NEW ENGLAND CENTER FOR STROKE RESEARCH

From Bench to Brain

In Situ Tissue Engineering for Brain Aneurysms

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Director, New England Center for Stroke Research

SIMI 2016 – 25th Anniversary; Buenos Aires

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Disclosures

- Research Grants
(last 12 months):
  - NINDS, NIBIB, NIA, NCI
  - Philips Healthcare
  - MicroVention/Terumo
  - Stryker Neurovascular
  - Codman Neurovascular
  - eV3 Neurovascular / Covidien
  - InNeuroCo Inc
  - Blockade Medical
  - CereVasc LLC
  - Gentuity
  - Cook Medical
  - Neuronal Protection Systems LLC
  - Spineology Inc
  - Silk Road
  - Wyss Institute
  - Neuravi

- **Consulting**
(fee-per-hour, last 12 months):
  - **Stryker Neurovascular**
  - **Codman Neurovascular**
- **Investment (Stocks)**
  - **InNeuroCo Inc**

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Patient-Specific Hemodynamic Analysis and Treatment Efficacy (Flow Diversion)

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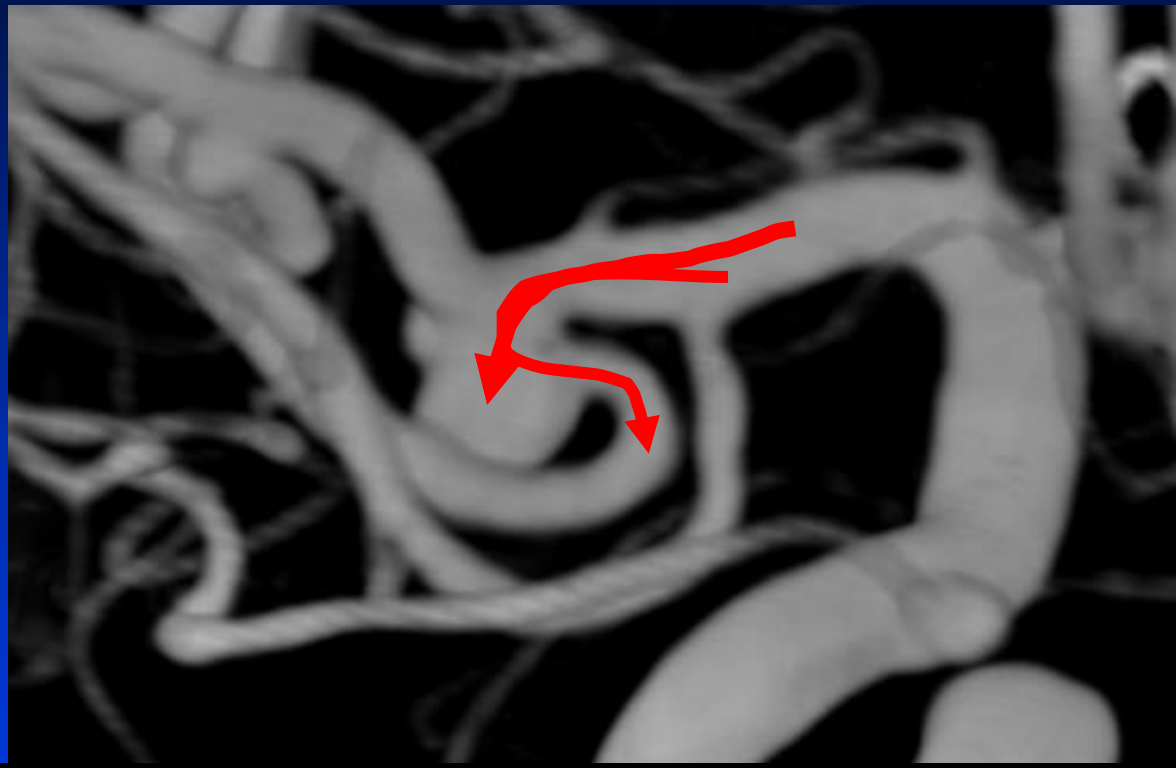
Flow Mechanics

Flow driven by ΔP

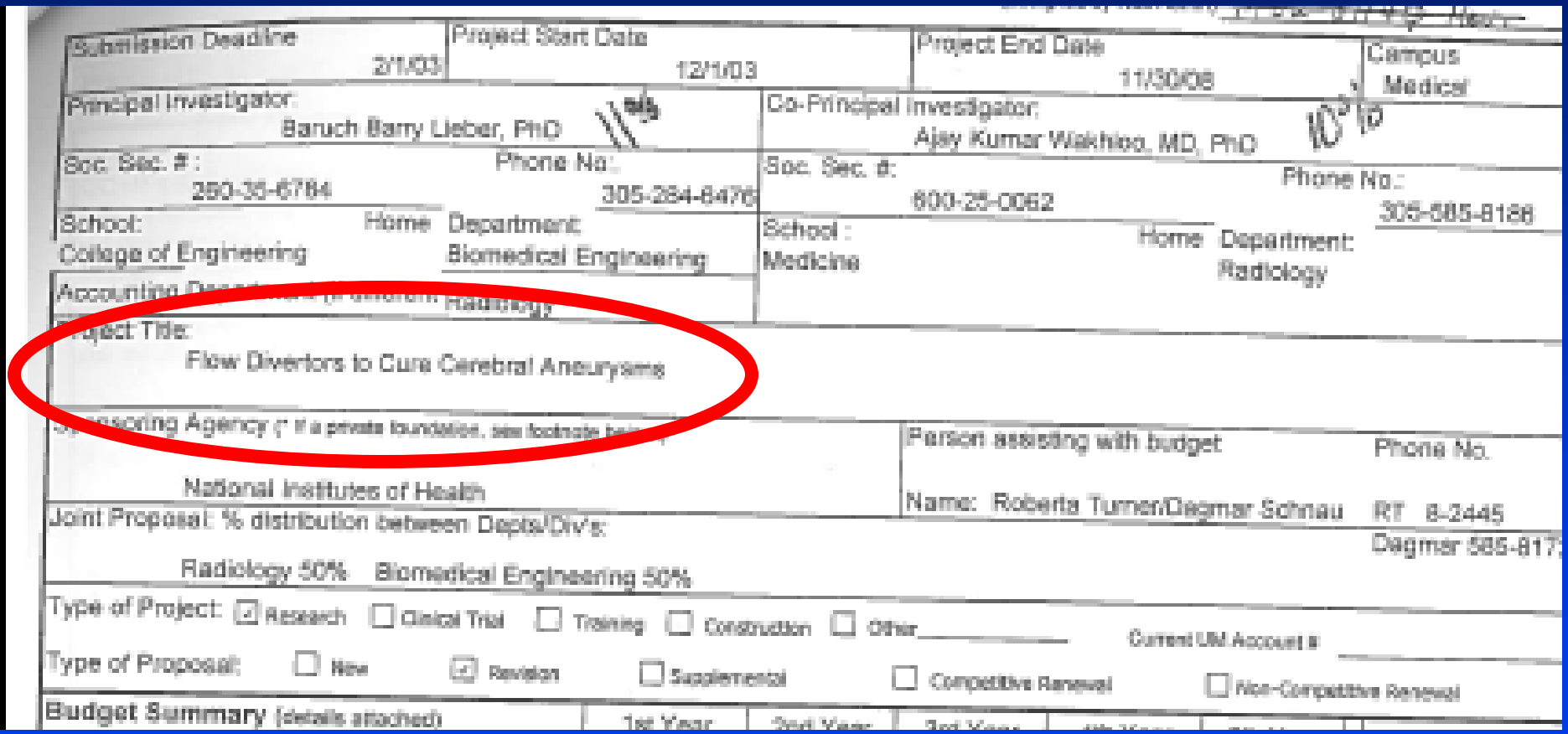
Momentum Transfer



Fundamental Goal: Design technology that can disrupt momentum transfer into the aneurysm producing exclusion from the circulation without occluding perforators/ jailed vessels

In a Word(s)…

- BETTER – in situ tissue engineering



Submission Deadline: 2/1/03
Project Start Date: 12/1/03
Project End Date: 11/30/08
Campus: Medical

Principal Investigator: Baruch Barry Lieber, PhD
Soc. Sec. # : 250-35-6784
Phone No: 305-284-8476
School: College of Engineering
Home Department: Biomedical Engineering

Co-Principal Investigator: Ajay Kumar Wakhloo, MD, PhD
Soc. Sec. #: 600-25-0062
Phone No.: 305-585-8188
School: Medicine
Home Department: Radiology

Project Title: Flow Diverters to Cure Cerebral Aneurysms

Sponsoring Agency (* If a private foundation, see footnote below.): National Institutes of Health

Person assisting with budget: Name: Roberta Turner/Dagmar Schnau
Phone No.: RT 8-2445 Dagmar 585-817

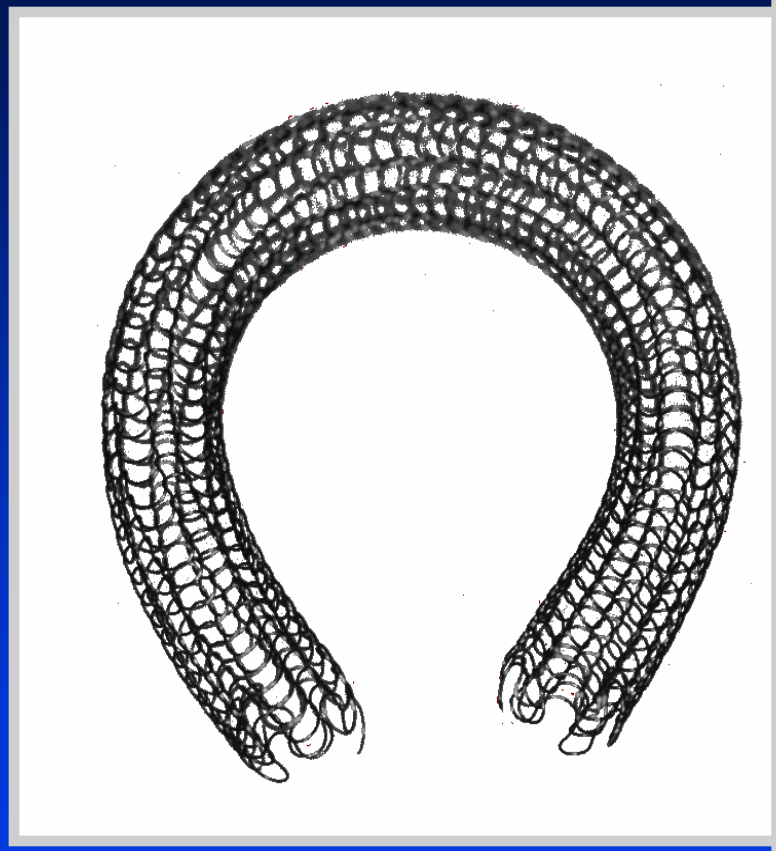
Joint Proposal: % distribution between Depts/Div's: Radiology 50% Biomedical Engineering 50%

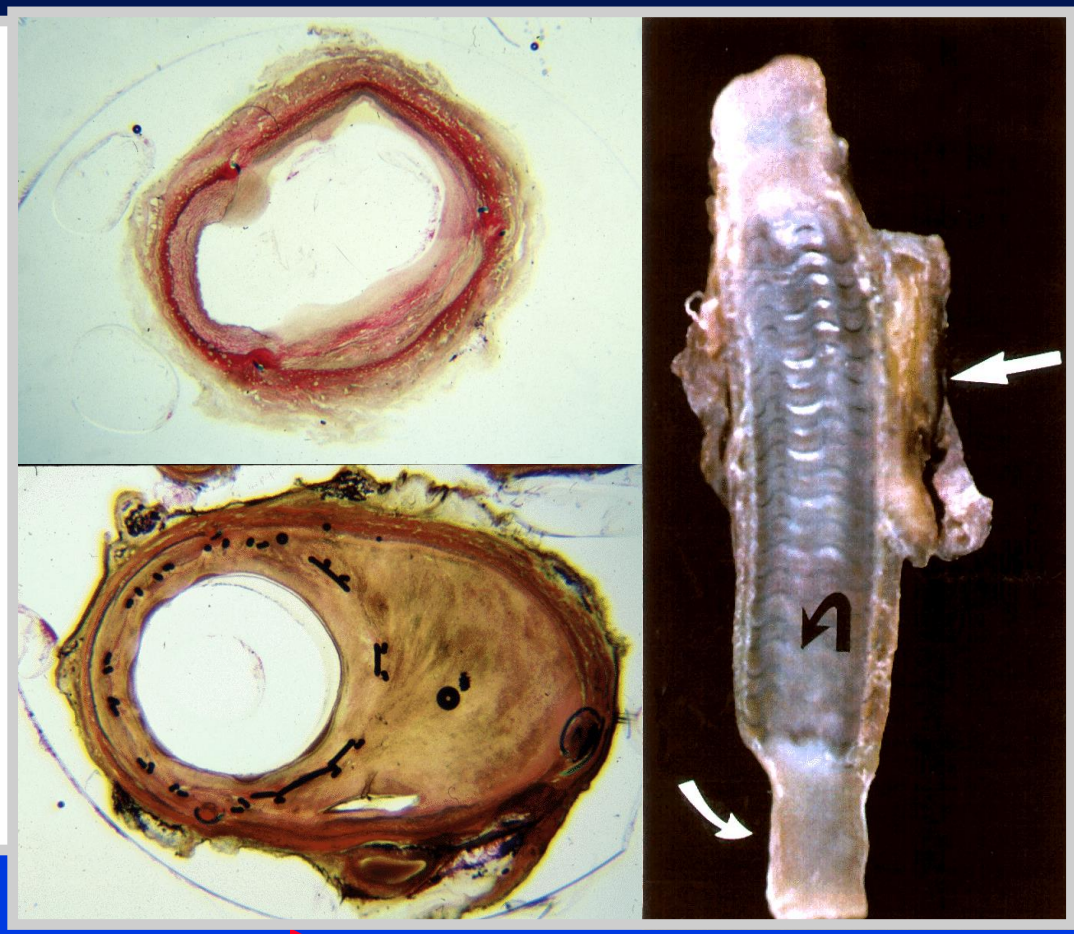
Type of Project: ☑ Research ☐ Clinical Trial ☐ Training ☐ Construction ☐ Other _____ Current UM Account #

Type of Proposal: ☐ New ☑ Revision ☐ Supplemental ☐ Competitive Renewal ☐ Non-Competitive Renewal

Budget Summary (details attached) 1st Year 2nd Year 3rd Year

The Observation





Neuroradiology 1992, AJNR 1994, 1995

Step 2: Tissue Engineering

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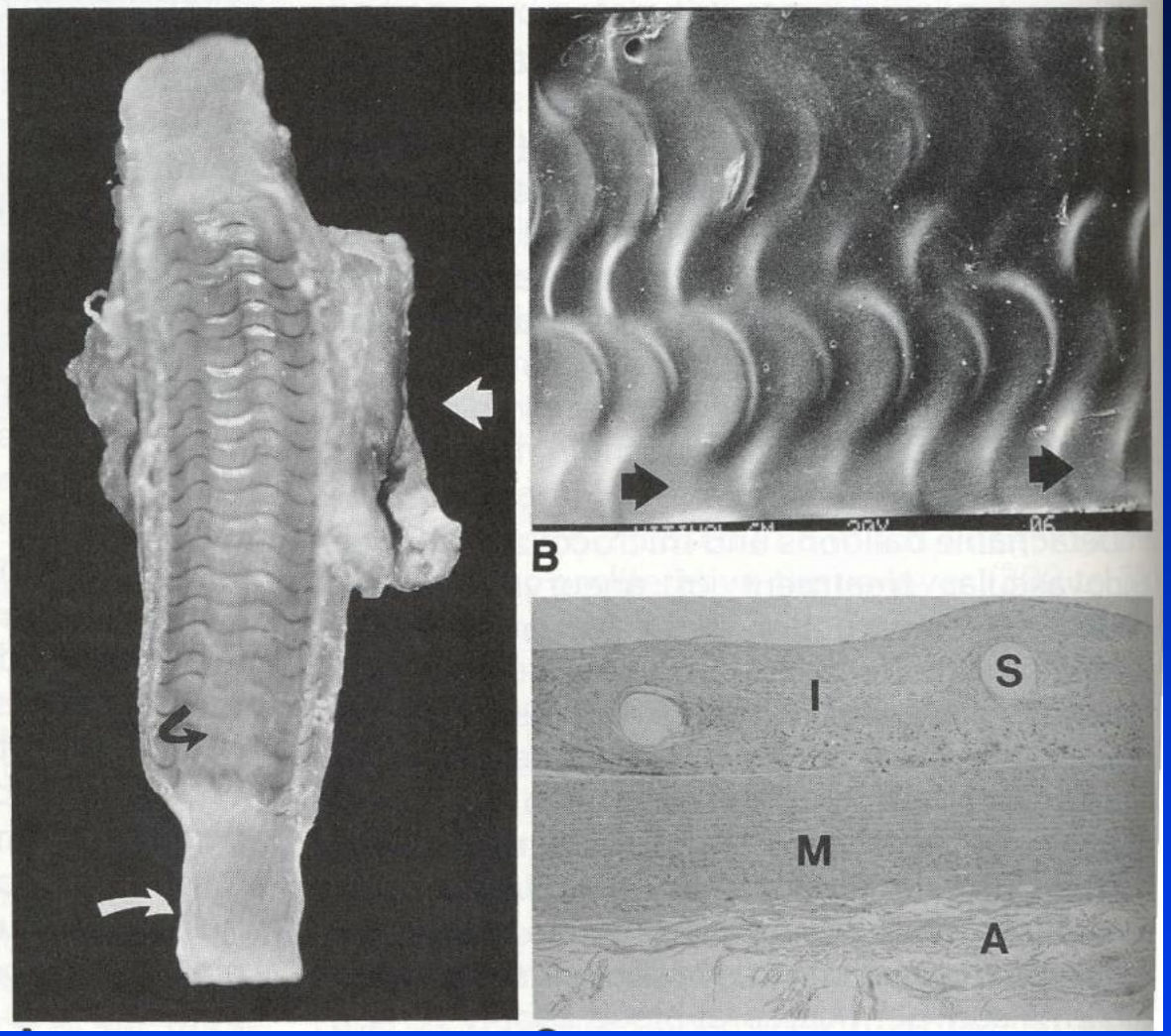
Fig. 7. Longitudinal section of a formaldehyde-fixated common carotid artery with thrombosed and organized lateral aneurysm (*short arrow*). The vessel (original diameter approximately 3.8 mm) was harvested 6 months after implantation of a heat-treated self-expanding nitinol stent (5-mm diameter in fully expanded state). A thin intimal fibrocellular tissue is covering the struts (*curved black arrow*). There is a markedly thickened vessel wall in the stented portion including the ostium of the aneurysm because of intimal proliferation. (Note the artificial reduction of the nonstented vessel segment after resection and fixation compared with the treated rigid part, *curved white arrow*).

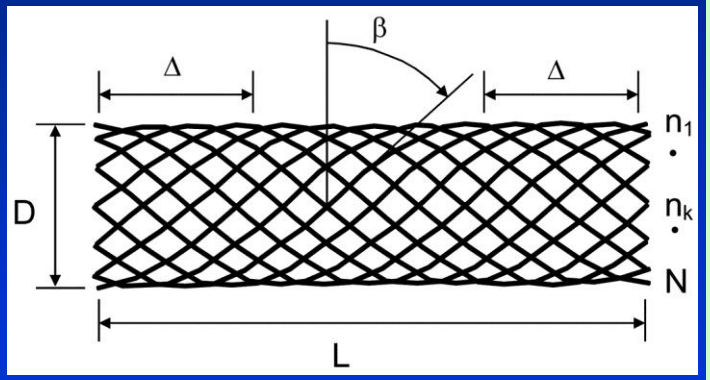
B, Scanning electron photomicrograph of a carotid artery harvested 6 months after nitinol stent placement demonstrates the flow-induced macroscopic architecture of the neointima (original magnification ×20).

