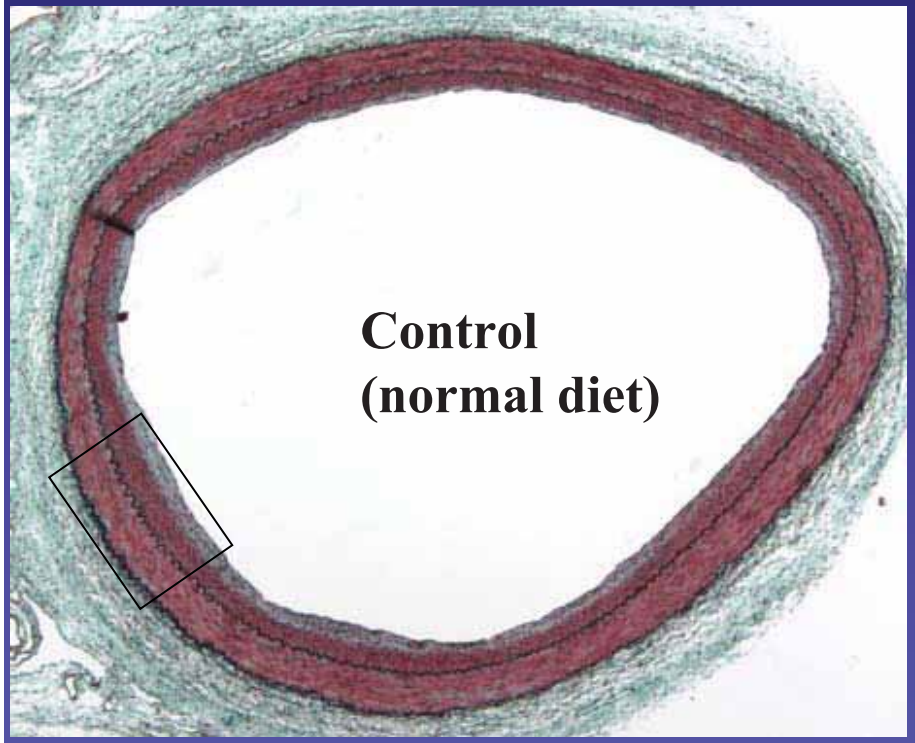


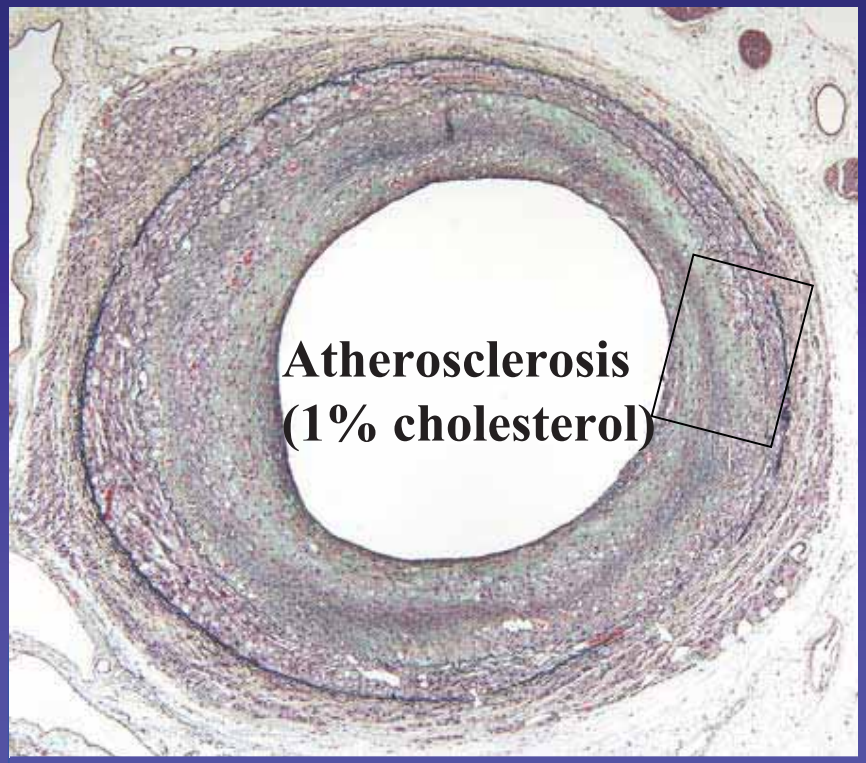
Balloon injury - cholesterol
Balloon injury + cholesterol

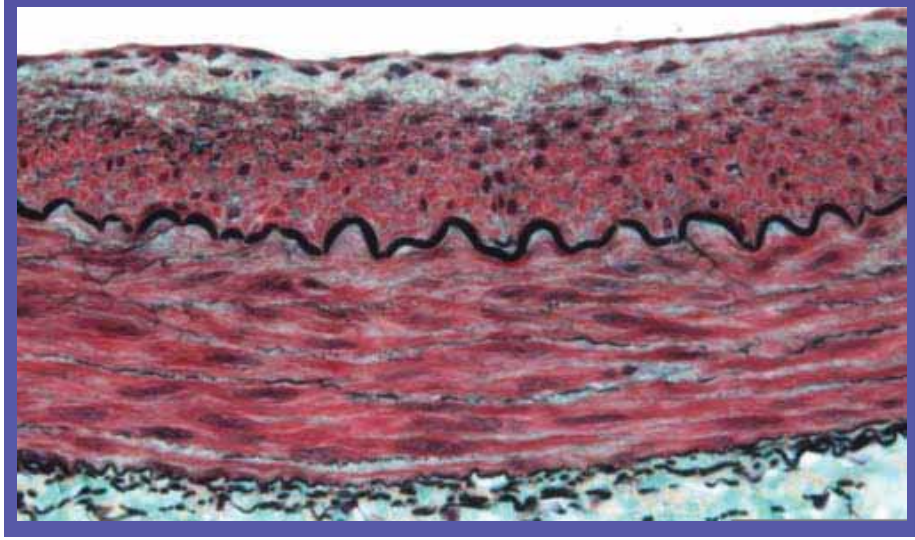


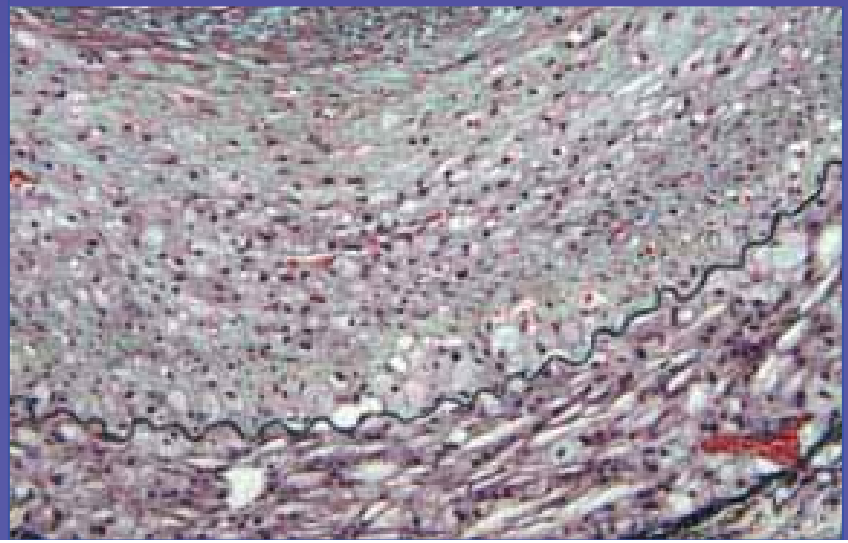
Stent area similar in the Atherosclerotic and Control

Atherosclerotic Iliac Arteries Prior to Stenting

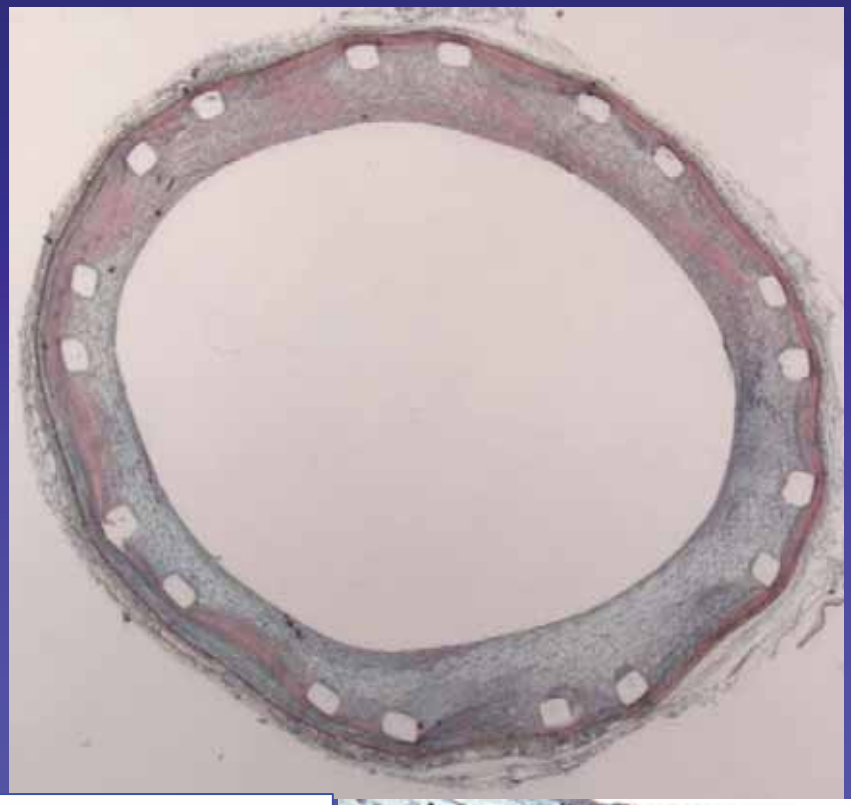


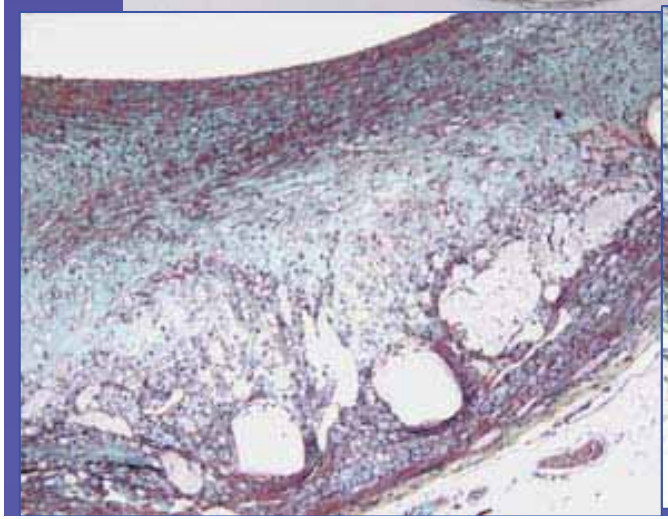


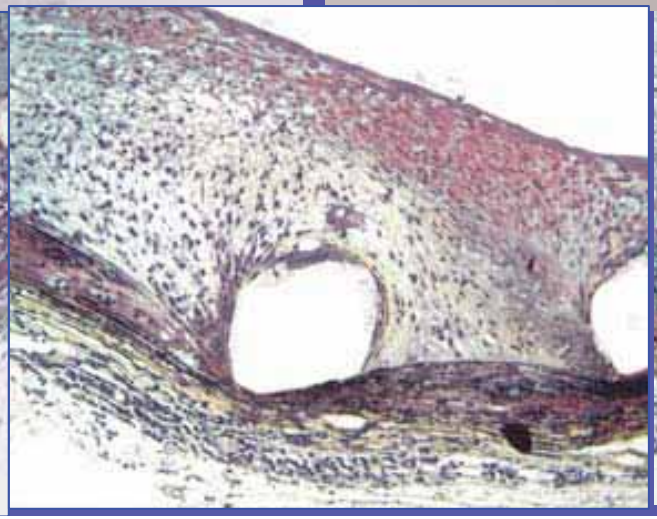


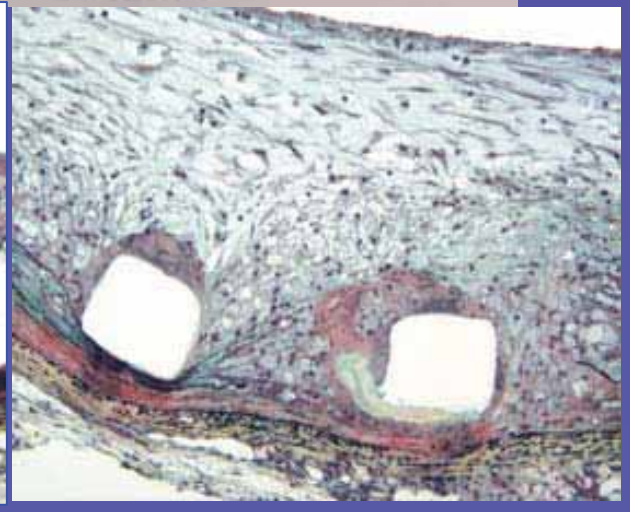


Stented Rabbit Atherosclerotic Iliac Arteries

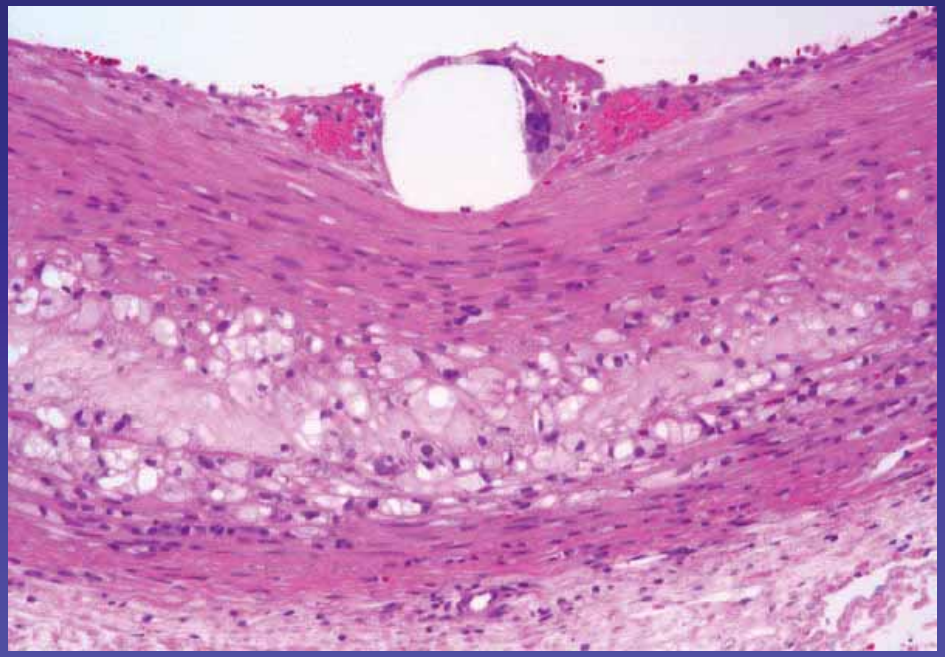


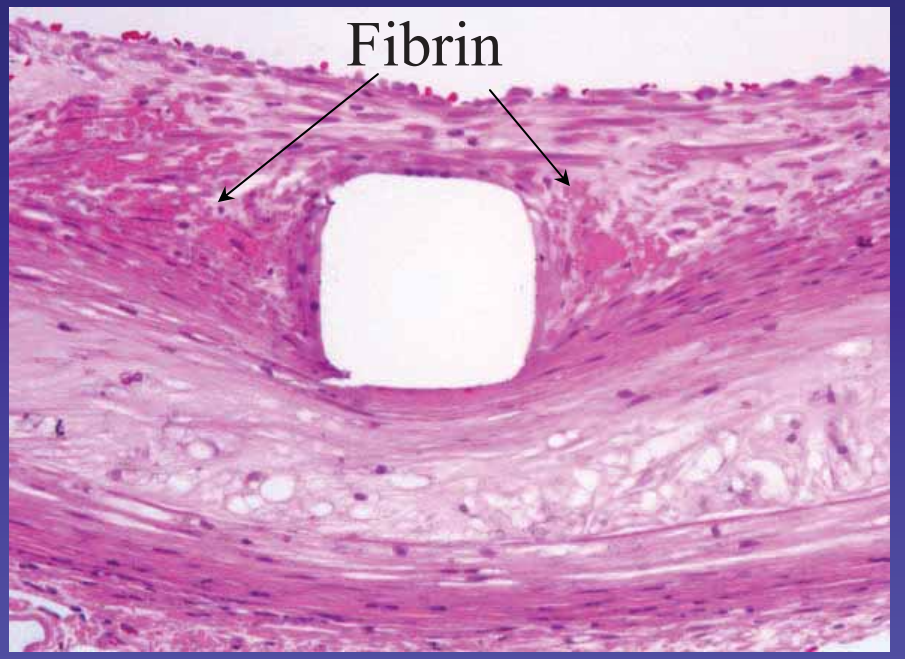




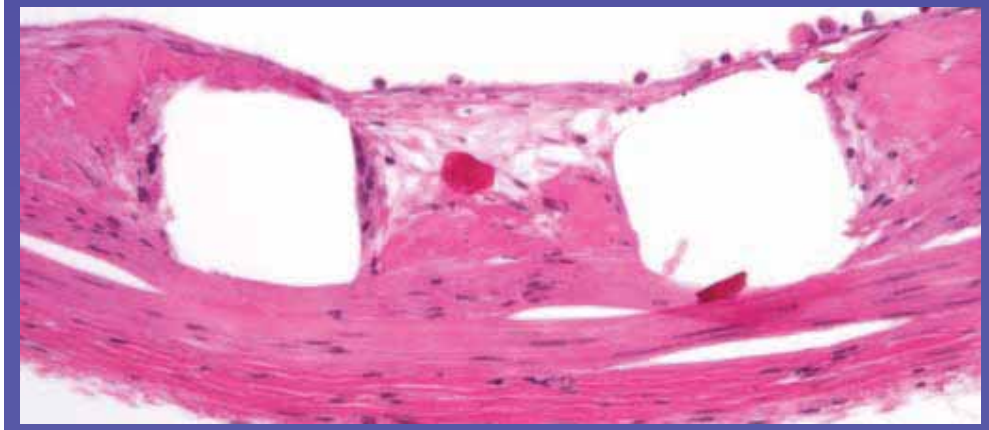


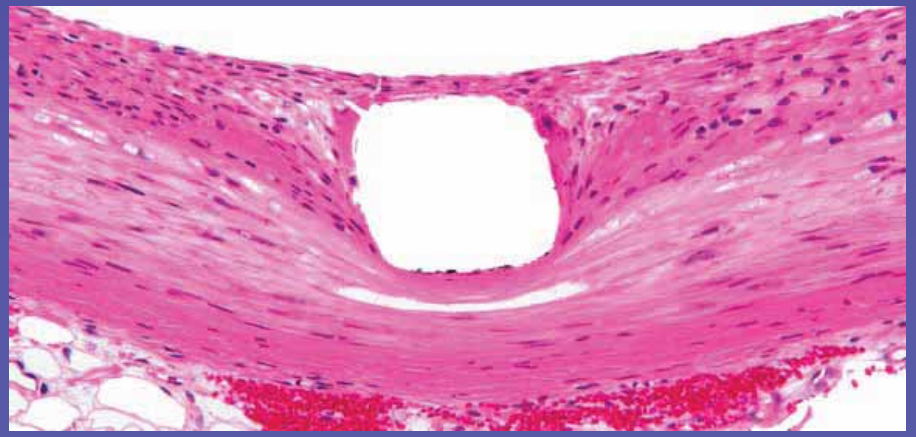
Atherosclerosis (Balloon injury + cholesterol)





