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1907-2002

A Tissue-engineered Stem Cell Vascular Graft

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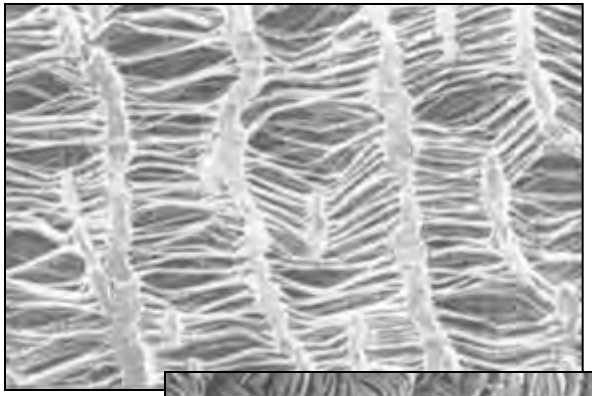
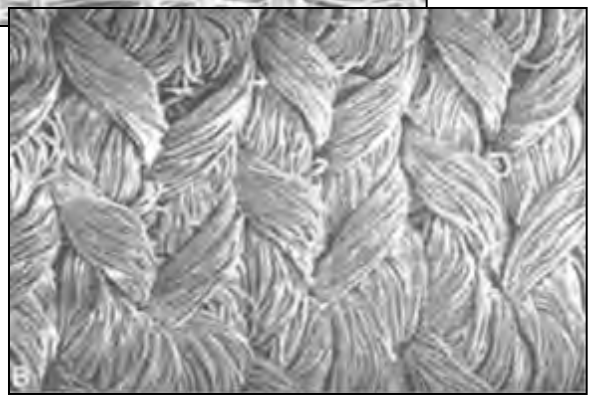
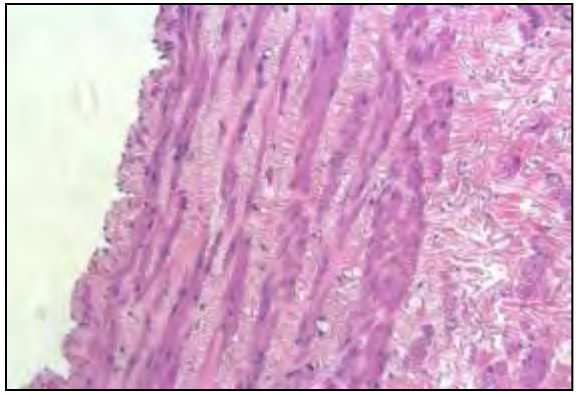
The Problem

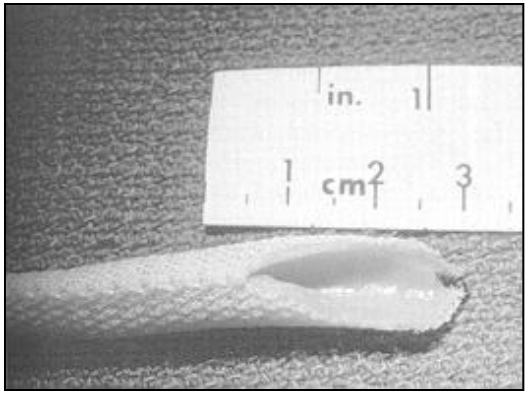
- Treatment of peripheral and coronary artery disease
  - Endovascular therapy
  - Bypass grafting
- Autologous tissue for bypass
  - Gold standard
  - Unavailable in 40% of patients

- Current alternatives
  - Prosthetic grafts
  - Biological grafts
  - Venous allografts

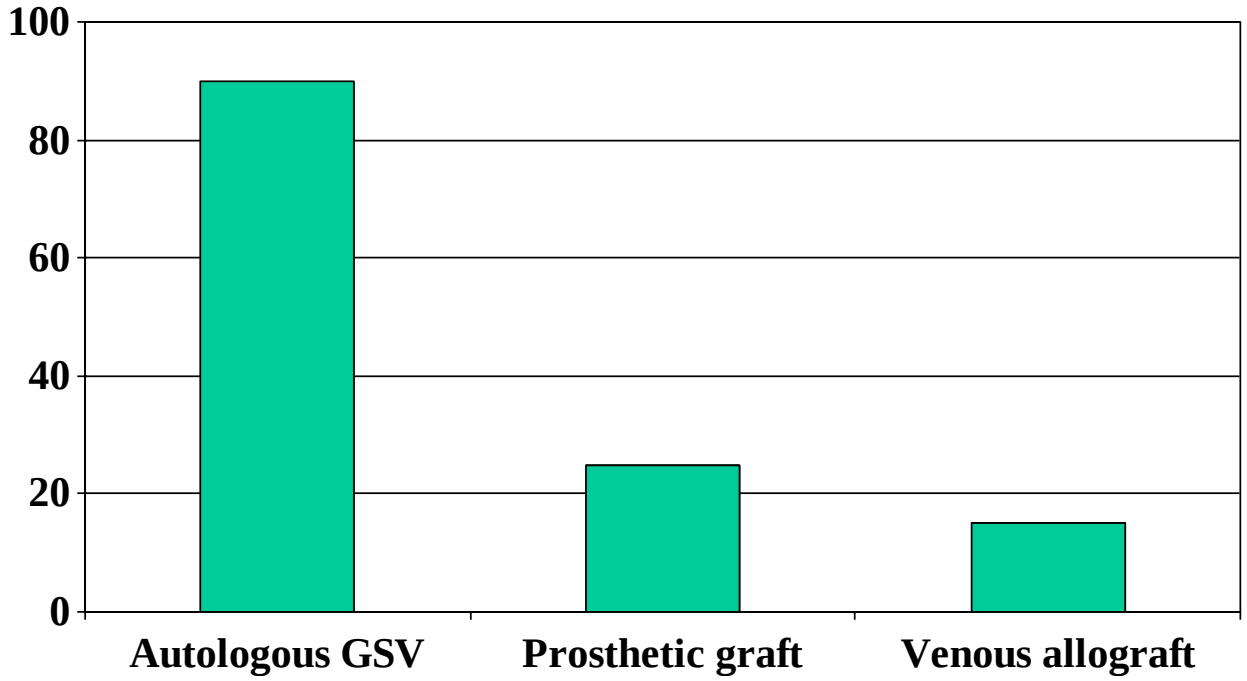








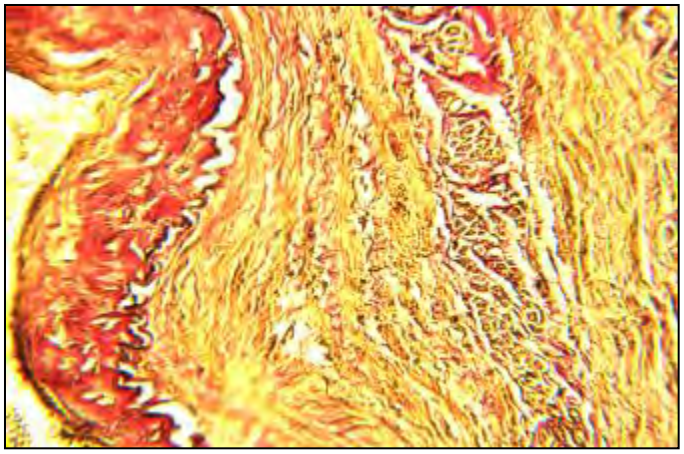
Patency @ 2 years

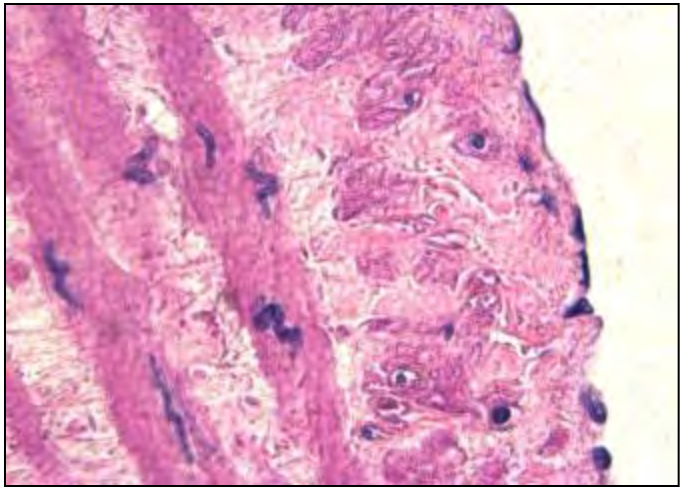


VASCULAR TISSUE ENGINEERING

Vascular Tissue Engineering

- Replication of vascular tissue
- Components of blood vessels:
  - Matrix (scaffolding)
    - Collagen
    - Elastin
  - Cells
    - Endothelial cells
    - Smooth muscle cells





Development of a Tissue-engineered Stem Cell Vascular Graft

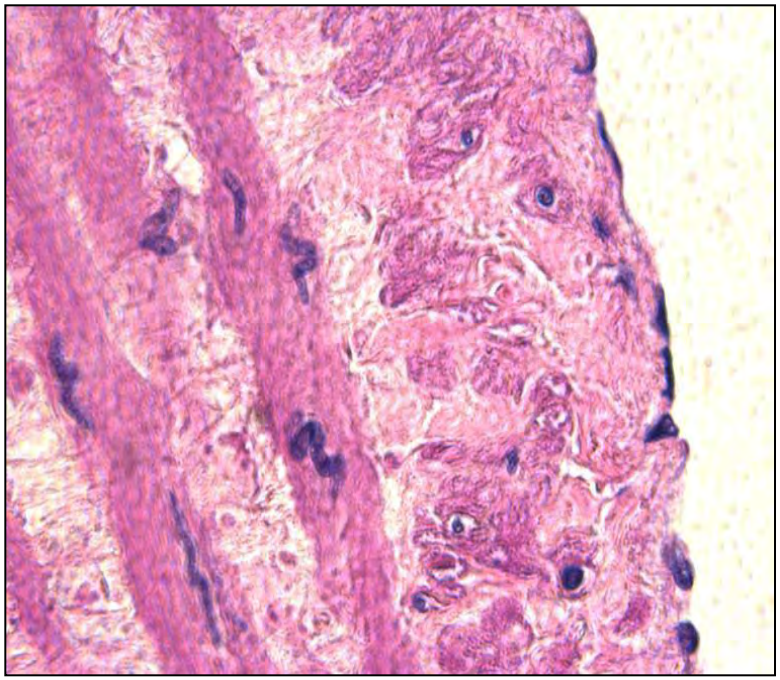
1. Matrix
2. Cells
3. Graft Creation
4. Graft Modifications
5. Future Directions

1. MATRIX

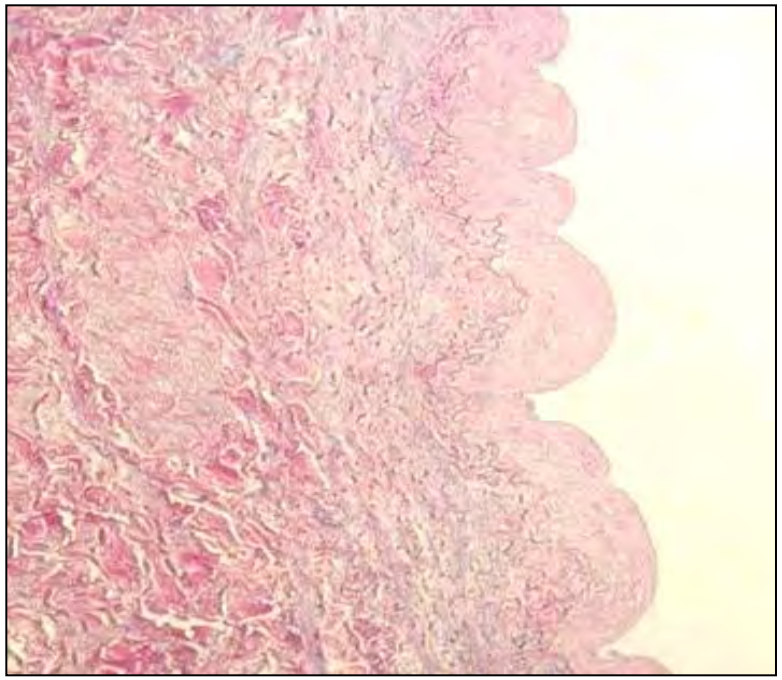
Matrix (scaffolding) options in Vascular Tissue Engineering

- Prosthetics
  - Non-absorbable: PTFE, Dacron grafts
  - Absorbable: PEG
- Collagen tubes
- Vascular allografts
  - Decellularized to reduce antigenicity
  - Veins or arteries