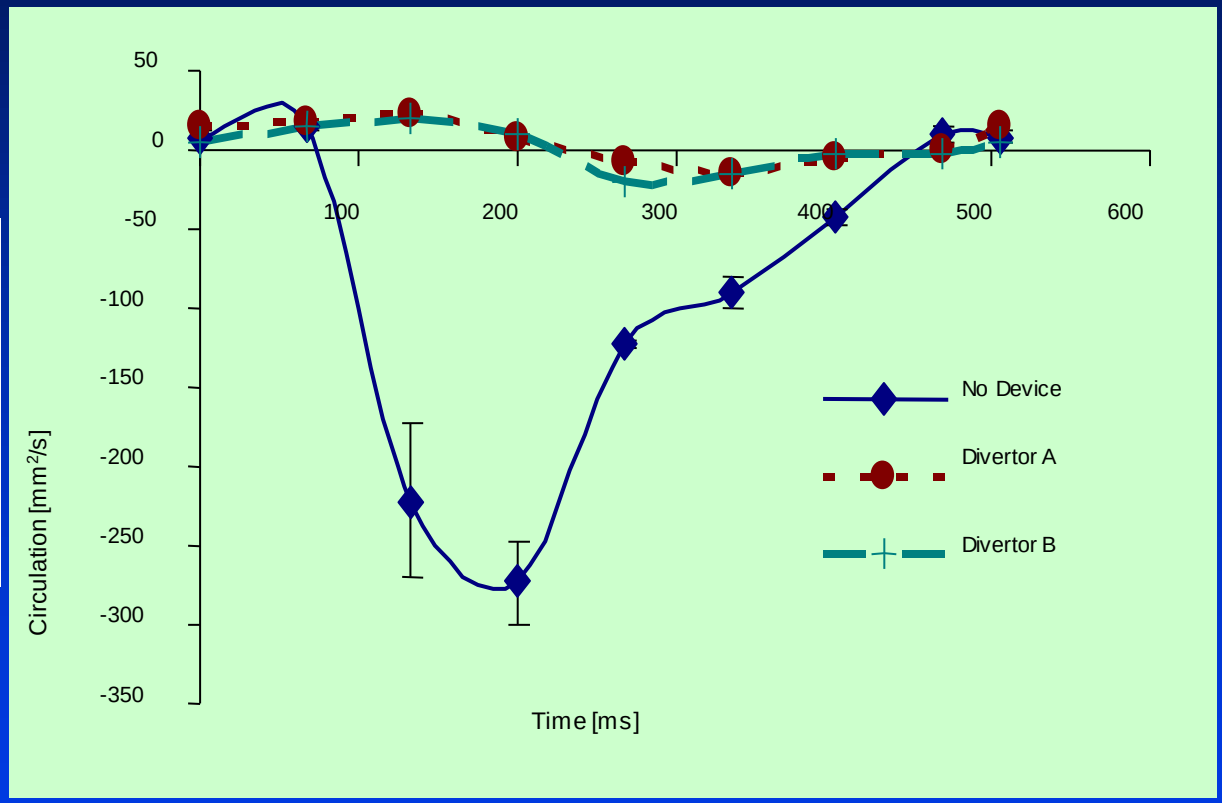
C, Transverse section of a common carotid artery 6 months after implantation of a nitinol stent (hematoxylin and eosin stain, original magnification ×25). I indicates intima; M, media; A, adventitia; S, empty space corresponding to stent filaments. Thickness of intima covering the filaments is approximately 80 μm and between the



Wakhloo et al. AJNR 1994

Mean Circulation: Function of FD Design





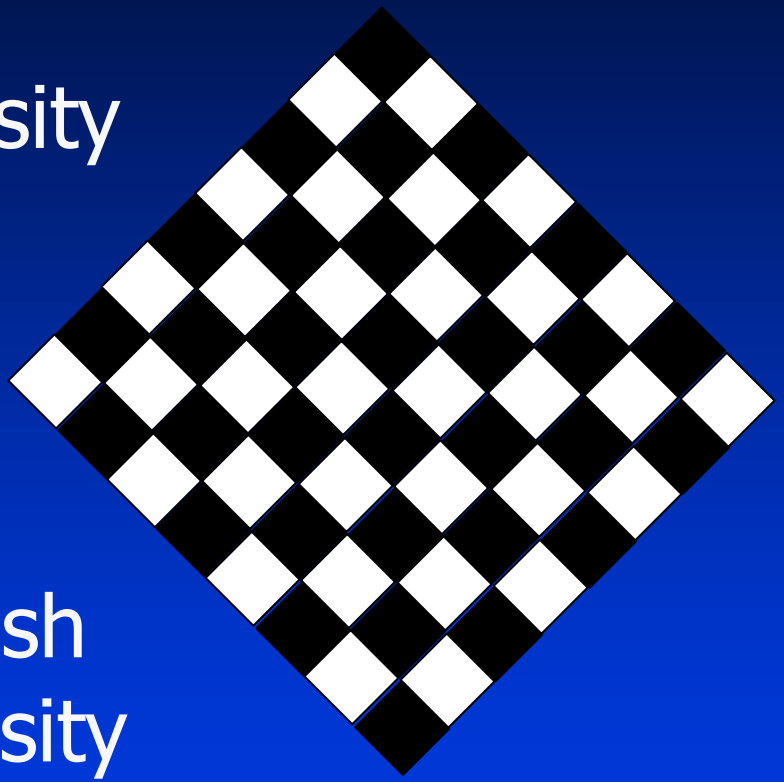
Sadasivan and Lieber, Stroke 2010

Seong, Lieber, Wakhloo. J Biomech Eng 2007

Porosity and Mesh Density

Porosity

=



Mesh Density

<

50% Metal Coverage
2 pores per diamond

50% Metal Coverage
32 pores per diamond

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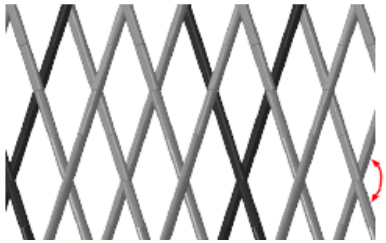
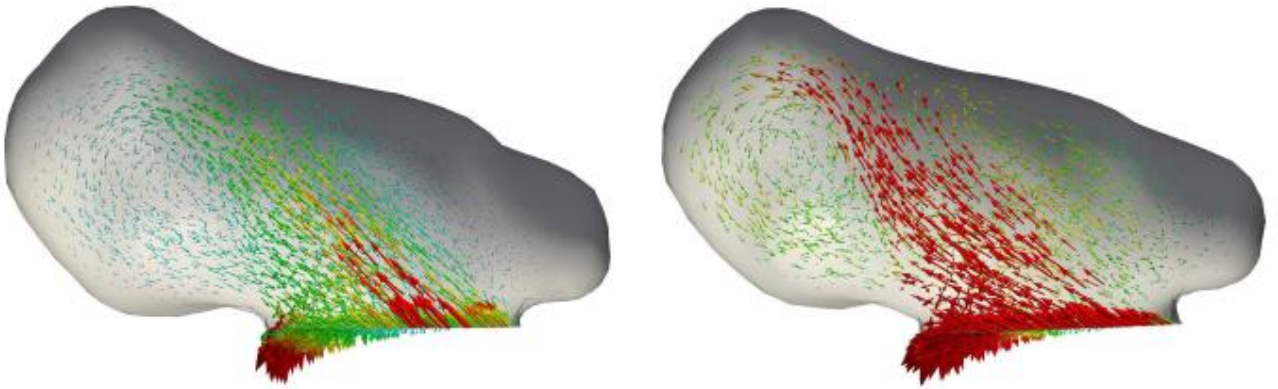
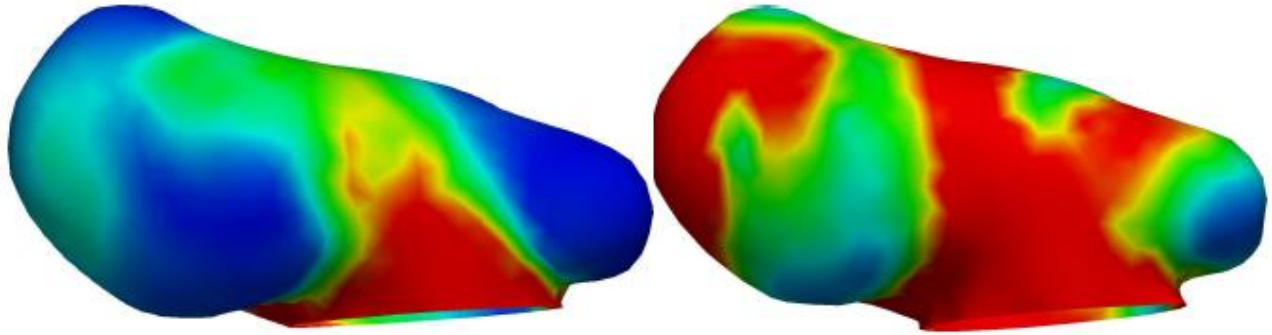




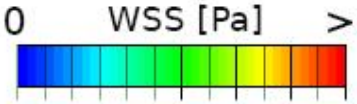
96 wires

48 wires



96 wires

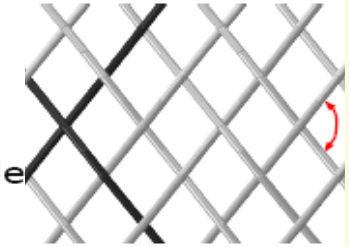
48 wires



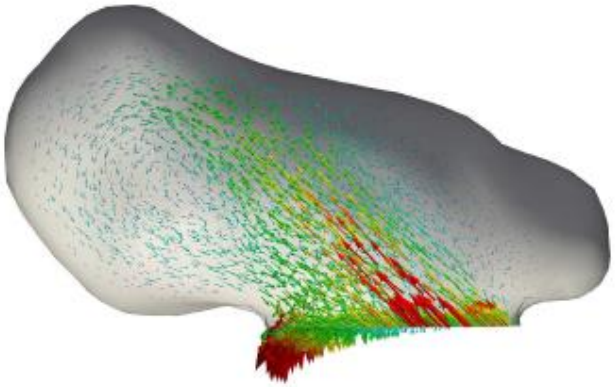
Courtesy of Matthieu De Beule, FEops

Braid angle
109°

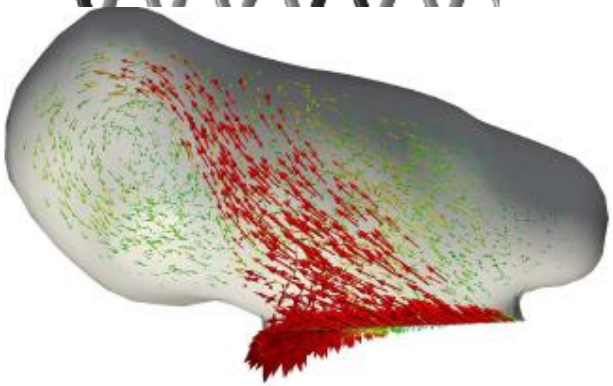
Braid angle
150°



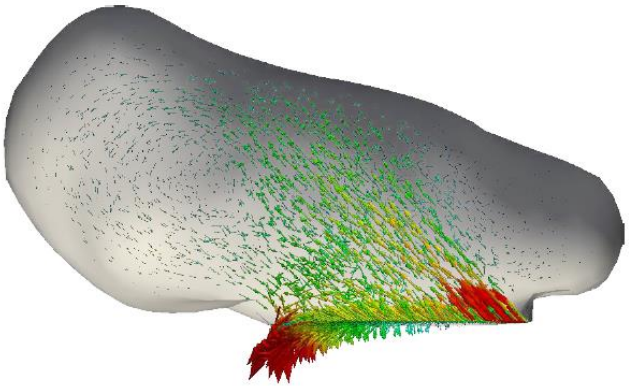
Braid angle 115°



96 wires



48 wires

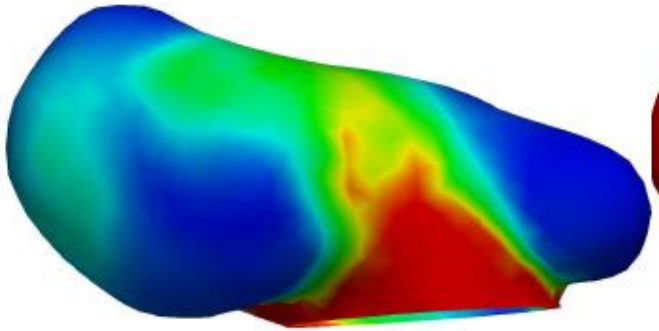


Velocity vectors

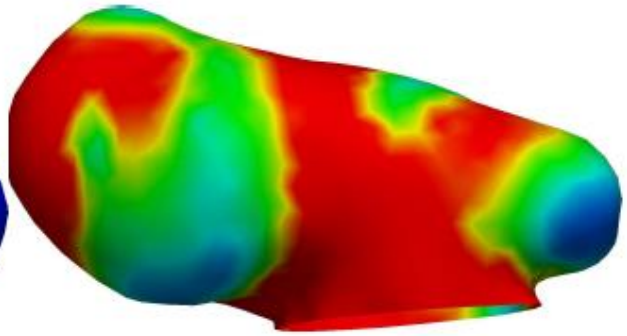
0 Velocity [m/s] >0.25

0 Velocity [m/s] >0.25

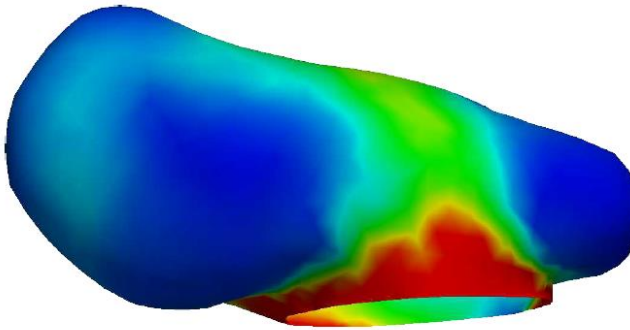
72 wires



96 wires



48 wires



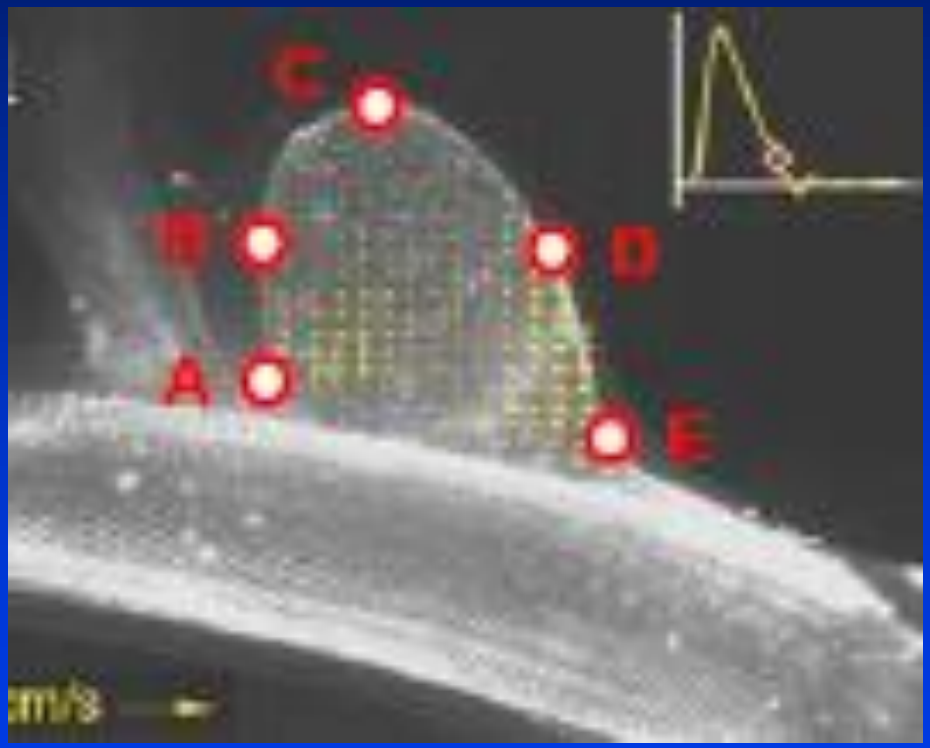
0 WSS [Pa] >2

0 WSS [Pa] >2

72 wires

Courtesy of Matthieu De Beule, FEops

Mean Circulation: Function of FD Design



Seong, Lieber, Wakhloo. J Biomech Eng 2007

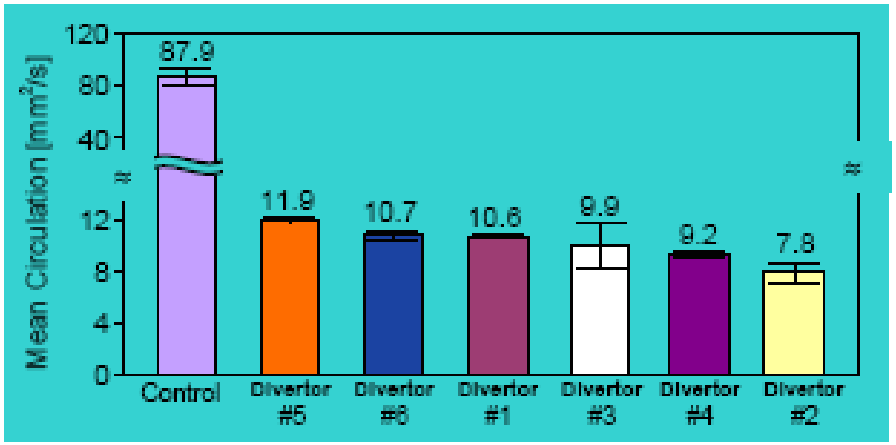


Figure 3. Mean hydrodynamic circulation inside the aneurysm before/after implantation of flow divertors.

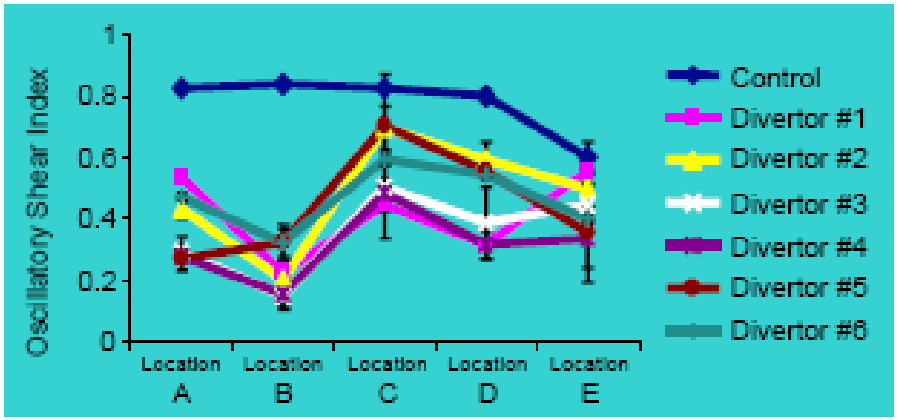
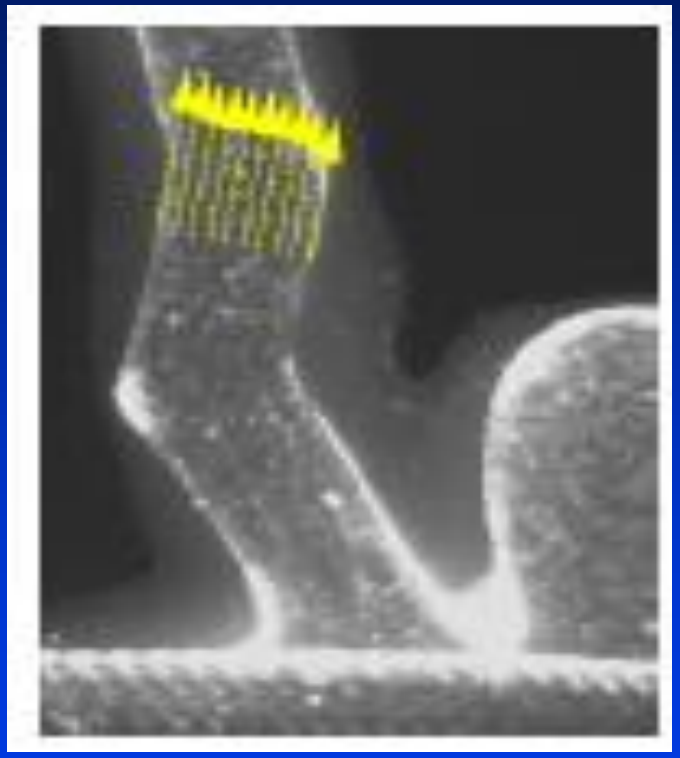


Figure 4. Oscillatory shear index (OSI) at five locations along the wall of the aneurysm sac for all divertors. Error bars denote standard error of mean (n=3).

Fate of Perforators/ Jailed Arteries



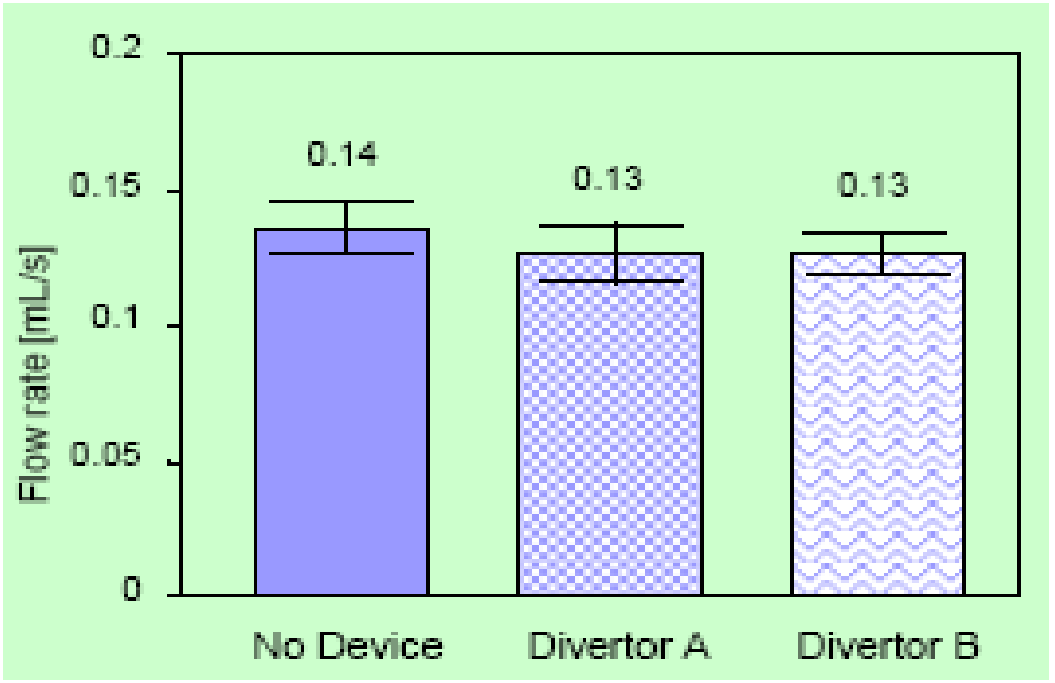


Figure 11: Mean flow rate in the vertebral artery before and after implantation of flow divertors.