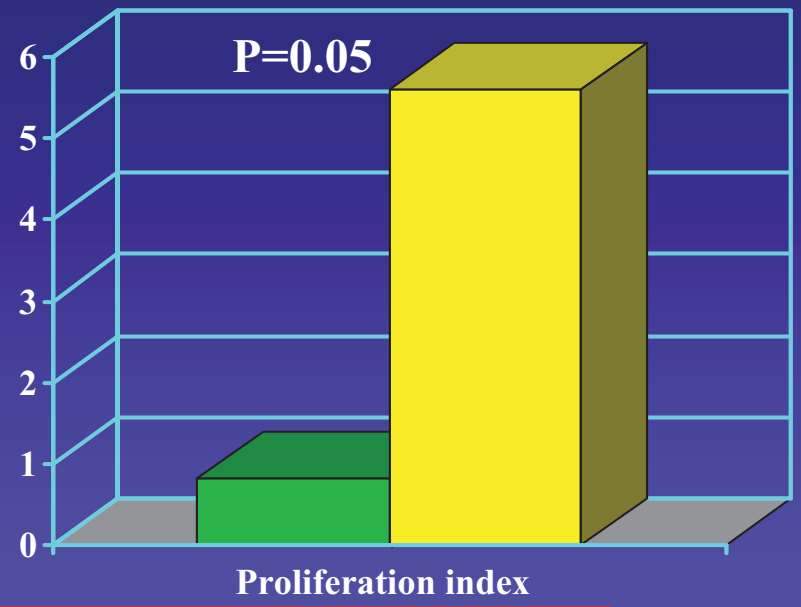
Control (Balloon injury only)



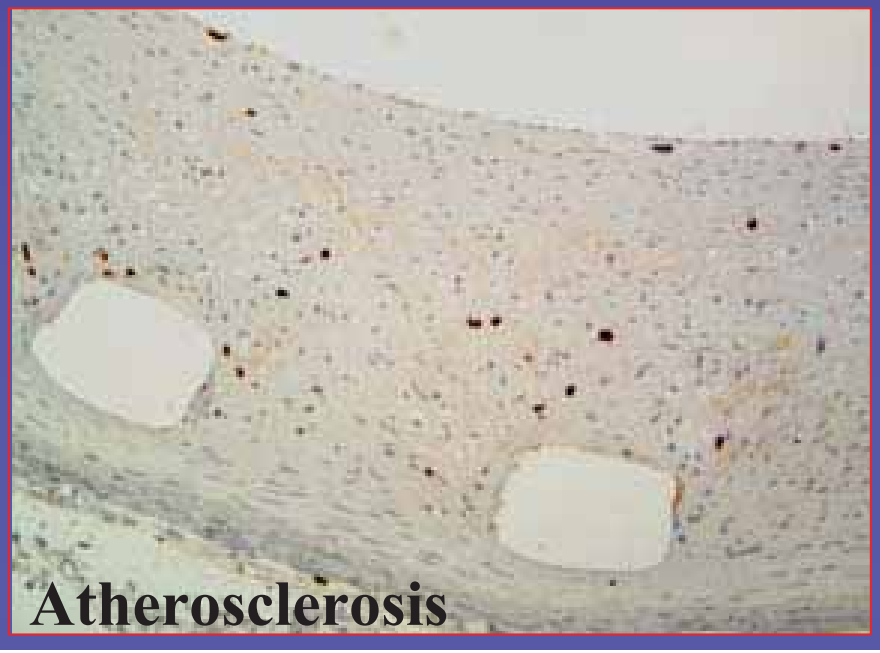


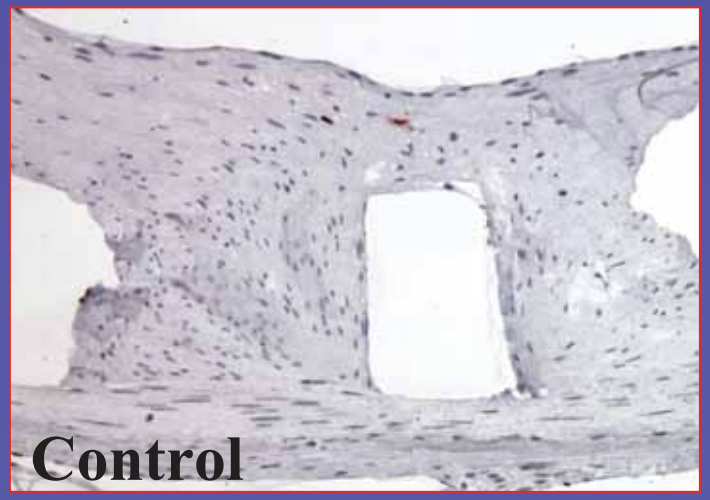
7 days **28 days**

Cell Proliferation (BrdU) in Stented Rabbit Iliac Arteries in The Presence and absence of atherosclerosis at 28 days



Normal Rabbit
Atherosclerotic





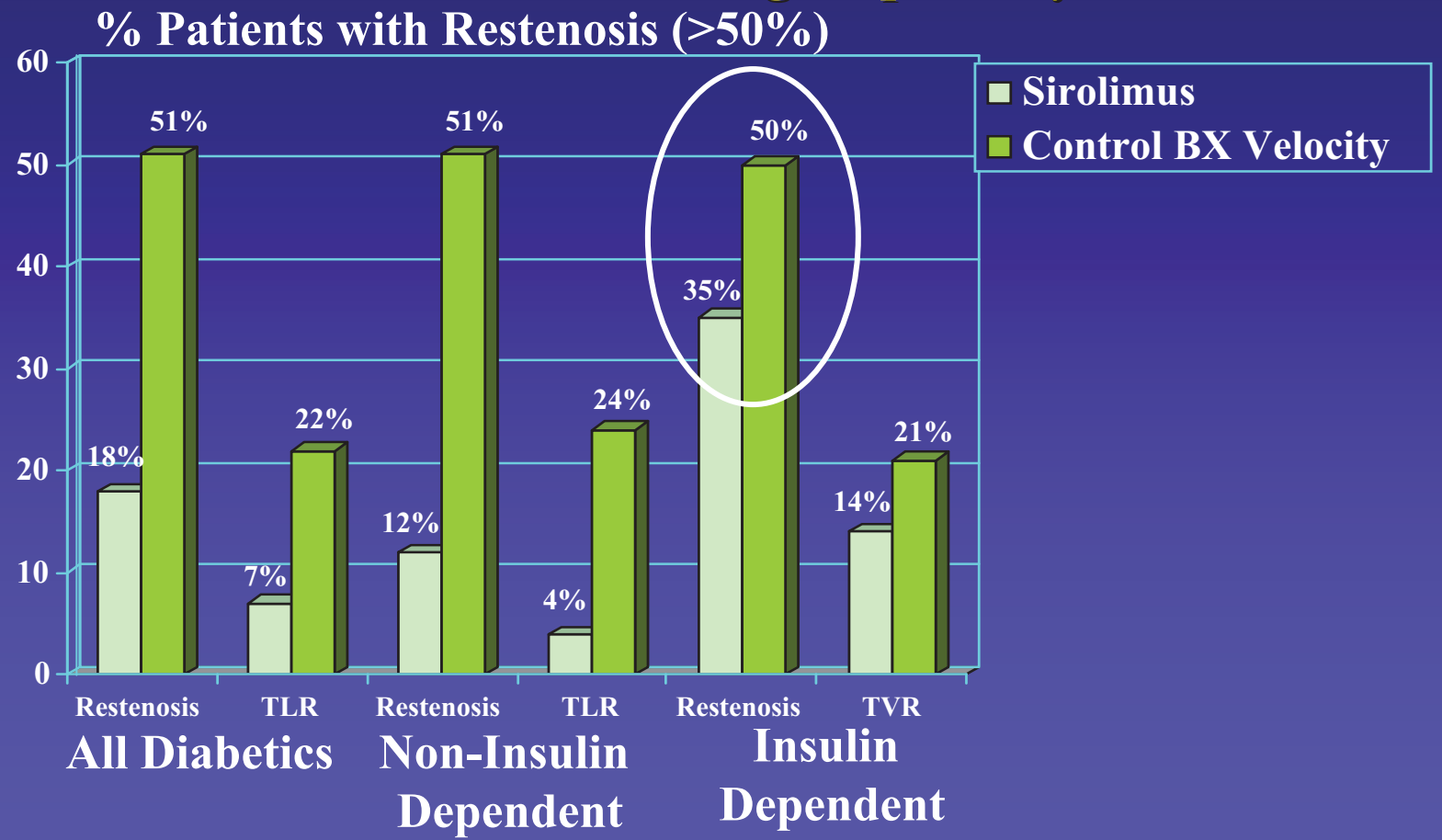
Influence of Atherosclerosis on Healing Following Stenting

- Greater in-stent restenosis compared to control
- Greater inflammation
- Persistence of fibrin
- Poor endothelialization
- Greater proliferation index

Delayed Healing

✓ Rabbit Model of atherosclerosis probably closer to man, but needs testing with Drug-Eluting Stents

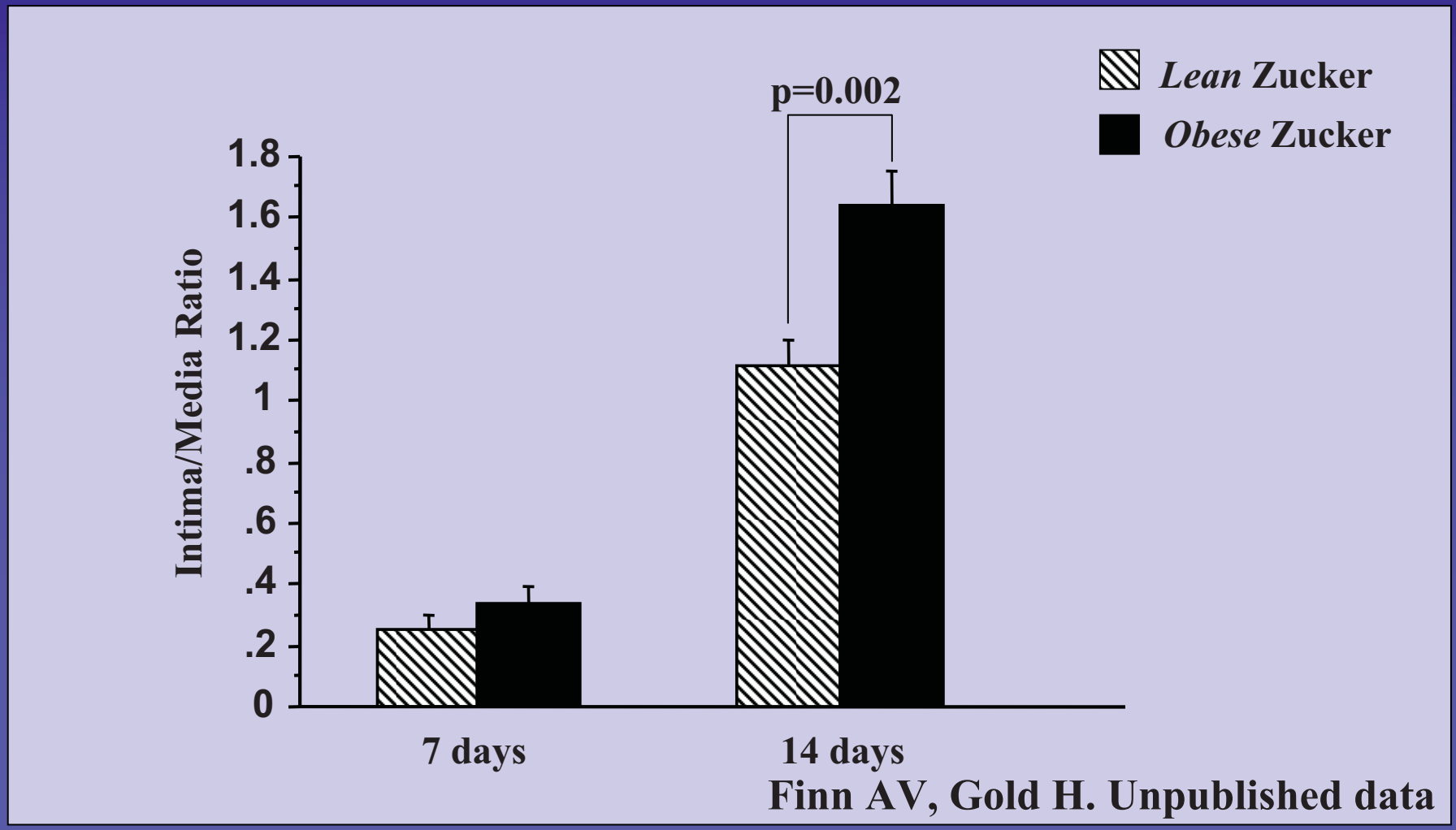
SIRUS Diabetic Subgroup Analysis



SIRUS trial New Eng J Med 2003

Can the Rat Model of Obesity be Used to determine why Diabetic Patients do poorly following PCI?

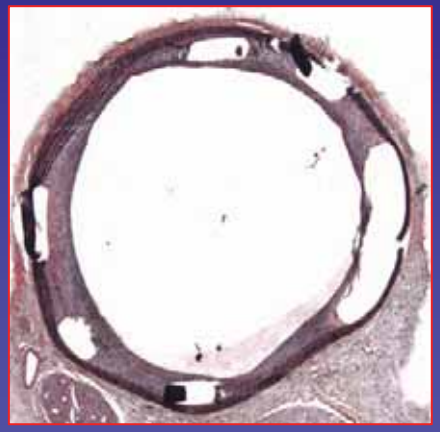
Neointimal Formation is Greater In Obese Zucker Rats when Compared with Lean Littermates

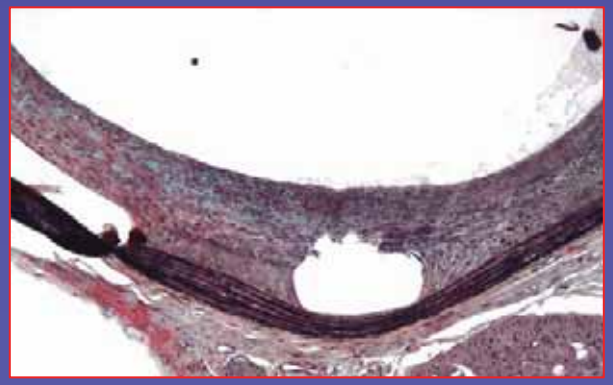


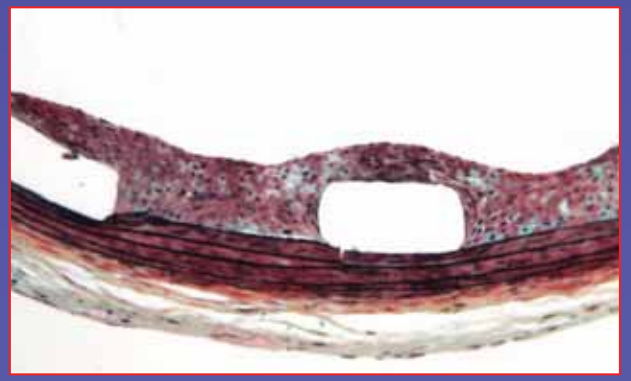
Finn AV, Gold H. Unpublished data

Obese Zucker Rats Develop More Neointima In Response to Arterial Stent Deployment