Human GSV
Decellularized with SDS

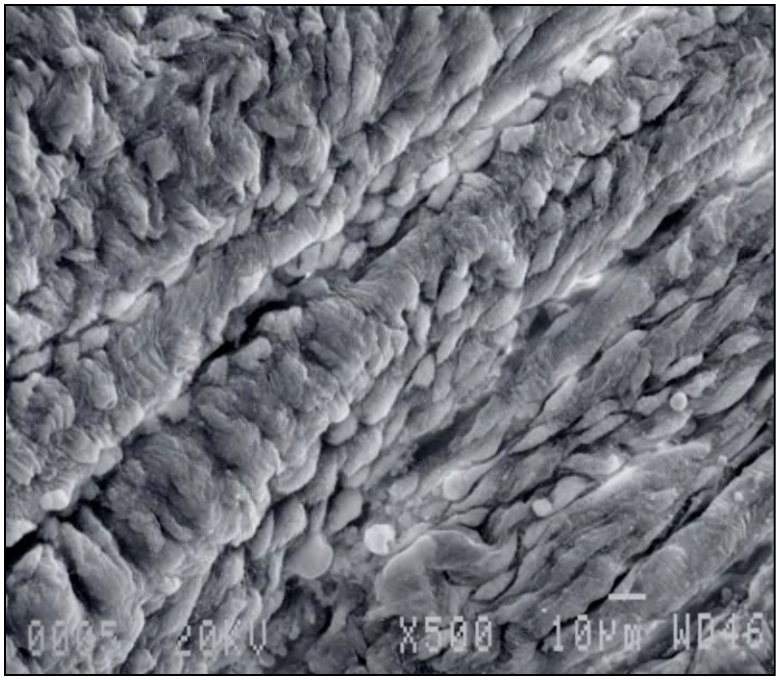


Fresh vein

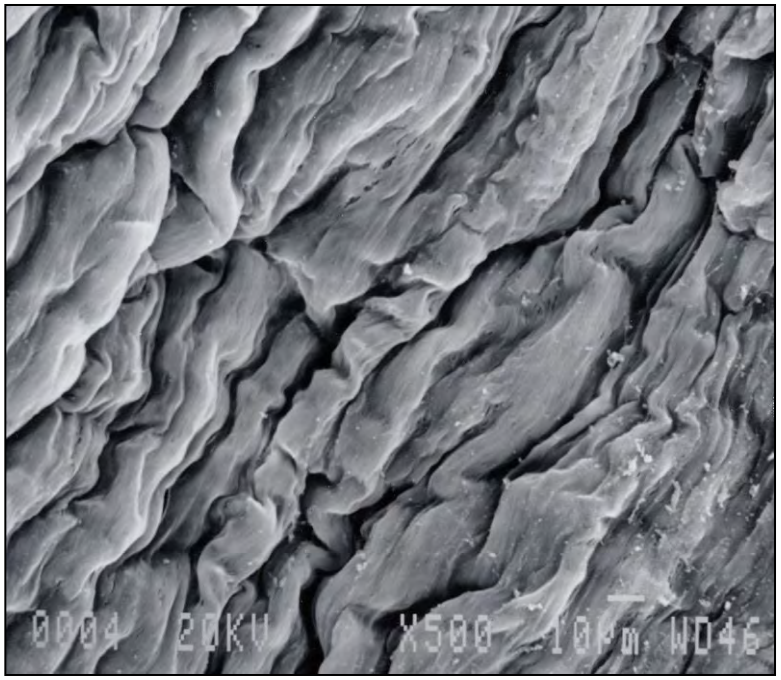


Decellularized vein

SDS removes all EC cells



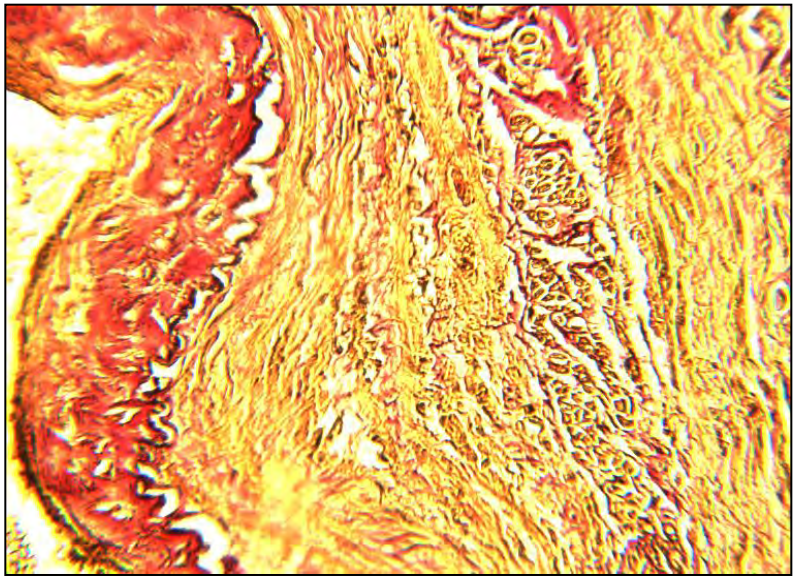
Fresh vein



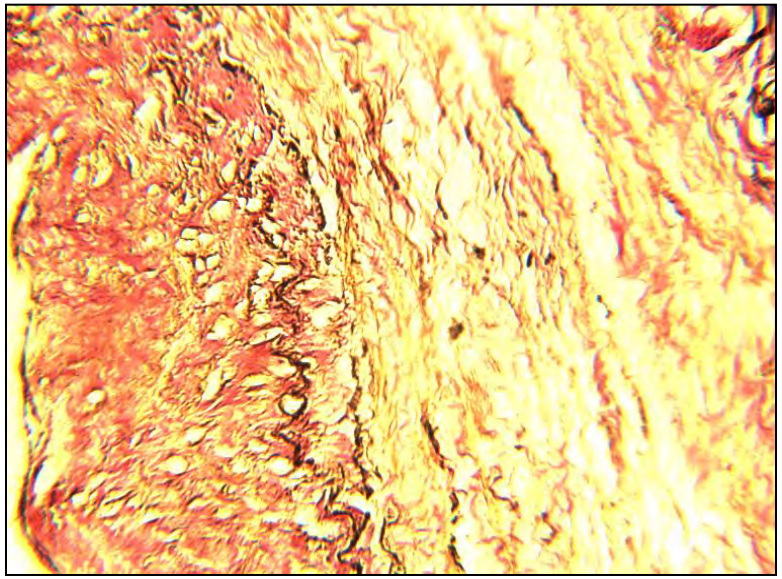
Decellularized vein

GSV matrix well-preserved after SDS decellularization

Fresh vein



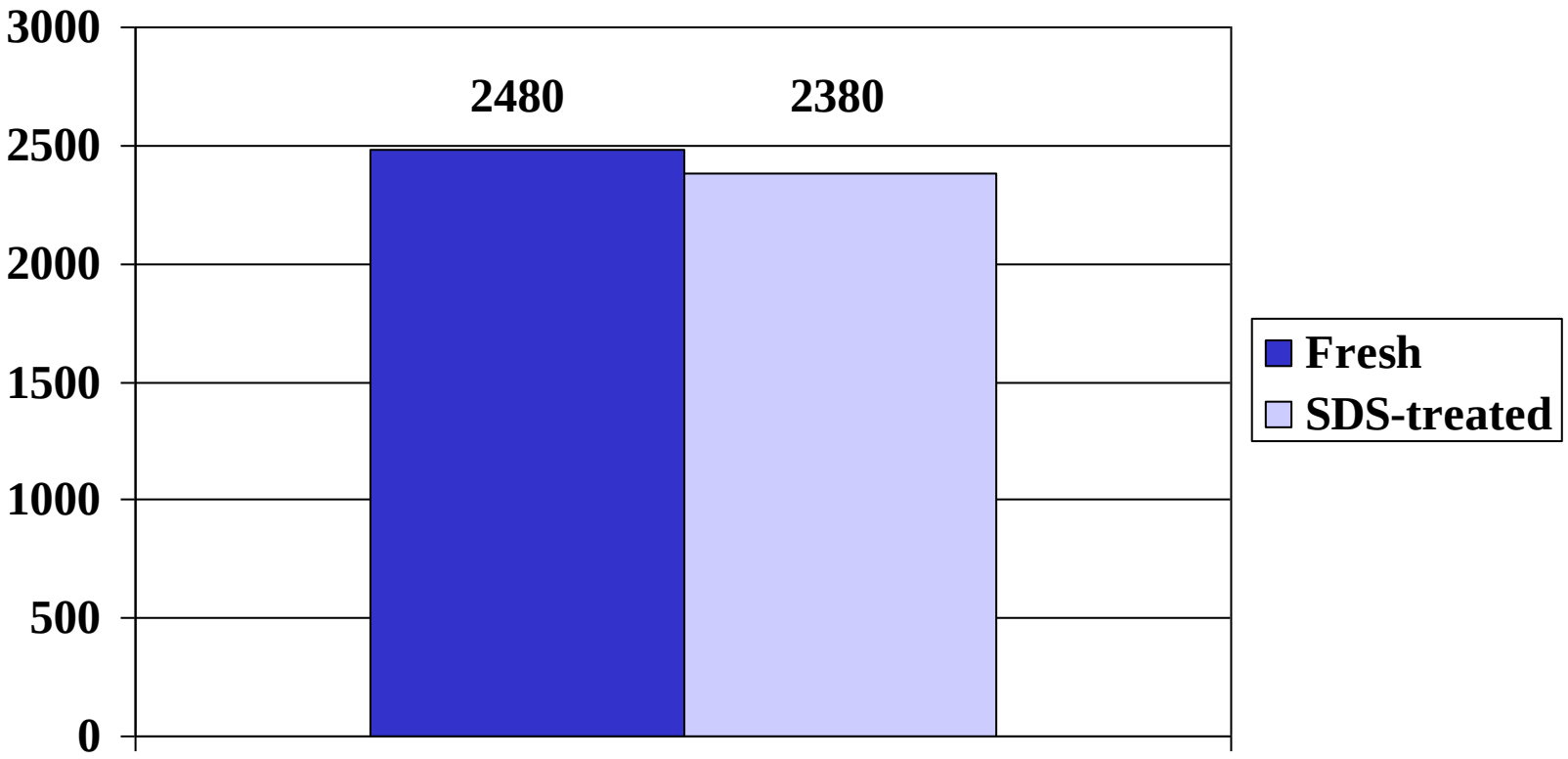
Decellularized vein



Collagen: 45 4% vs 41 7% (P>0.05)

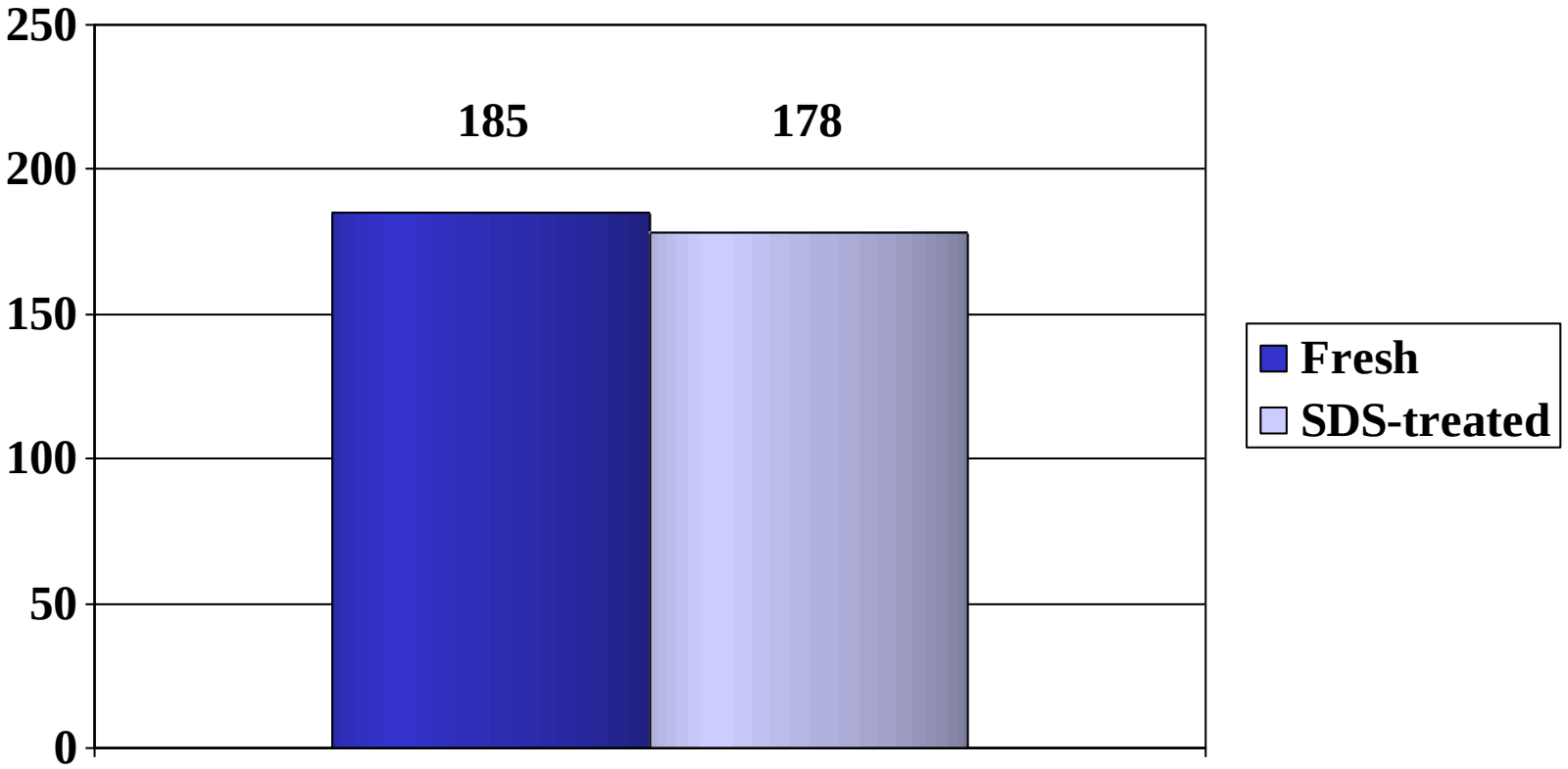
Elastin: 10 4% vs 8 3% (P=0.02)

Burst Strength is preserved



2480 460 vs 2380 620 mmHg (n=10, P>.05)

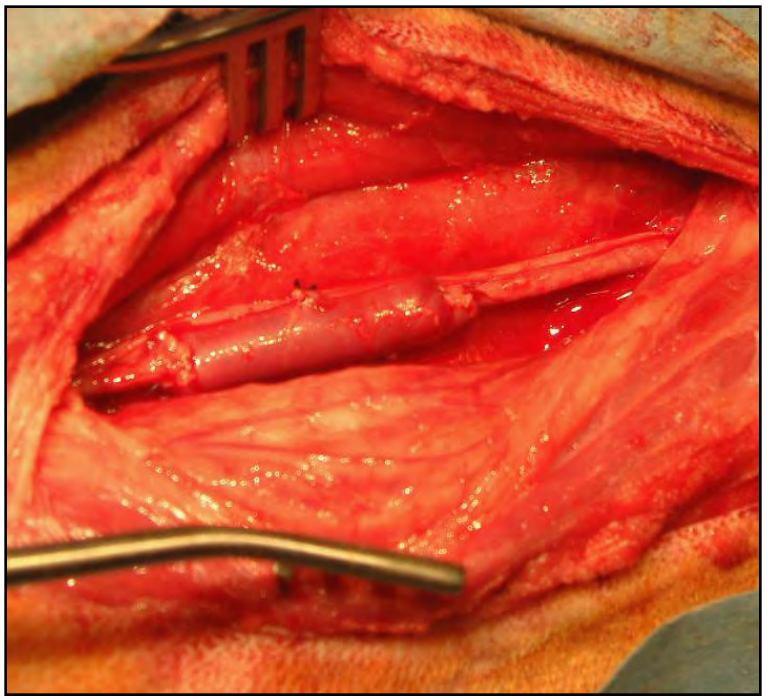
Suture Holding Strength is preserved



185 30 vs. 178 66 g (n=10, P>.05)

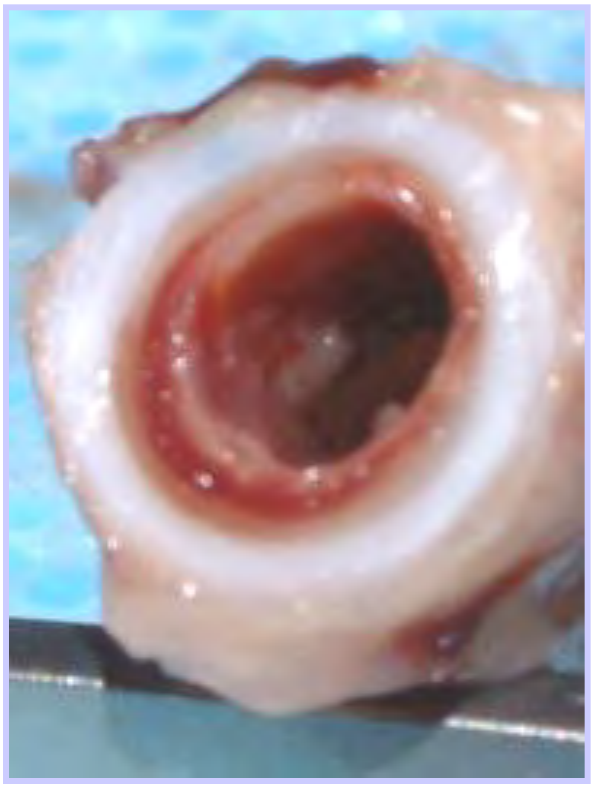
In vivo testing of decellularized vein

- Canine model
  - Jugular vein
  - Carotid interposition graft
- Groups
  - Autograft (n=22)
  - Allograft (n=10)
  - Decell. Allograft (n=12)