Seong, Lieber, Wakhloo. J Biomech Eng 2007

Do Engineering Models Translate to In Vivo

FD – Not Really About Flow…

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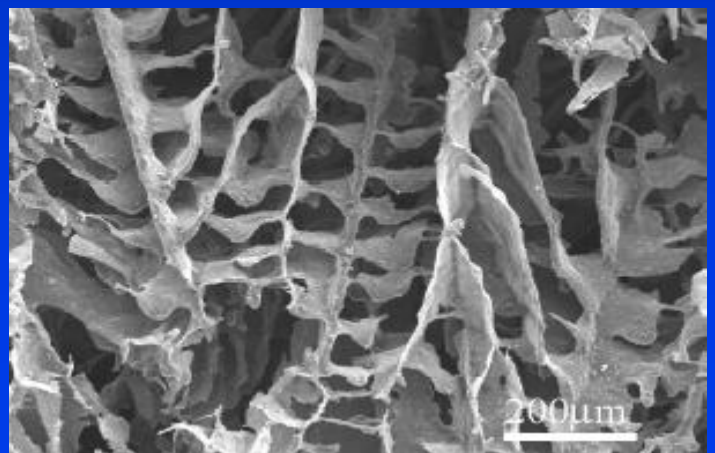
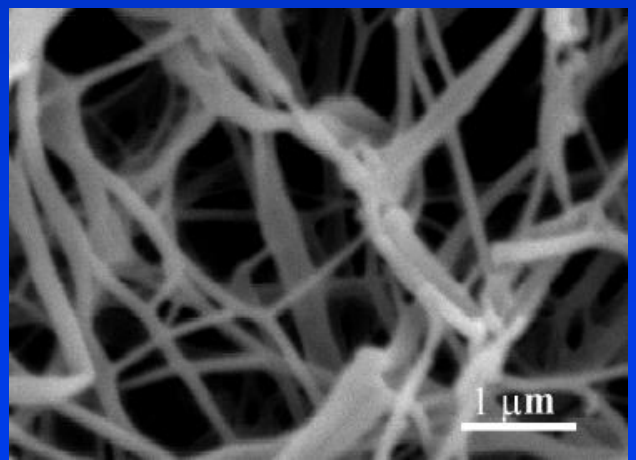
- **Hypothesis: FDs with high/uniform pore density accelerate cell growth (formation of the neointima).**
- **Goal: to demonstrate formation of the basement membrane and subsequent endothelization rates after FD implant**





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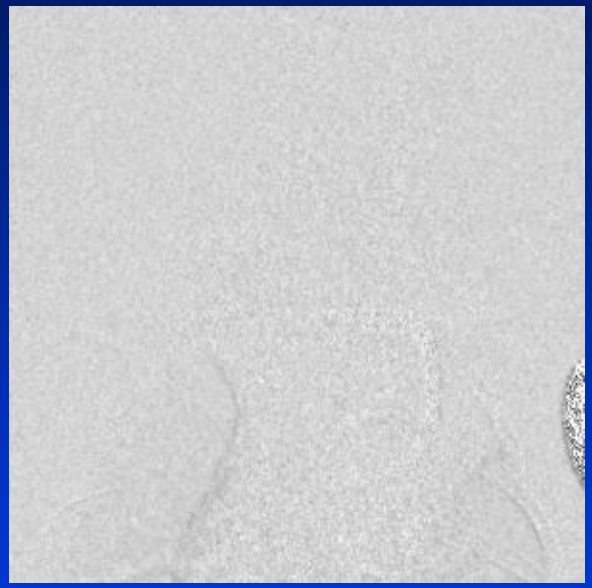
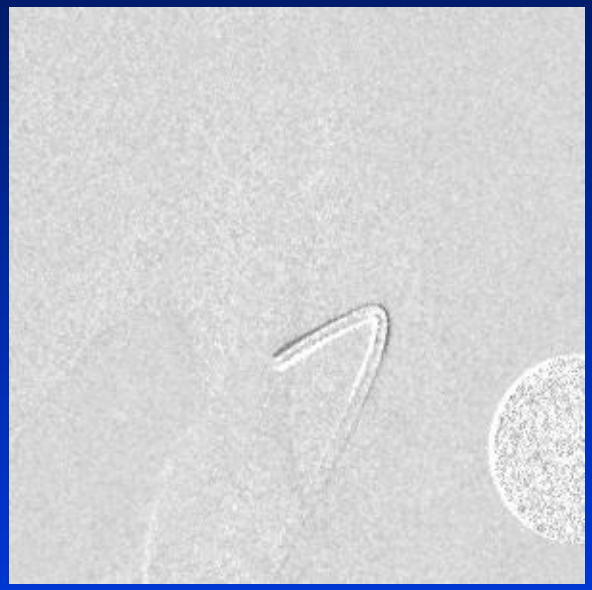
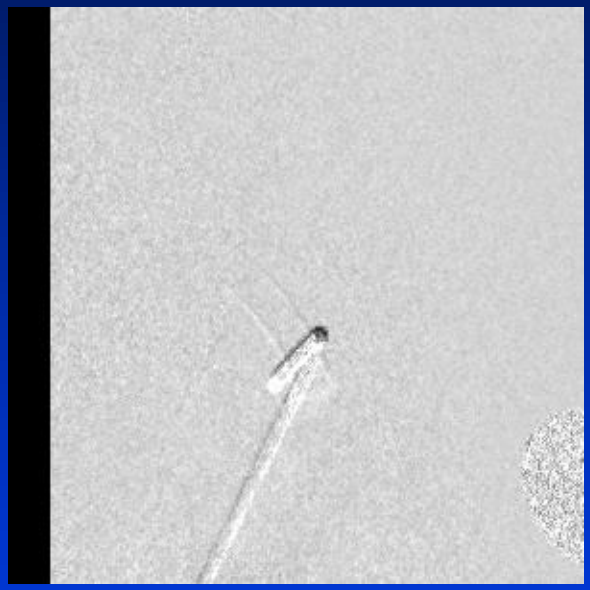
Rabbit Aneurysm Model



Pre



Post



FU – 1 wk

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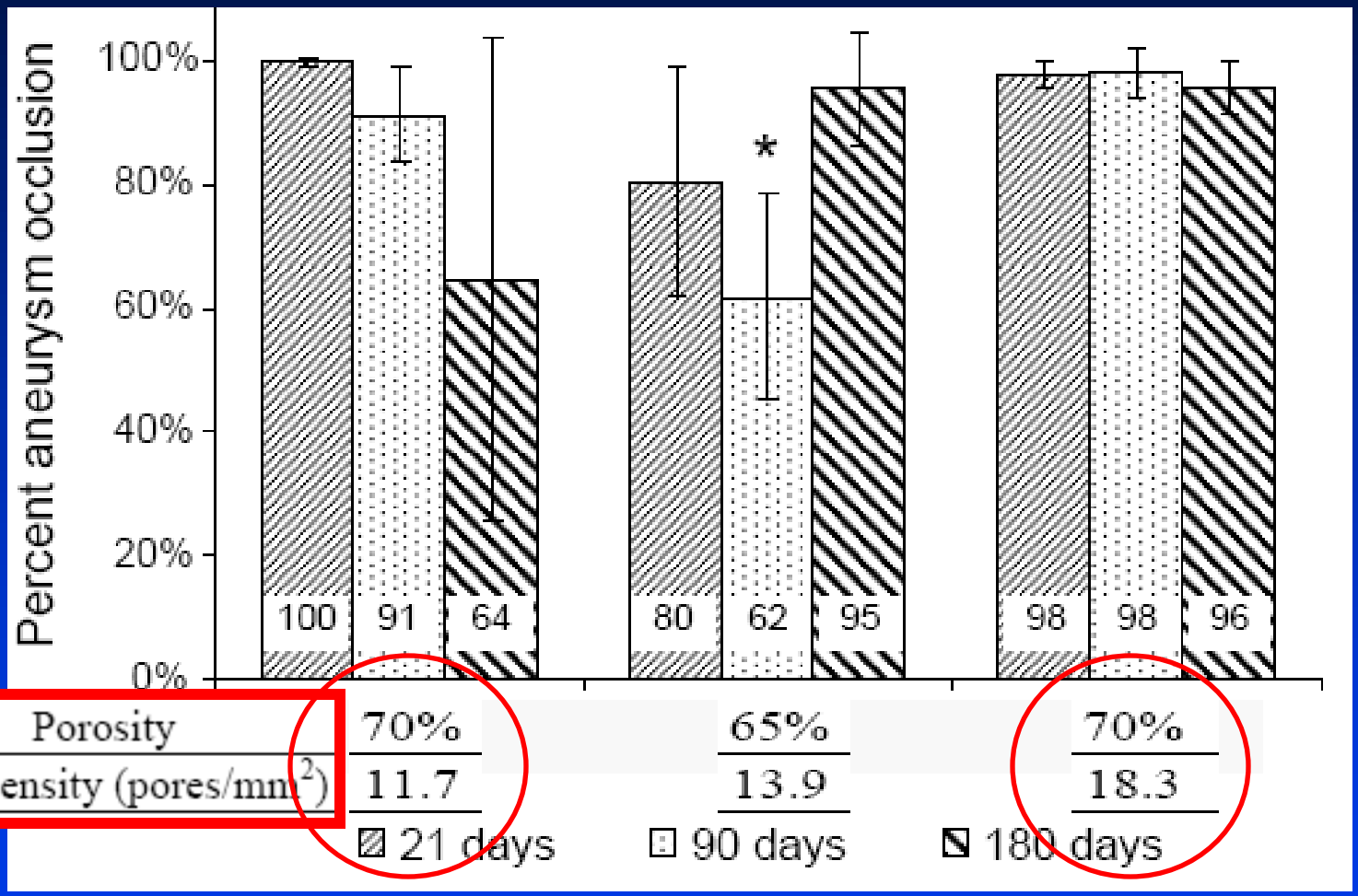
Angiographic Aneurysm Occlusion at Different Time Points



Sadasivan, Cesar, Seong, Rakian, Hao, Tio, Wakhloo, Lieber Stroke 2009

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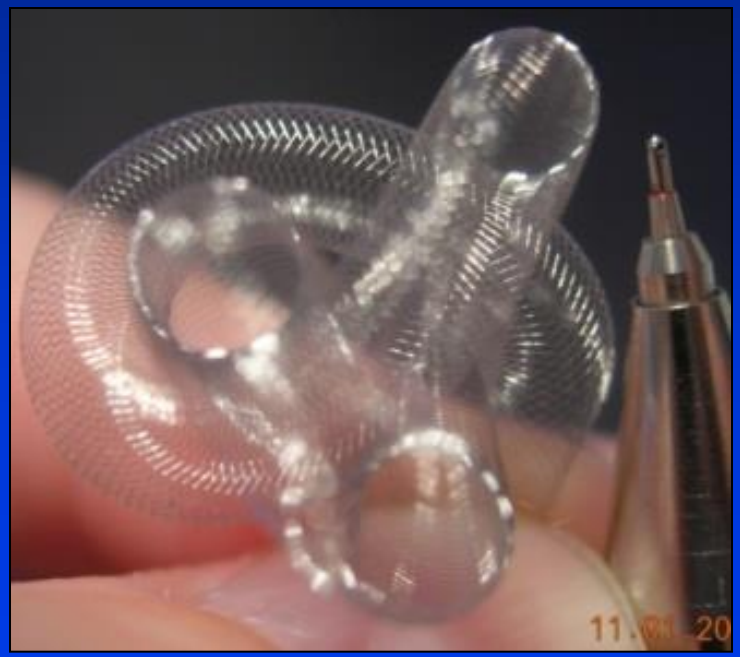
Tissue Engineering: A Function of FD Design?



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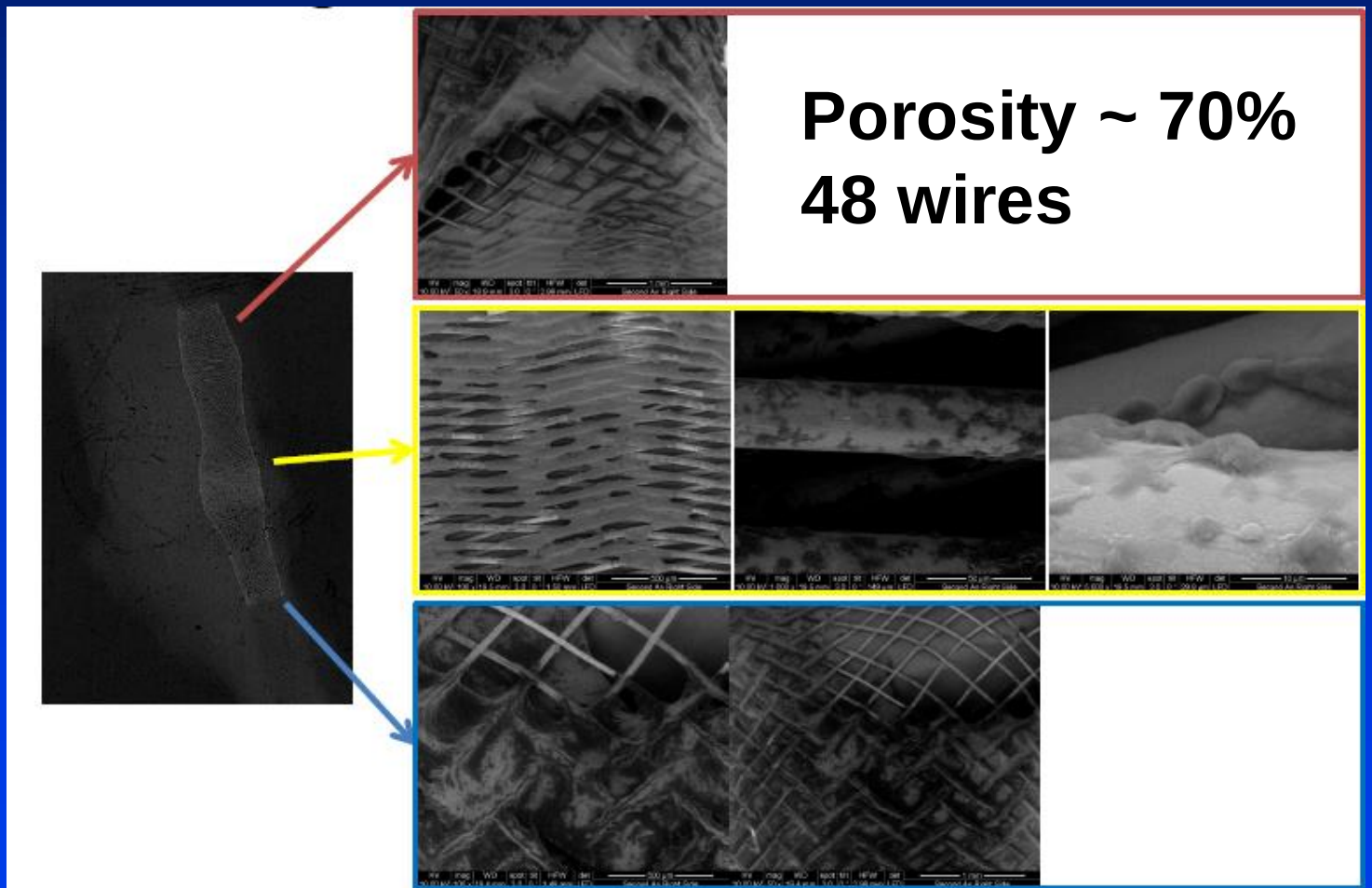
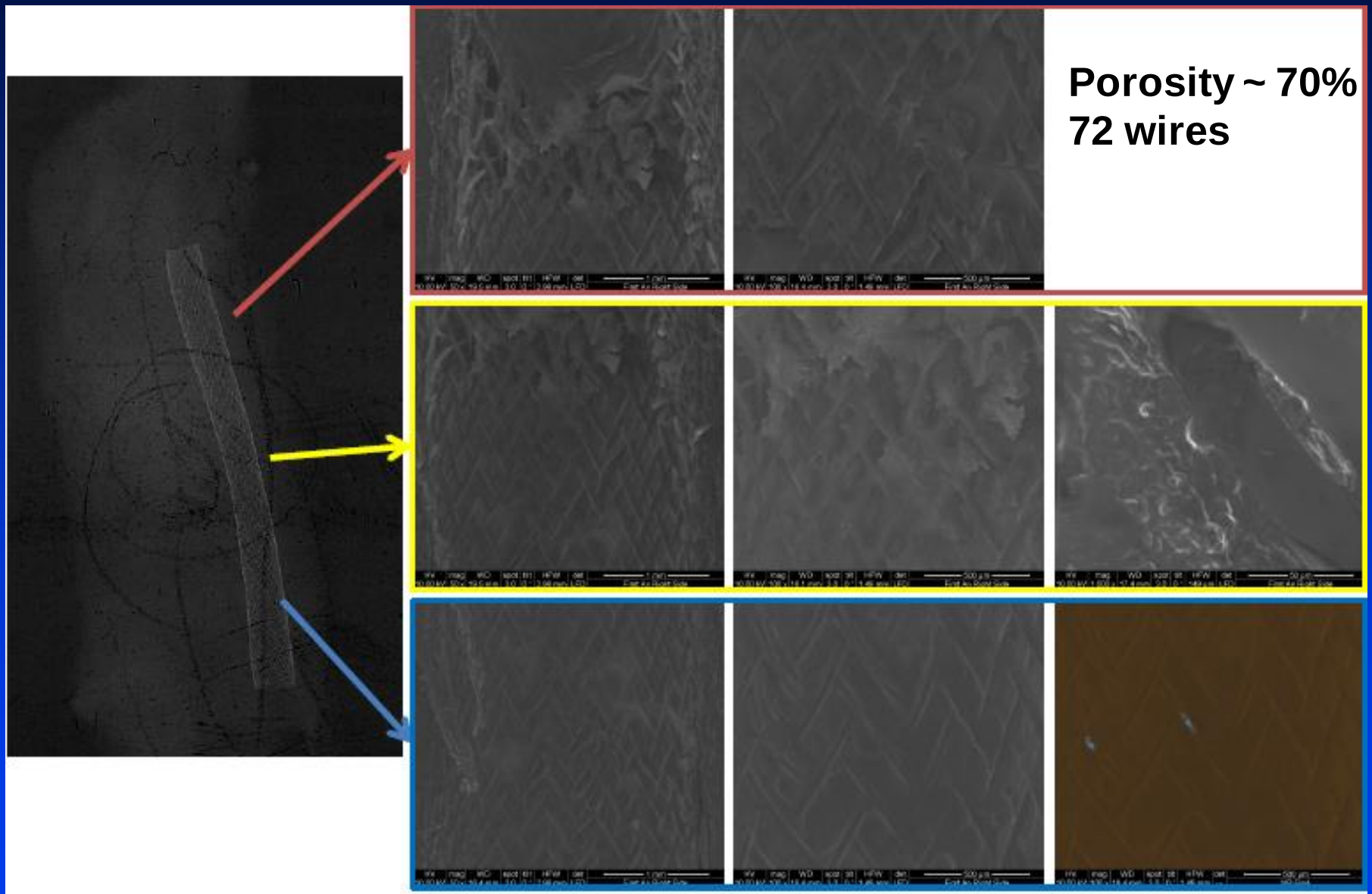
In Situ Tissue Engineering

- Canine, side-wall aneurysm – ***7 days*** post FD implant



In Situ Tissue Engineering



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In Situ Tissue Engineering

- **The objective of this study:**
  - to demonstrate formation of the basement membrane and subsequent endothelialzation rates after flow diverter stent implant

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Methods

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Rabbit Elastase Induced Aneurysm Model

- 24 extracranial (innominate artery) aneurysm
  - **Efficacy:**
    FD endothelial coverage – histology, SEM
    aneurysm occlusion rate – DSA, MR
  - **Safety (complications)**
    local: FD occlusion, stenosis
- 2 different type of FD:
  - 48-Wire Device
  - 72-Wire Device
- Periprocedural medication (based on literature review)
  - 10mg/kg clopidogrel and
  - 1mg/kg ASA