14 Day Time Point







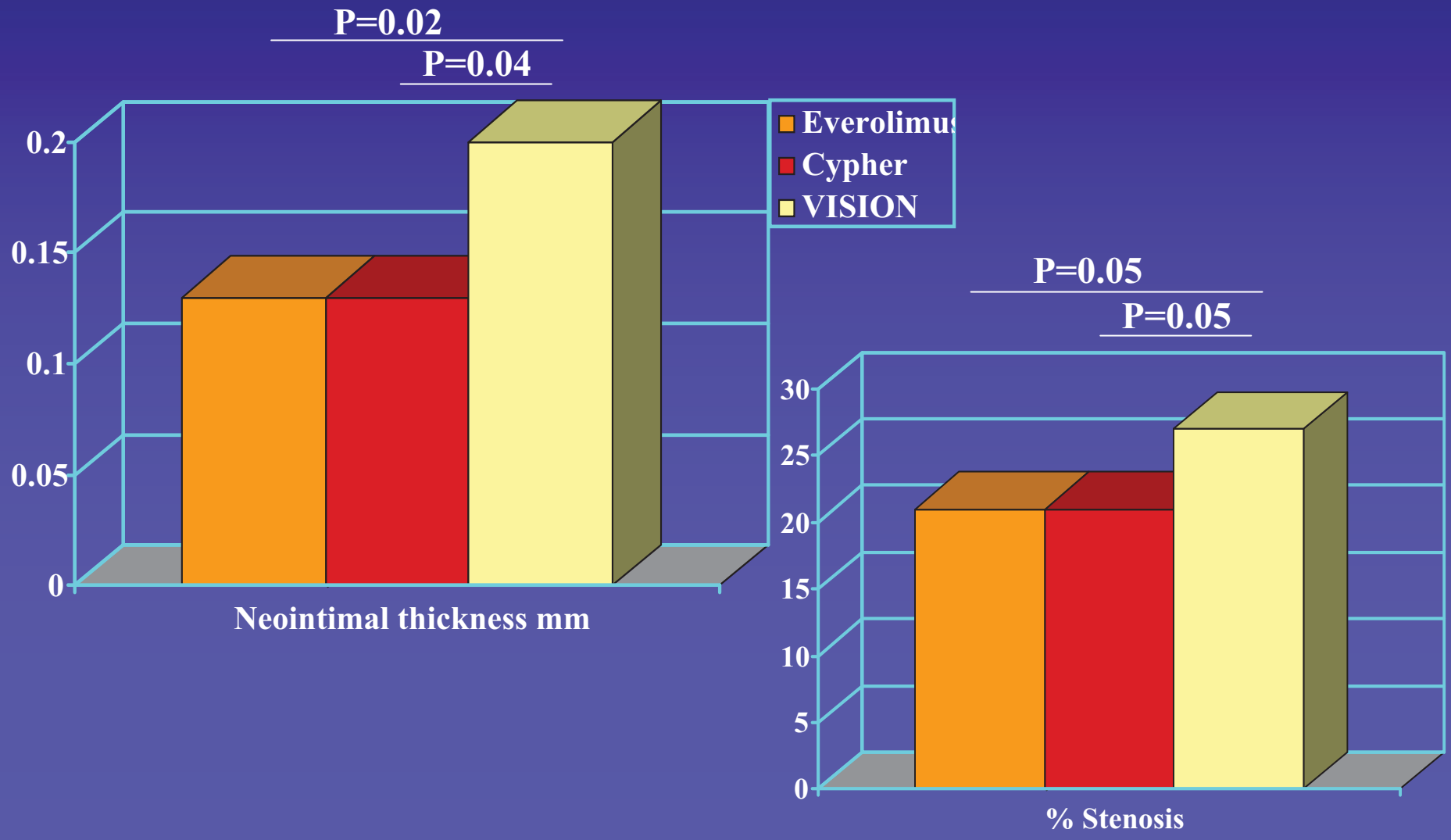
Obese Zucker

Lean Zucker

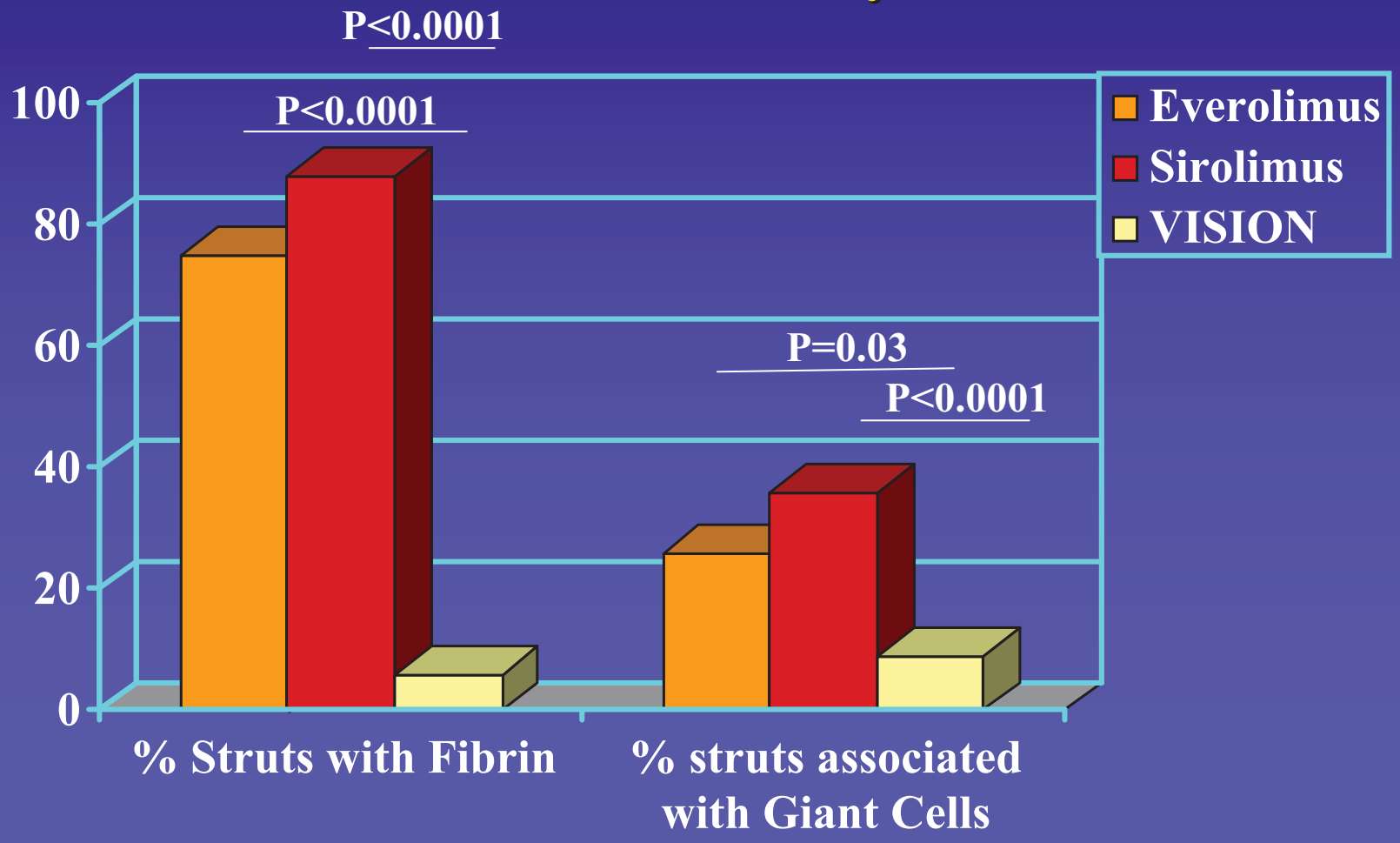
Finn AV, Gold H. Unpublished data

Effect of Drug Eluting Stent (CYPHER and Everolimus) on the Neointima Formation, and Vascular Healing in the Porcine Model at 28 days

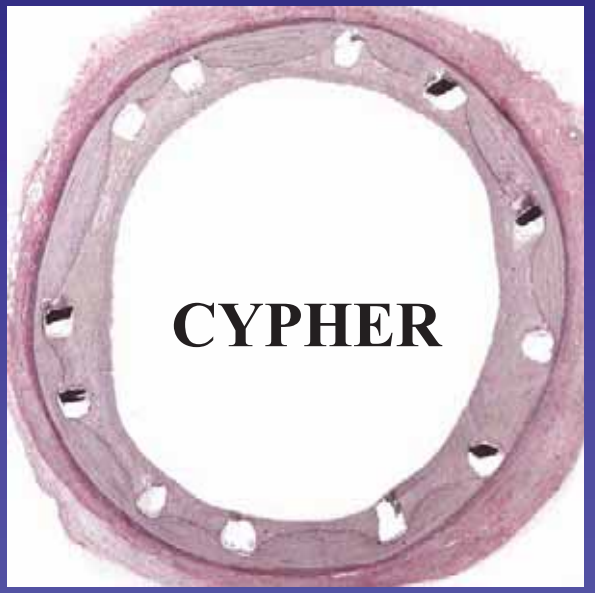
Comparison of Intimal thickness and % Stenosis with Everolimus, Sirolimus (CYPHER) and VISION Stents in Pig Coronary arteries at 28 days

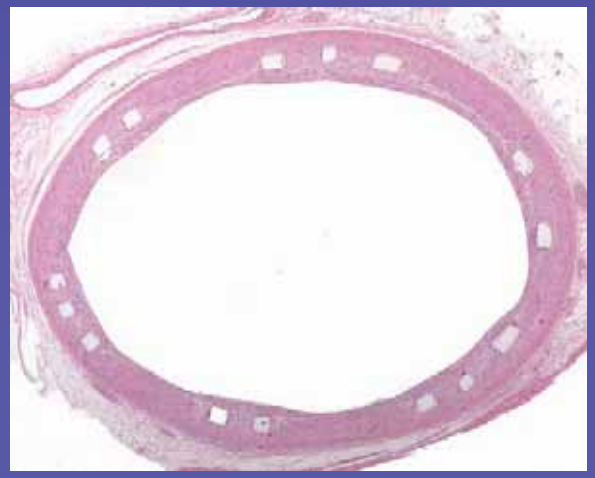


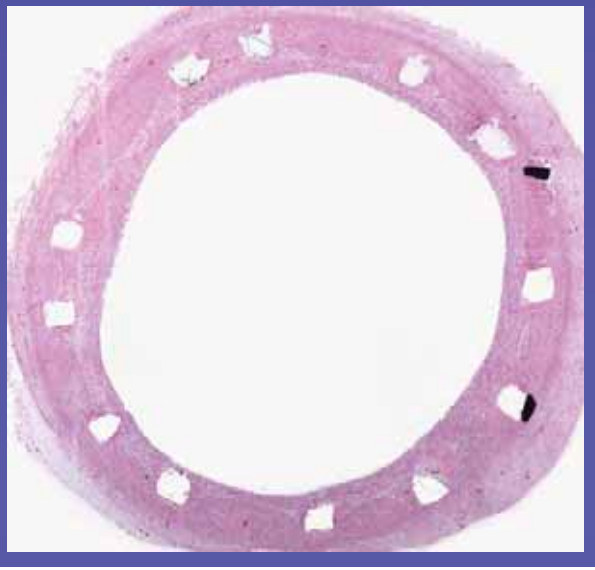
Comparison of % Struts showing Presence of Fibrin and Giant Cells with Everolimus, Sirolimus (CYPHER) and VISION Stents in Pig Coronary arteries at 28 days



Comparison of VISION with and without Everolimus to CYPHER in Pig coronary arteries at 28 days