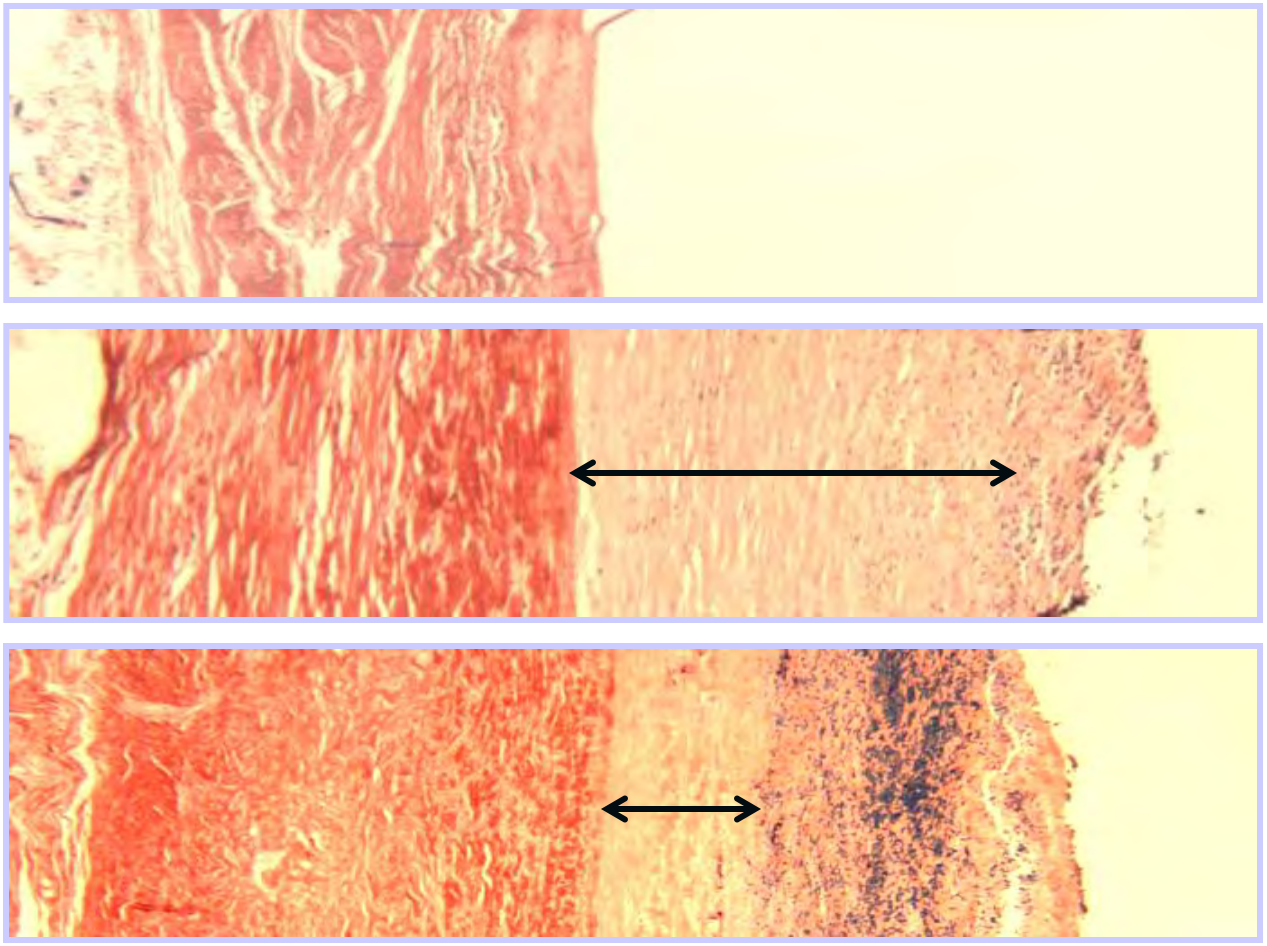


Gross Appearance @ 2 months

Autograft	Allograft	Decell. Allograft

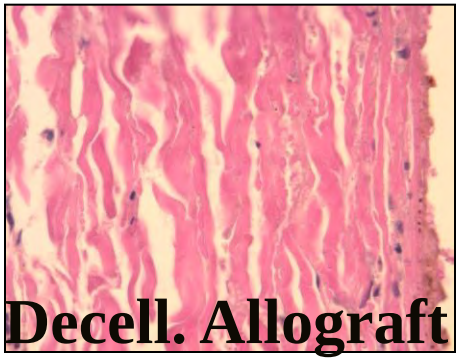


Histologic Appearance @ 2 months



Autograft
Allograft
Decell. Allograft

Immunologic Response @ 2 weeks



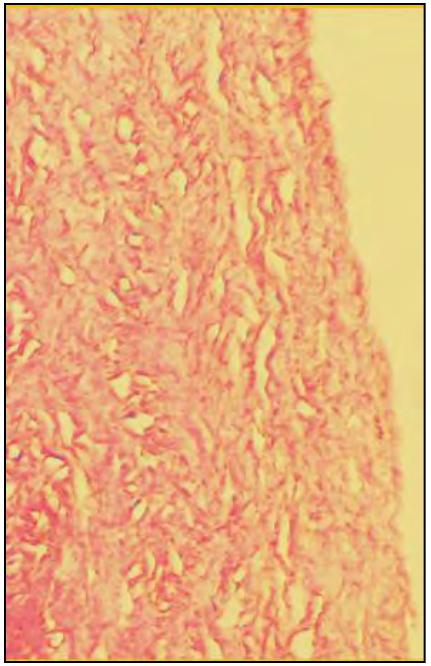
Mononuclear cell infiltrate (cell/hpf):

Autograft:	19±8
Allograft:	59±24*
Decell. Allograft:	19±15

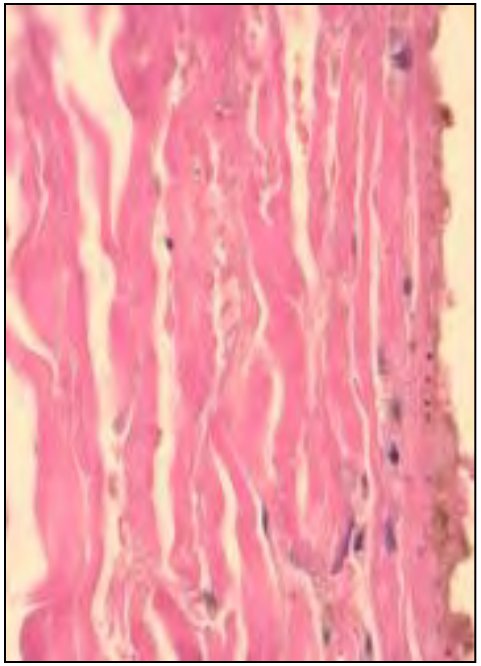
(*n=4, P<.05)

Cellular Repopulation of Decellularized Vein in vivo

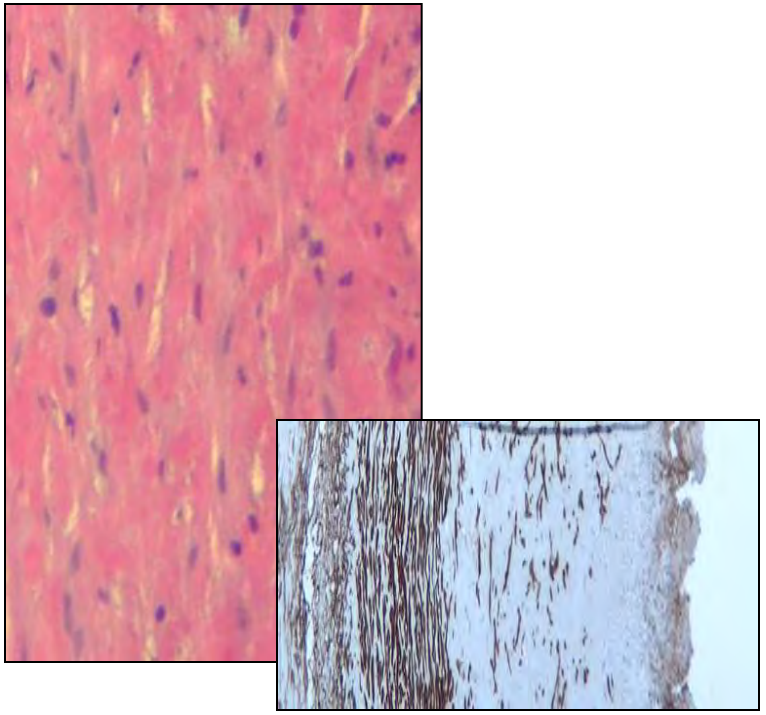
Implantation



2 wk



2 mo



SMC α-actin MAb

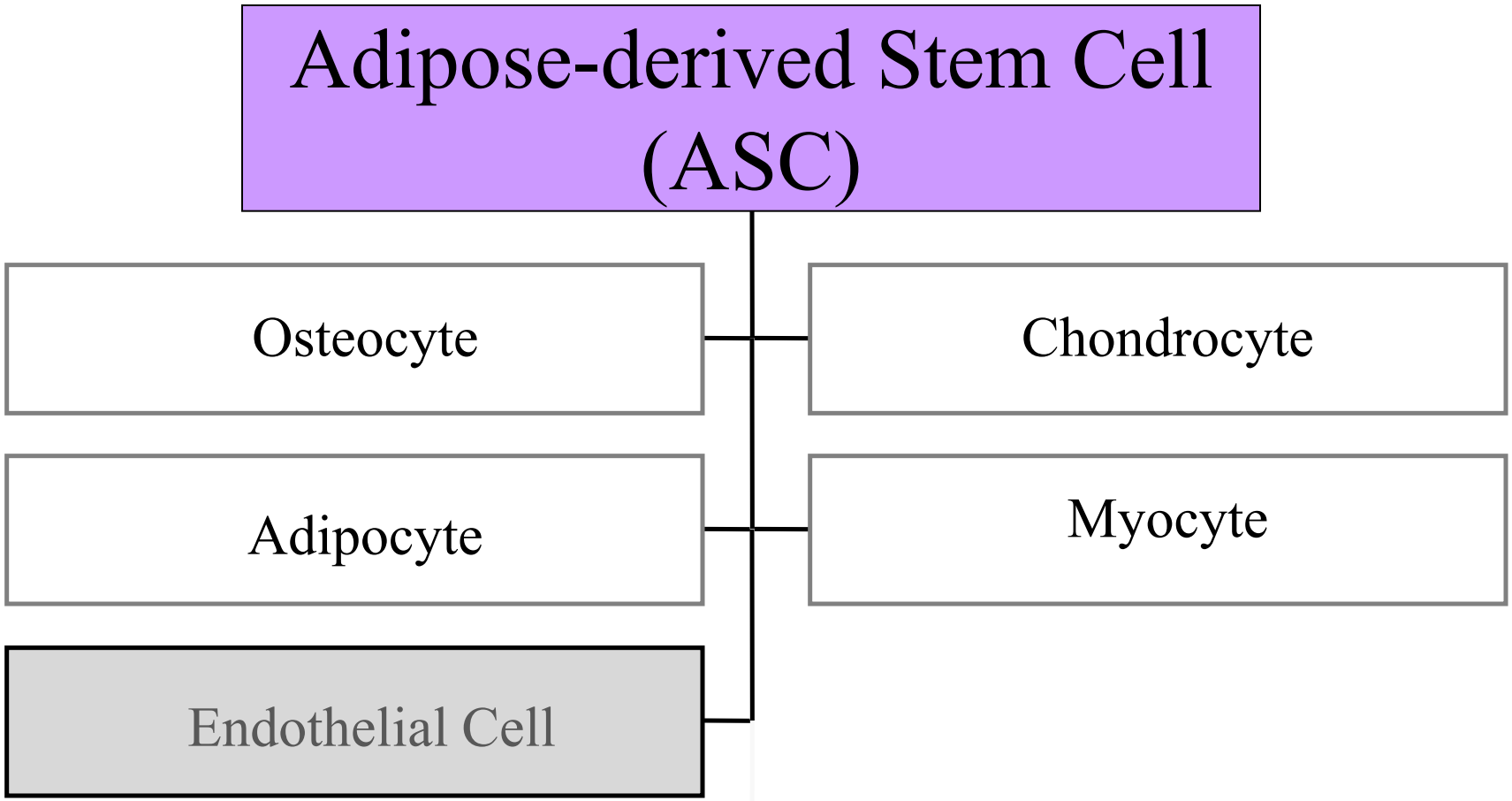
Summary:
Decellularized vein allograft

- Readily available and easy to produce
- Similar strength to natural vein
- Reduced antigenicity
- Luminal surface remains thrombogenic

2. CELLS

Cell options for Vascular Tissue Engineering

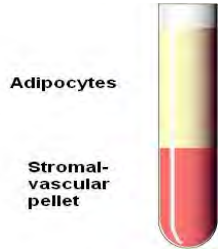
- Endothelial cells (EC)
  - Large and small vessel EC
  - Endothelial progenitor cells (from blood)
  - Mesenchymal stem cells (bone marrow, fat, amnionic fluid)
- Smooth muscle cells


Adipose-derived Stem Cell (ASC)
Osteocyte
Chondrocyte
Adipocyte
Myocyte
Endothelial Cell

ASC Isolation Technique

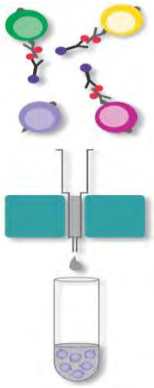
1. Liposuction

2. Collagenase



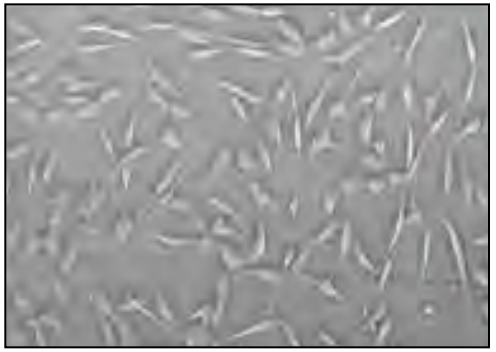
3. Centrifugation

4. Cell Culture



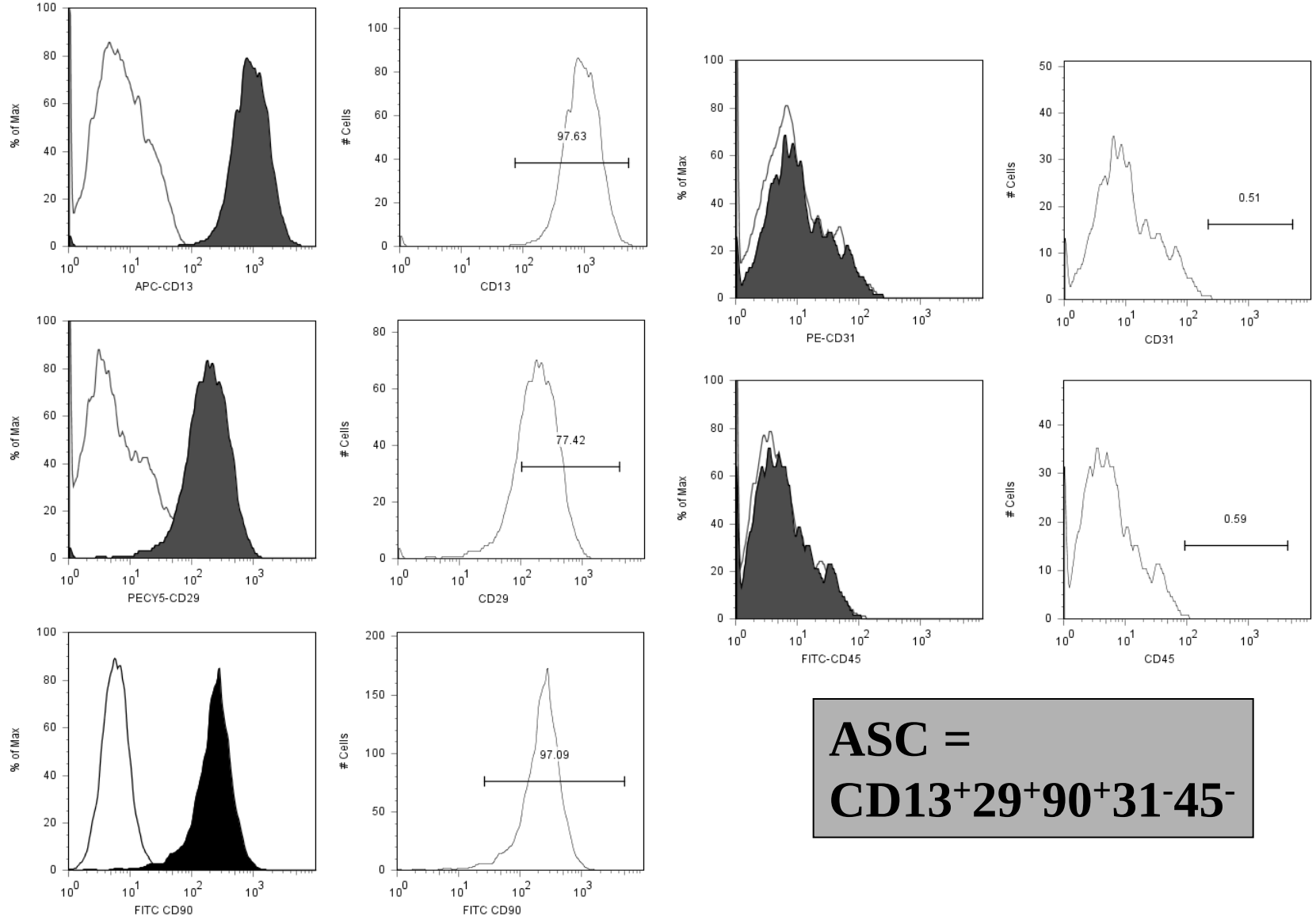
Negative selection: **CD31,45**

5. MACS



ASC

ASC Surface Markers



ASC = $CD13^{+}29^{+}90^{+}31^{-}45^{-}$

Differentiation of ASC into EC

- Chemical stimulus
  - Endothelial cell growth supplement
- Physical stimuli
  - Shear stress
  - Contact with basement membrane