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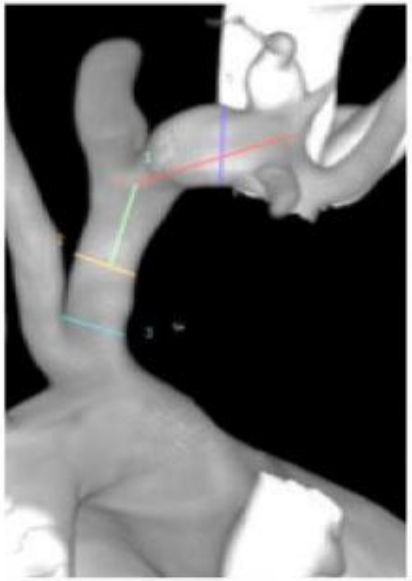
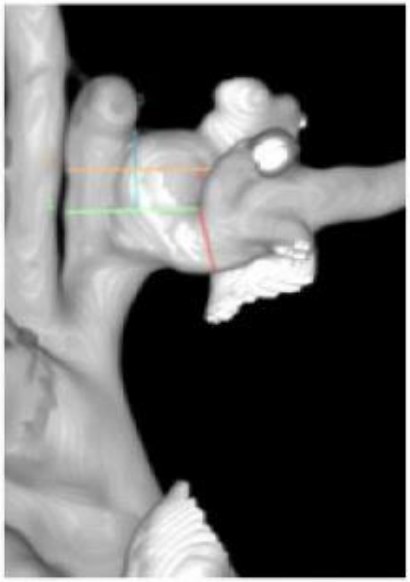
Study Design

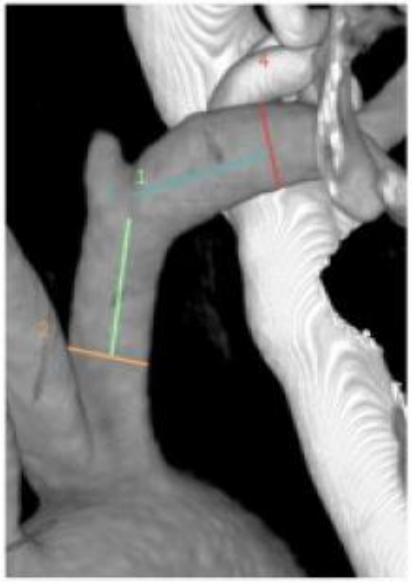
Animal grouping	Number of 72-wire FDs	Number 48-Wire FDs	FD implant procedure	Duration
Group 1	2	2	4	**10 (± 1) days**
Group 2	2	2	4	**20 (± 2) days**
Group 3	2	2	4	**30 (± 2) days**
Group 4	2	2	4	**60 (± 2) days**
Totals	8	8	16	



wide neck aneurysm along with distal fusiform vessel



complex, multilobular aneurysm

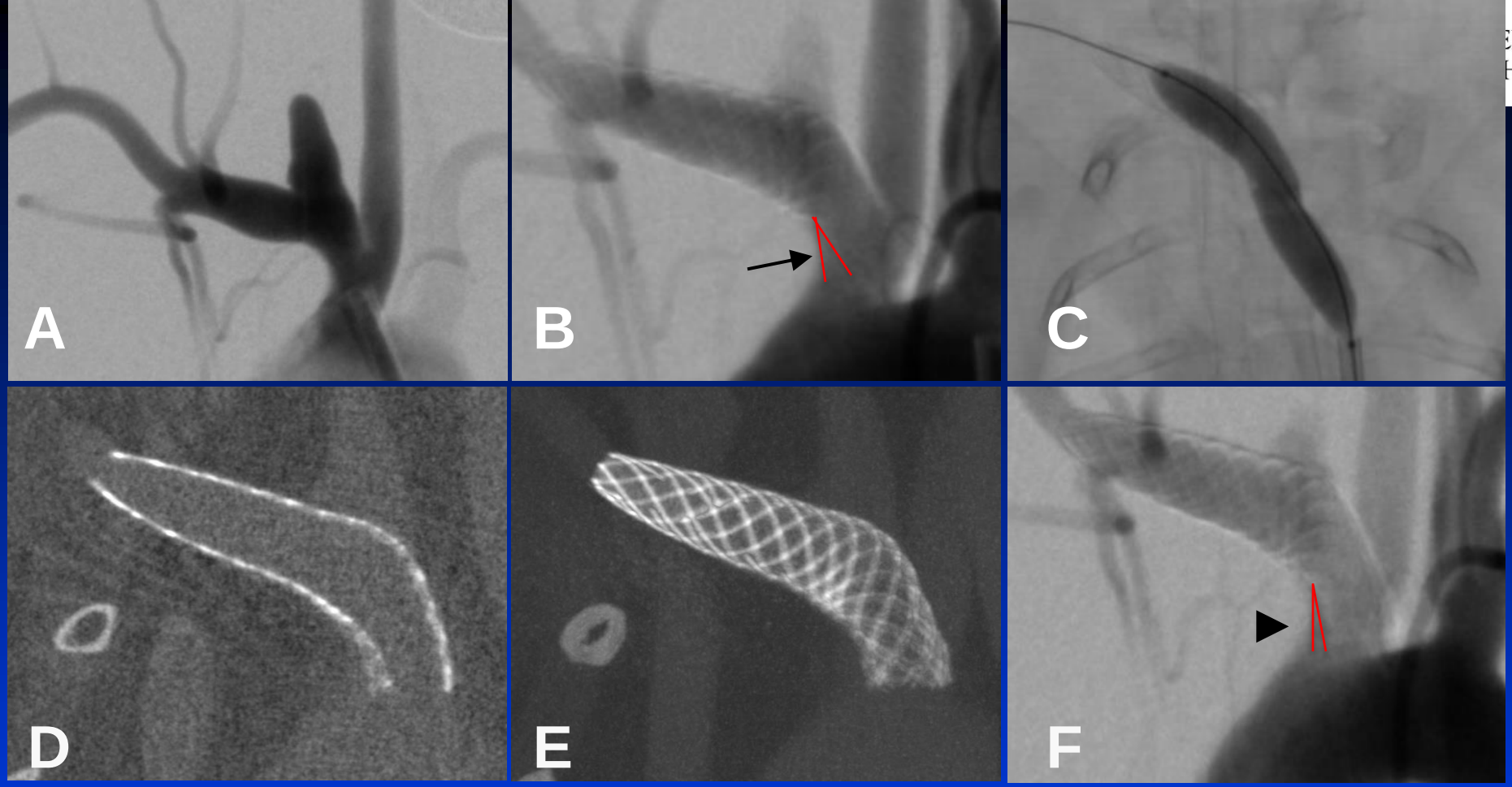


small aneurysm with wide neck and distal fusiform vessel

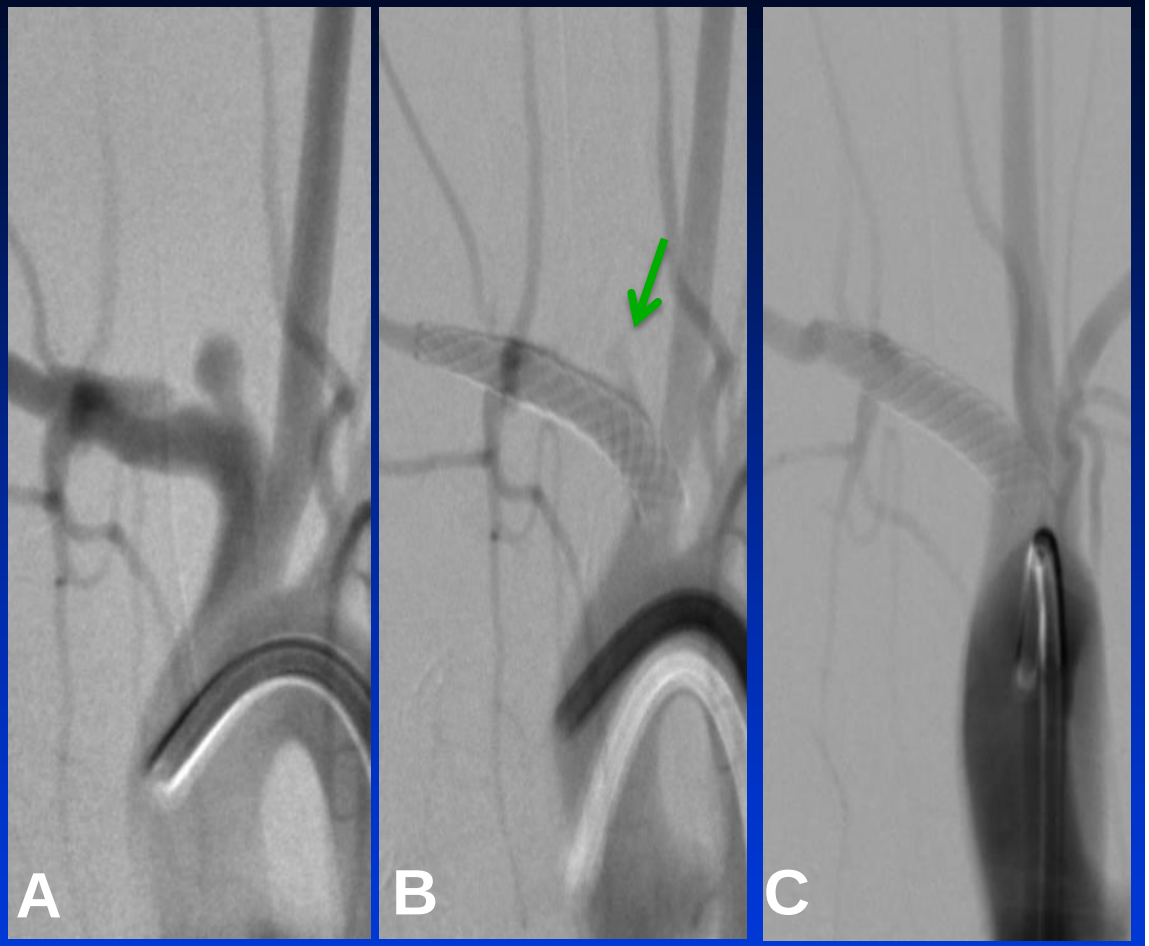
small neck aneurysm along with distal fusiform vessel

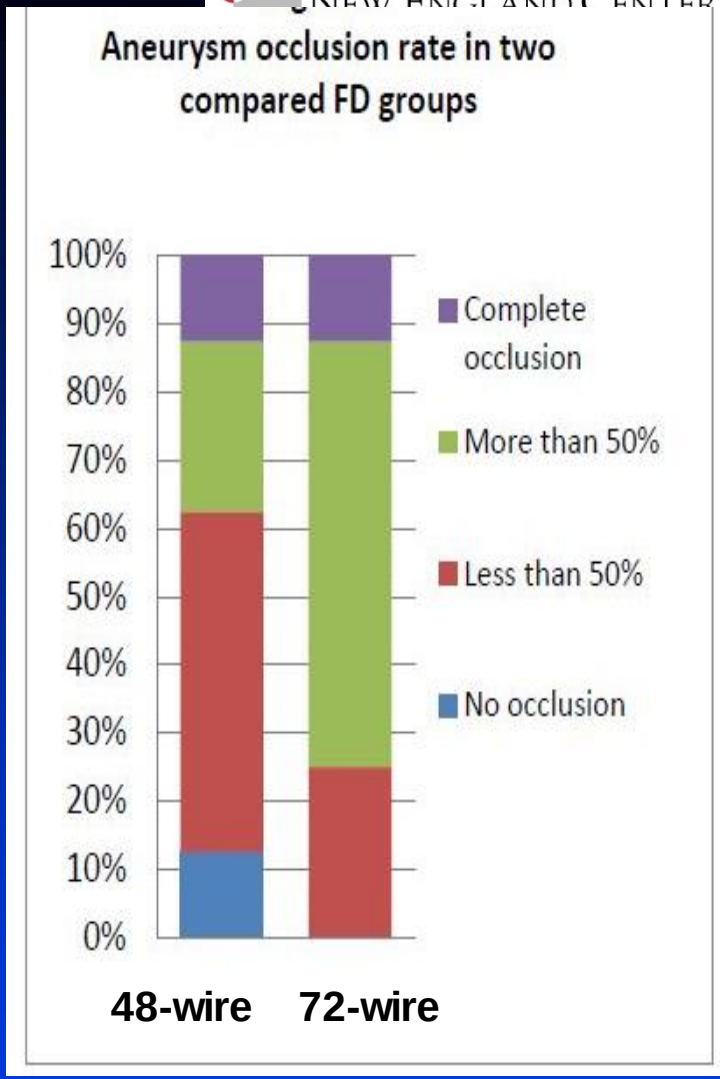
- **Grouping of aneurysm was based on:**
  - **aneurysm morphology**
  - **Vessel diameter proximal and distal to the aneurysm**
  - **Length of proximal segment of the vessel – landing zone!!**

	48-wire	72-wire	p-value
aneurysm height	6.9 ±1.8	7.1 ±1.6	0.86
aneurysm width	5.5 ±2.3	5.0 ±1.9	0.64
aneurysm neck	5.3 ±1.9	4.6 ±1.4	0.47
aspect ratio	1.4 ±0.5	1.6 ±0.4	0.42
parent vessel diameter 5mm distal the aneurysm	4.6 ±1.0	4.4 ±0.6	0.64



A.) Pre-procedural DSA, frontal view
B.) Post-implant angiography, FD is not apposed at the proximal site;
C.) angioplasty
D-E.) VasoCT, distal end of FD slightly compressed (deployed into a 2.5mm vessel), part bad apposition proximally
F.) after 2 attempt of angioplasty DSA showed improved apposition (arrow-head)





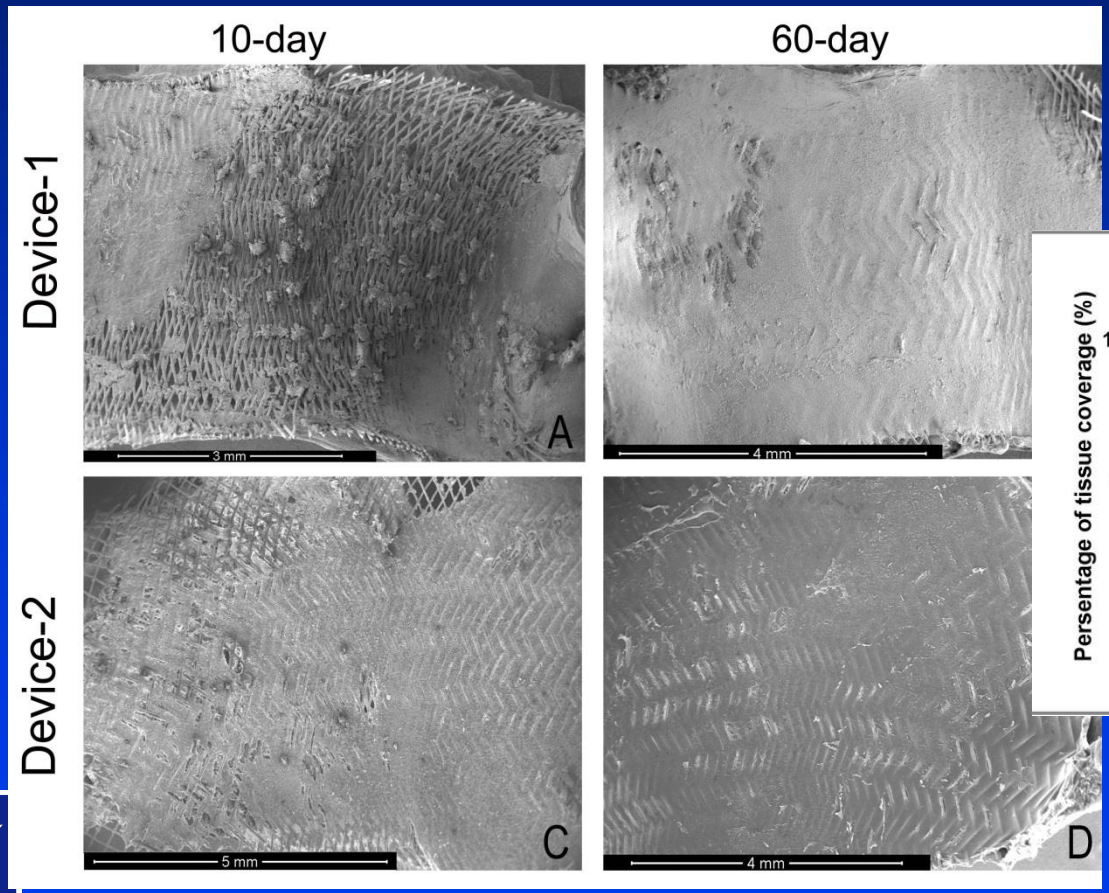
A.) DSA prior FD implant shows a small neck aneurysm with a distally dilated parent-vessel

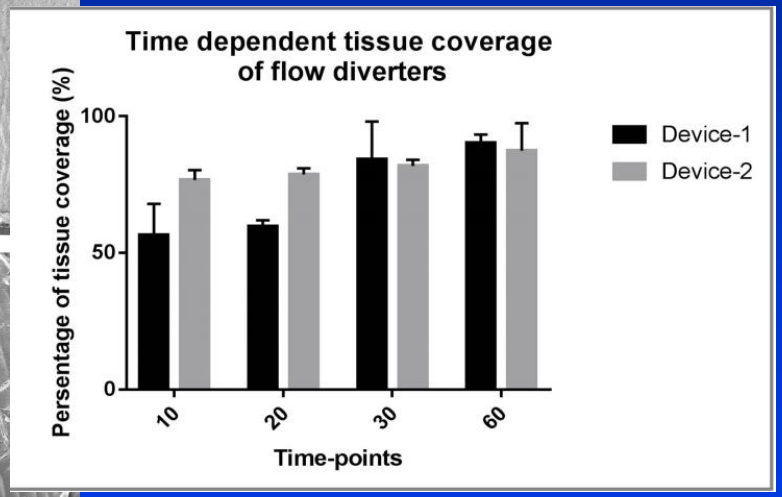
B.) After NEG implant, some contrast inflow is still present on DSA (arrow),

C.) 30 days follow up DSA indicates complete aneurysm occlusion.

Basement Membrane

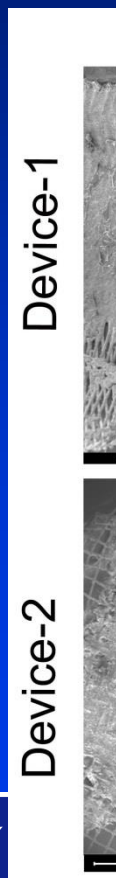
- Important first step, forms substrate for endothelialization