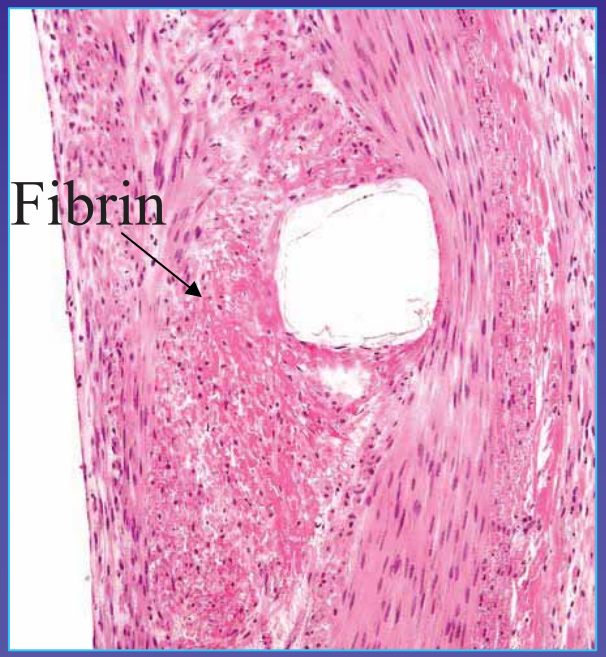




Histologic appearance at 28 days

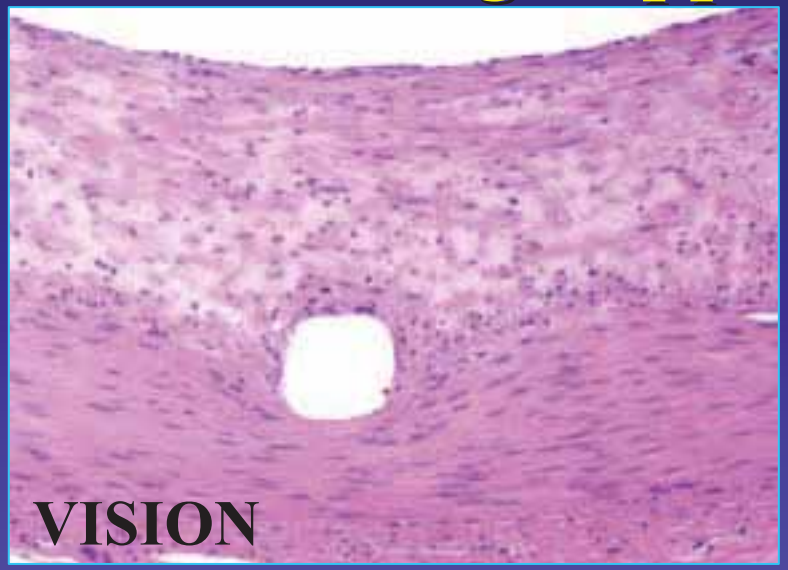
VISION™

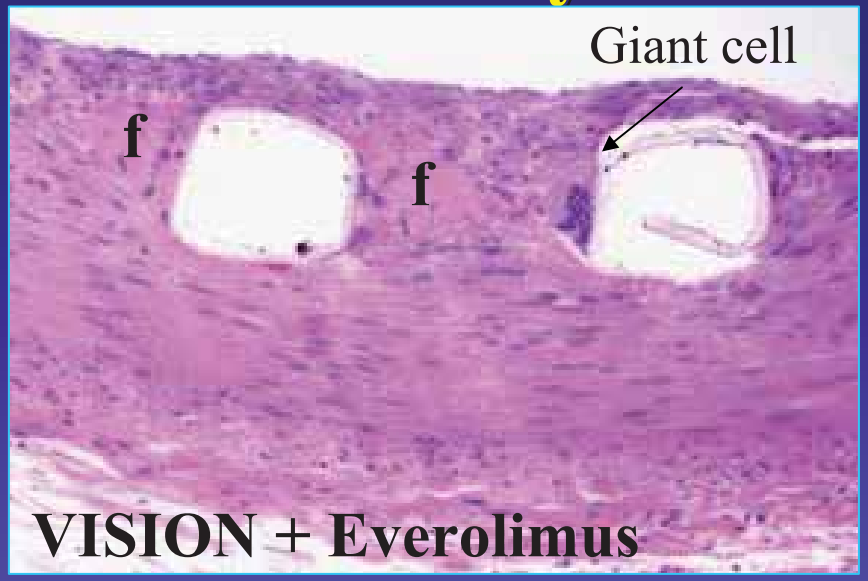
VISION™ +Everolimus

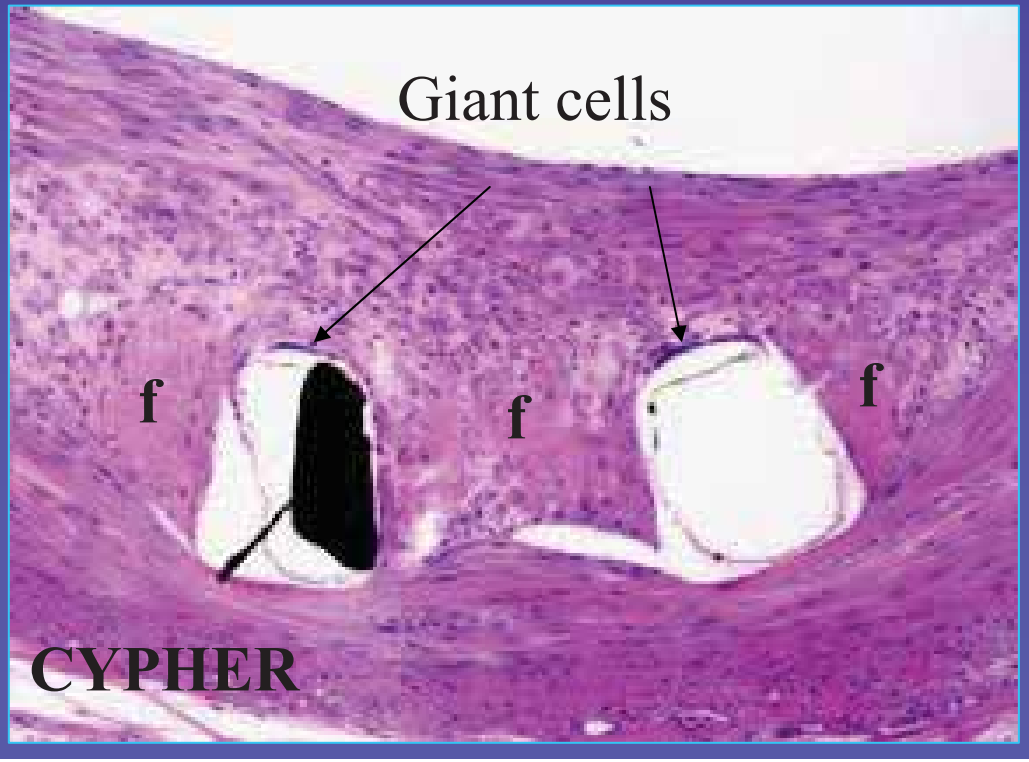


CYPHER™

Histologic appearance at 28 days



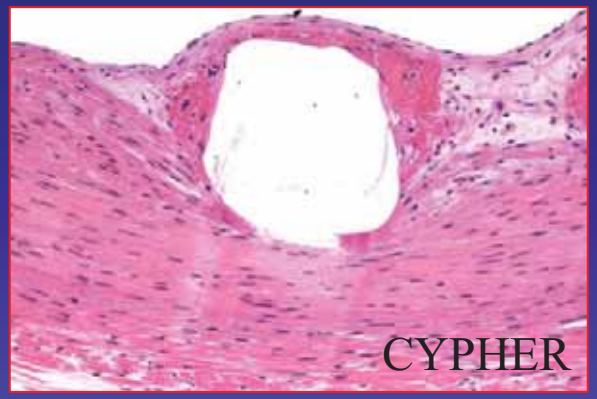


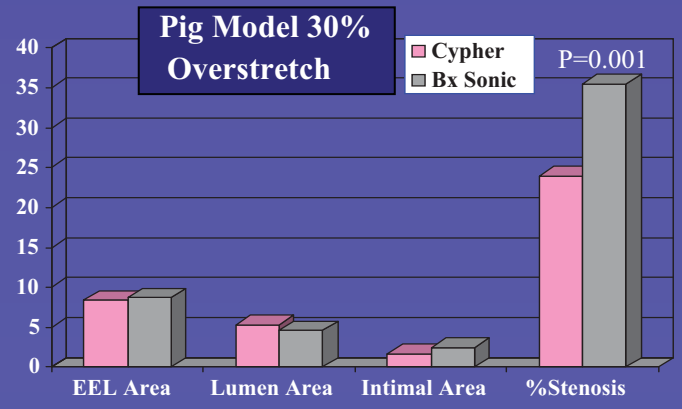


Fibrin = f

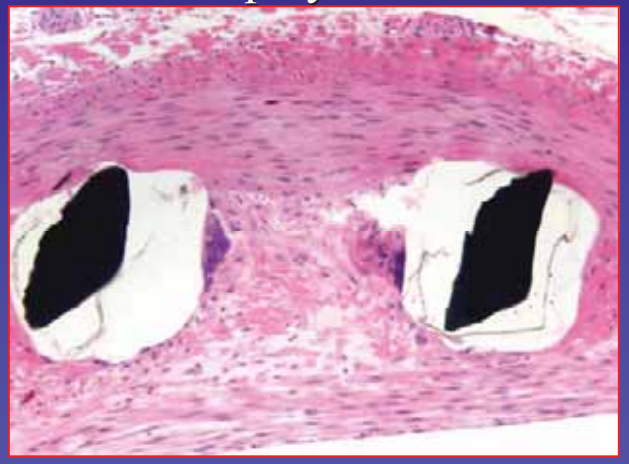
Lessons from Animal Studies with CYPHER

28 days



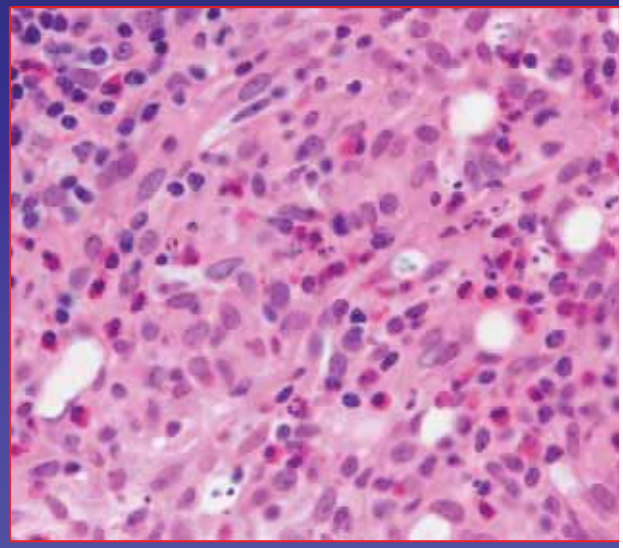


Giant cell reaction to polymer

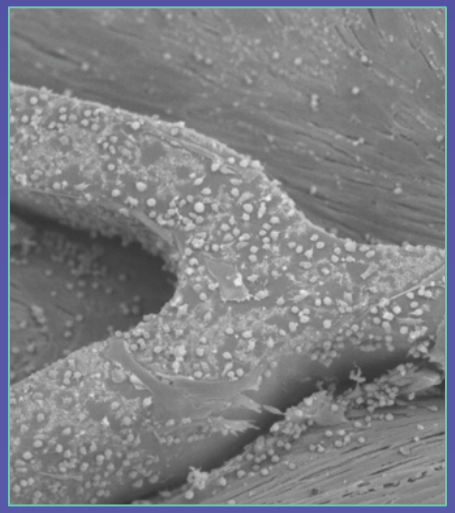


Inc. cell proliferation

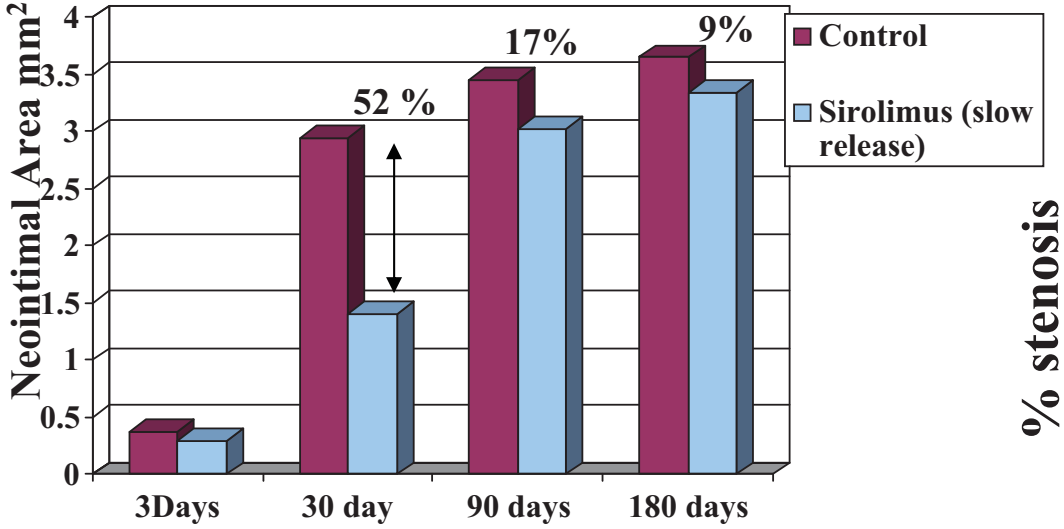
Hypersensitivity reaction with eosinophilia



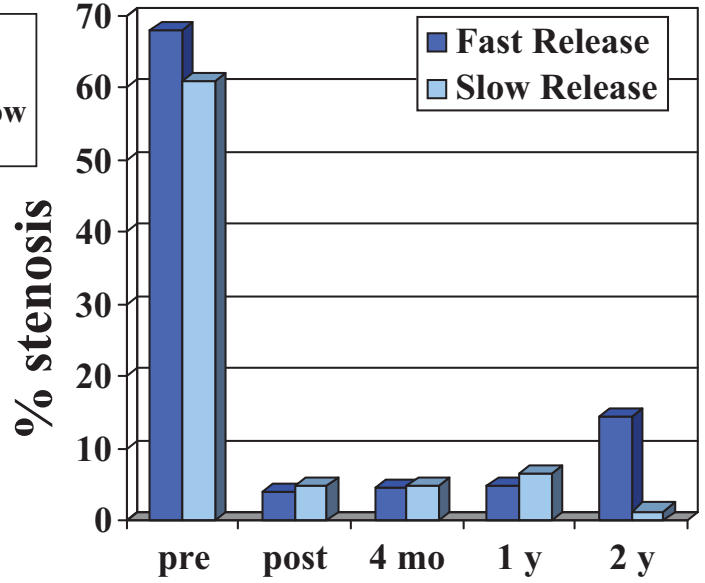
Incomplete Endothelialization (Everolimus)



Comparison of the Animal Data with the Sirolimus First in Man Study



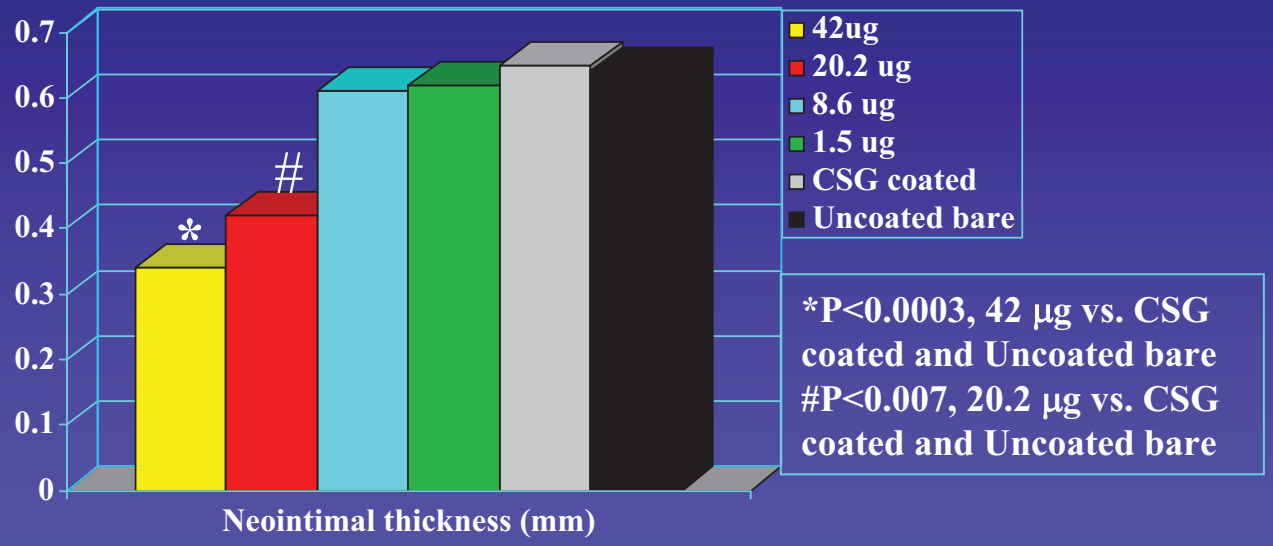
SIROLIMUS - PIG STUDY



SIROLIMUS FIRST IN MAN

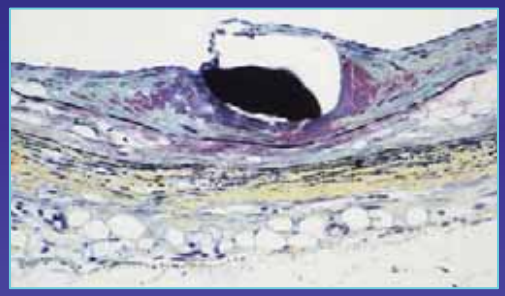
- RAVEL at 6 months % stenosis 15% vs. 37% (sirolimus vs. Bx, p<0.0001) percent reduction is 59%
- If the increase is at the rate shown from the First in Man trial then 15% at 6 mo will become much higher and therefore the benefit will be lost beyond 2 years.

Neointimal thickness 28 days post-deployment of Chondroitin sulfate and gelatin (CSG) coated Stents Containing Varying Concentrations of Paclitaxel

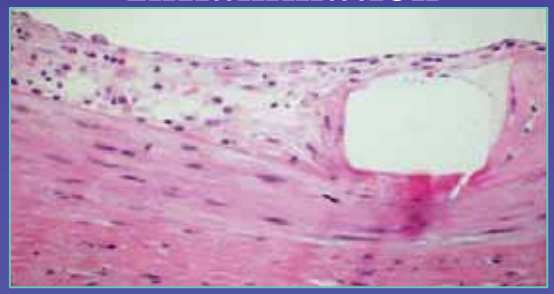


*P<0.0003, 42 μg vs. CSG coated and Uncoated bare
#P<0.007, 20.2 μg vs. CSG coated and Uncoated bare