Response to:
ECGS

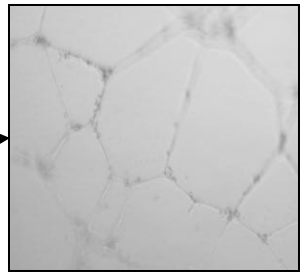
Cell line	CD31	eNOS	vWF	Matrigel Cords
EC	+	+	+	
SMC	-	-	-	
ASC	-	-	-	

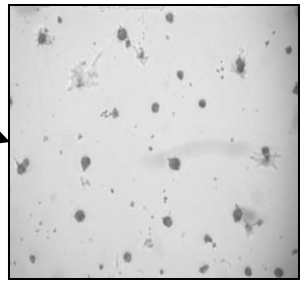
Response to:
ECGS

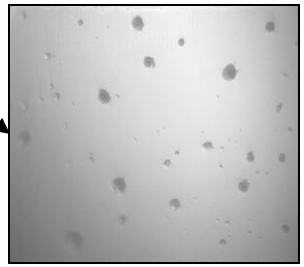
Cell line	CD31	eNOS	vWF	Matrigel Cords
EC	+	+	+	
SMC	-	-	-	
ASC	-	-	-	
ASC +ECGS	+	-	+	

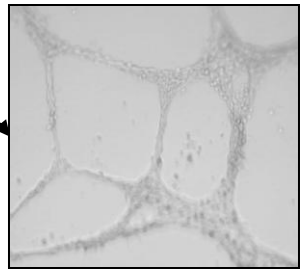
Cell line	CD31	eNOS	vWF	Matrigel Cords
EC	+	+	+	+
SMC	-	-	-	-
ASC	-	-	-	-
ASC +ECGS	+	-	+	+

Response to:
ECGS



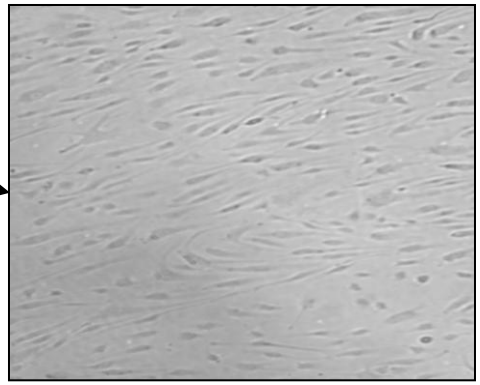


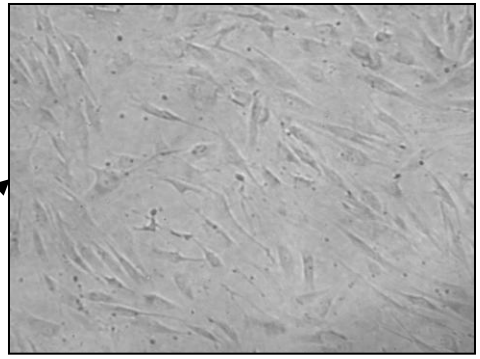


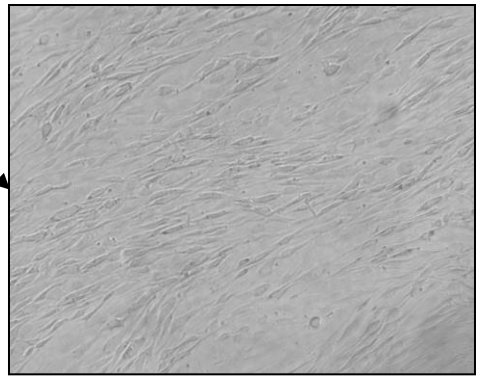


Cell line	CD31	eNOS	vWF	Alignment
EC	+	+	+	+
SMC	-	-	-	-
ASC	-	-	-	-
ASC +ECGS	+	-	+	+

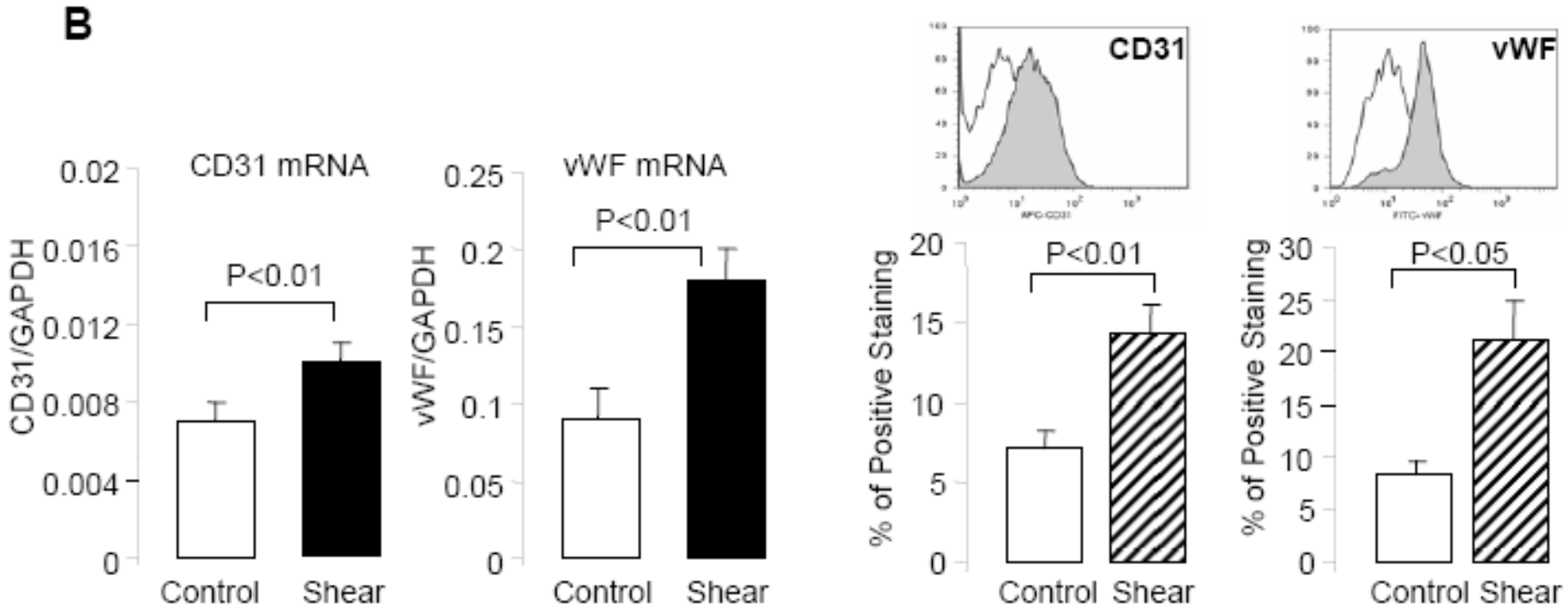
Response to:
Shear Force



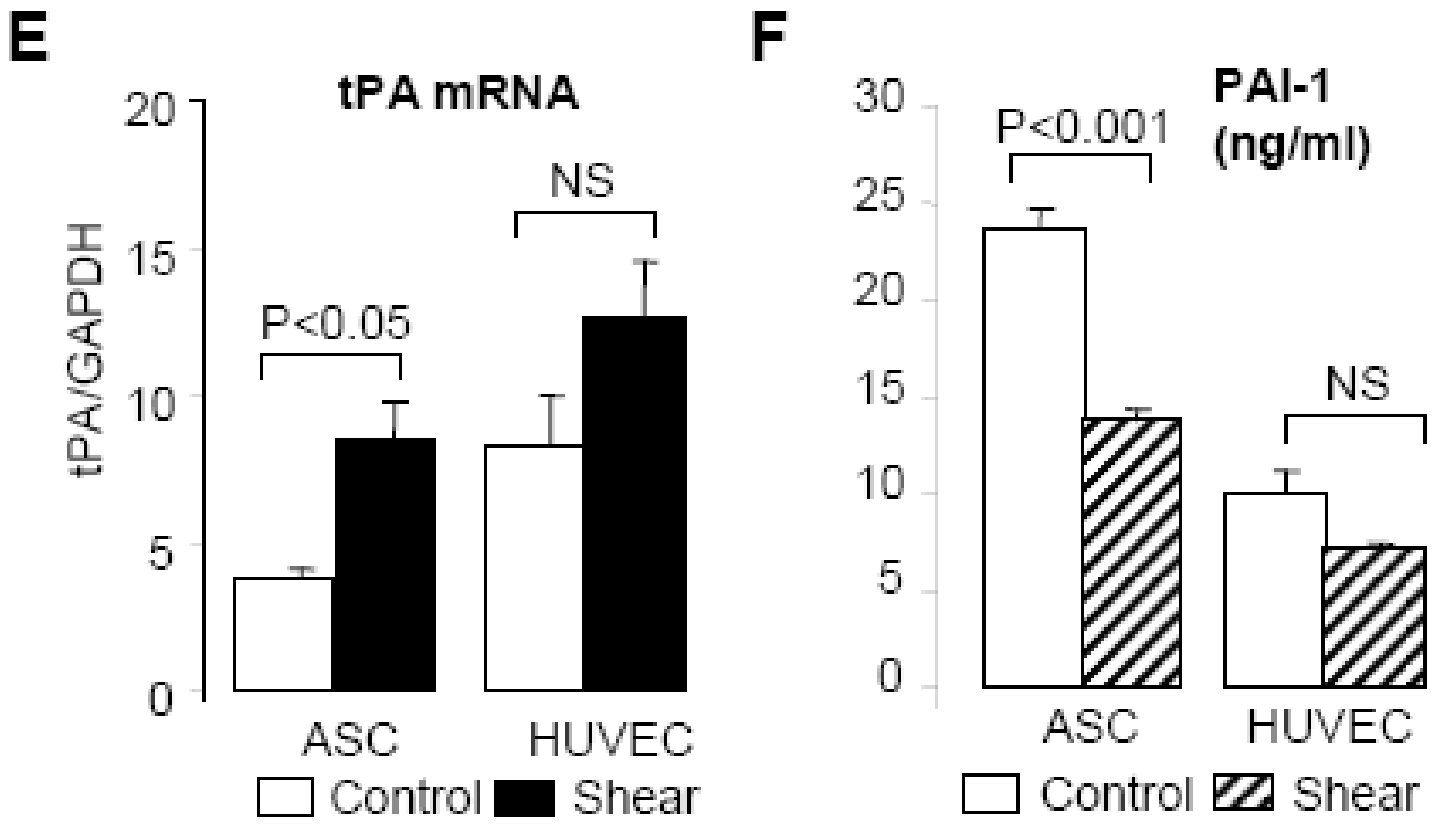




Shear upregulates EC molecular markers



Shear promotes non-thrombogenic phenotype



Cell Summary: Endothelial differentiation of ASC

- Promoted by growth factors, shear
- Acquisition of morphological features in response to Matrix, shear
- Acquisition of key molecular markers, but not eNOS
- Readily available in elderly patients with vascular disease

3. GRAFT CREATION

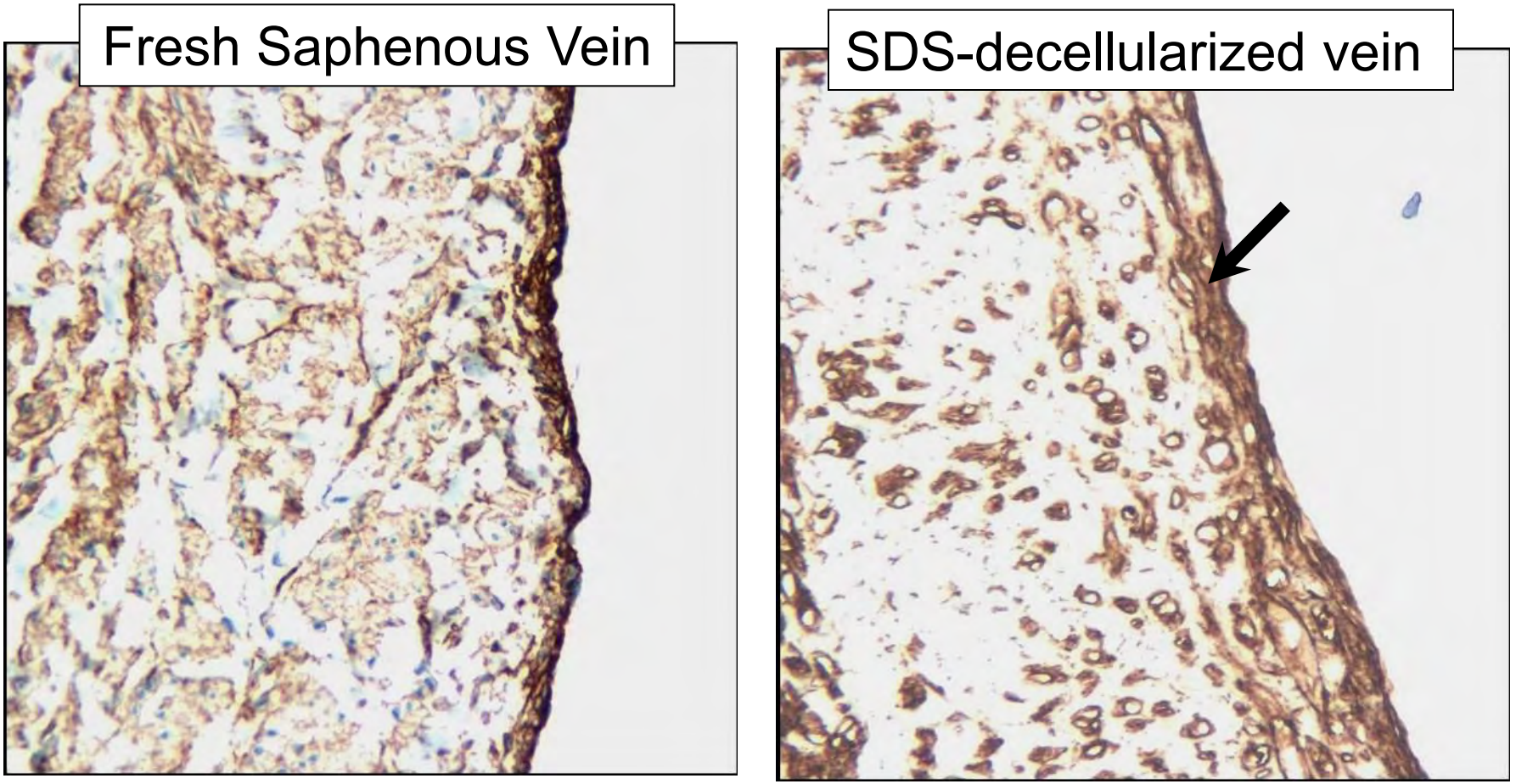
Endothelial cell seeding

- Mansfield, 1970: growth of endocardial cells on Dacron suture
- Herring, 1978: seeding of Dacron graft with EC
- Weinberg, Bell, 1986: collagen tube seeded with EC
- Jarrell & Williams, 1985: single step pressure "sodding" PTFE
- Zilla, 1990s-present: two step EC-PTFE graft culture