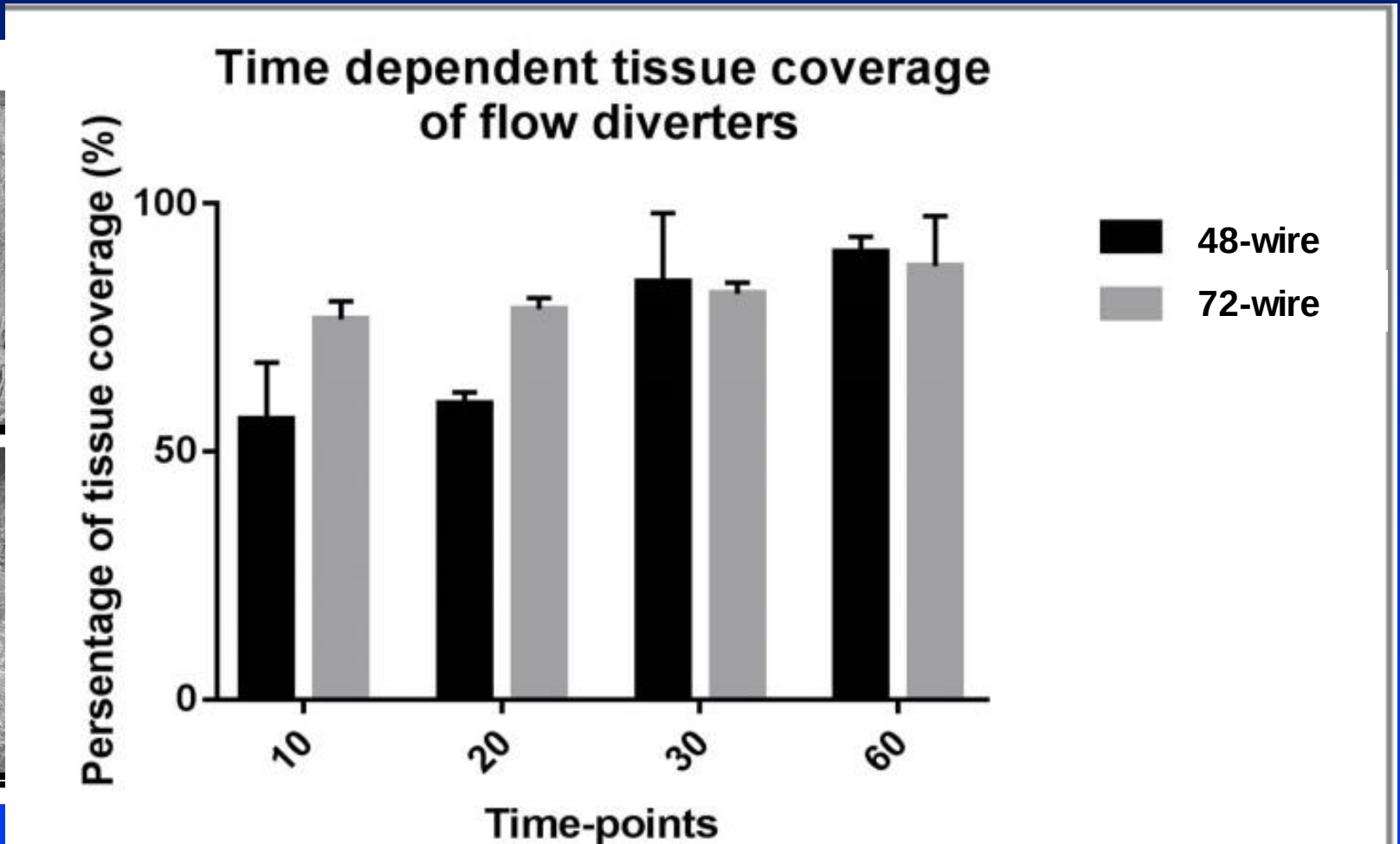


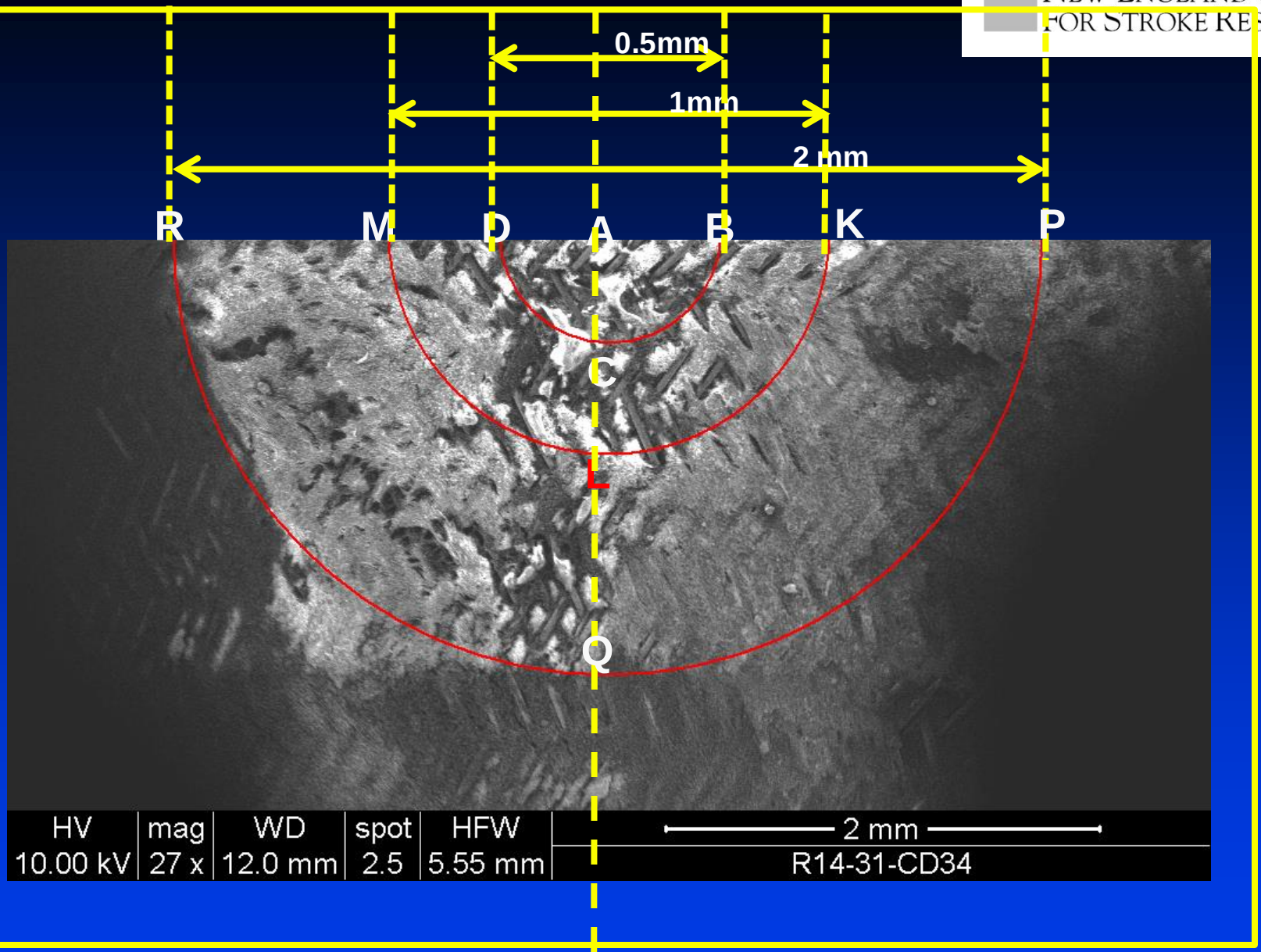


Basement Membrane

- Important first step, forms substrate for endothelialization






0.5mm
1mm
2 mm
R
M
D
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B
K
P
C
L
Q
HV
10.00 kV
mag
27 x
WD
12.0 mm
spot
2.5
HFW
5.55 mm
2 mm
R14-31-CD34
X
Y
5 mm
10 mm

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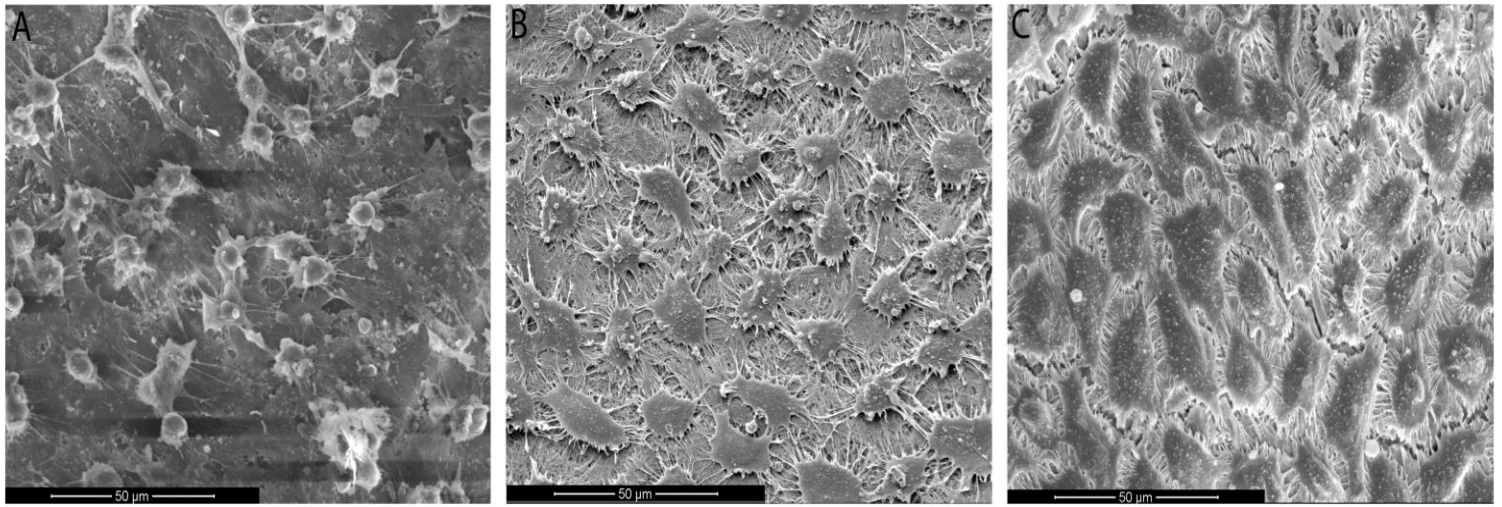
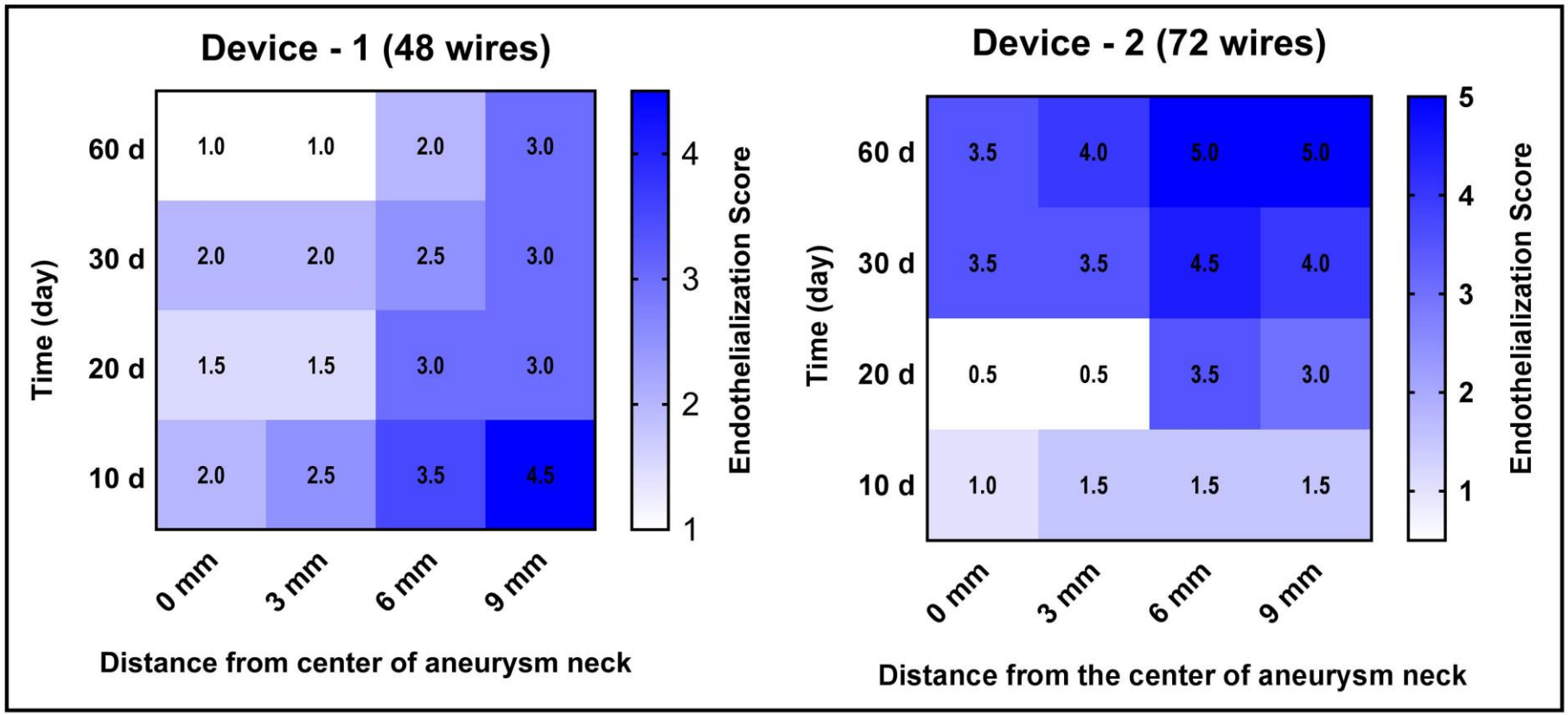
Table 1. Scoring system for assessing the rate of flow diverter endothelialization (S-FDE)

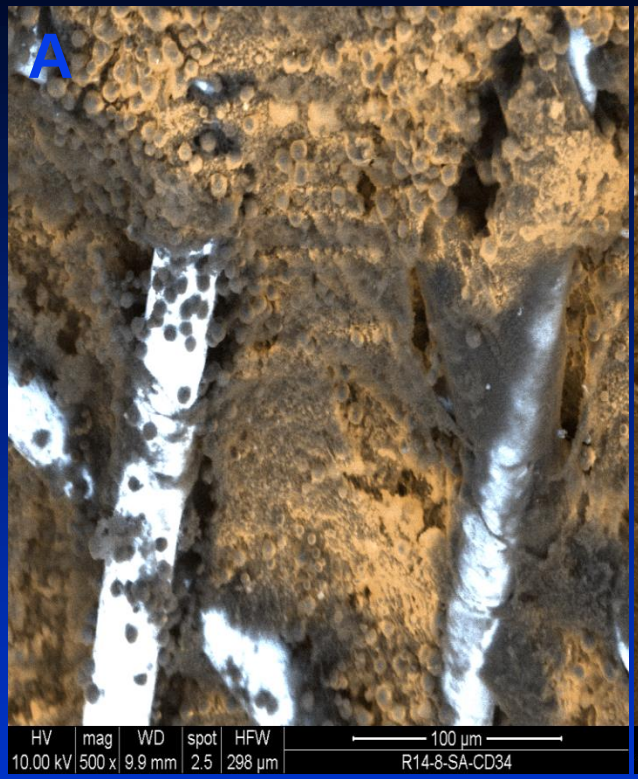
Score	Coverage of Struts	Description of Coverage
0	0%	No coverage
1	1-25%	Contains EPCs, inflammatory cells, red blood cells, proteins, and other components such as fibrin and collagen
2	26-50%	Contains EPCs, inflammatory cells, red blood cells, proteins, and other components such as fibrin and collagen for the beginning of the basement membrane
3	51-75%	Contains EPCs, inflammatory cells, red blood cells, proteins, and other components such as fibrin and collagen creating the basement membrane
4	76-99%	Contains EPCs and/or endothelial cells along with the components of the basement membrane
5	100%	Fully Endothelialized

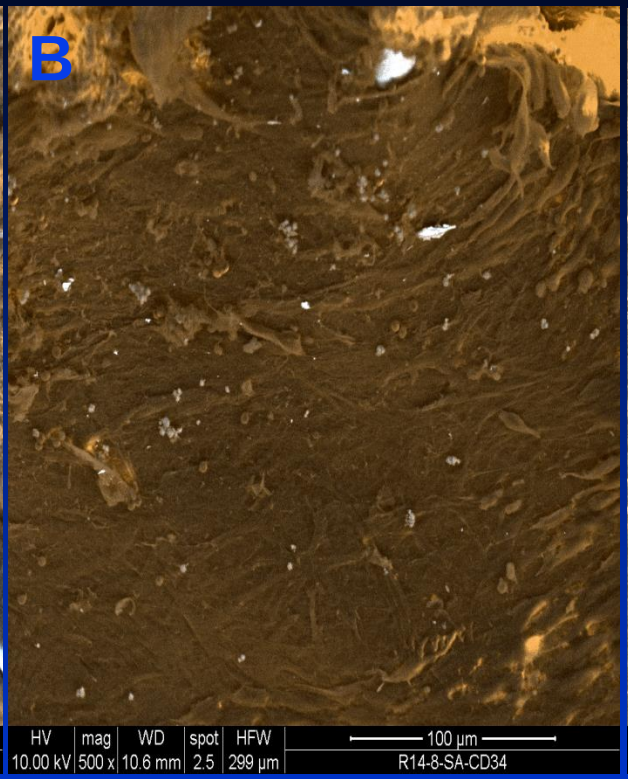


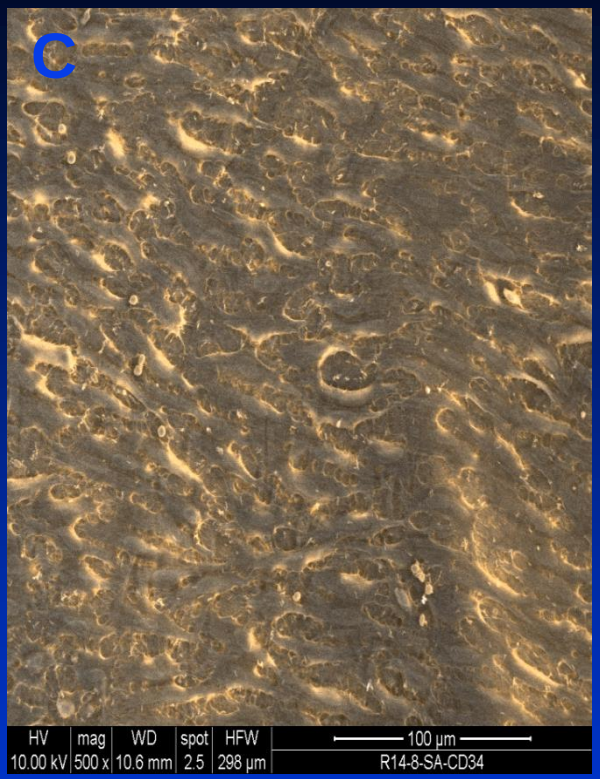
Endothelialization



- 48-Wire (Device-1): EC scores related to location (p=0.083)
- 72-Wire (Device-2): EC scores are function of time (p=0.013)







500x mag.

A.) center of the aneurysm neck, partial coverage of struts

B.) 2mm proximal to image A, disorganized cell network on the surface of basal membrane

C.) 5mm proximal to image A, endothelial cells are evenly distributed

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Immuno-gold labeling for SEM