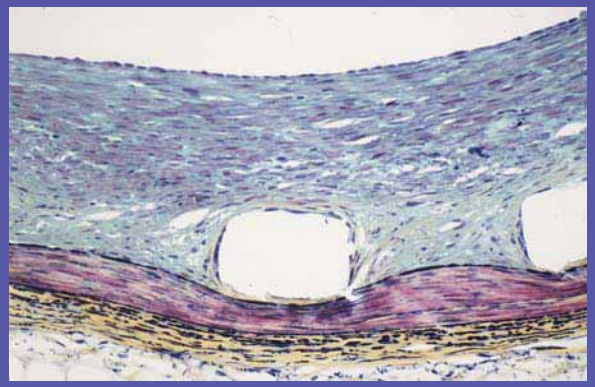
Necrosis



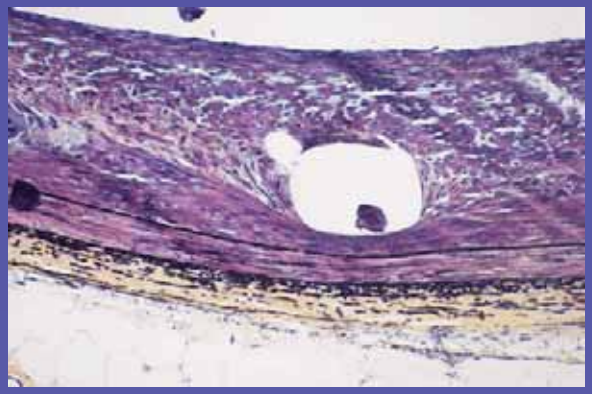
Inflammation



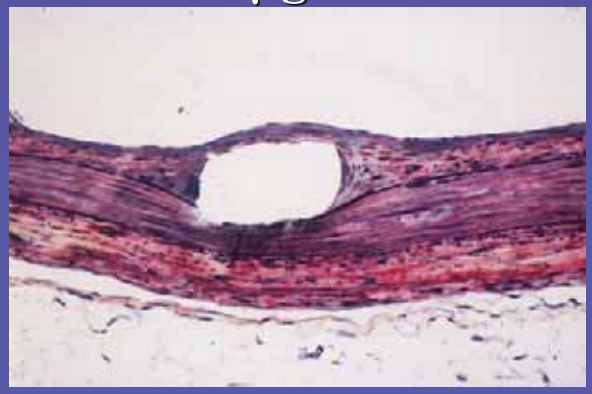
Uncoated



CGS Coated

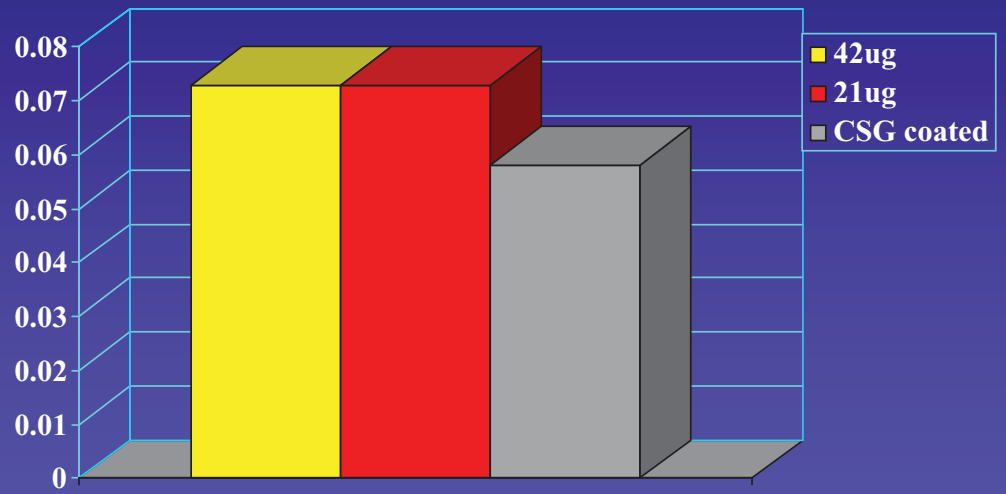


42μg

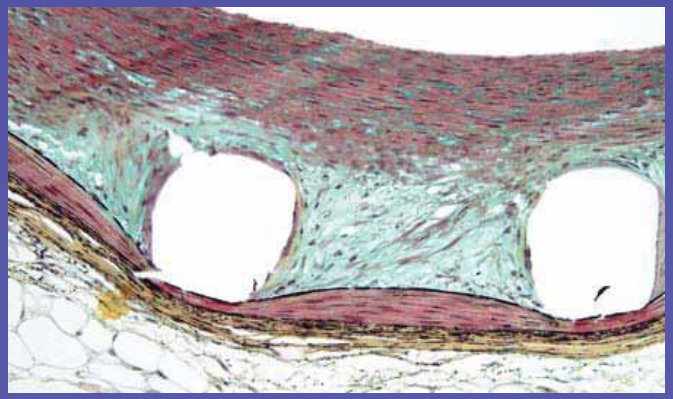


Lessons From Preclinical Studies of DES

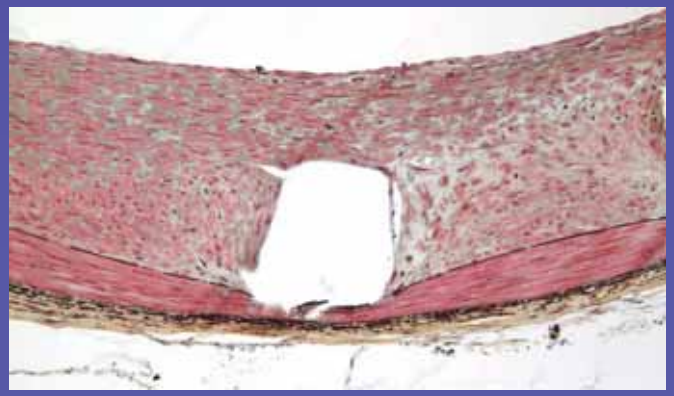
Neointimal Catch-Up = Delayed Late Loss



Control



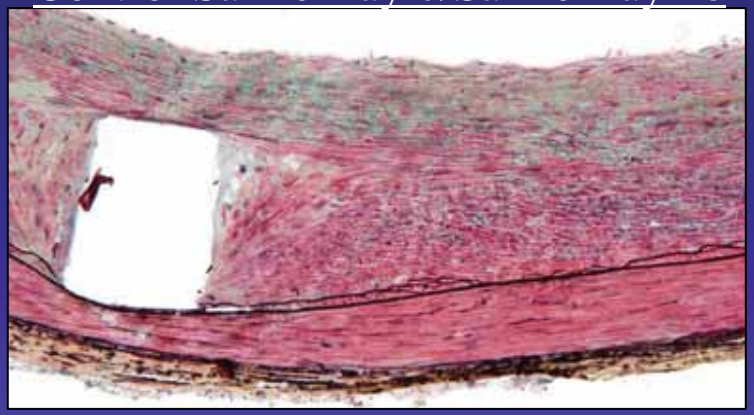
Paclitaxel DES



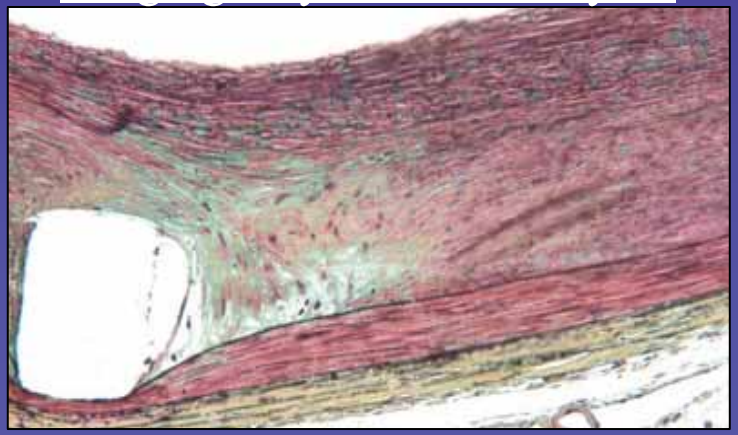
90 Days

Repeat Dosing of Paclitaxel

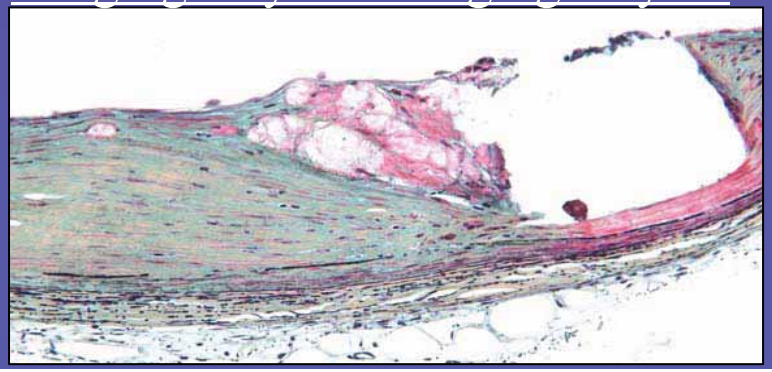
Control Saline Day 0/Saline Day 28



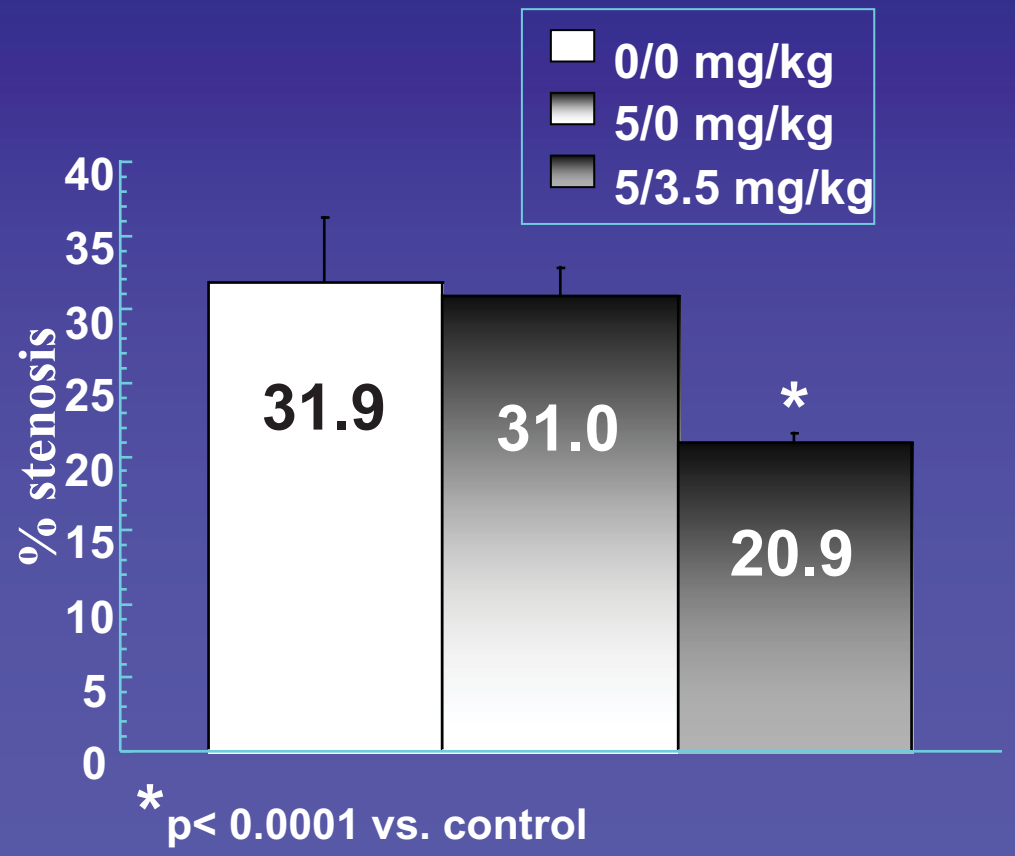
5 mg/kg Day 0/ Saline Day 28



5 mg/kg Day 0/3.5 mg/kg Day 28



Analysis 90-Days Post-Stenting

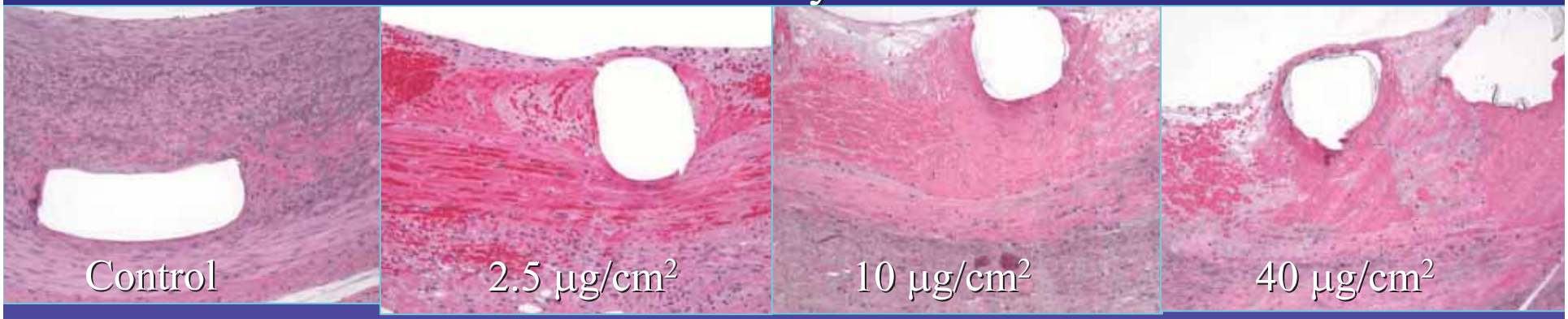


Kolodgie, Circ 2002: 106; 1195

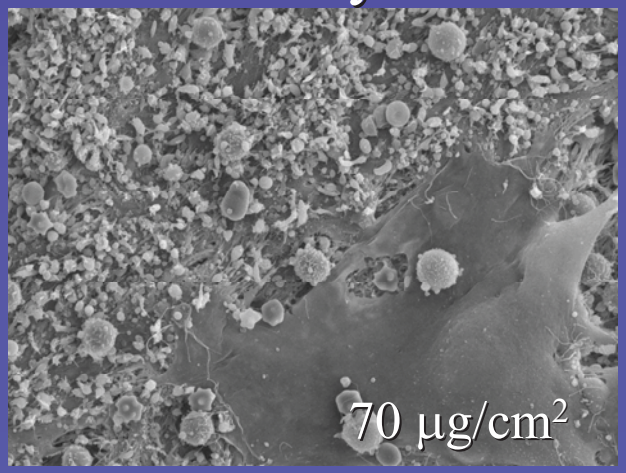
Actinomycin-D Eluting Stents

Preclinical Assessment

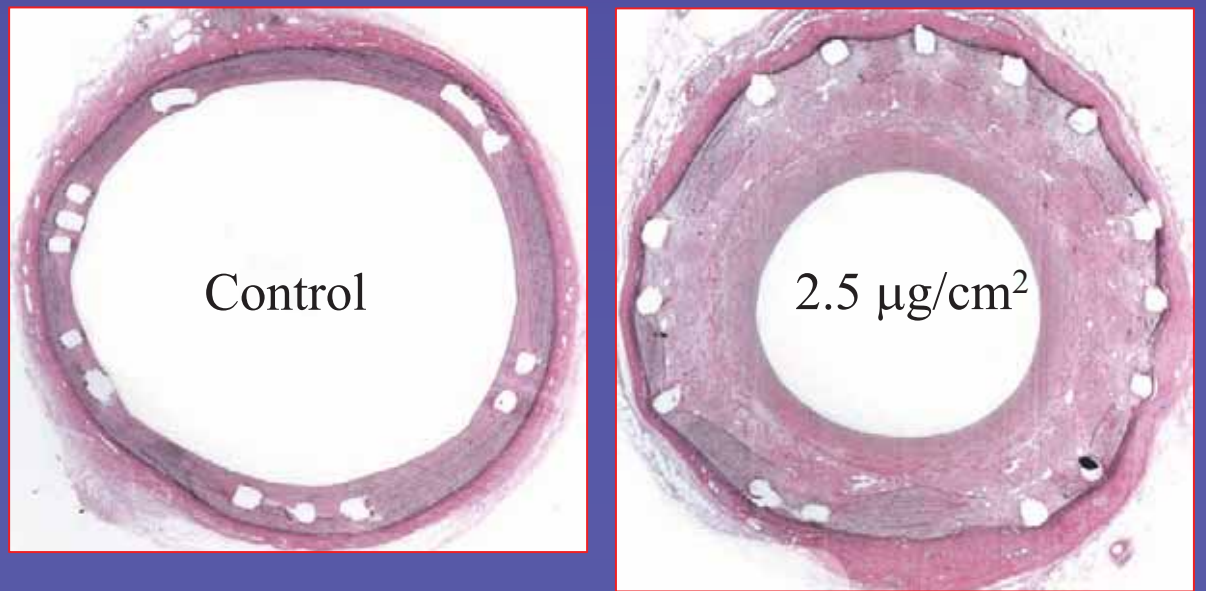
28 Days



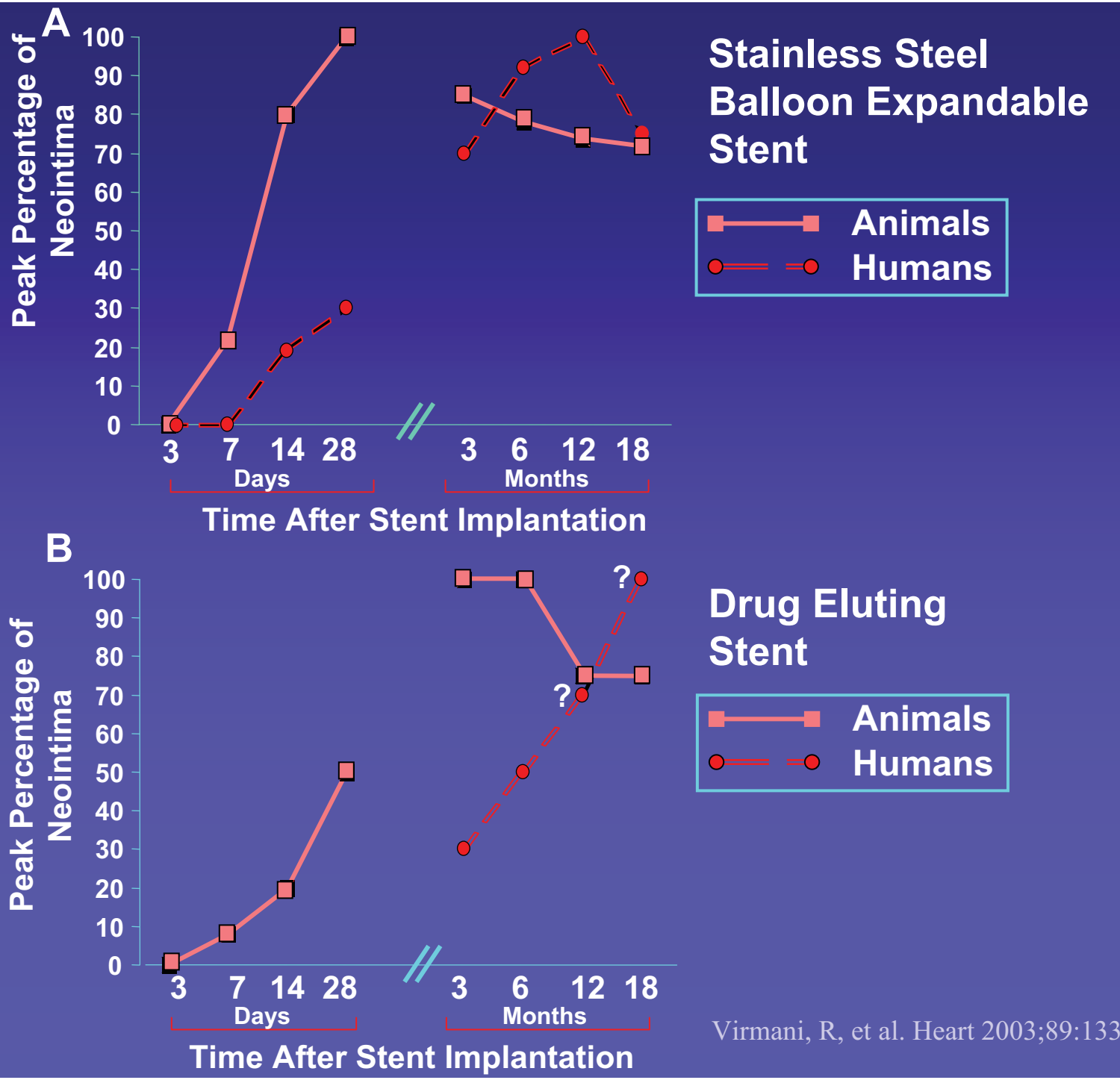
28 Days



180 Days



Arterial Healing Following Stainless Steel and Drug Eluting Stent Placement in Animals and Humans



Virmani, R, et al. Heart 2003;89:133

The Value of Animal Models in Evaluating Pathobiologic Effects of Drug-Eluting Stents: *Insights from past successes and failures*

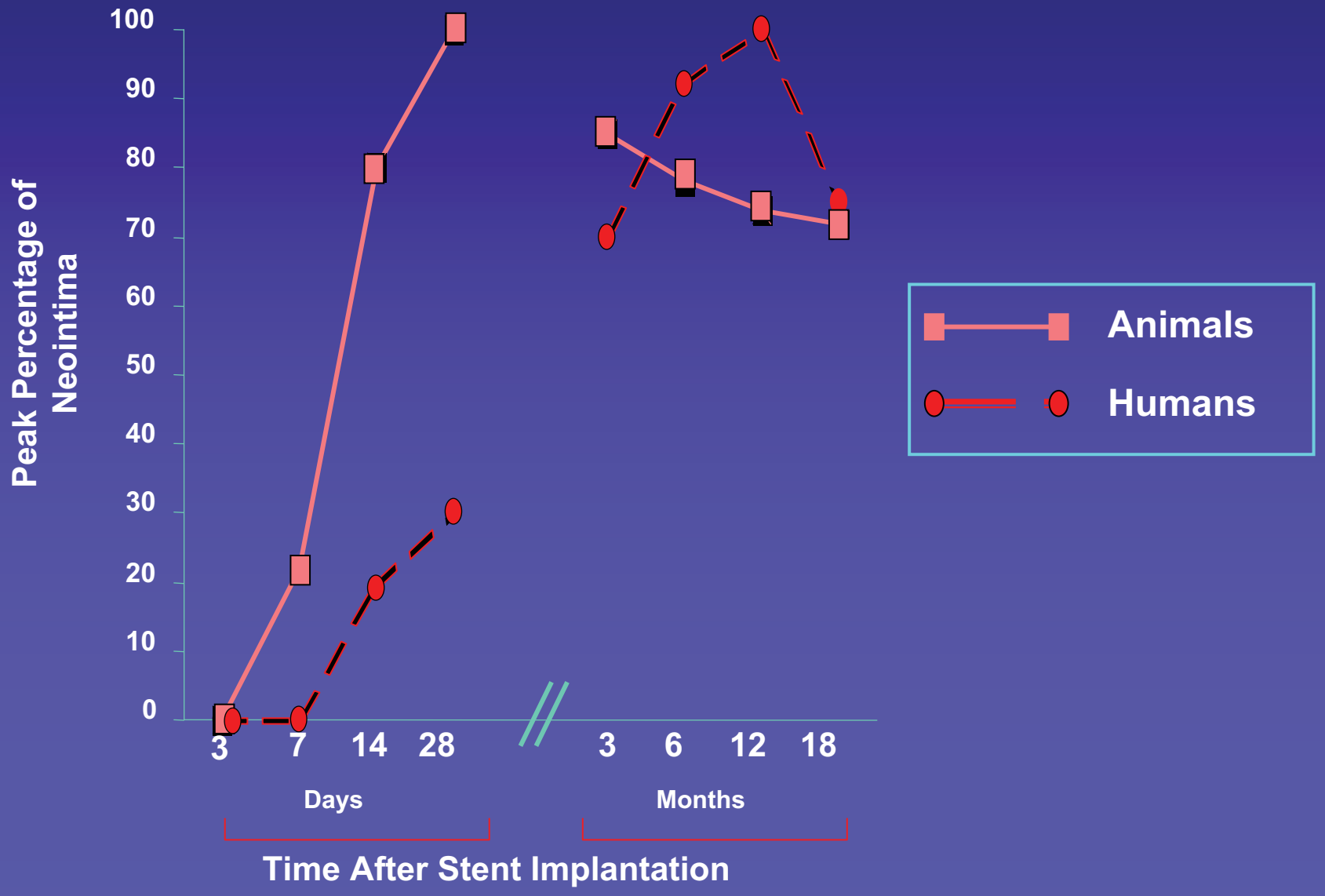
Summary:

- Do animal models predict systemic drug therapy failures? Would have, if only our understanding of restenosis was better e.g., drugs that are effective must show biologic effects on healing.
- Animal models did predict failure of QuaDs-QP2 (Taxane high dose) and Actinomycin-D, and hypersensitivity but some did not know how to evaluate the outcome in animals.
- But we need appropriate models that more closely simulate human disease - atherosclerosis with or without underlying diabetes

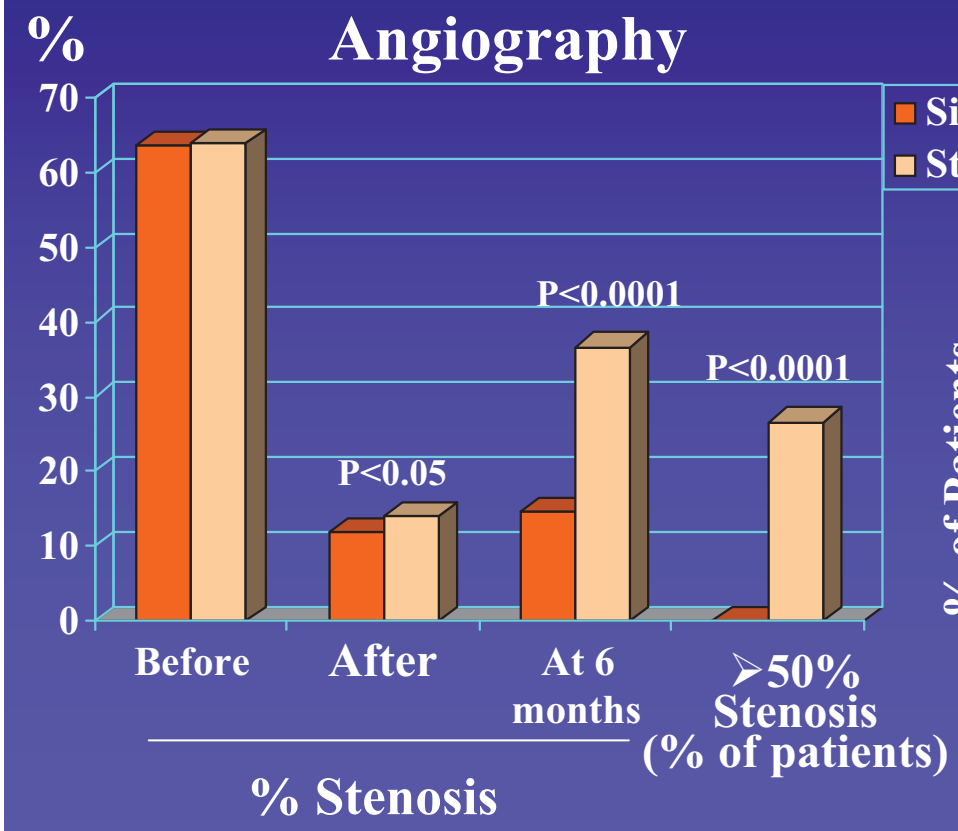
Acknowledgments

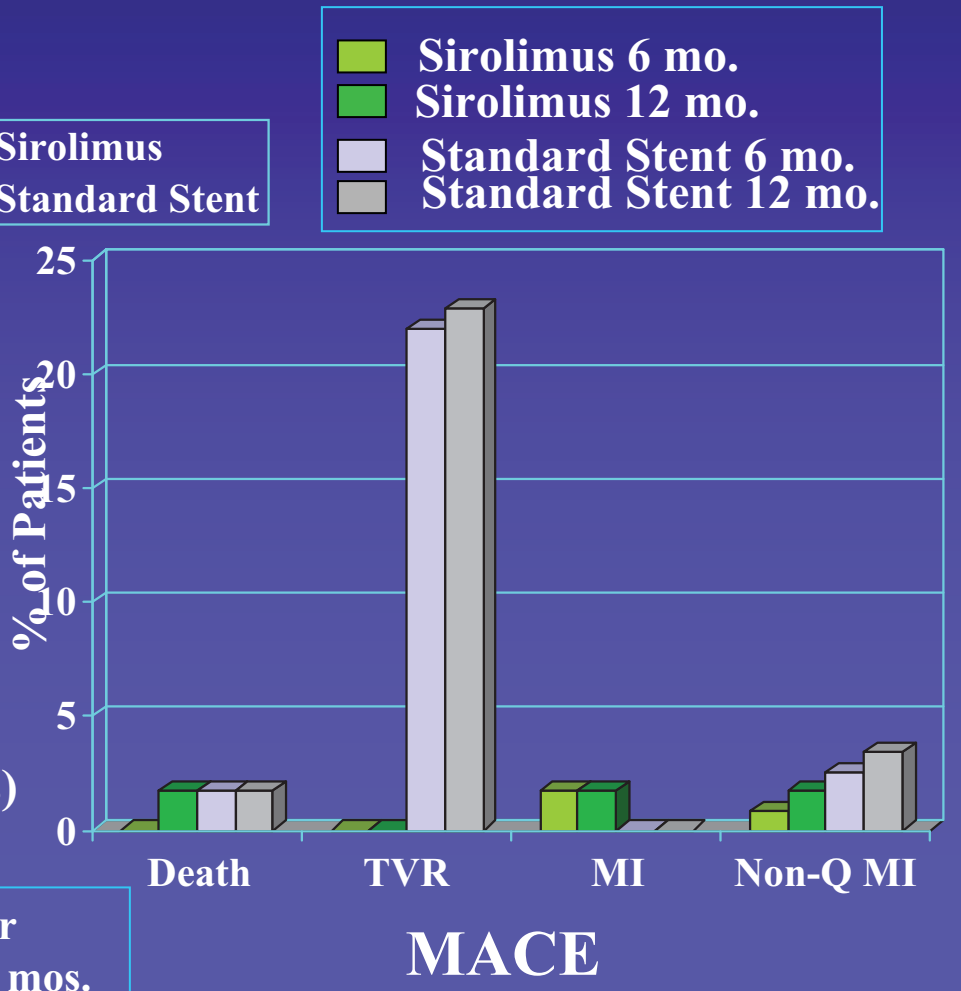
- Andrew Farb, M.D.
- Frank Kolodgie, Ph.D.
- Allen Burke, M.D.
- Russ Jones
- Edward Campado, D.V.M.
- Robert Kutz, M.S.
- Deena Weber, M.S.
- You-hui Liang, M.D.
- Hedwig Avallone
- Lila Adams