Seeding decellularized vein

- Does the luminal surface have preserved basement membrane for the attachment of the ASC (EC)?
- Variables for seeding:
  - Time for attachment
  - Role of pre-coating

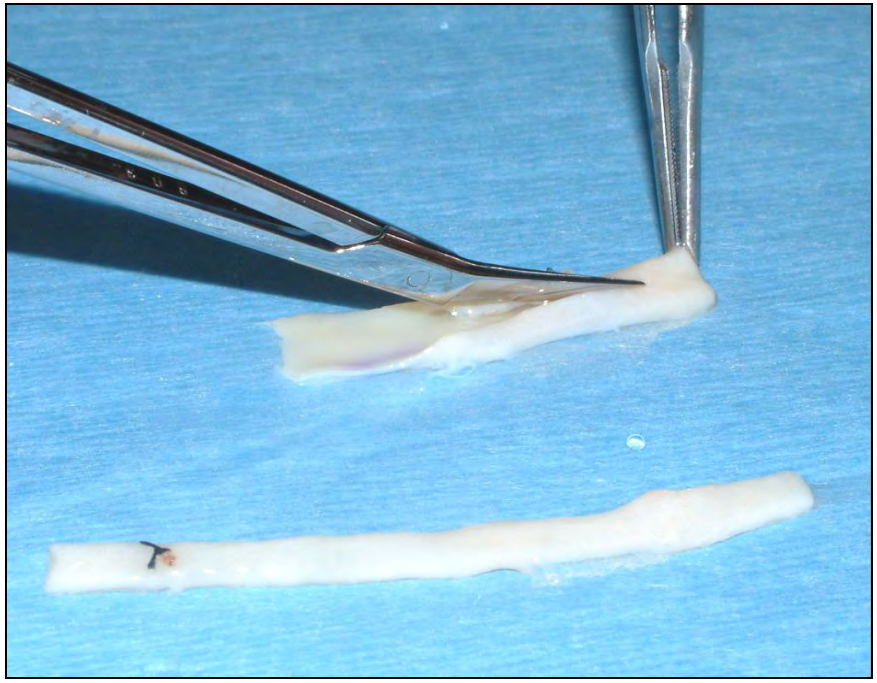
Decellularized Vein has Preserved Basement Membrane

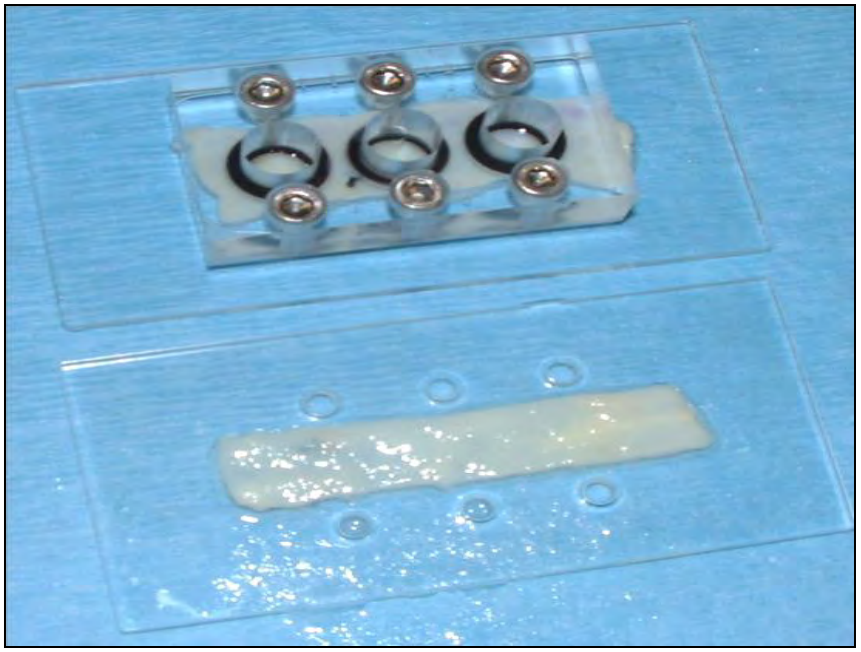


Type IV Collagen MAb

Experimental Model

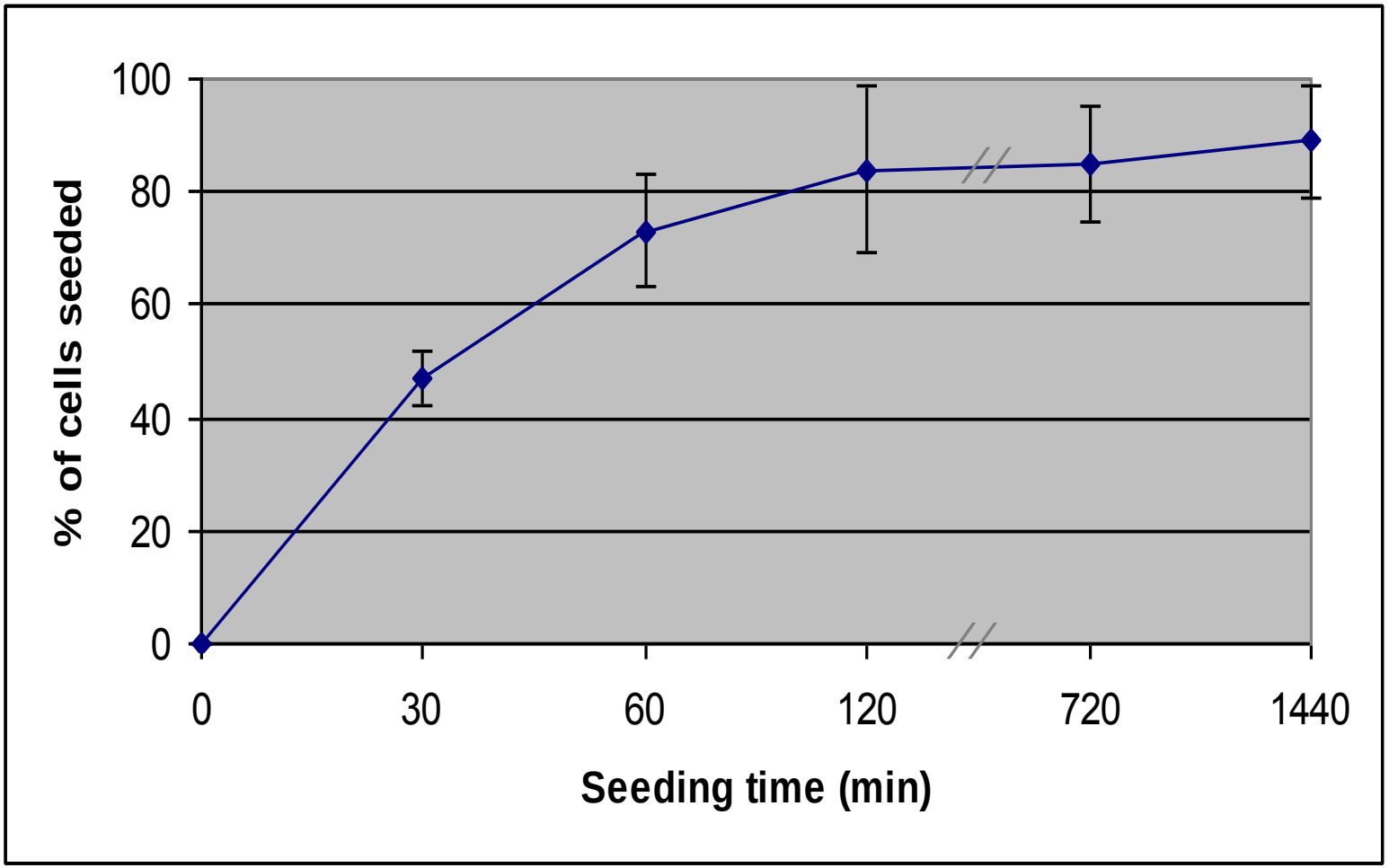
In vitro



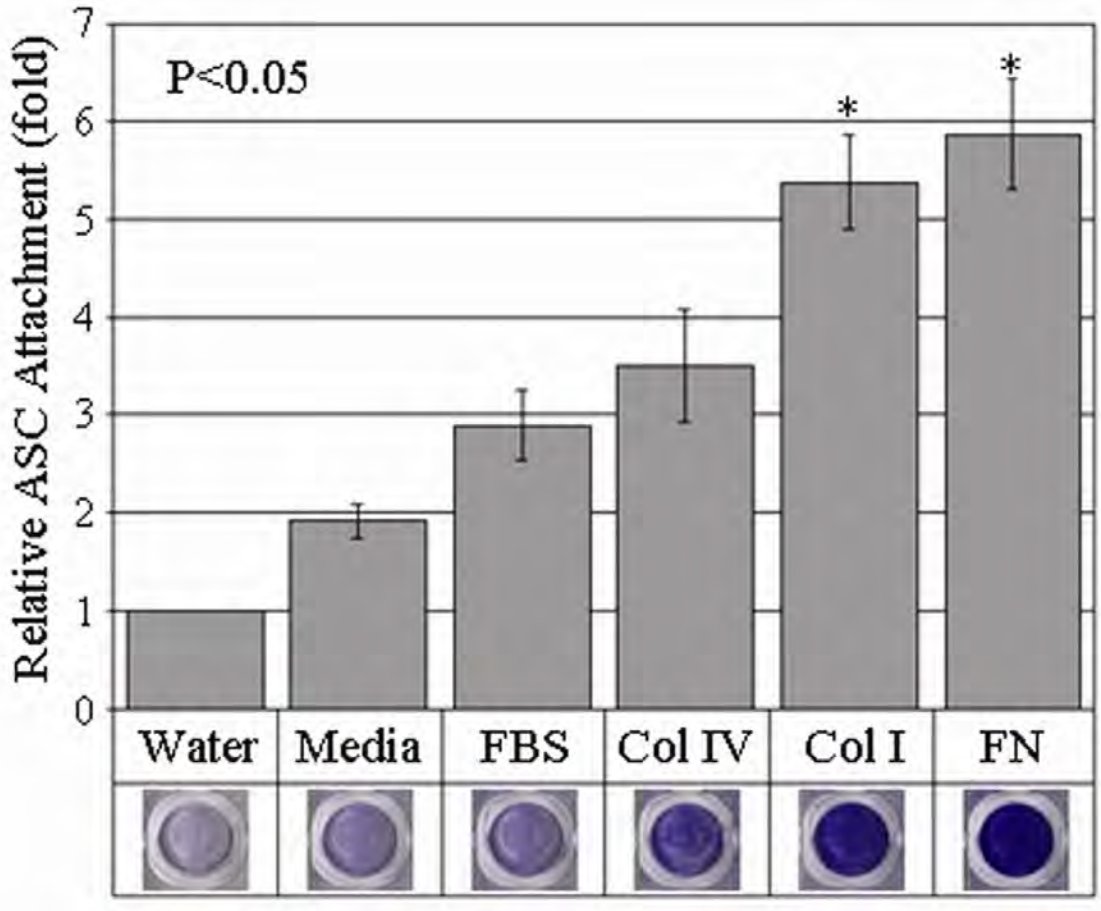


Cell Attachment vs Time

(n=12)