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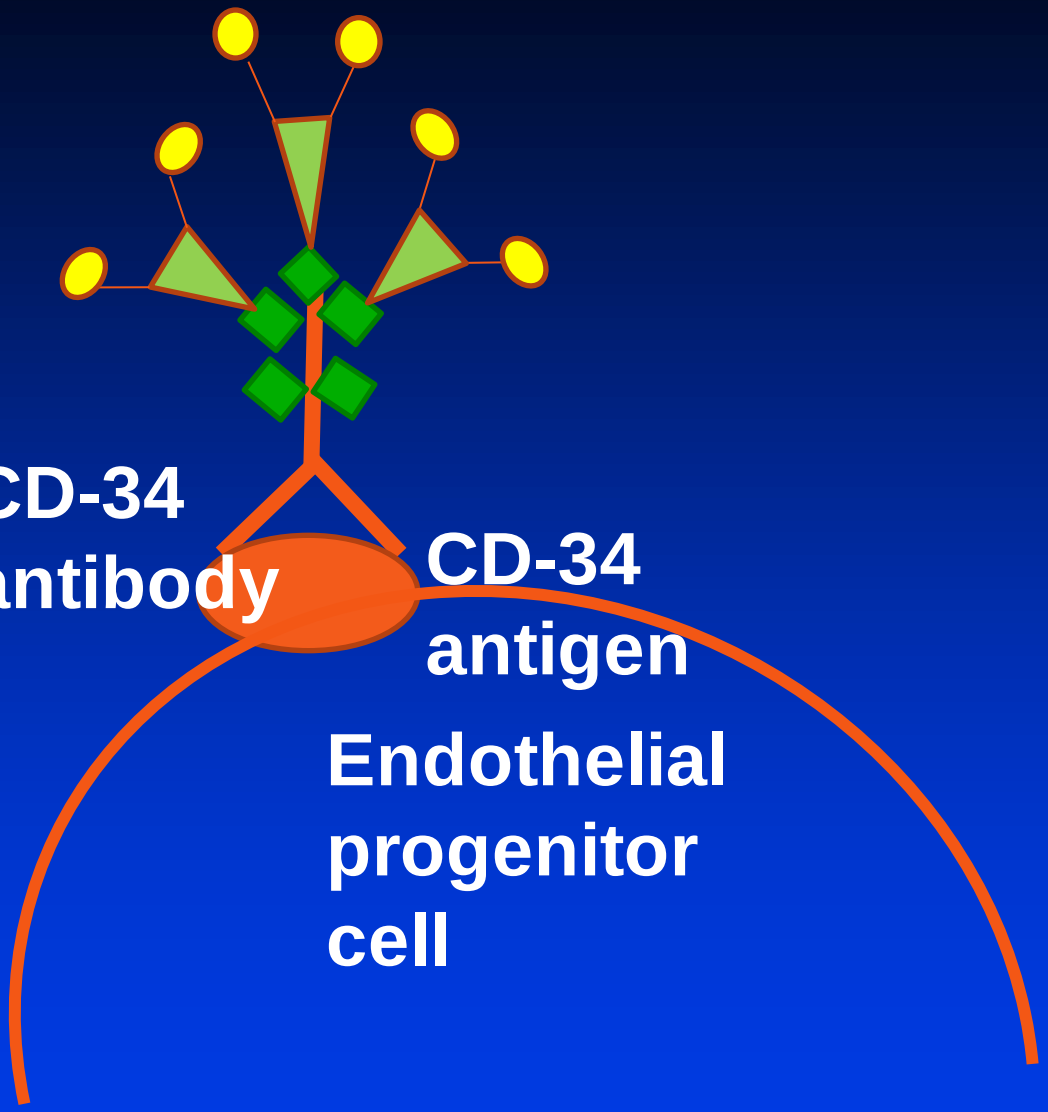
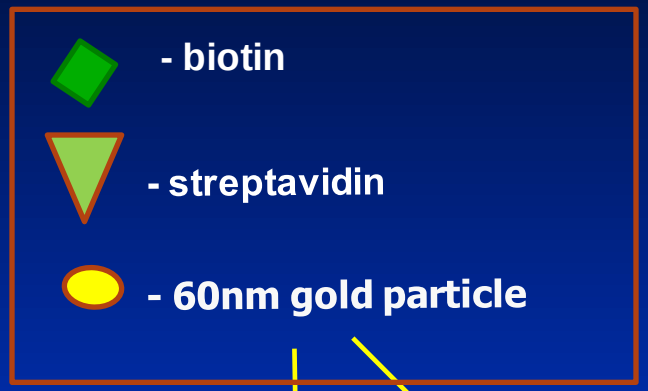
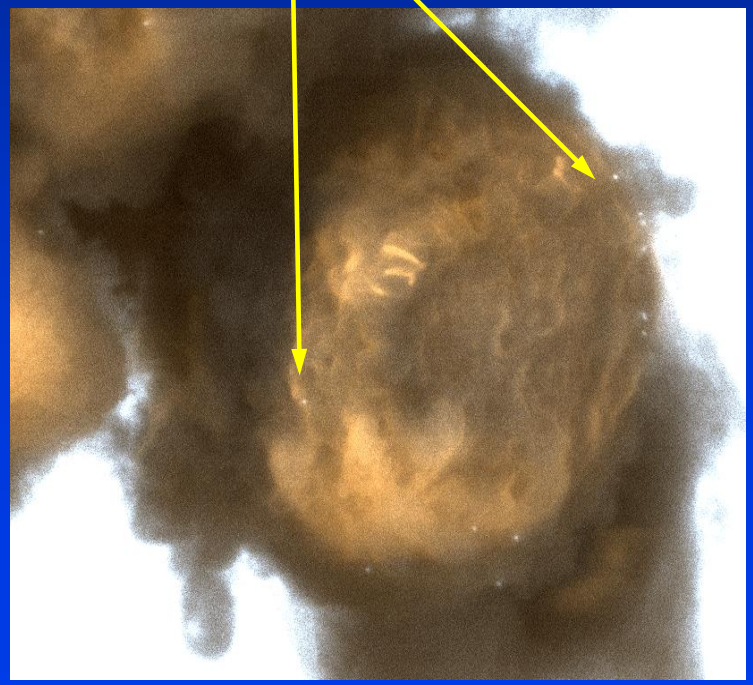
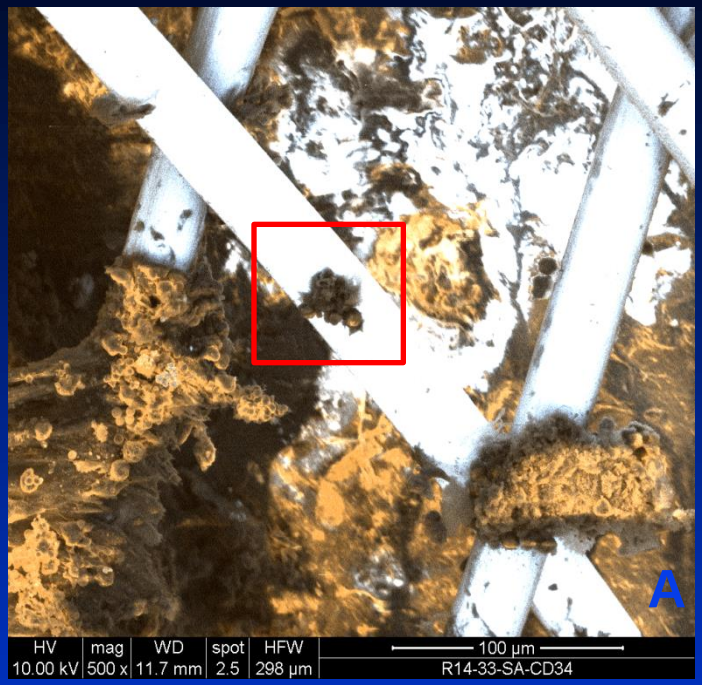
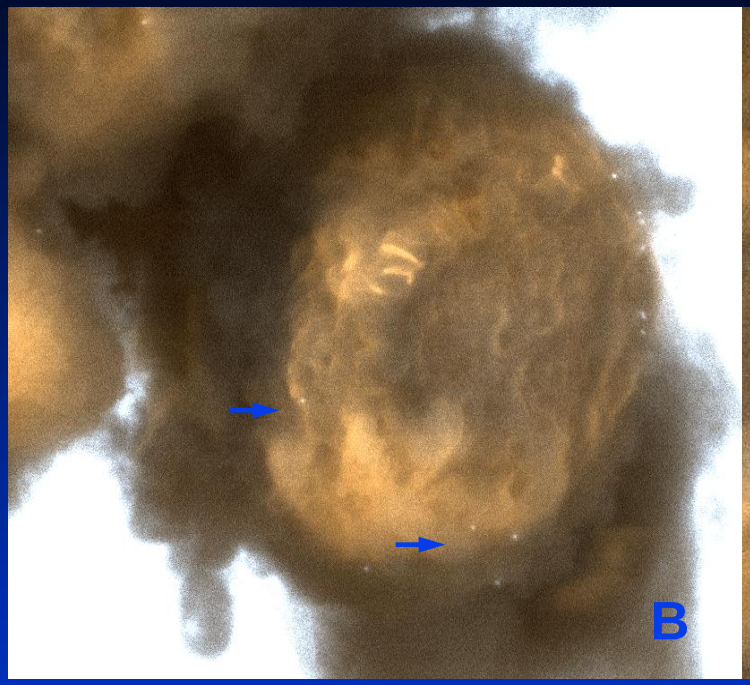


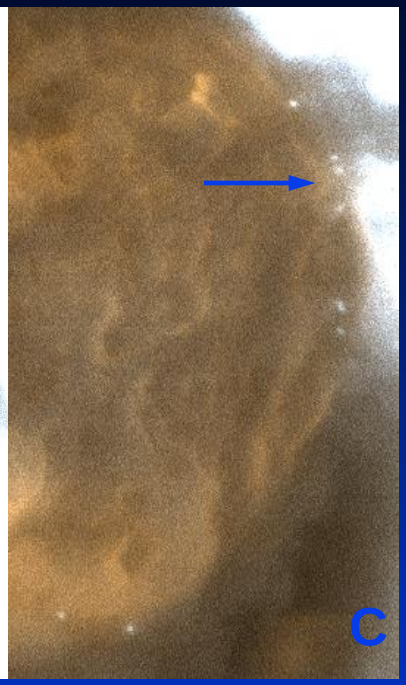




UMASS







A.) 500x, image of the inner surface of the NEG implant, 10days after implantation

B.) 10,000x, the immuno-gold labeling on the surface of the cell (white arrows)

C.) manually zoom of the image B for better visualization of the gold nanoparticles

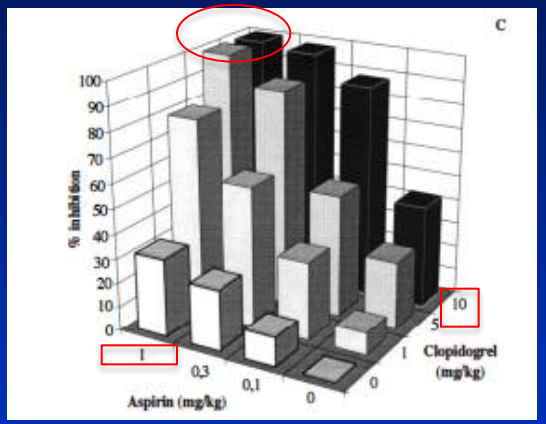
Preliminary results – anti-platelet drugs activity tests and APD (anti-platelet drug) dosing strategy

- sample collection: femoral artery
- timing: prior terminal angiography
- test: clopidogrel and aspirin activity – VerifyNow (PRU-P2Y12 Reaction Units)
- data interpretation according HUMAN studies:
  - P2Y12 Reaction Units (PRU) result of ≥208 were at a significantly increased risk of cardiovascular events
  - and patients with a PRU of < 95 were receiving virtually no additional protection from cardiovascular events, but at a significantly increased risk of bleeding

Thromb Haemost 1998; 80: 512–8

The Antiaggregating and Antithrombotic Activity of Clopidogrel Is Potentiated by Aspirin in Several Experimental Models in the Rabbit

Jean-Marc Herbert, Frédérique Dol, André Bernat, Robert Falotico[1], Alain Lalé, Pierre Savi



N=16	PRU (Clopidogrel test)	ARU (Aspirin Test)
results	102 (61-129)	652 (636-664)

N=16	In-stent stenosis	In-stent thrombosis
results	0/16 (0%)	0/16 (0%)

Flow Diversion: Summary

- Evidence: curative treatment of brain aneurysms
  - Treats diseased segment of the blood vessel
  - Endoluminal reconstruction is ideal
- Engineer construct and surface properties to promote rapid endothelialization
- Need to remove dependency on dual antiplatelet medication
- Need imaging tools developed specifically for technology to ensure proper deployment

NECStR

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- **UMass Collaborations**
  - Marc Fisher, MD
  - Neil Aronin, MD
  - Alexei Bogdanov, PhD
  - Greg Hendricks, PhD
  - Guanping Gao, PhD
  - Miguel Esteves, PhD
  - Linda Ding, PhD
  - Srinivasan Vedantham, PhD
  - John Weaver, MD
- **Collaborations**
  - Alex Norbash, MD – BU
  - Thanh Nguyen, MD - BU
  - Italo Linfante, MD - Baptist
  - Guilherme Dabus, MD - Baptist
  - Don Ingber, PhD – Harvard
  - Netanel Korin, PhD - Technion
  - Johannes Boltze, MD, PhD – Frauhofer Institute
  - Raul Nogueira, MD - Emory
  - Ajay Wakhloo, MD, PhD
  - Ajit Puri, MD
  - Juyu Chueh, PhD
  - Miklos Marosfoi, MD
  - Martijn van der Bom, PhD
  - Kajo van der Marel, PhD
  - Anna Kühn, MD, PhD
  - Ivan Lylyk, MD
  - Frédéric Clarençon, MD, PhD
  - Bo Hong, MD
  - Mary Howk, MS, CRC
  - Thomas Flood, MD, PhD
  - Erin Langan, BS
  - Olivia Brooks
  - Conrad Bzura, BS
  - Chris Brooks, PA
  - Mary Perras, NP
  - Shaokuan Zheng, PhD

Mean Rate of Angiographic Aneurysm Occlusion

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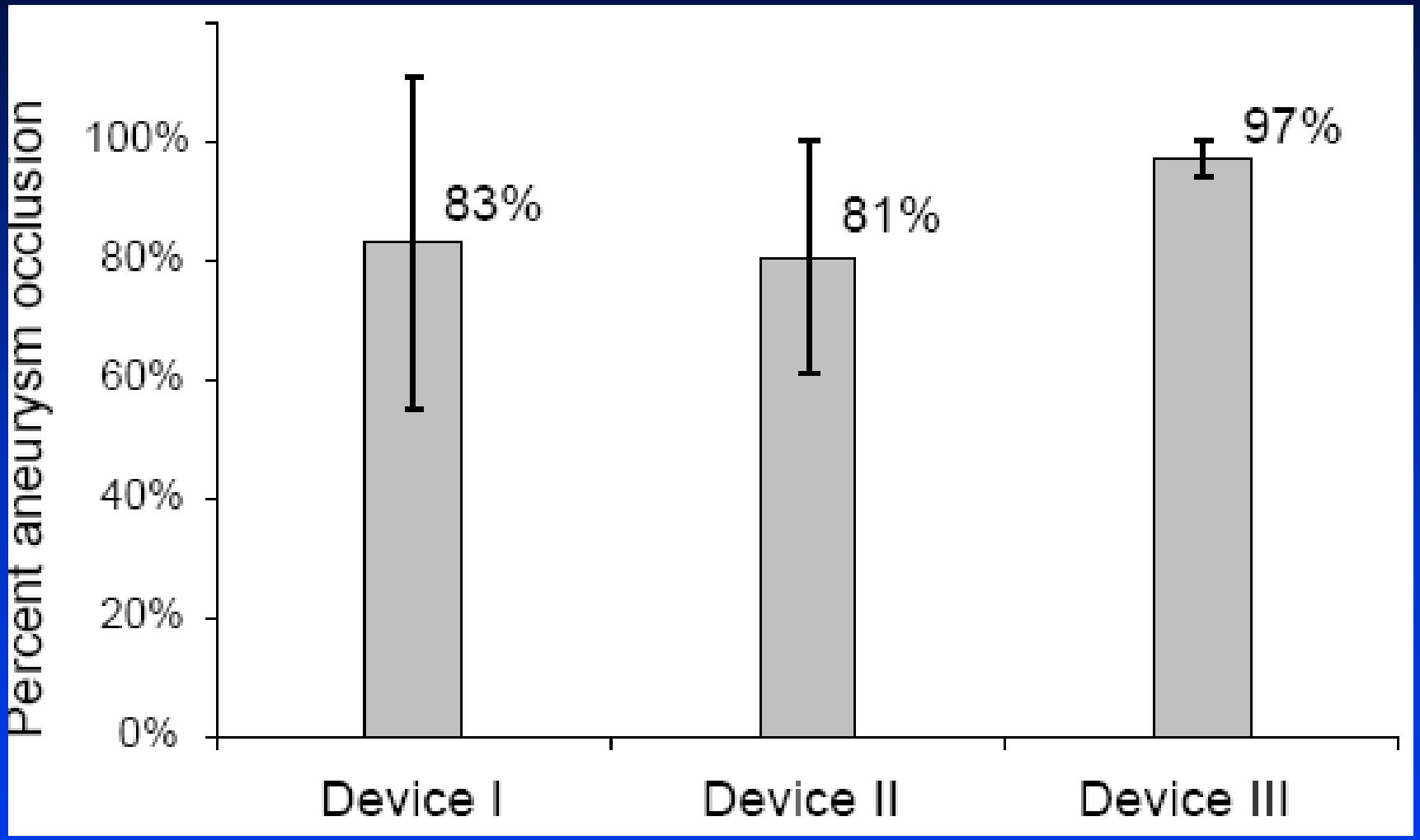

Sadasivan, Cesar, Seong, Rakian, Hao, Tio, Wakhloo, Lieber Stroke 2009

Histology – Progressive Occlusion – Rabbit Elastase Aneurysm Model

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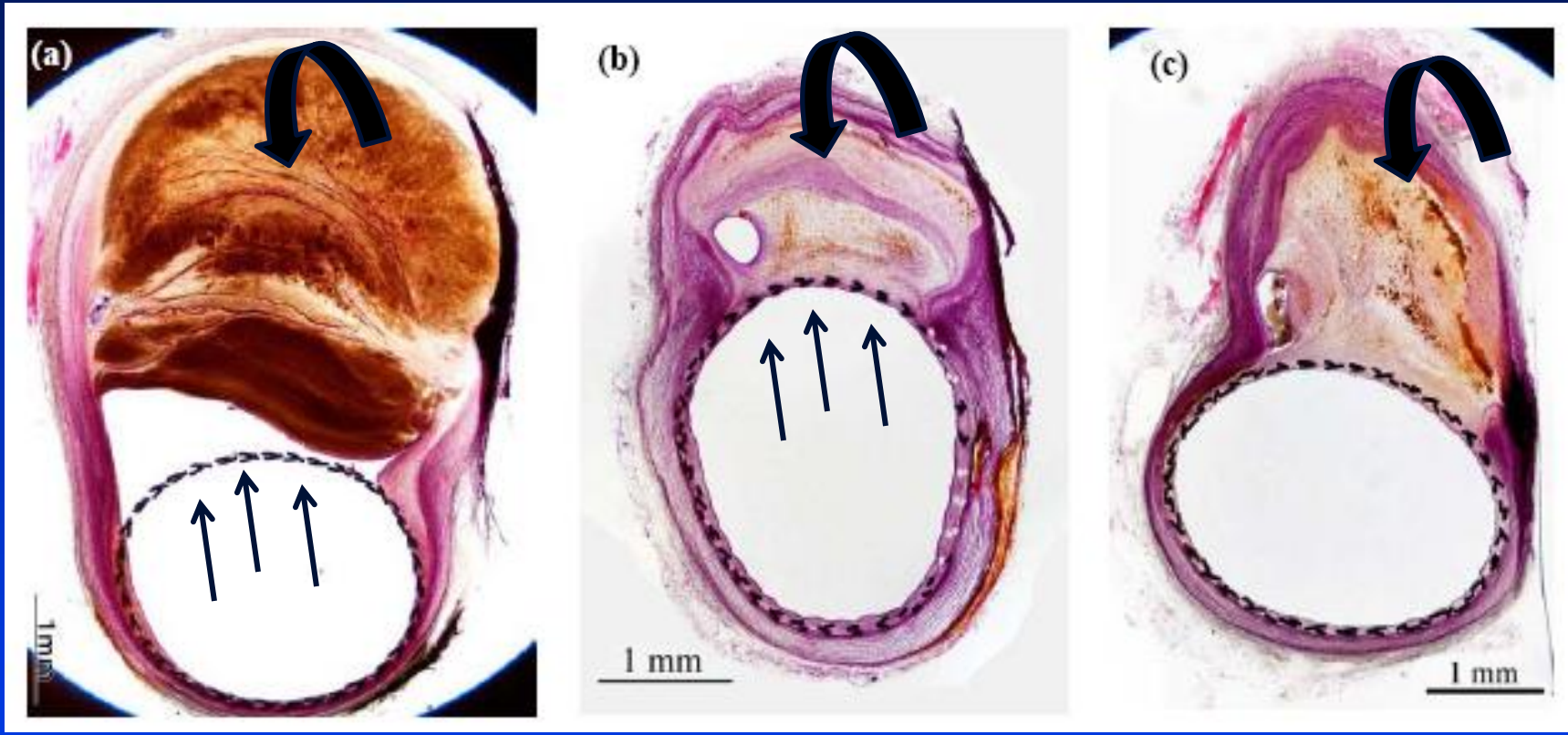
Amorphous clot -Organizing clot Collagen formation and Endothelialization

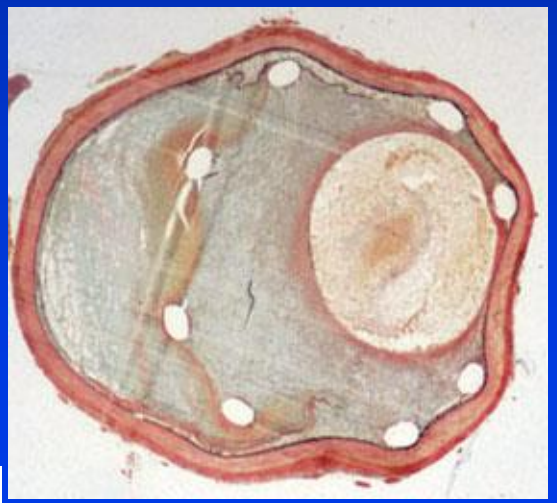


21 days 90 days 180 days

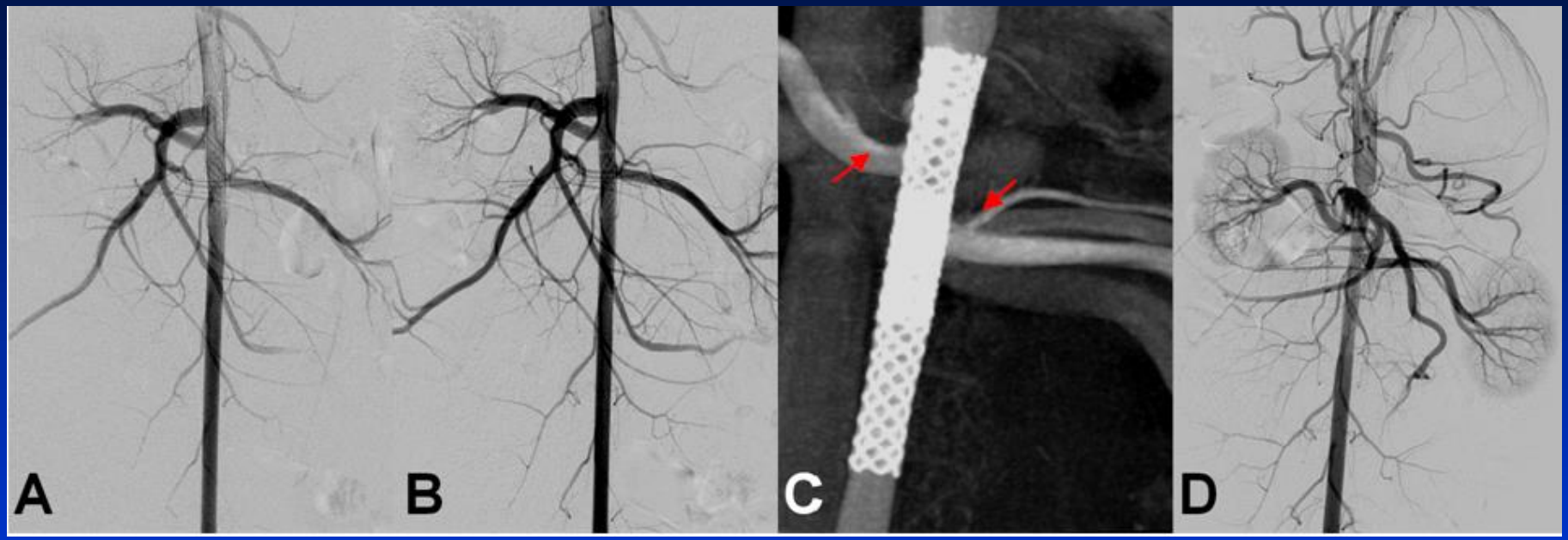
Sadasivan, Cesar, Seong, Rakian, Hao, Tio, Wakhloo, Lieber Stroke 2009