Arterial Healing Following Stainless Steel Balloon Expandable Stent Placement in Animals and Humans



Results from RAVEL Trial: Angiogram and MACE





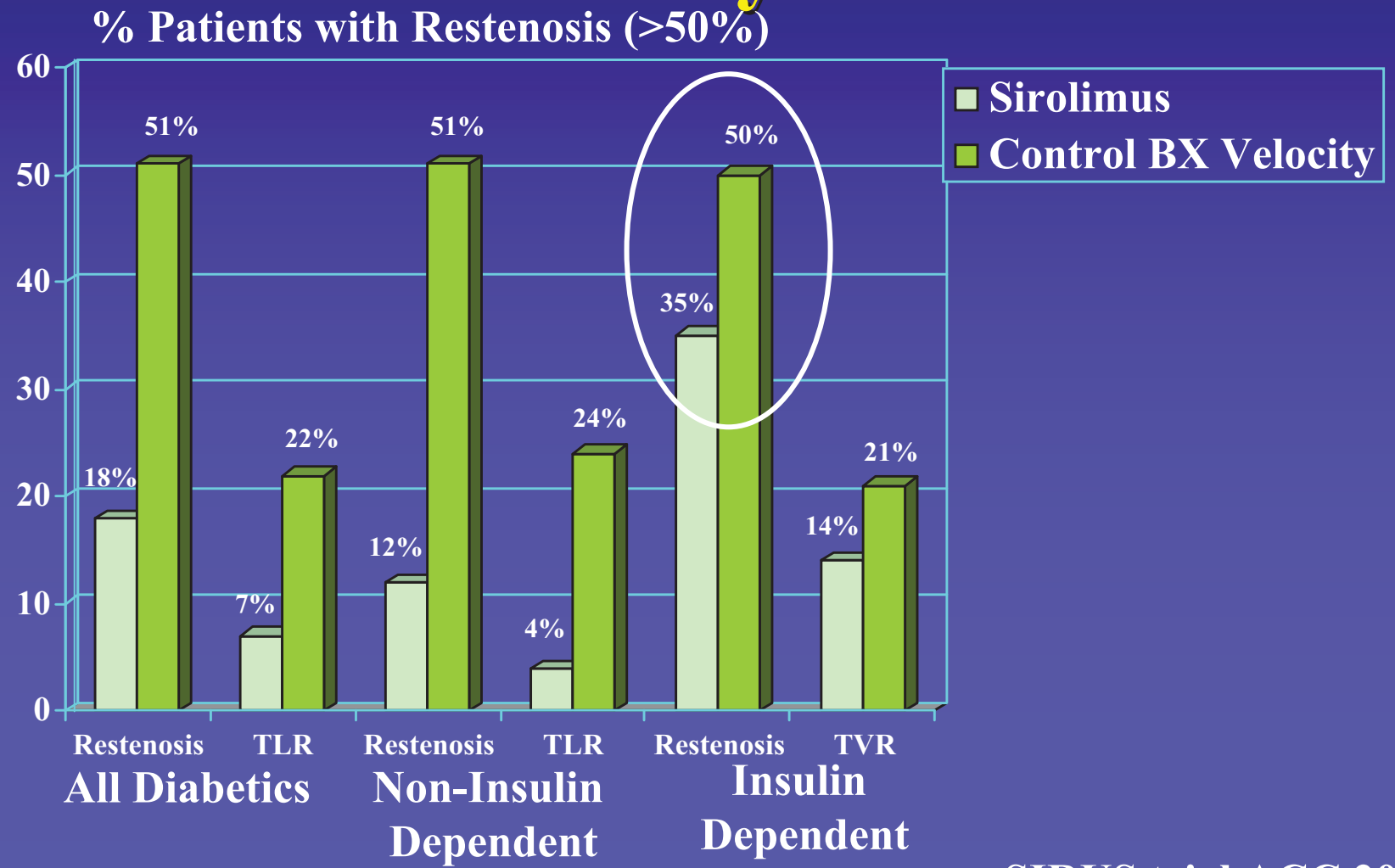
3 patients in the sirolimus group had death, MI, or Non-Q MI at 6 mos. compared to 6 patients at 12 mos.

5 patients in the Control group had death, MI, or Non-Q MI at 6 mos. compared to 6 patients at 12 mos.

Are there other models that may better predict time lines seen in humans?

Stenting of Rabbit Iliac Arteries With and without Balloon Injury give Different results as far as Neointimal thickness and Cell Proliferation

SIRUS Diabetic Subgroup Analysis



SIRUS trial ACC 2003

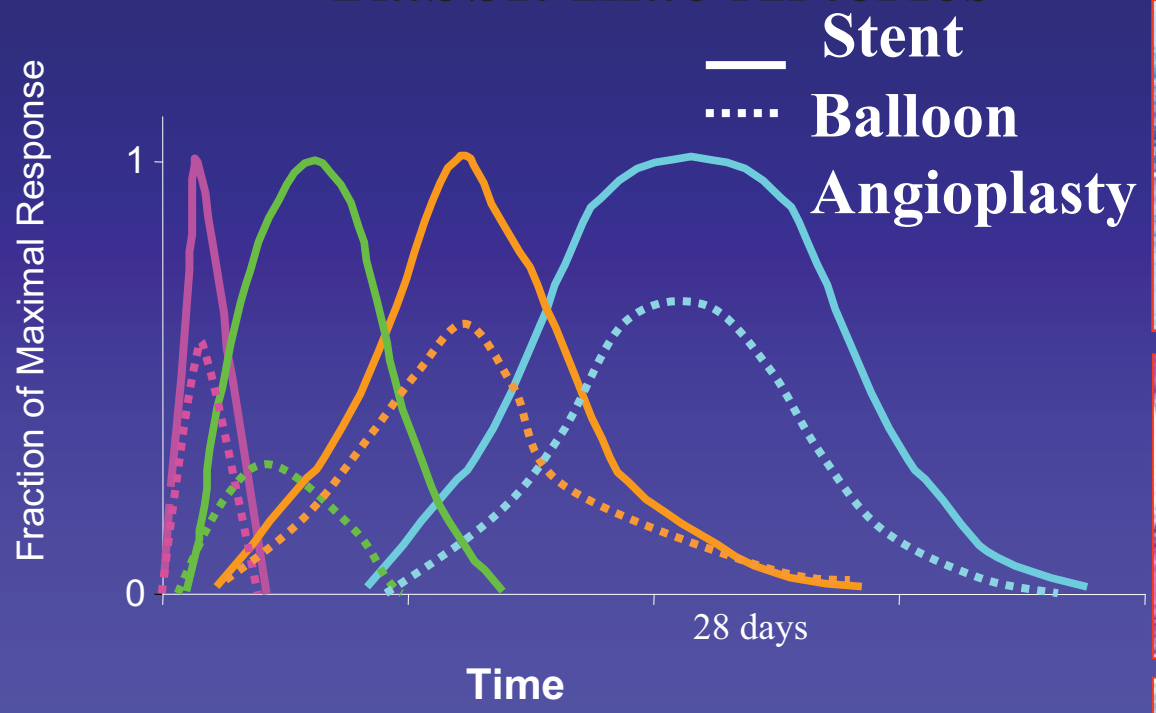
The Value of Animal Models in Evaluating Pathobiologic Effects of Drug-Eluting Stents:

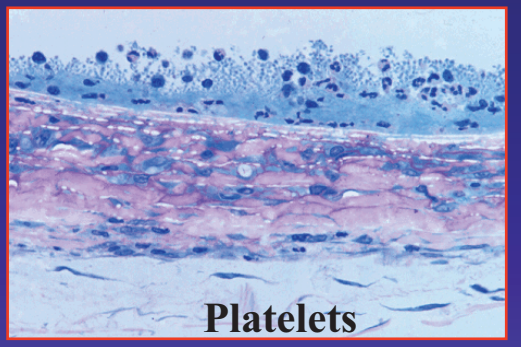
Insights from past successes and failures

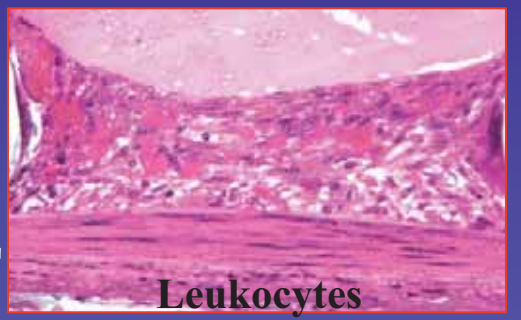
CONCLUSIONS

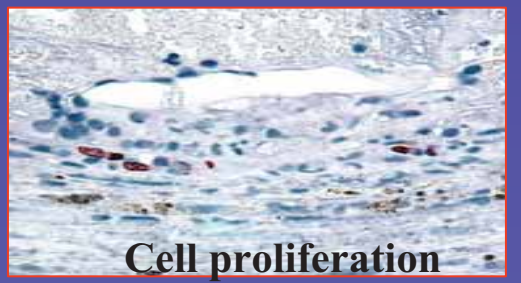
- Animal models show both safety and efficacy for drug eluting stents (DES), but do not mimic human disease.
- Currently DES are deployed in normal arteries either in pigs or rabbits (with or without injury) and have not been studied in the setting of atherosclerosis.
- An atherosclerotic background is more representative of human disease and will influence drug pharmacokinetics and arterial healing.
- A diabetic model will enable us to further determine the influence of insulin resistance on restenosis.
- Without meeting these challenges we will be frustrated and will fail to make progress.

Cascade of Events Following Stent Placement In Rabbit Iliac Arteries



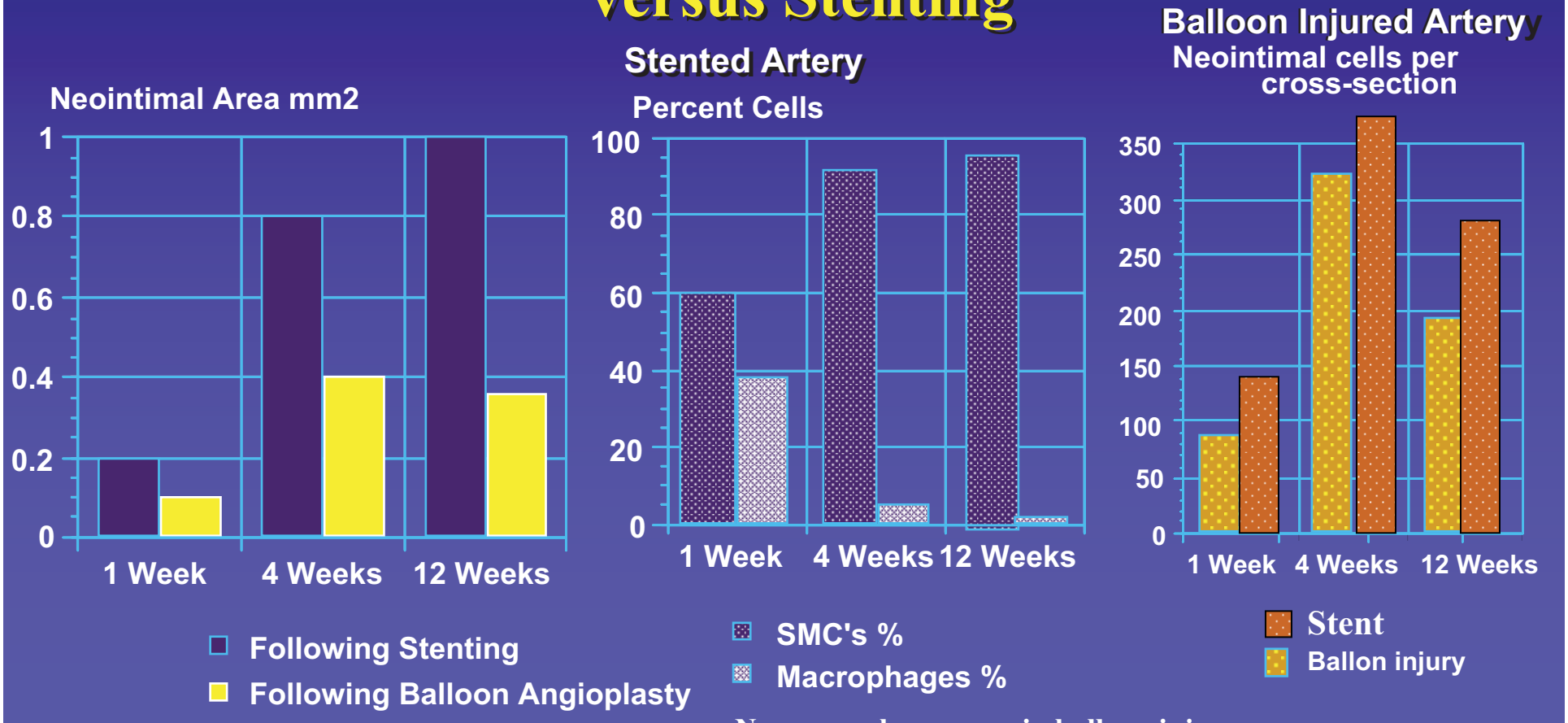






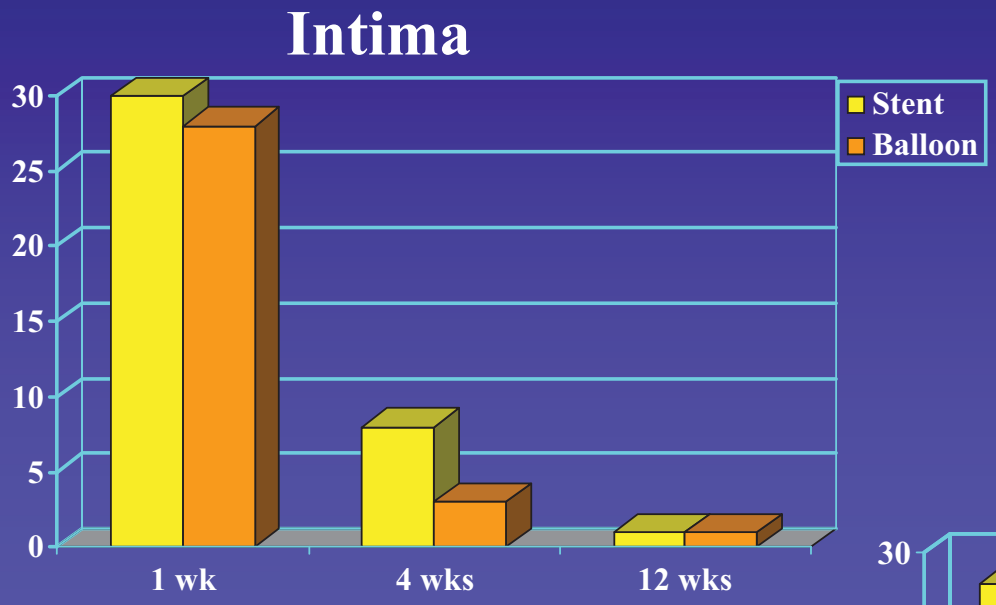
- Platelet Deposition
- Leukocyte recruitment
- VSMC proliferation /migration
- Matrix deposition

Comparison of the Neointimal Area (mm^2) in the Rabbit Iliac Artery Following Balloon Angioplasty versus Stenting

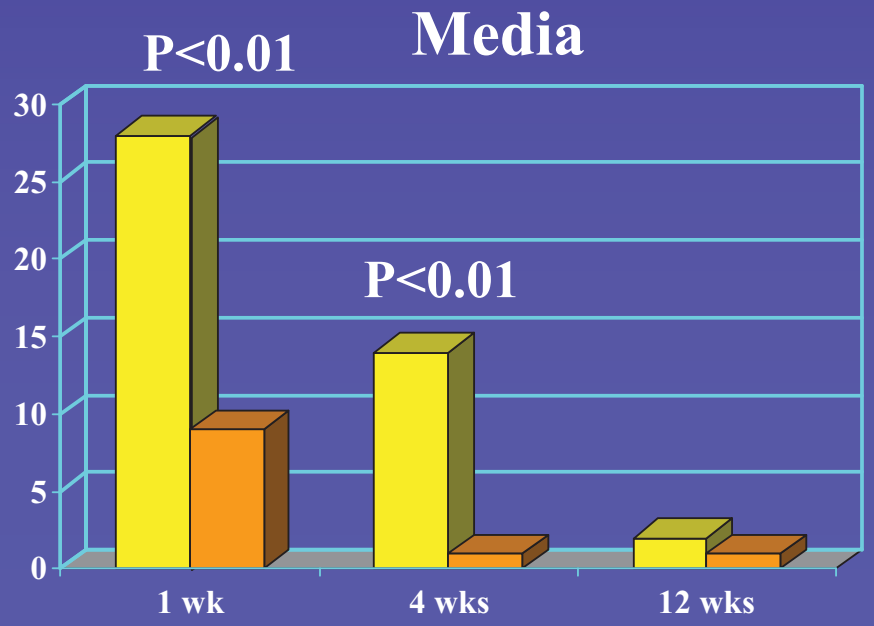


Kollum M, et al. Arterioscler Thromb Vasc Biol. 1997;17:2383-2388.