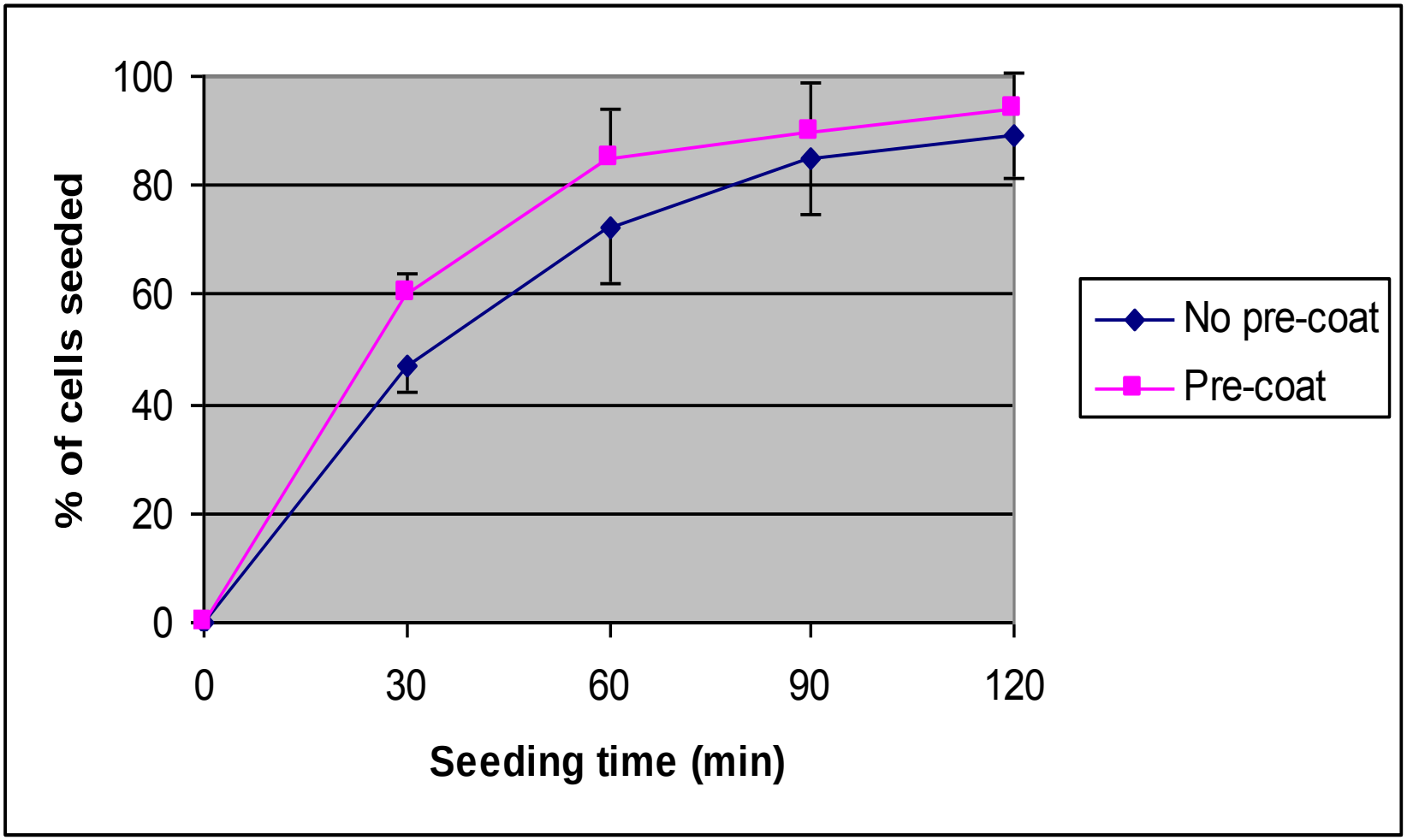


Candidate materials for Pre-coating

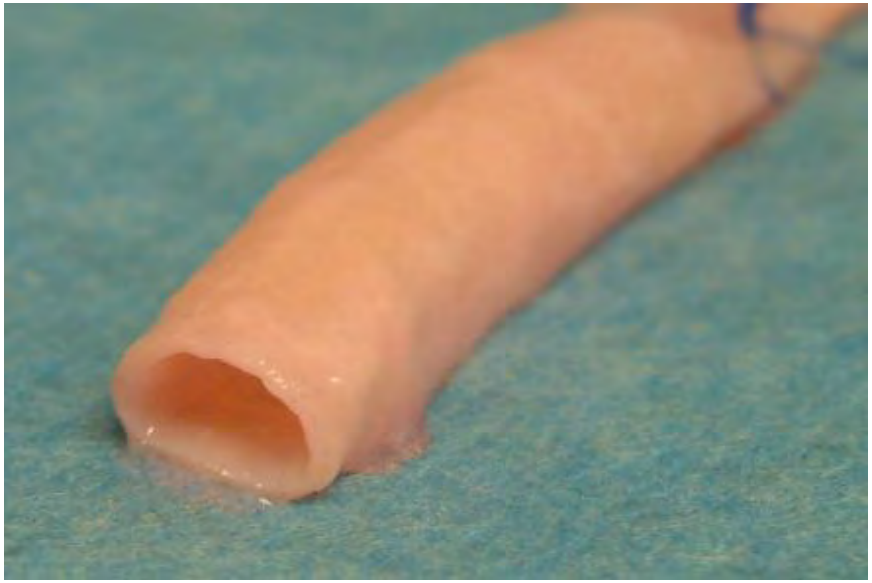


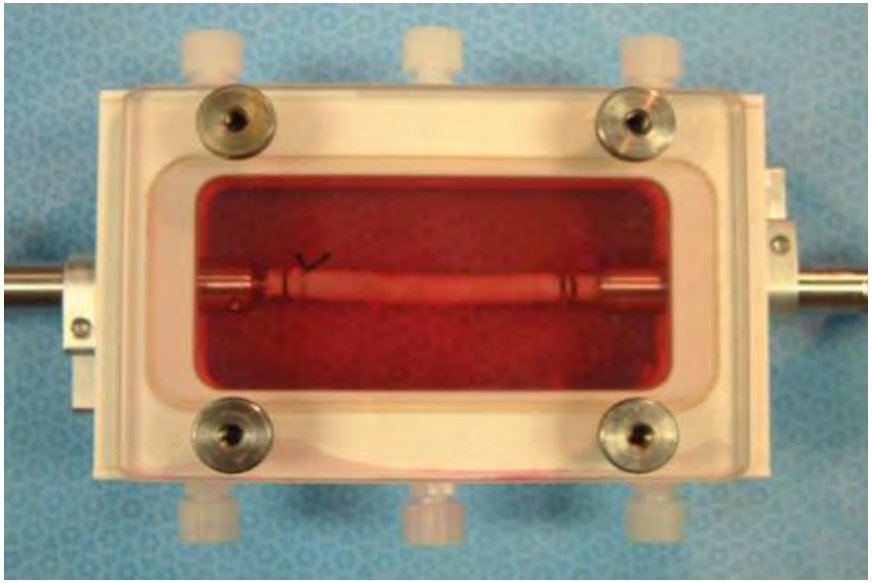
Effect of Pre-coating

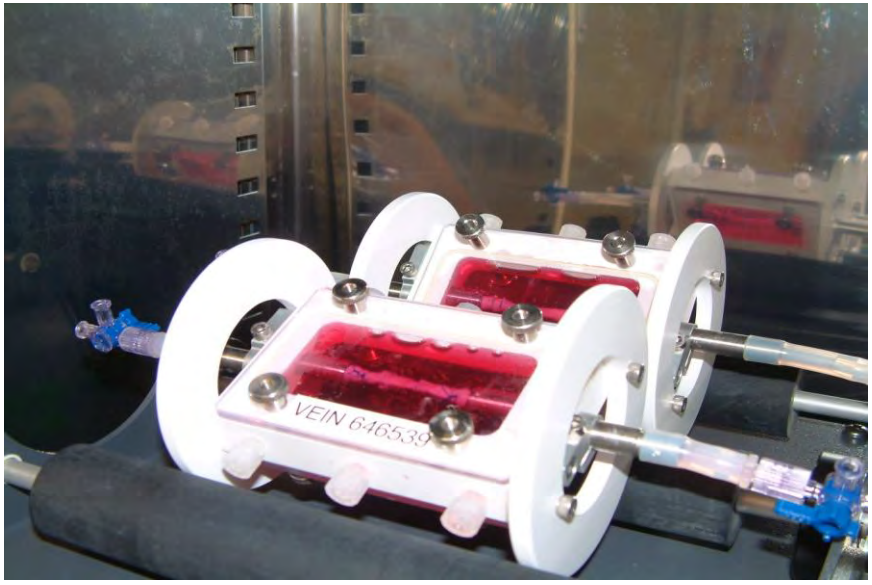
(n=6)



Vascular graft bioreactor

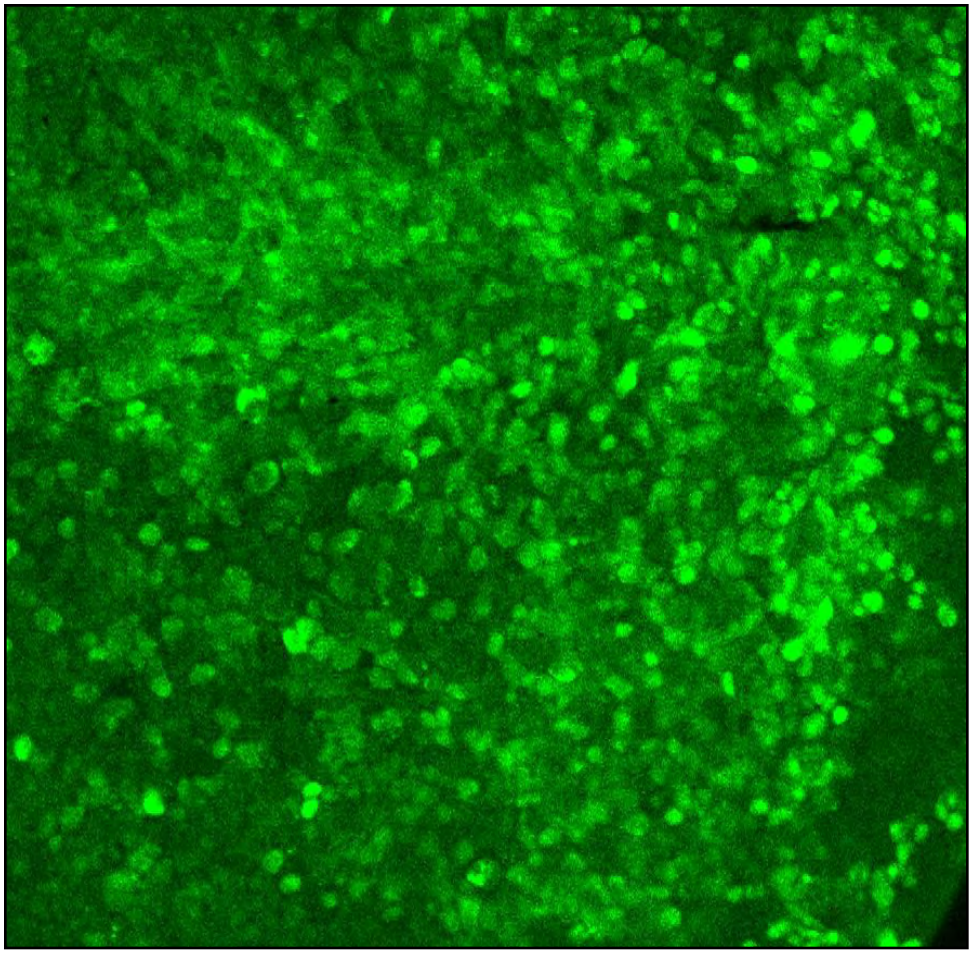




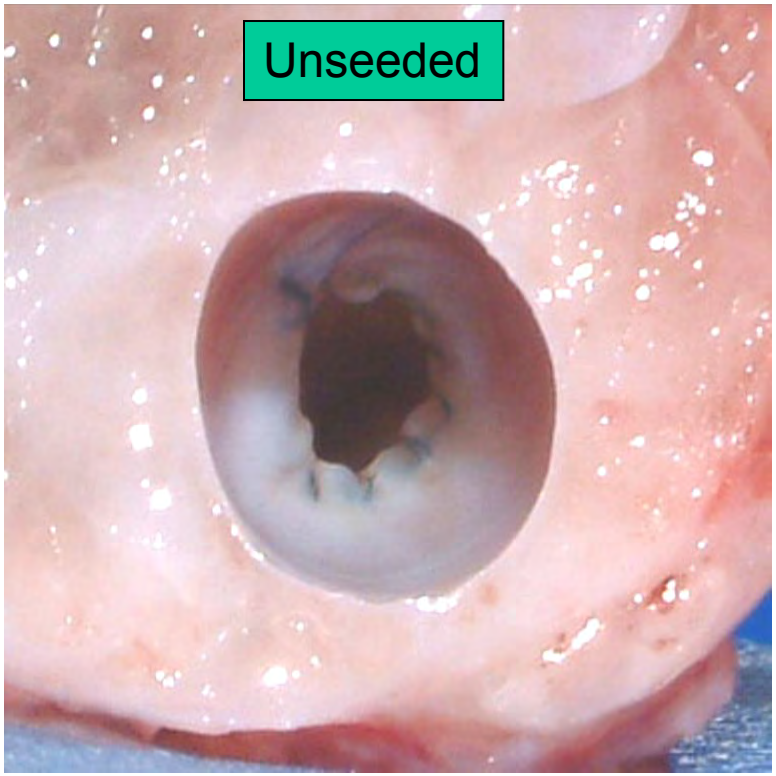


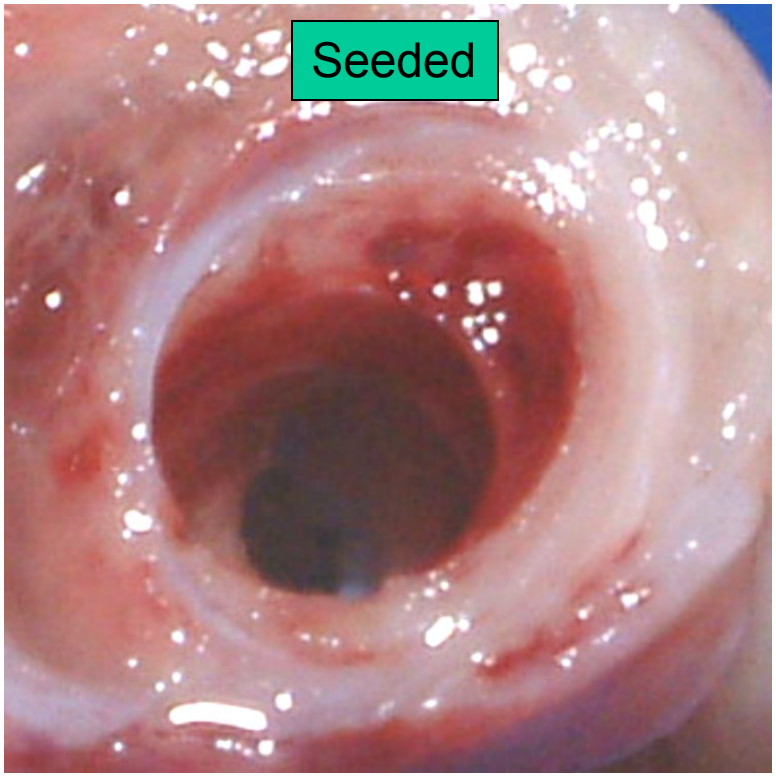
Initial graft creation

- Decellularized vein allograft
- Pre-coated with fibronectin
- Seeded with autologous stem cells
- Cultured in bioreactor x 3 days