Perforators

- Large struts that cover approximately >50% of the ostium increase resistance to flow and can lead to perforator thrombosis



Perforators/ Jailed Arteries



- Model: Rabbit Aorta w/ covered Lumbar Arteries and Renal Arteries
  - Test propensity to shed emboli to kidney – both with single and double FD coverage
  - Test risk of perforator occlusion

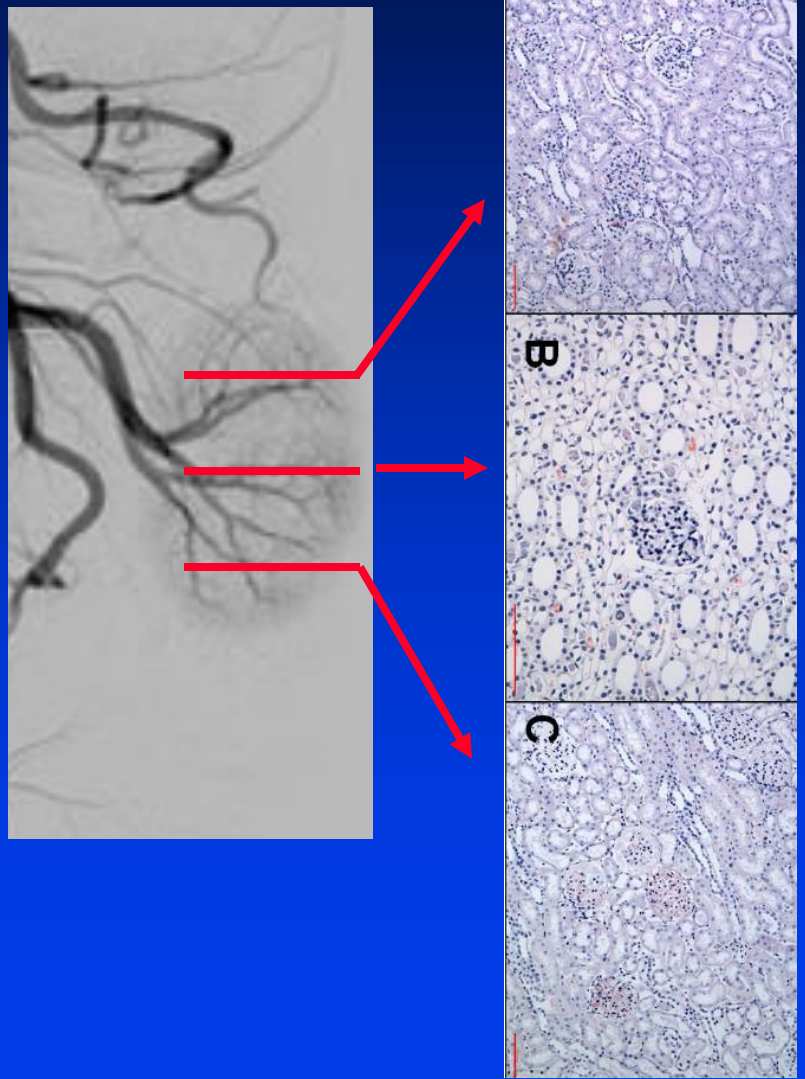
Gounis and Wakhloo, in preparation 2015

Study Design

- 45 Animals: 5 Timepoints – 7, 28, 90, 180 and 365 days
  - Per Timepoint: 6 animals for histology, 2 animals for SEM, 1 Naïve Control
  - Antiplatelet: ASA (10mg) and Clopidogrel (10mg) 4 days prior to implant, continued for 30 days
- Endpoints:
  - Vascular Response to Implants
  - Kidney histopathology
  - Perforator (lumbar arteries) patency

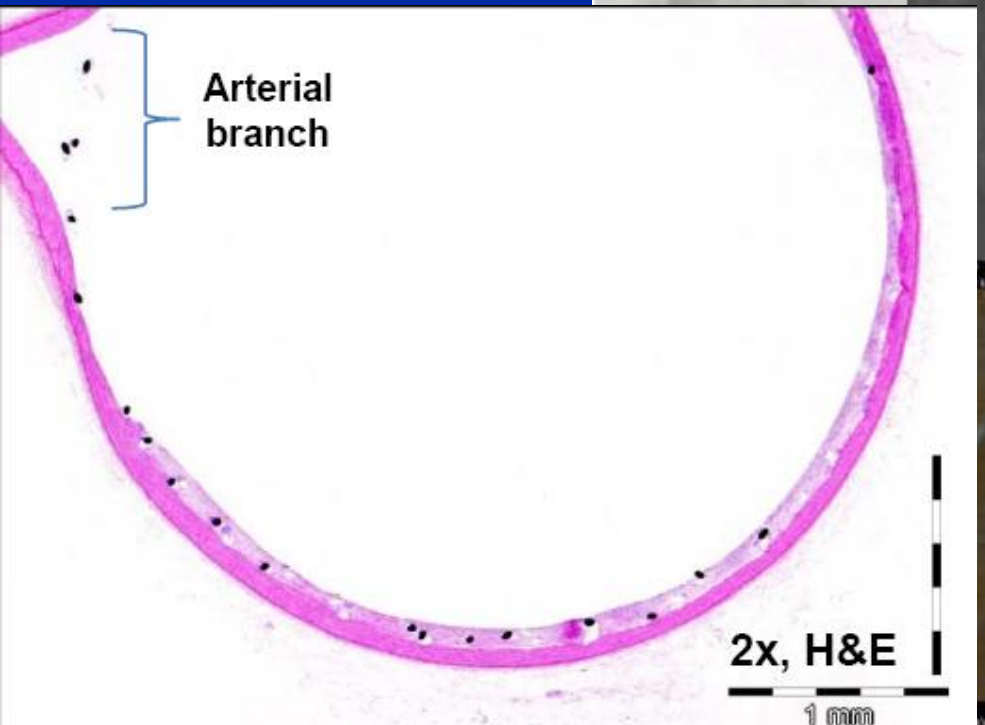
Thromboembolic Events

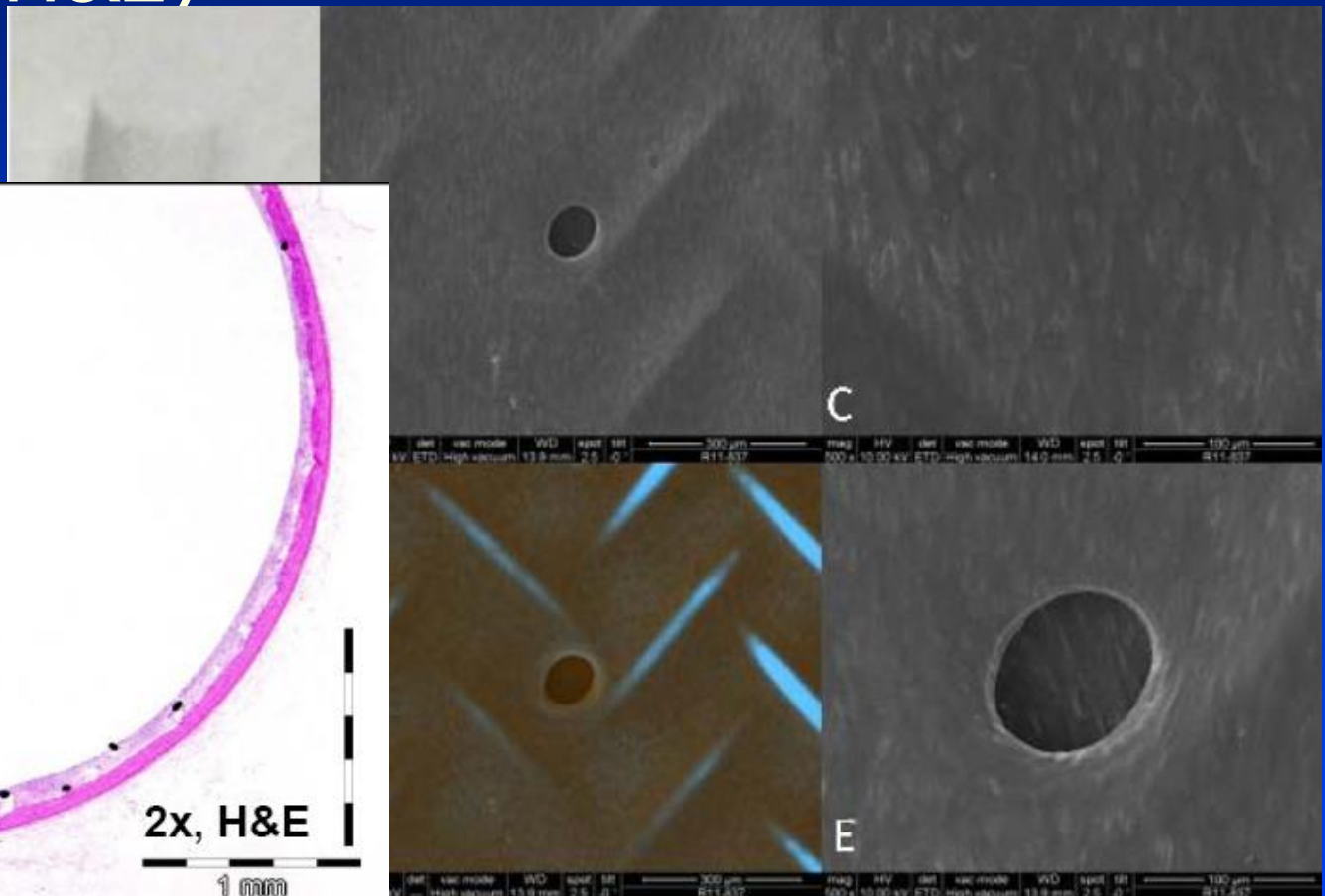
- Kidneys bread-loafed, 1 section each from cranial, mid and caudal aspects analyzed by light microscopy for ischemic changes
- 0 ischemic events



Perforator Patency

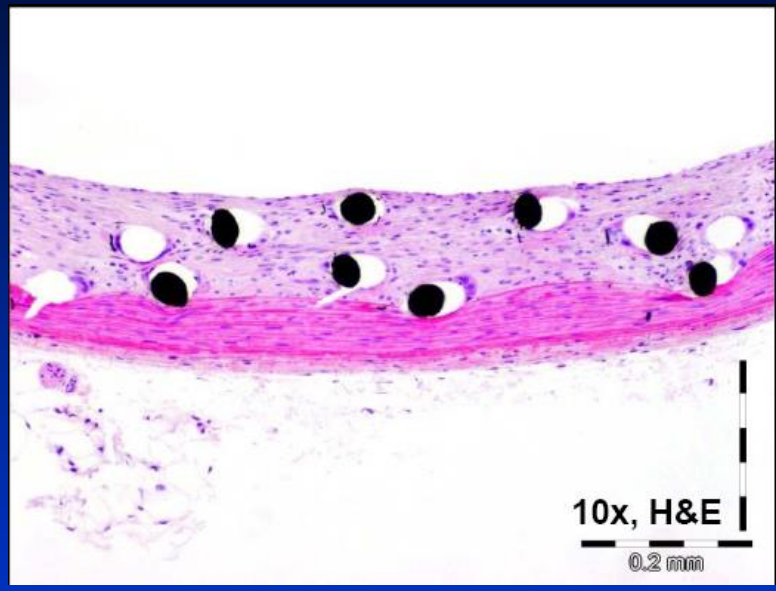
- All lumbar arteries remained patent (angio, SEM, H&E)





Vascular Response

- Pathology report:
  - "Histomorphometric analysis showed neointimal proliferation to be negligible at all five timepoints."
  - "Inflammation, injury, and neointimal fibrin was overall minimal to mild in the Endograft group"
  - "showed acceptable vascular healing and produced a minimal tissue response"



- "There was complete or nearly complete endothelial-ization and neointimal maturation at the 28-day time point."

Future…

Step 1: Aneurysm Thrombosis

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- Patient-Specific Hemodynamics is **ONE-THIRD** of Aneurysm Thrombosis

GESAMMELTE ABHANDLUNGEN

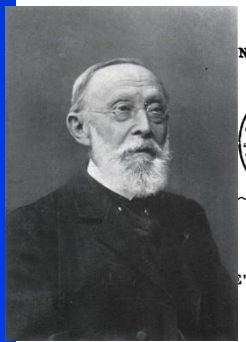
ZUR

WISSENSCHAFTLICHEN MEDICIN

VON

RUDOLF VIRCHOW,

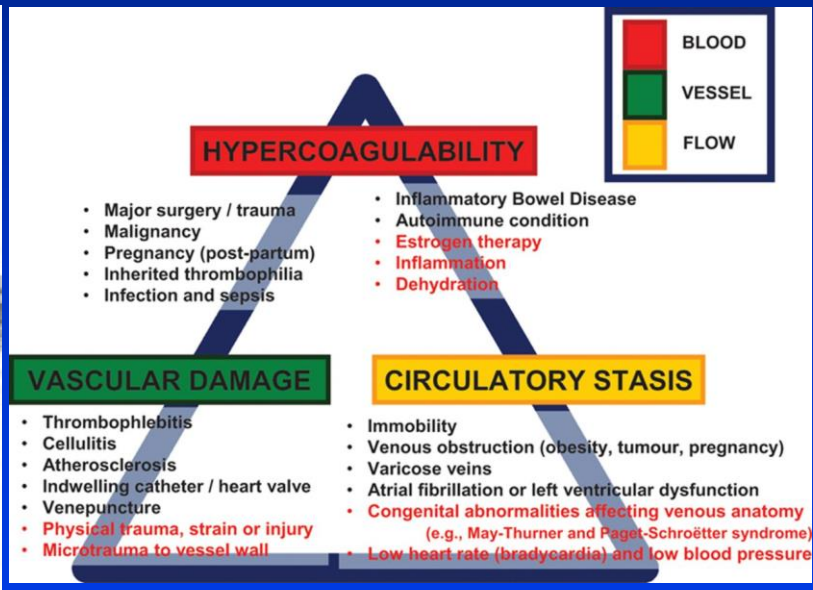
PROFESSOR AN DER UNIVERSITÄT ZU BERLIN.



NVERÄNDERTE AUSGABE.

HAMM,
'SCHE BUCHHANDLUNG
(C. MÜLLER).
1862.

Fig. 1.



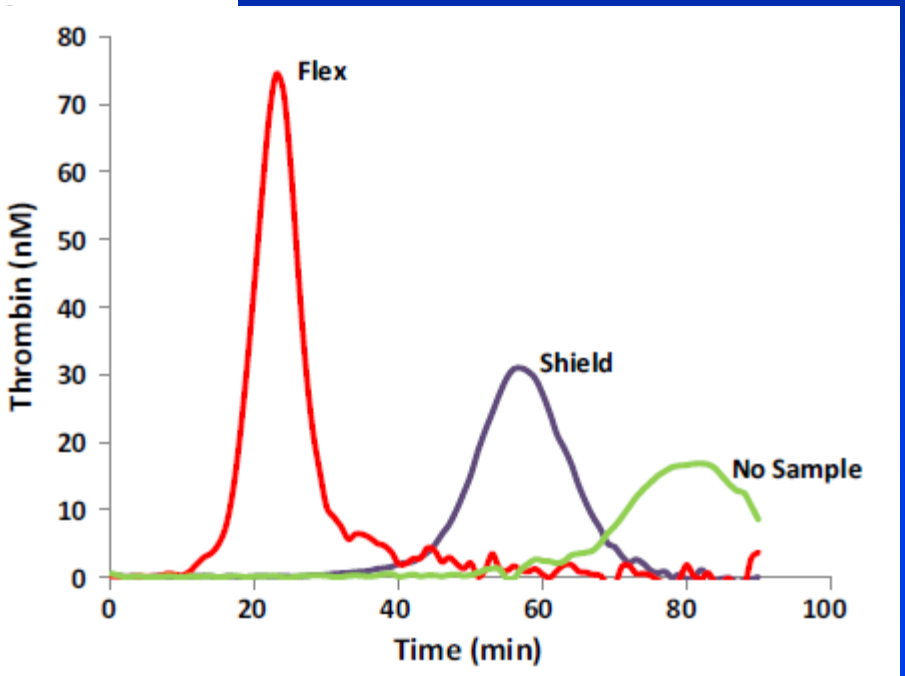
Hull and Harris, Circulation 2013

Platelets, Platelets, Platelets….

J Thromb Thrombolysis
DOI 10.1007/s11239-015-1228-0

In-vitro thrombogenicity assessment of flow diversion and aneurysm bridging devices

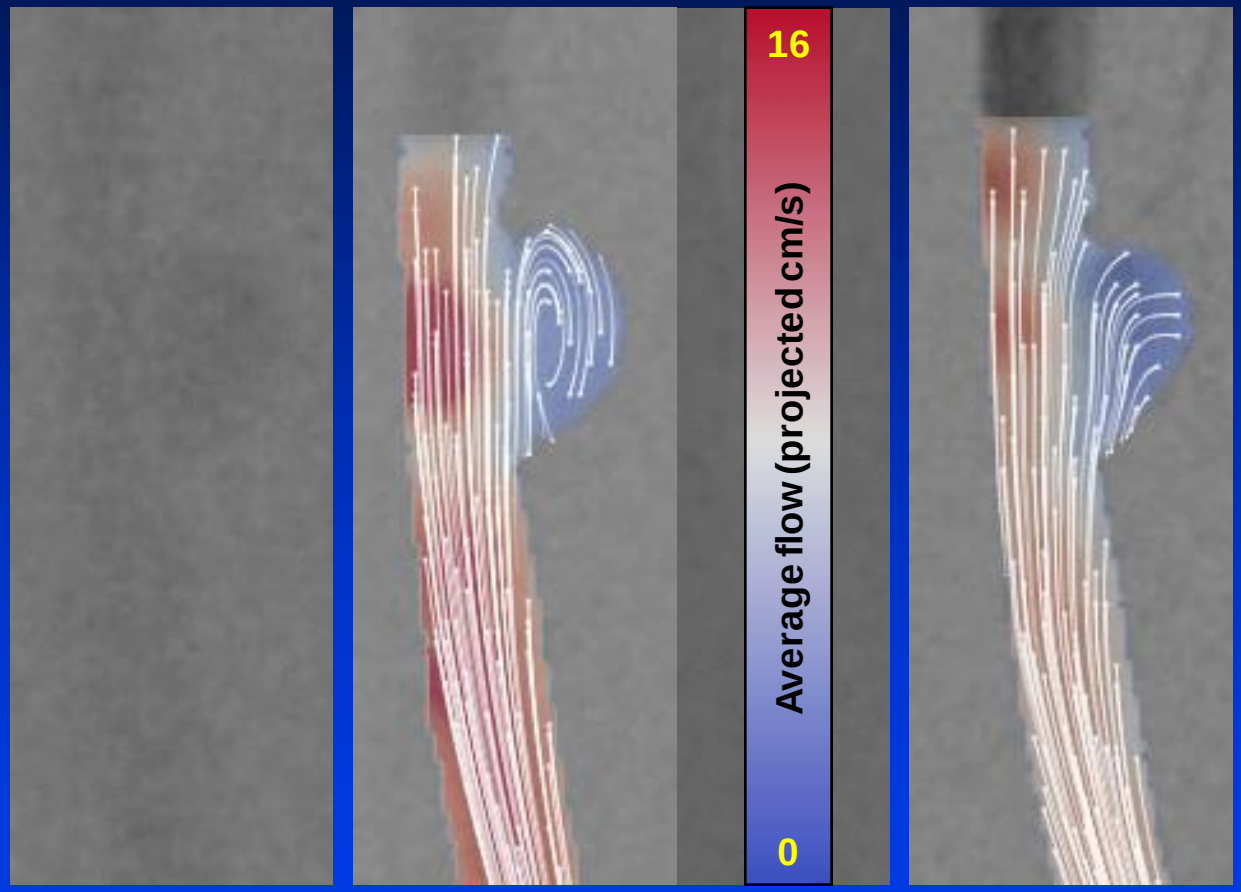
Gaurav Girdhar[1] · Junwei Li[2] · Larisa Kostousov[3] · John Wainwri
Wayne L. Chandler[4]



DSA-Based Intra-Aneurysmal Flow

Baseline

After FD implantation



DSA (60 fps)

Average flow (projected cm/s)

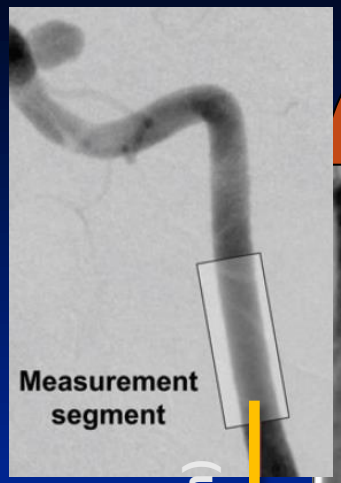
DSA (60 fps)