Proliferation by PCNA staining of Neointimal and Medial cells Following Balloon Angioplasty Versus Stenting

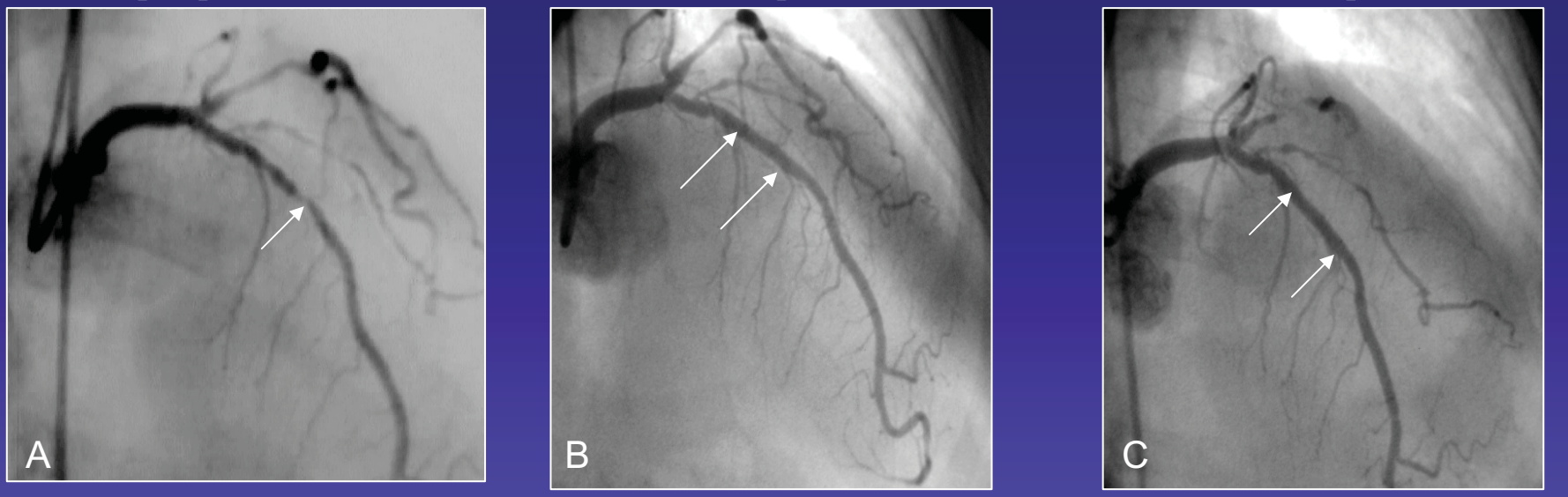


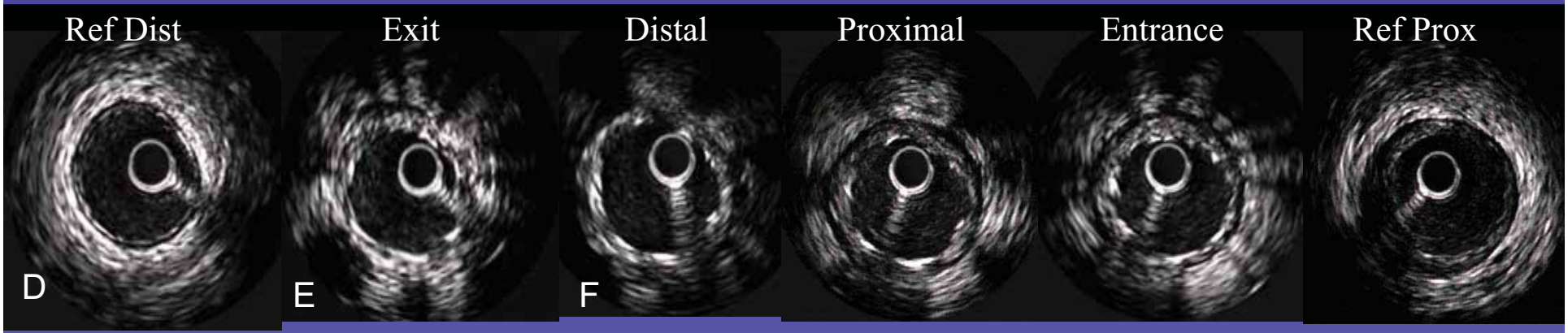
No significant differences were observed between the groups



Pathology of Cypher Stents in Humans

A rare glimpse!!

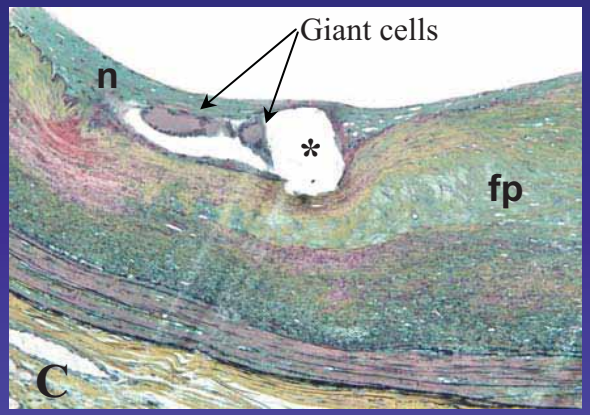


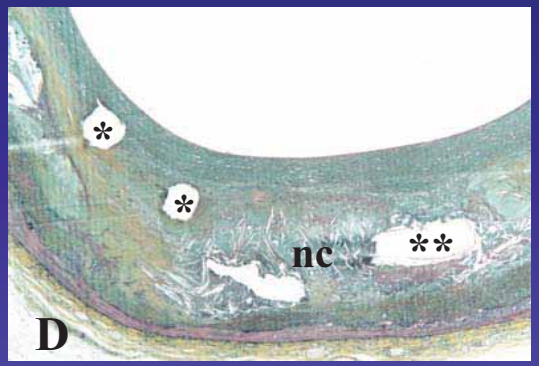


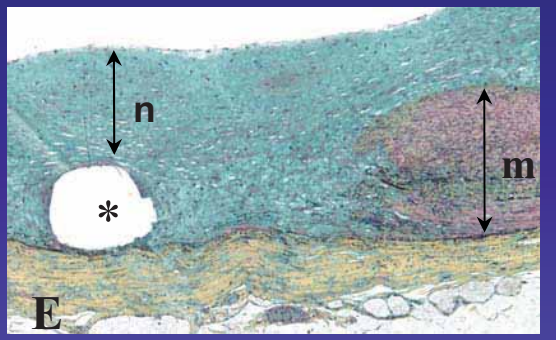
Sirolimus-Eluting Stent Implanted in Human Coronary Artery for 16 Months: Pathologic Findings

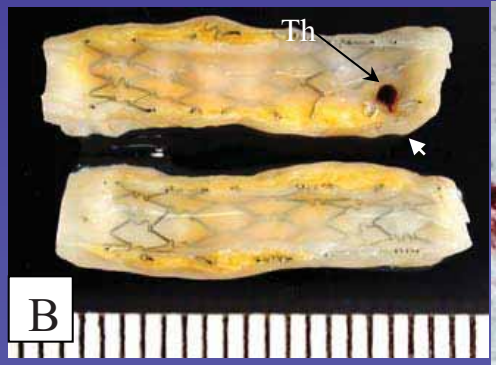
Giulio Guagliumi, Andrew Farb, Giuseppe Musumeci
Orazio Valsecchi, Maurizio Tespili, Teresio Motta,
Renu Virmani, M.D.

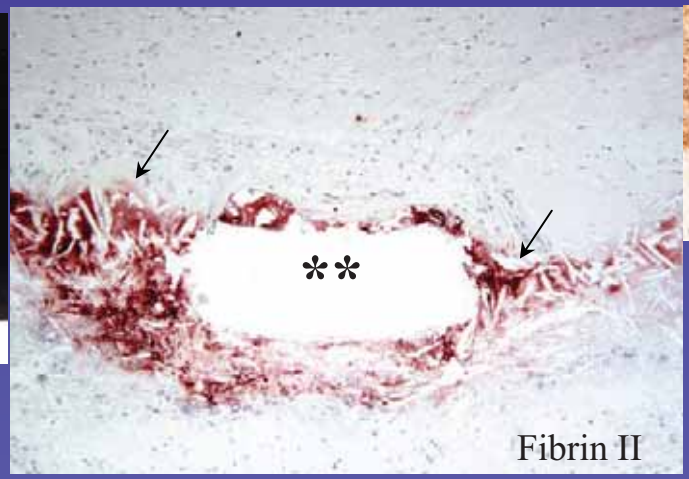
Sirolimus-Eluting BX Velocity Stent
--implanted for 16 months in LAD--

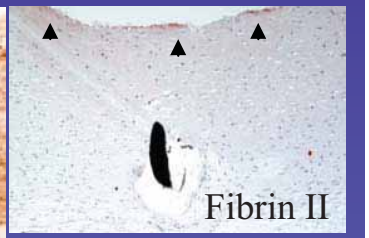










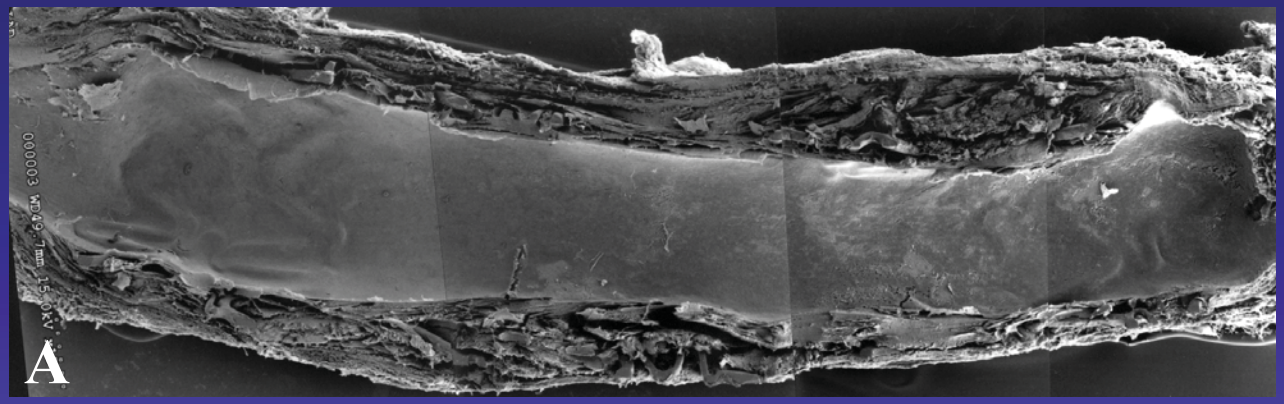


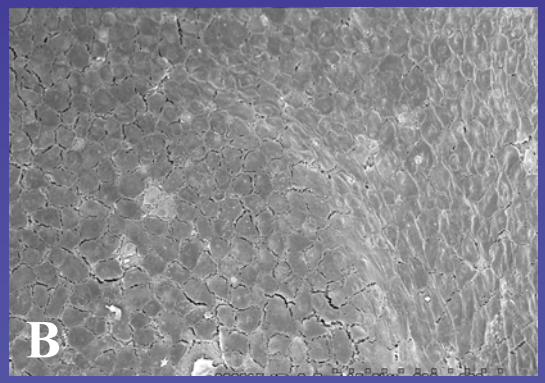
- Well-healed neointima
- Very small thrombus at side branch

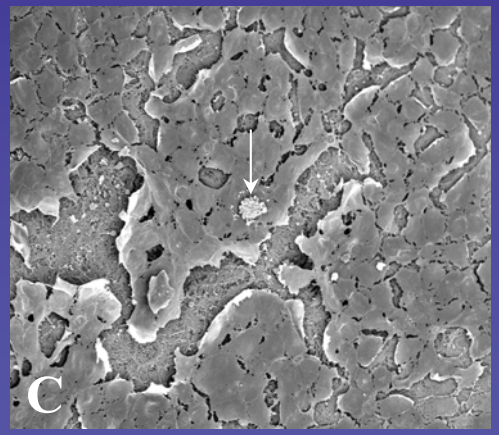
- Fibrin w/strut embedded in core
- Minimal inflammation

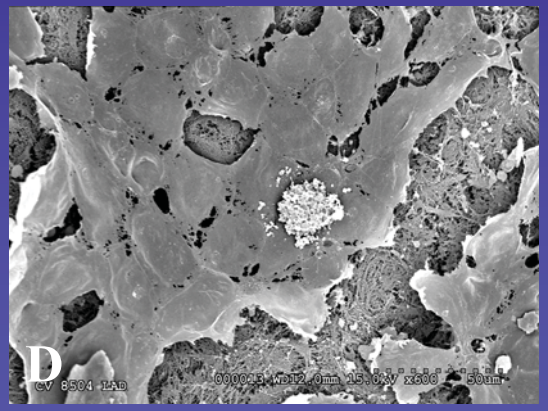
- SMC-rich neointima
- Minimal surface fibrin

Sirolimus-Coated Stent implanted for 16 months in LAD: SEM





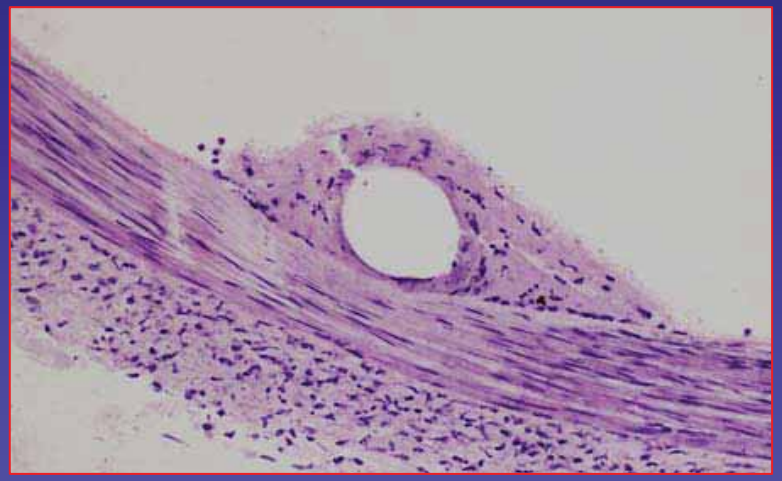




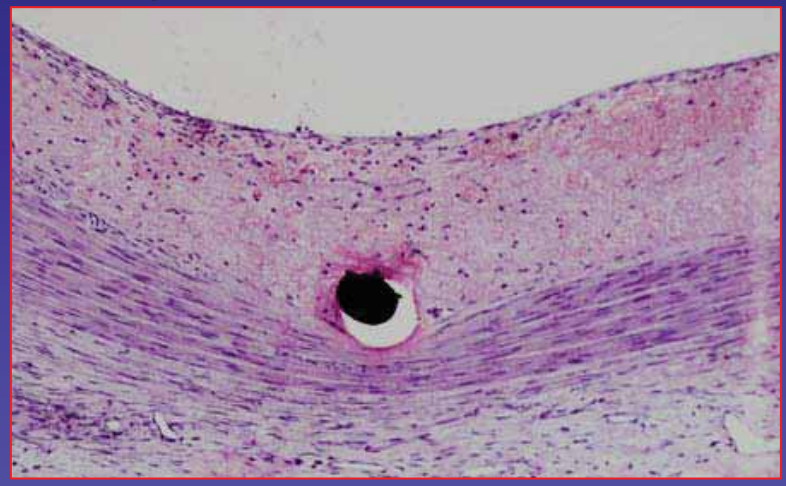
- >80% surface endothelialization
- Loose intercellular junctions
- Rare minute platelet aggregates

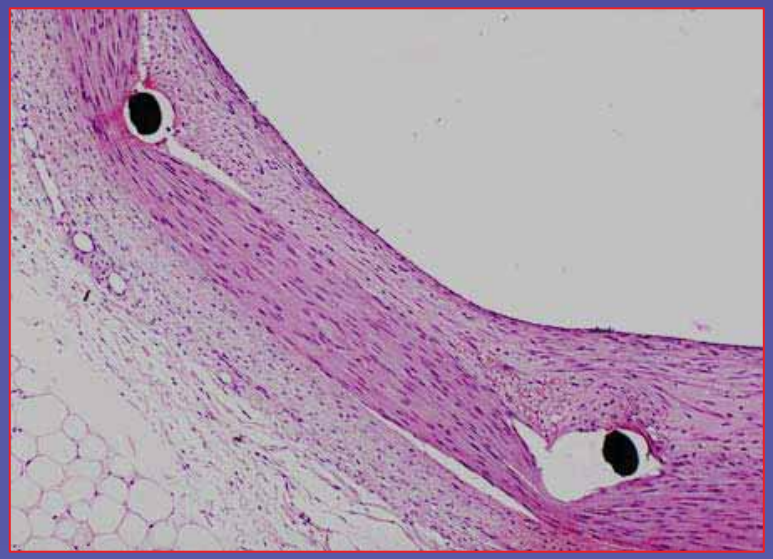
Balloon Expanded Stents in Pig Coronary Arteries