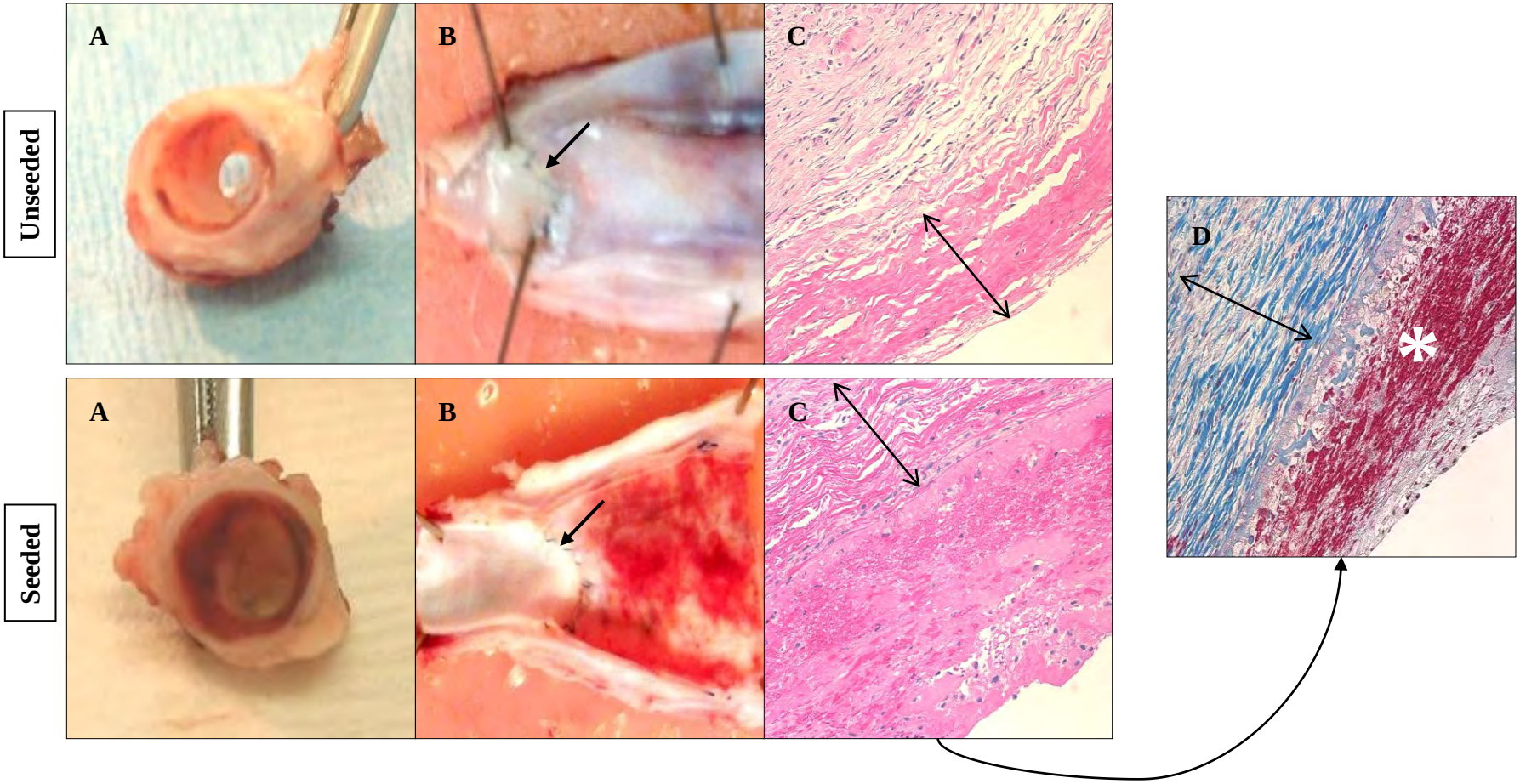
Gross examination @ 2 weeks





Histology @ 2 weeks

Stem Cell Graft:	# of GRAFTS PATENT (2wk)
Unseeded	6/7
Seeded	6/7



Initial Graft Summary

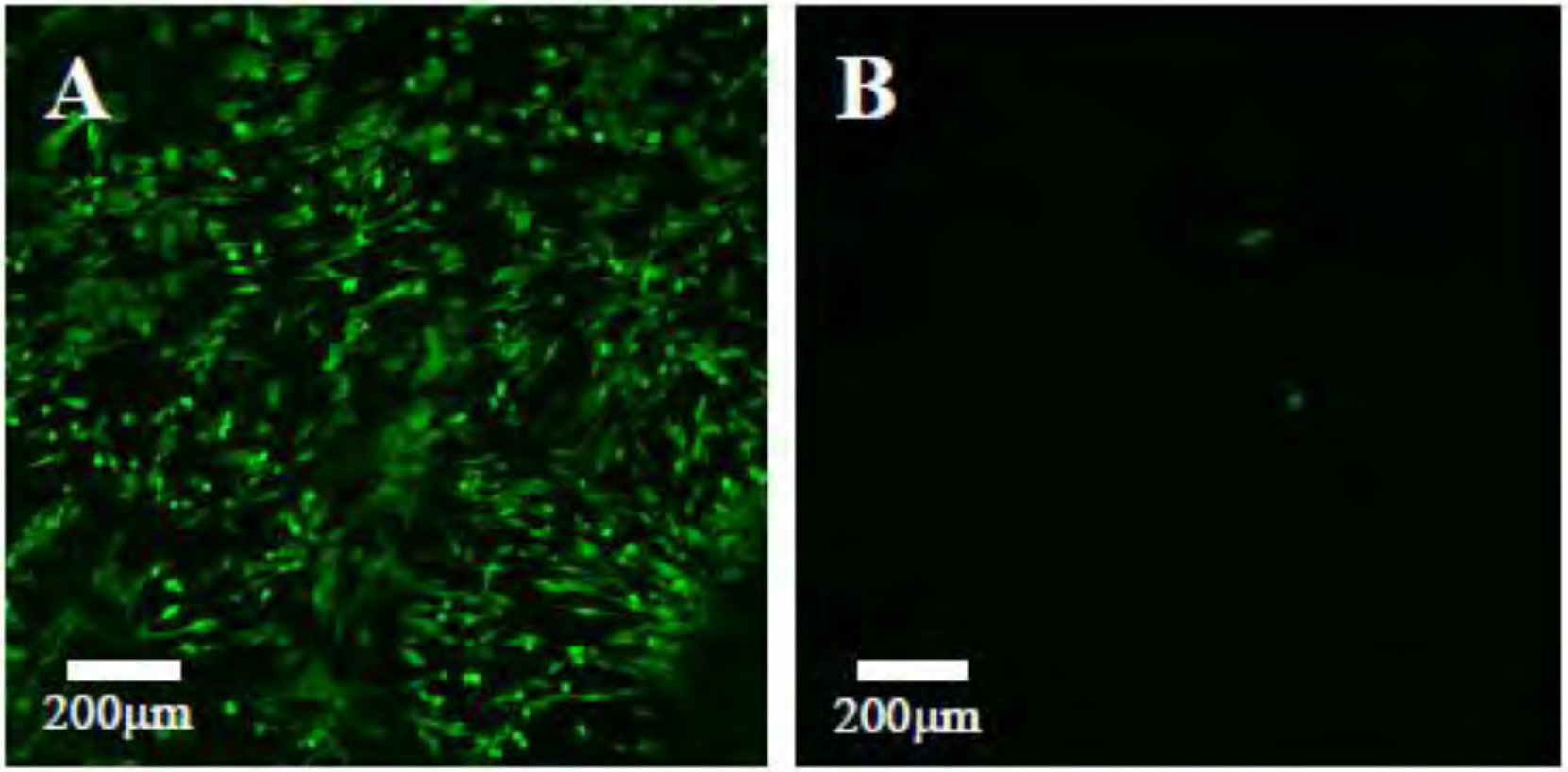
- Success achieved in the various steps of graft creation without:
  - Infection
  - Rupture
- Although patent at 2 weeks, the lumen of the stem cell-seeded graft appeared thrombogenic

4. GRAFT MODIFICATIONS

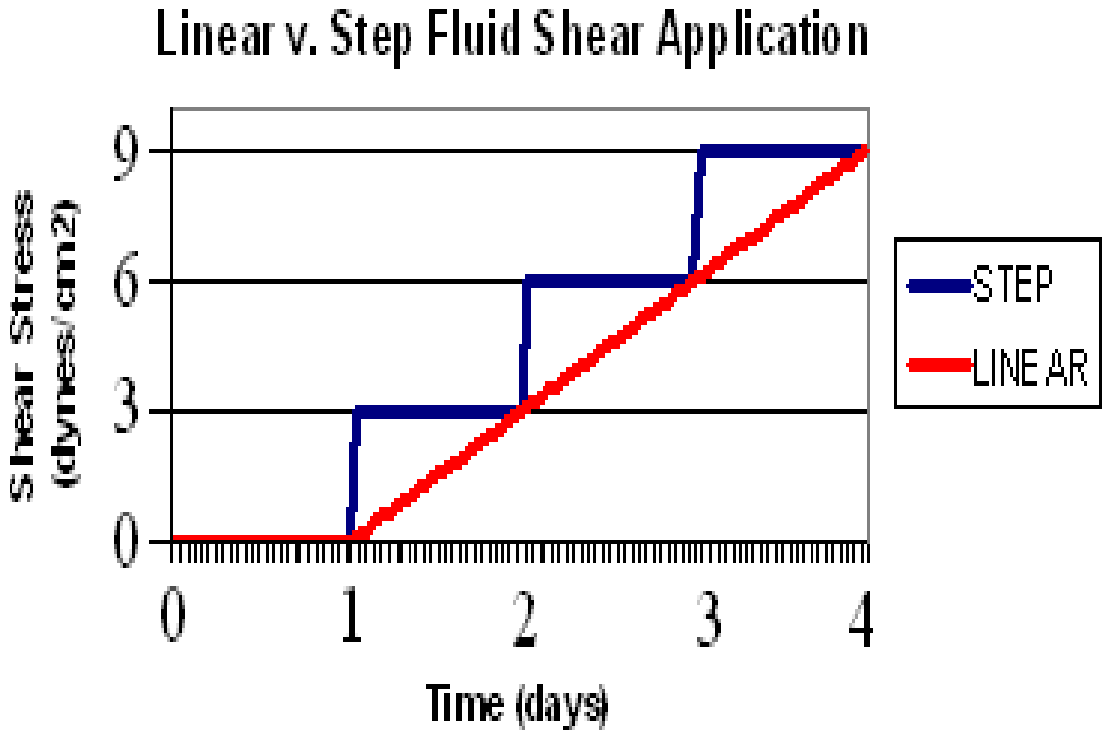
Graft modifications

- Was the initial "failure" due to poor stem cell retention?
  - Evaluate retention ex vivo and modify seeding technique using flow conditioning
- Or due to poor stem cell function?
  - Evaluate nitric oxide production (NO) and transduce the cells with endothelial nitric oxide synthase (eNOS)

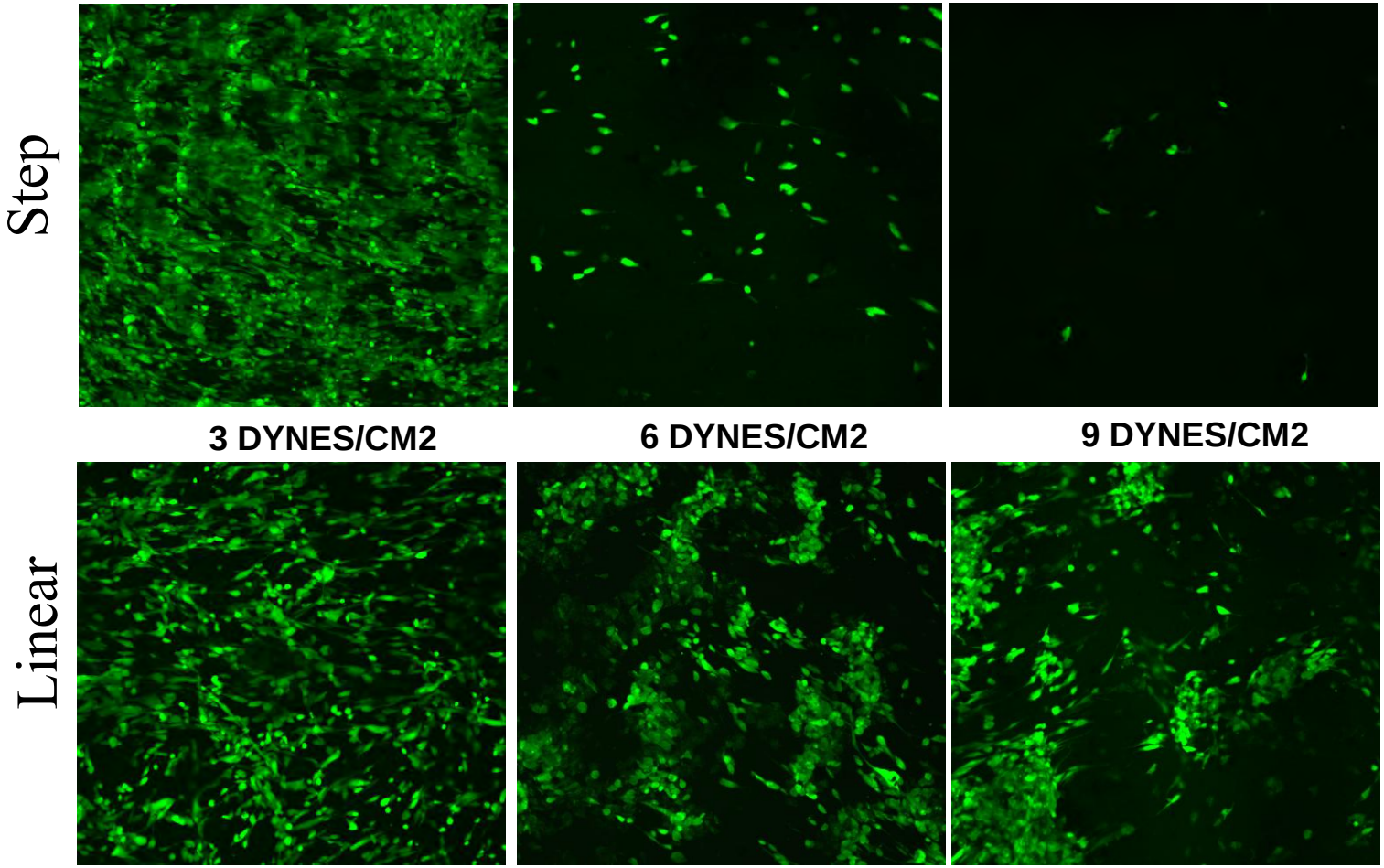
Acute increase in shear dislodges seeded stem cells from graft



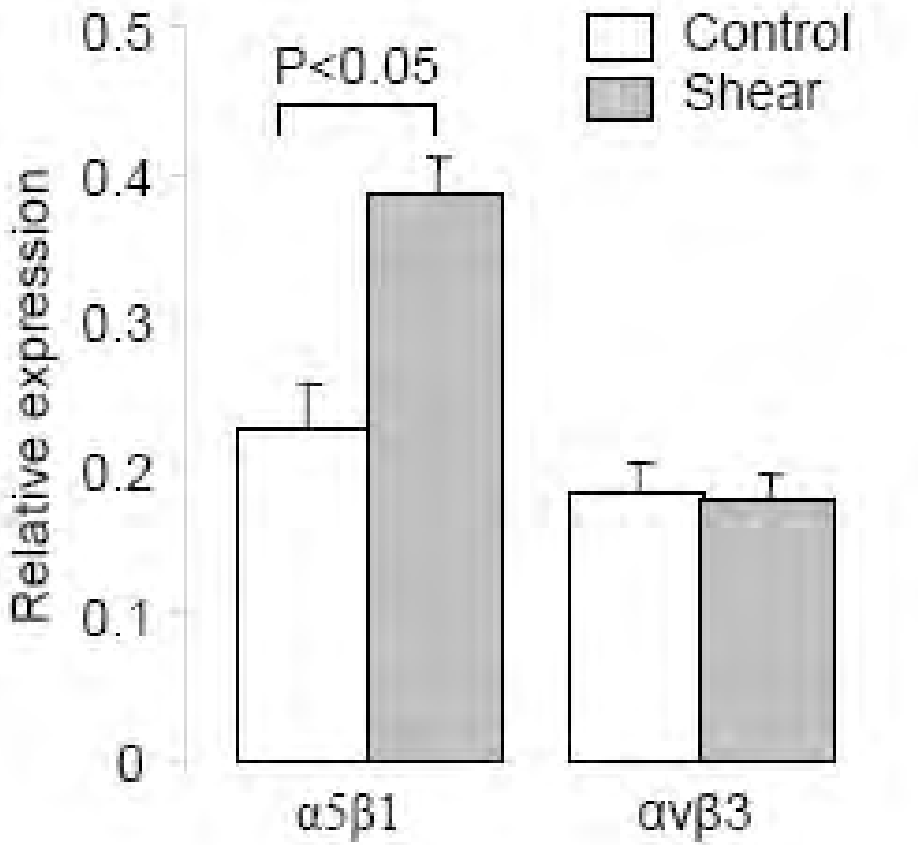
Flow conditioning to improve retention of seeded cells