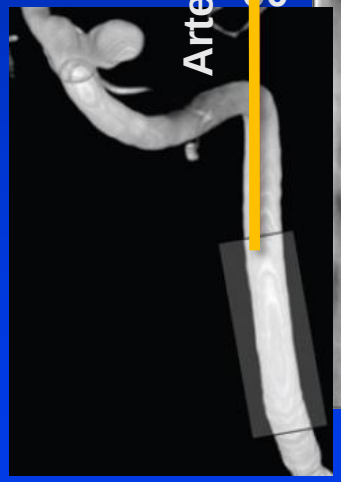
Average flow (projected cm/s)

2014; 35: 156-163

DSA

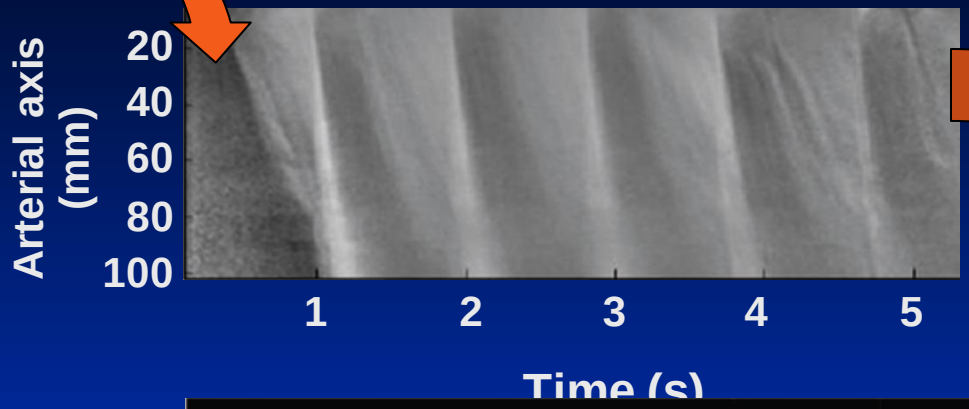
Contrast wave map



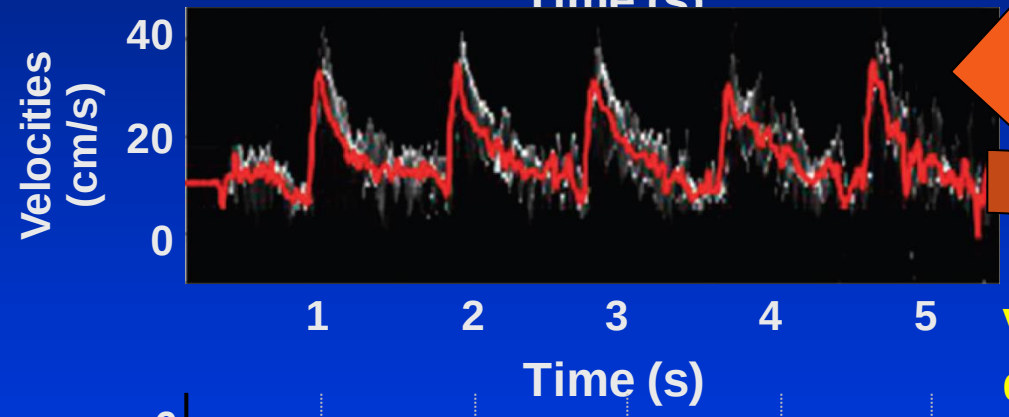


Warped 2D - 3D Vessel Registration

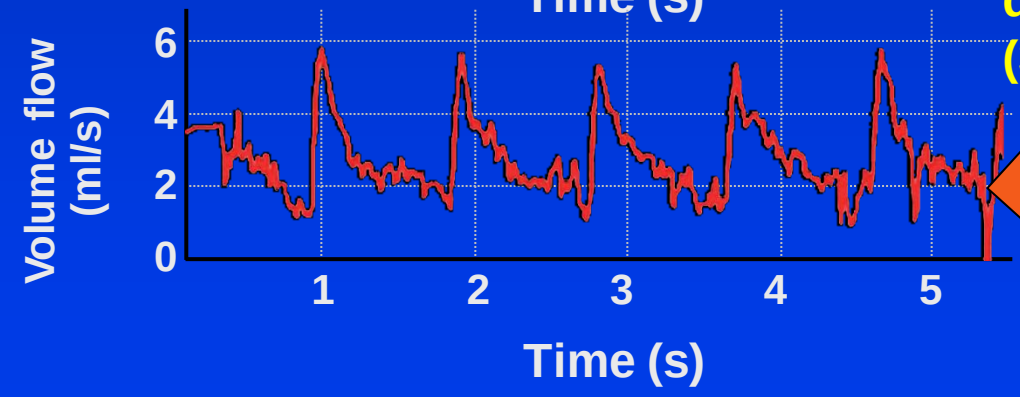
3D reconstruction



Optical Flow Tracker

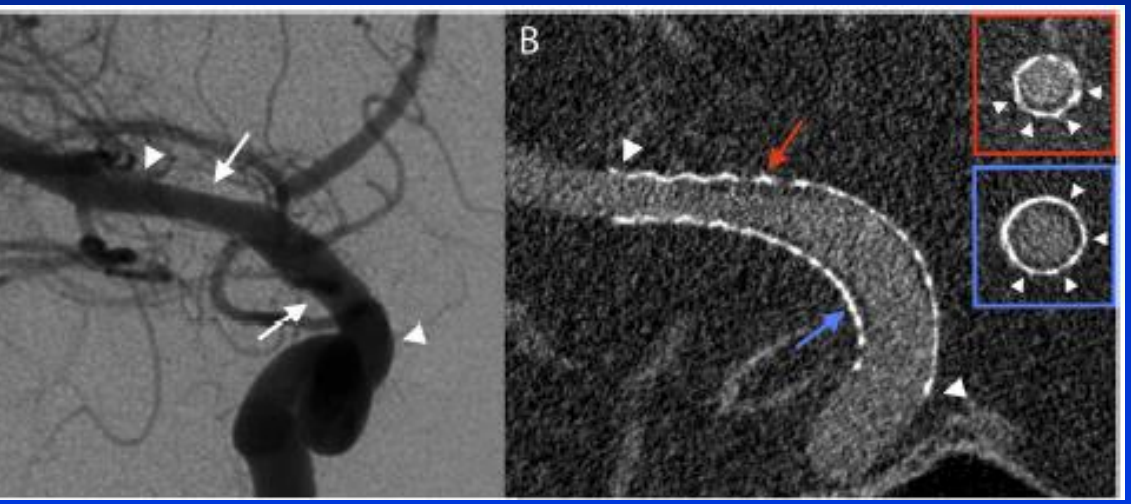


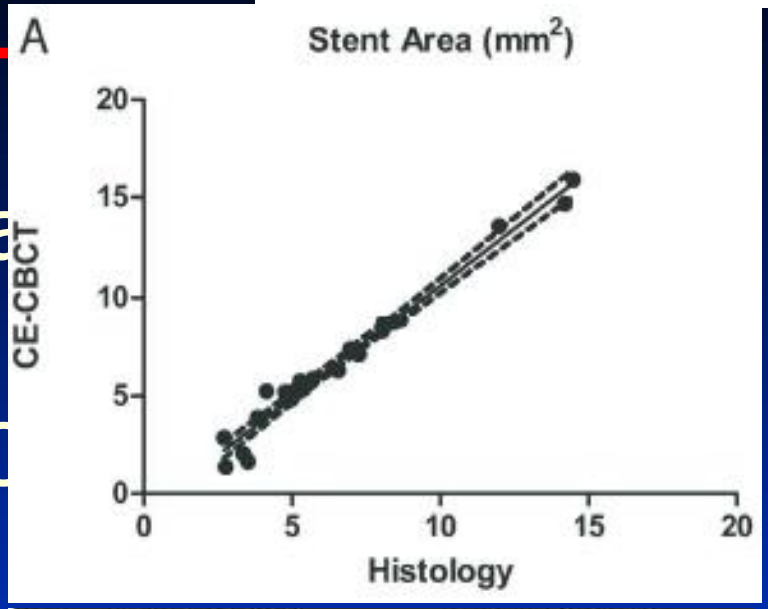
Vessel diameter (3DRA)

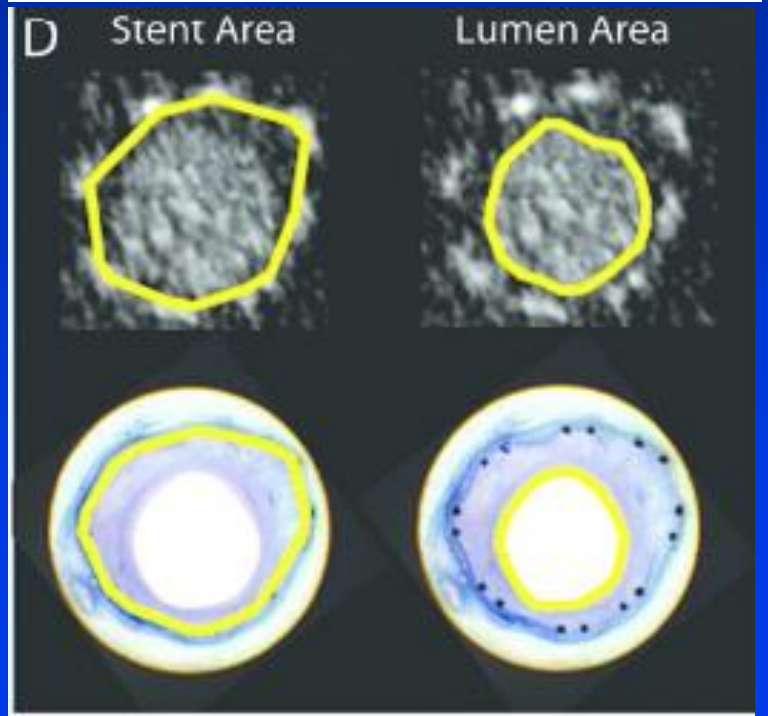


Apposition – Assumed!

- All models assumed device a
  wall
- Non-binned, small FOV CE-C

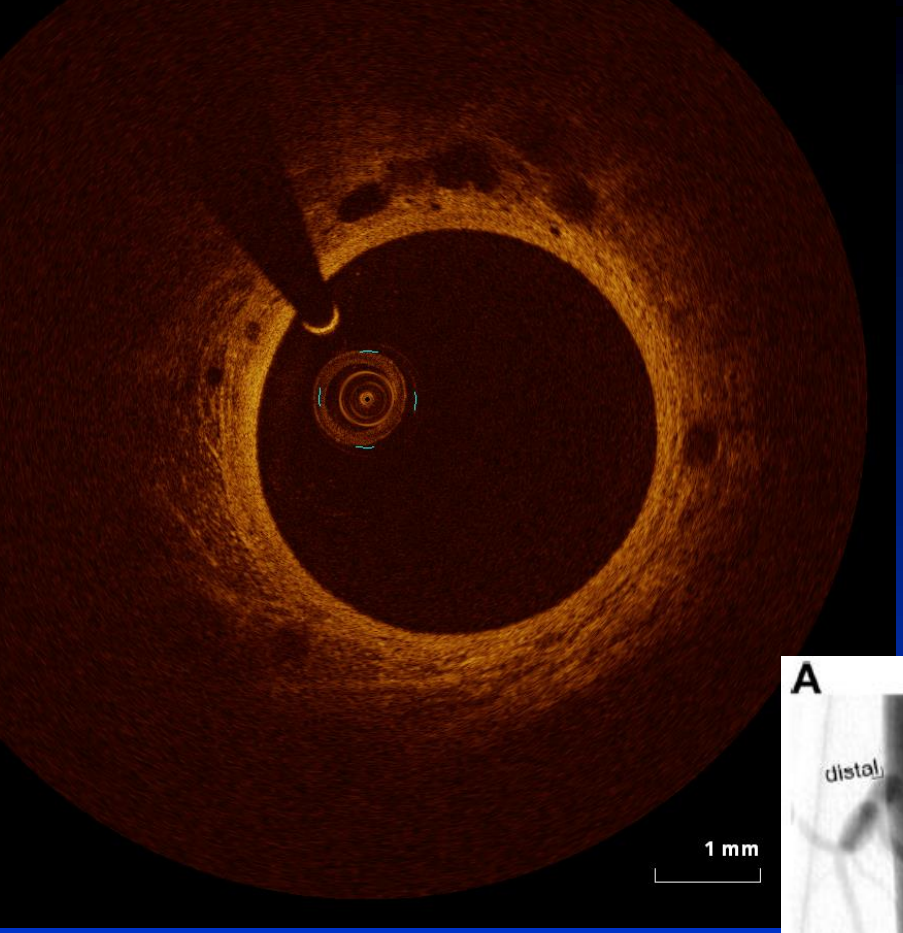




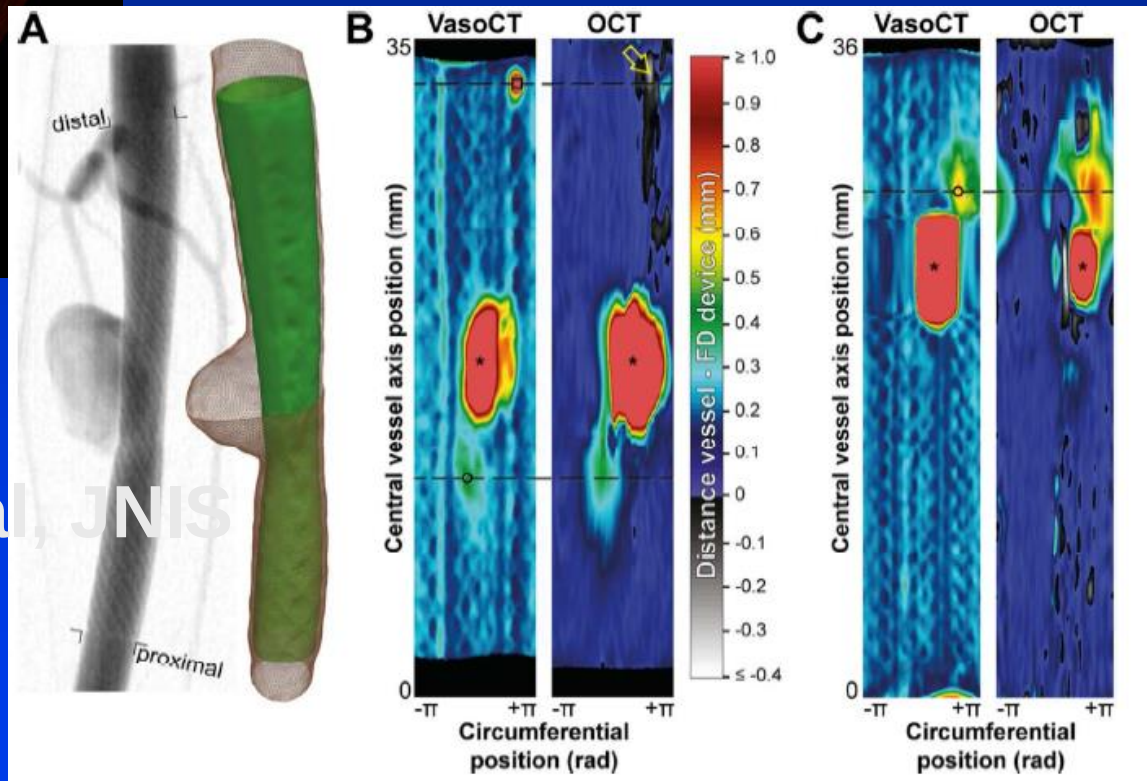


Flood et al JNIS 2014

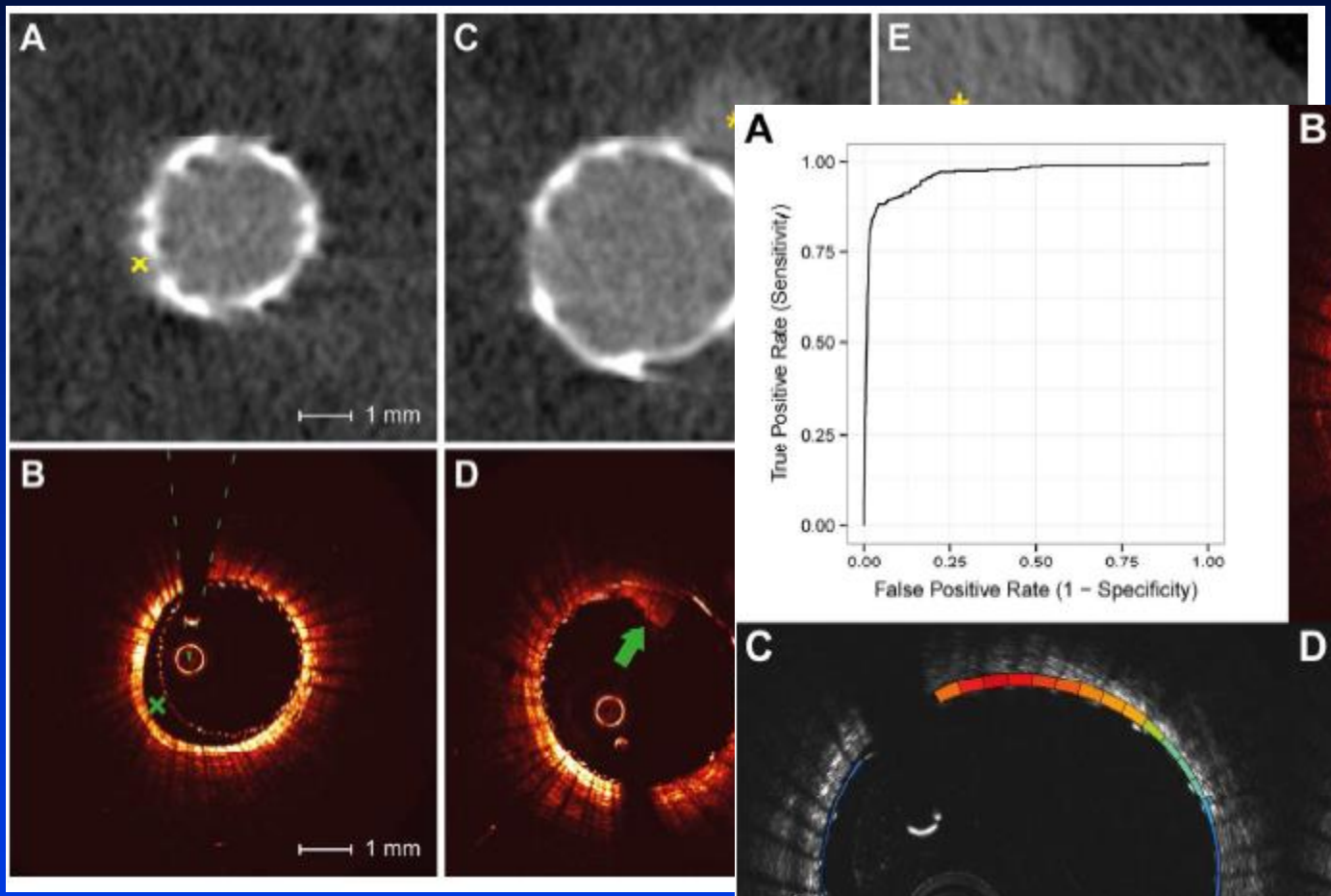
Intravascular Imaging

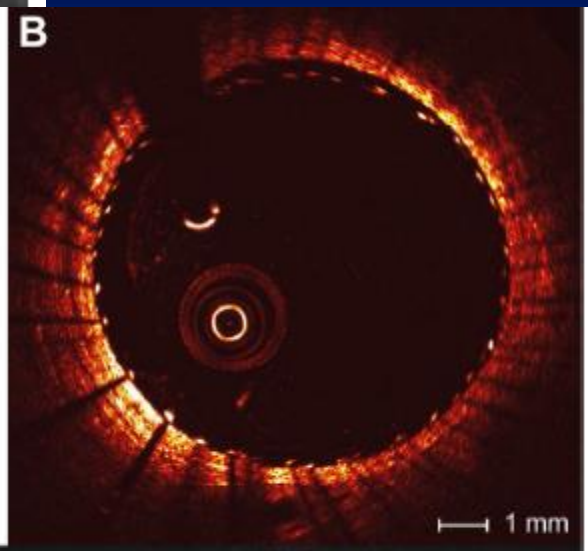


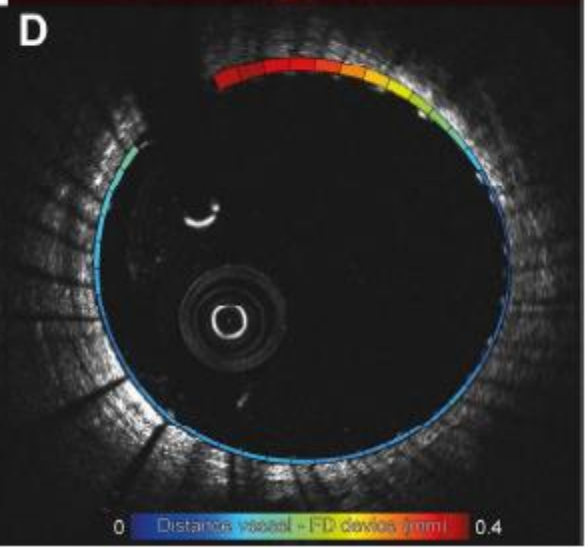
K van der Marel, et al, JNIS Accepted



Intravascular Imaging







K van der Marel, et al, JNIS Accepted

UMASS