24 hours

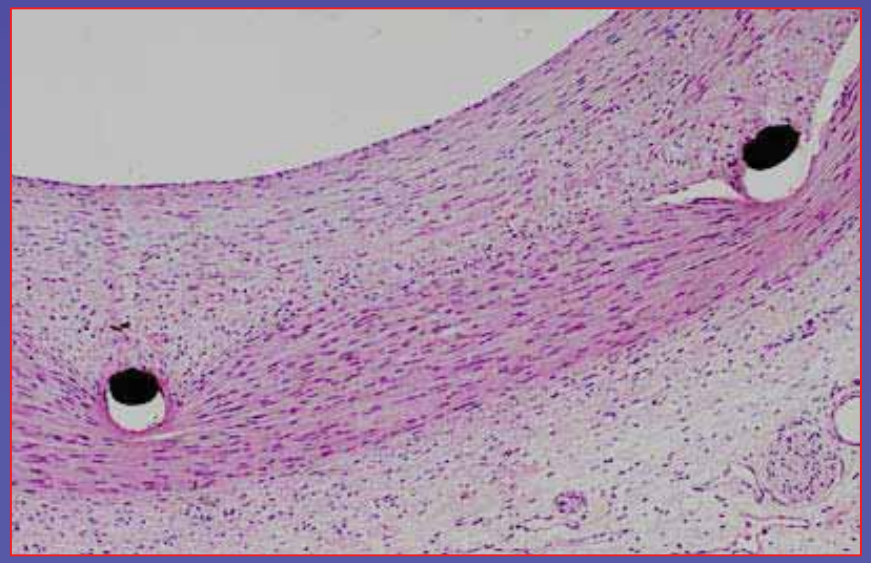


7 days



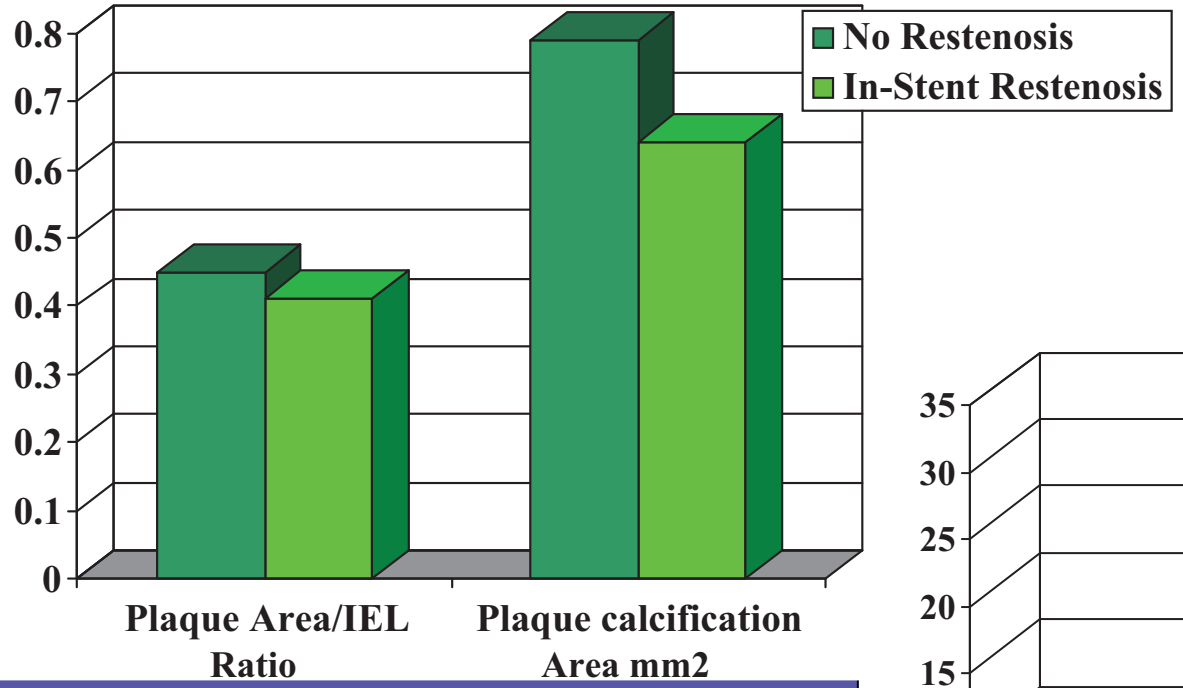


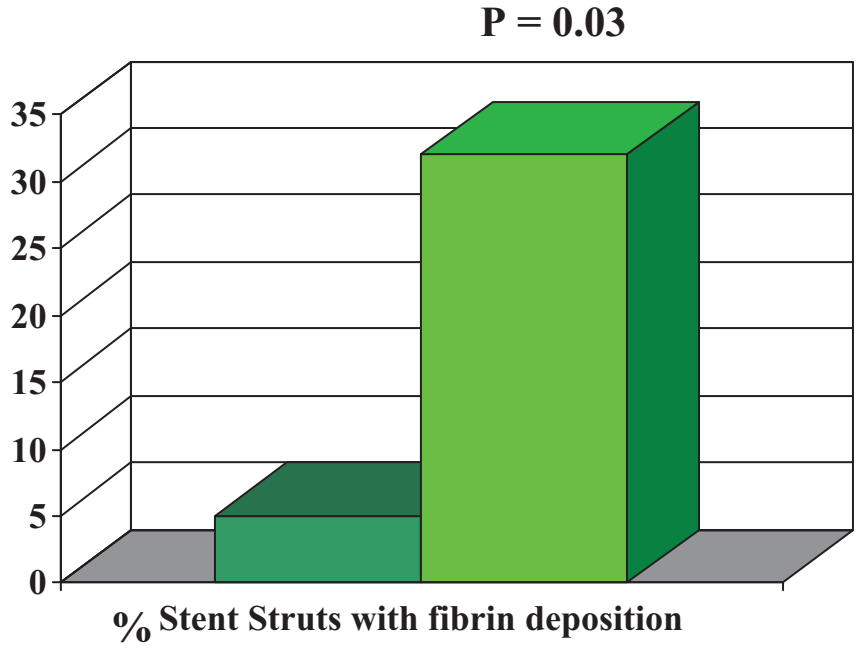
2 weeks



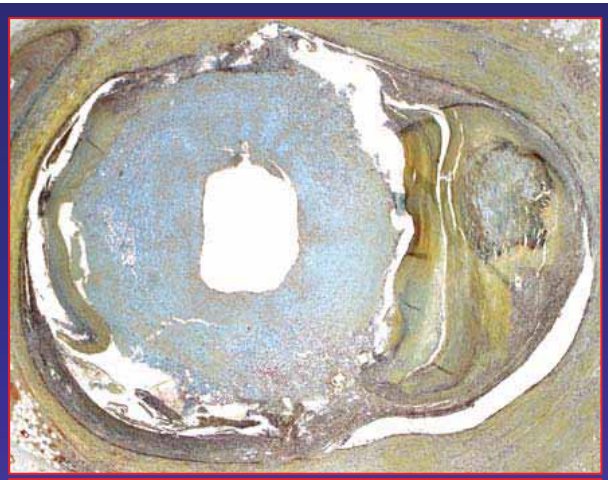
4 weeks

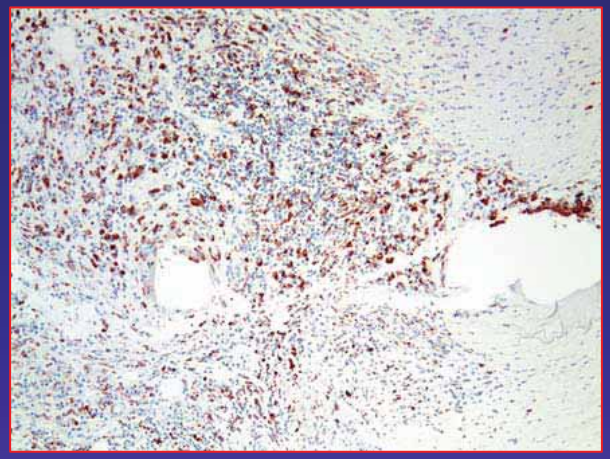
Basic Morphology: Long-Term Stent Patency Versus In-Stent Restenosis

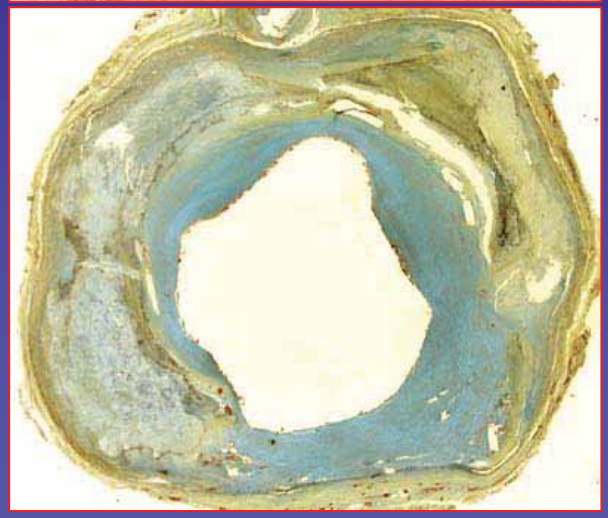


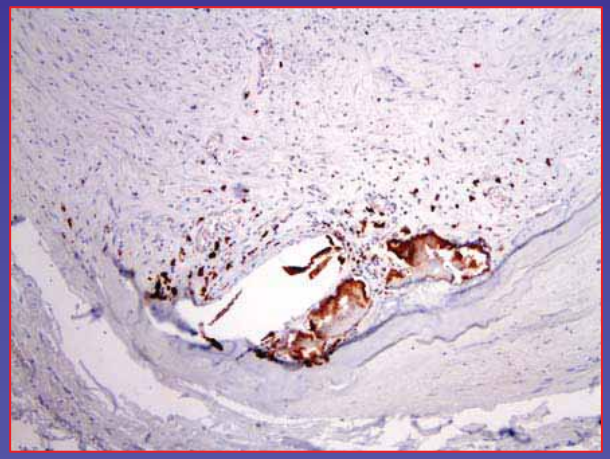


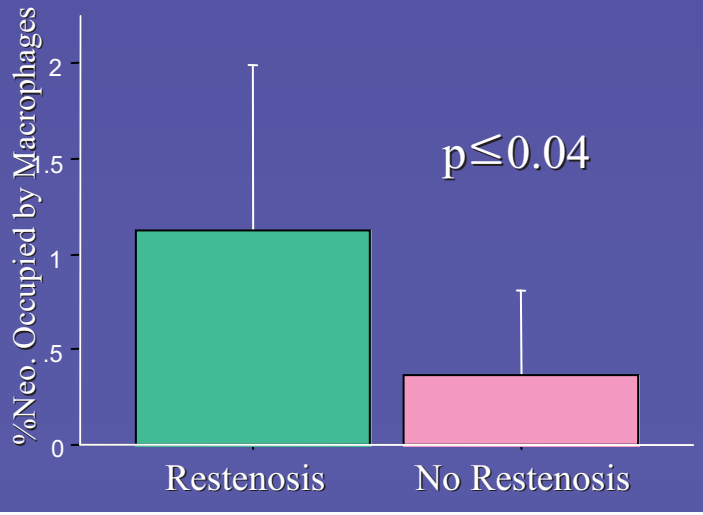
Neointimal Macrophages & Restenosis in Human Coronary Arteries











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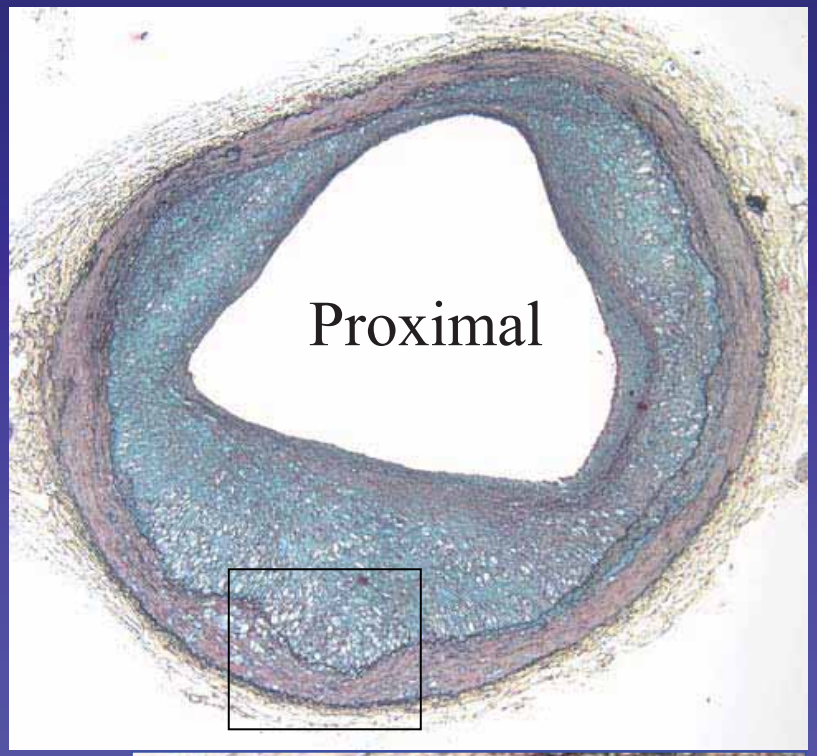
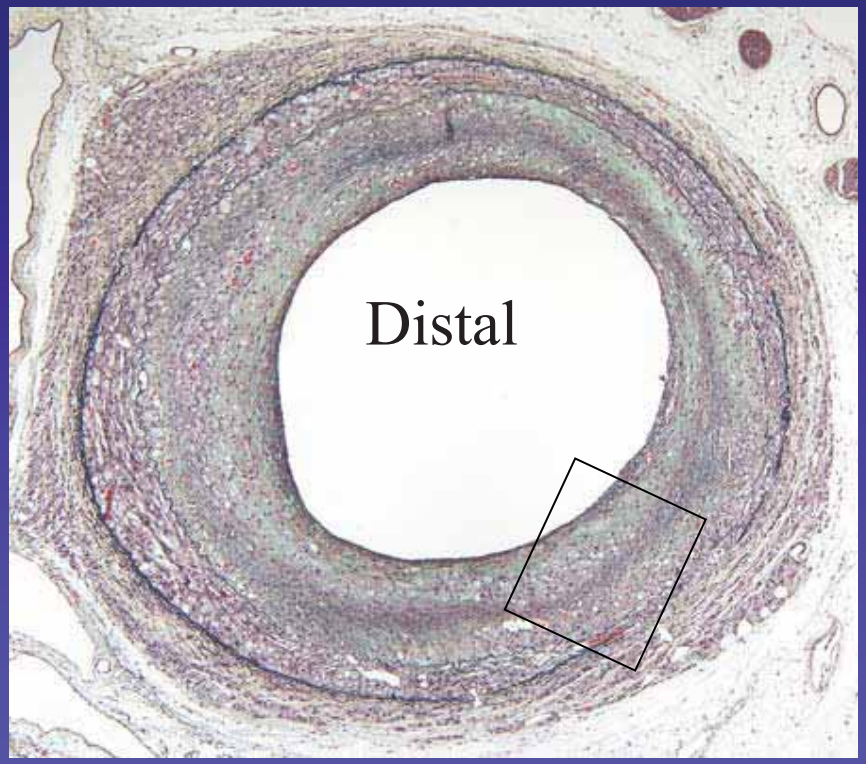
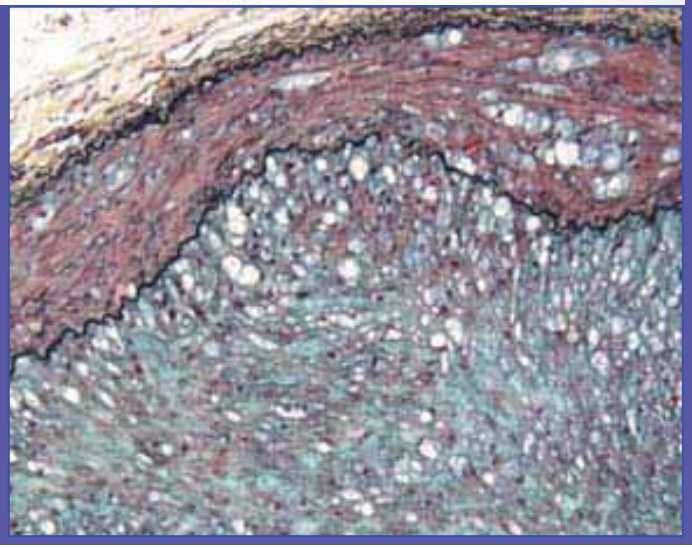
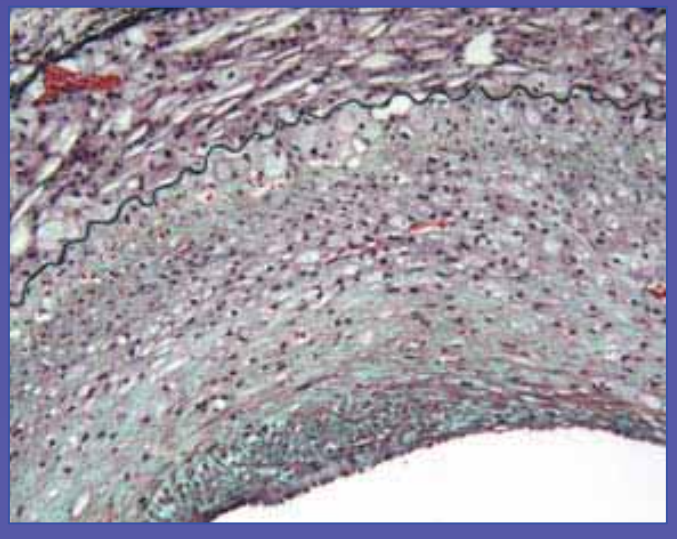
Atherosclerotic Iliac Arteries Prior to Stenting

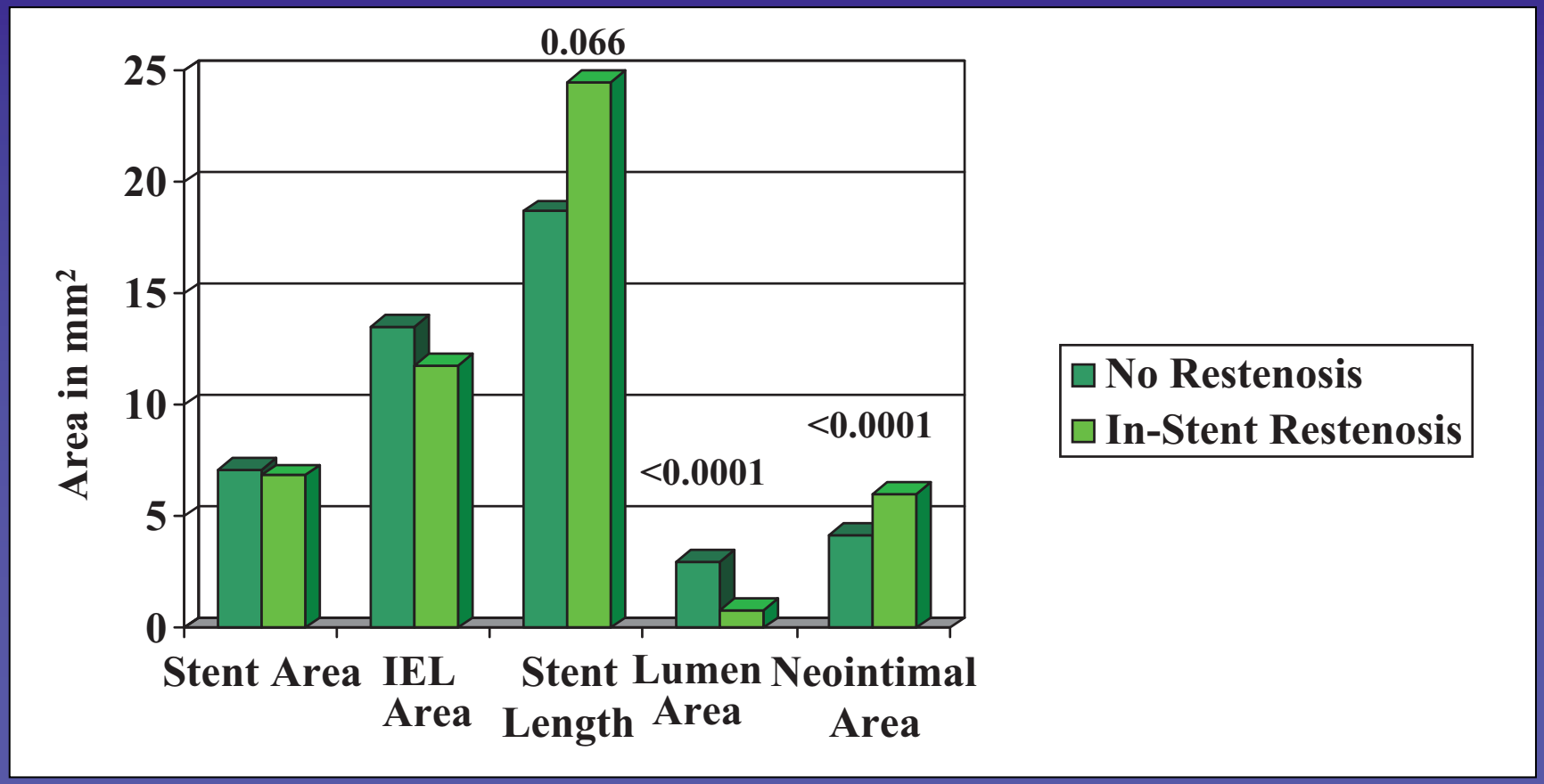








Basic Morphology: Long-Term Stent Patency Versus In-Stent Restenosis



56 individuals (mean age 59±13 years) with coronary artery stents in place for 10±7.1 months (range 3 to 36 months) with 116 stents