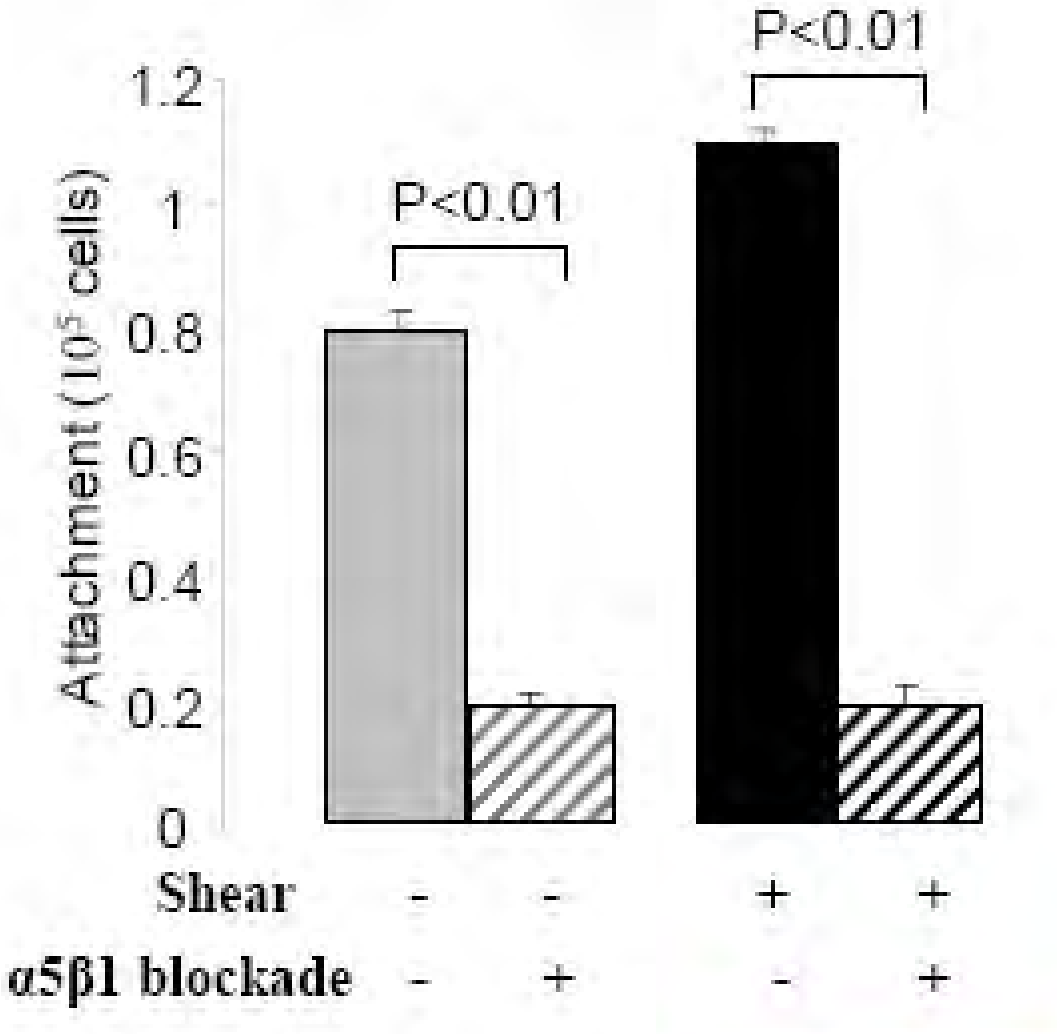
“Linear” flow conditioning improves cell retention



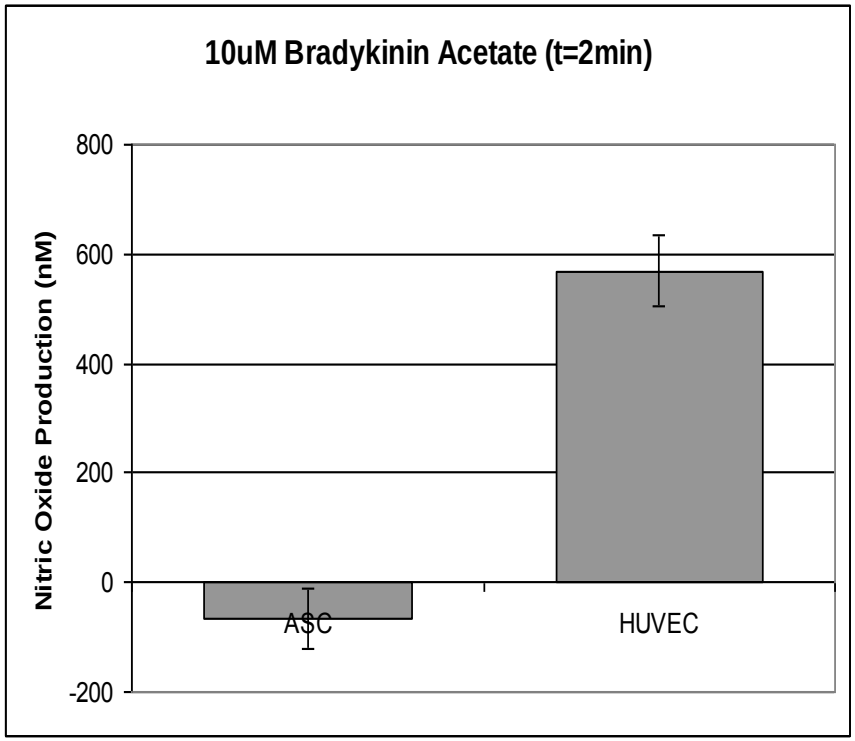
Mechanism: Shear stimulates integrin expression

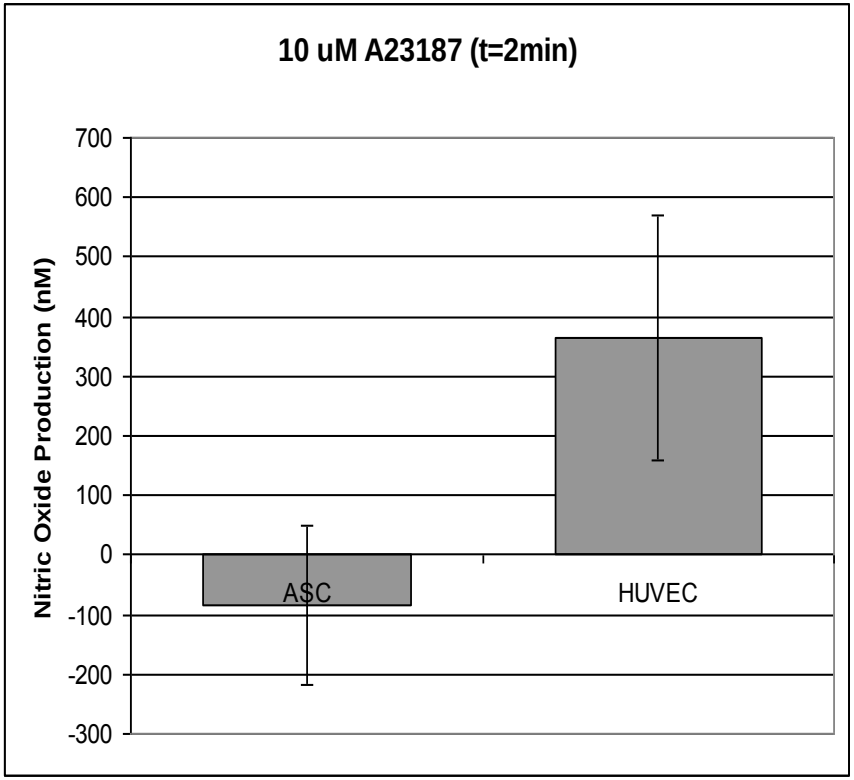


$\alpha_5\beta_1$ important for stem cell attachment

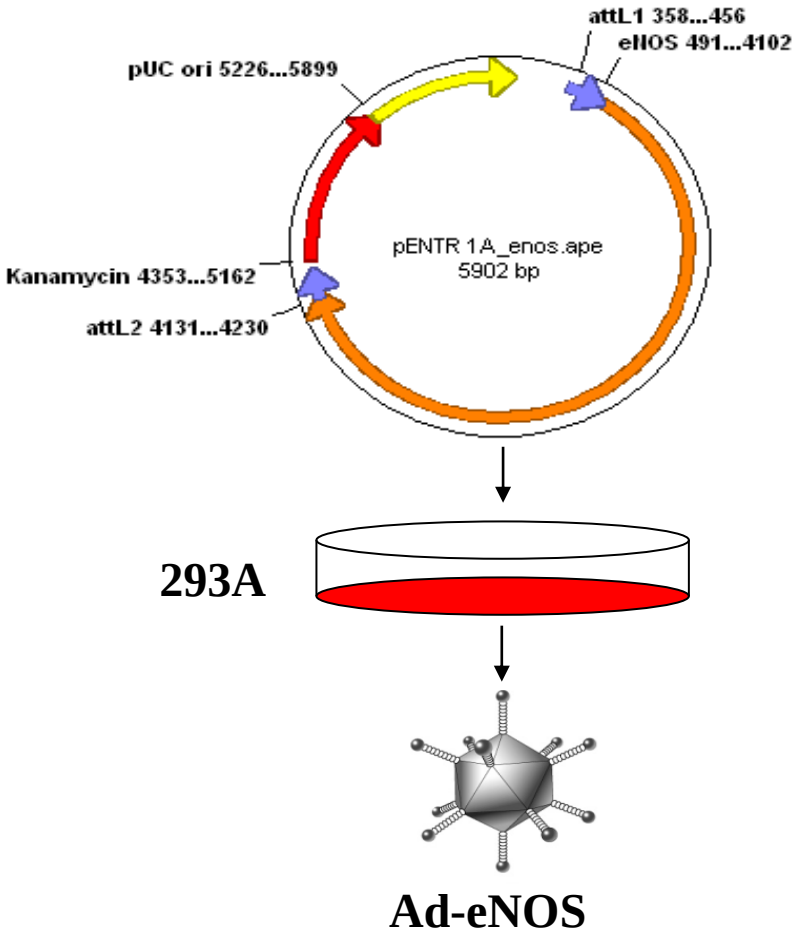


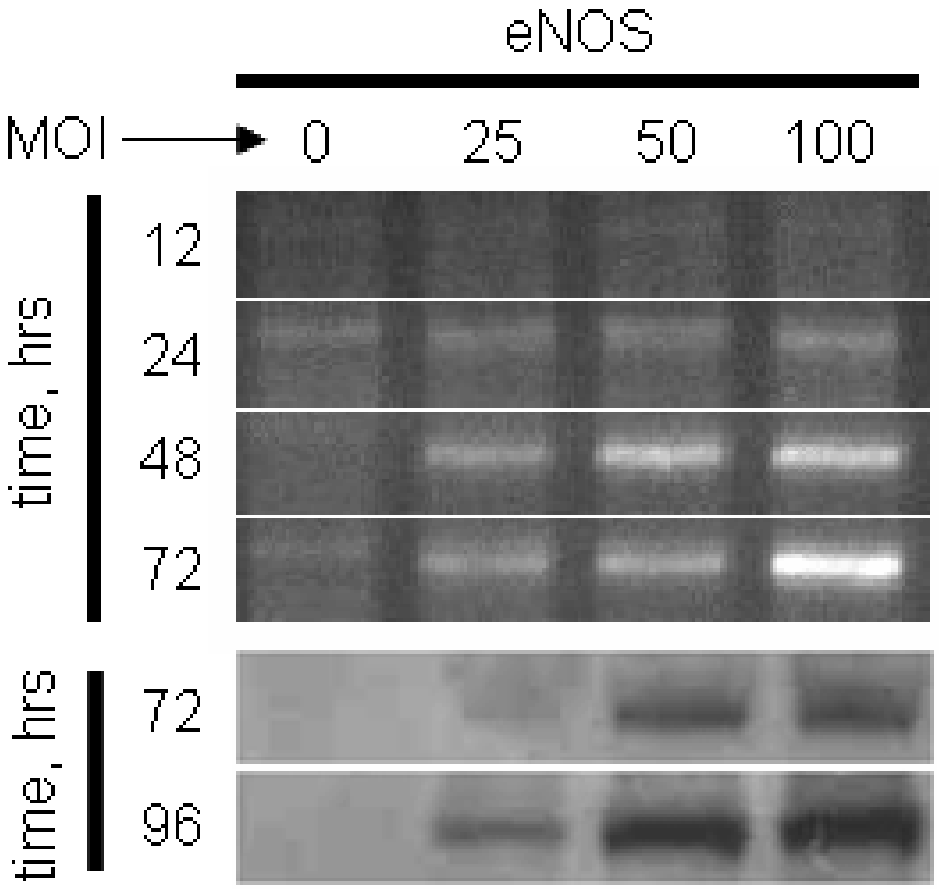
ASC do not produce NO



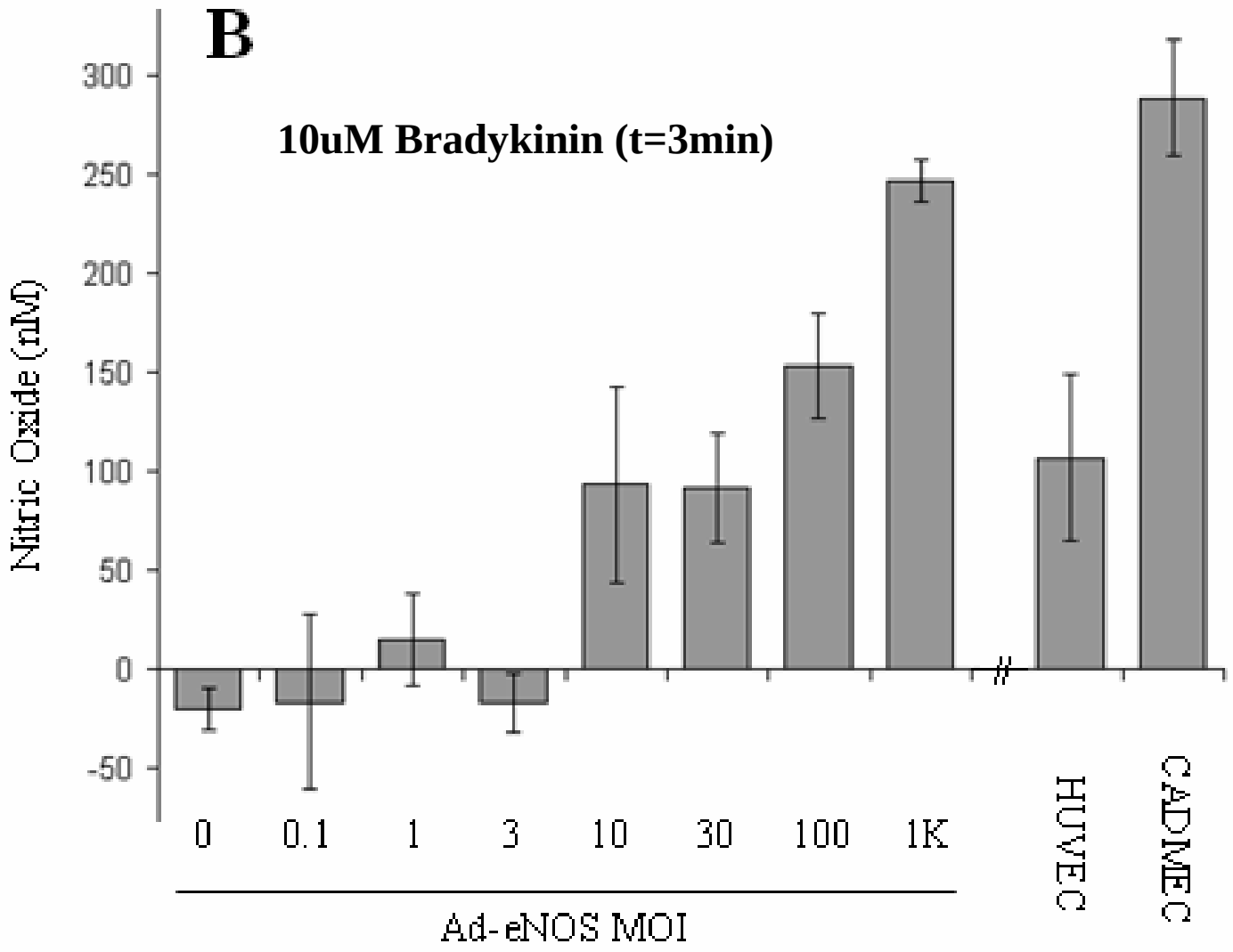


Adenoviral transfection of ASC with human eNOS gene

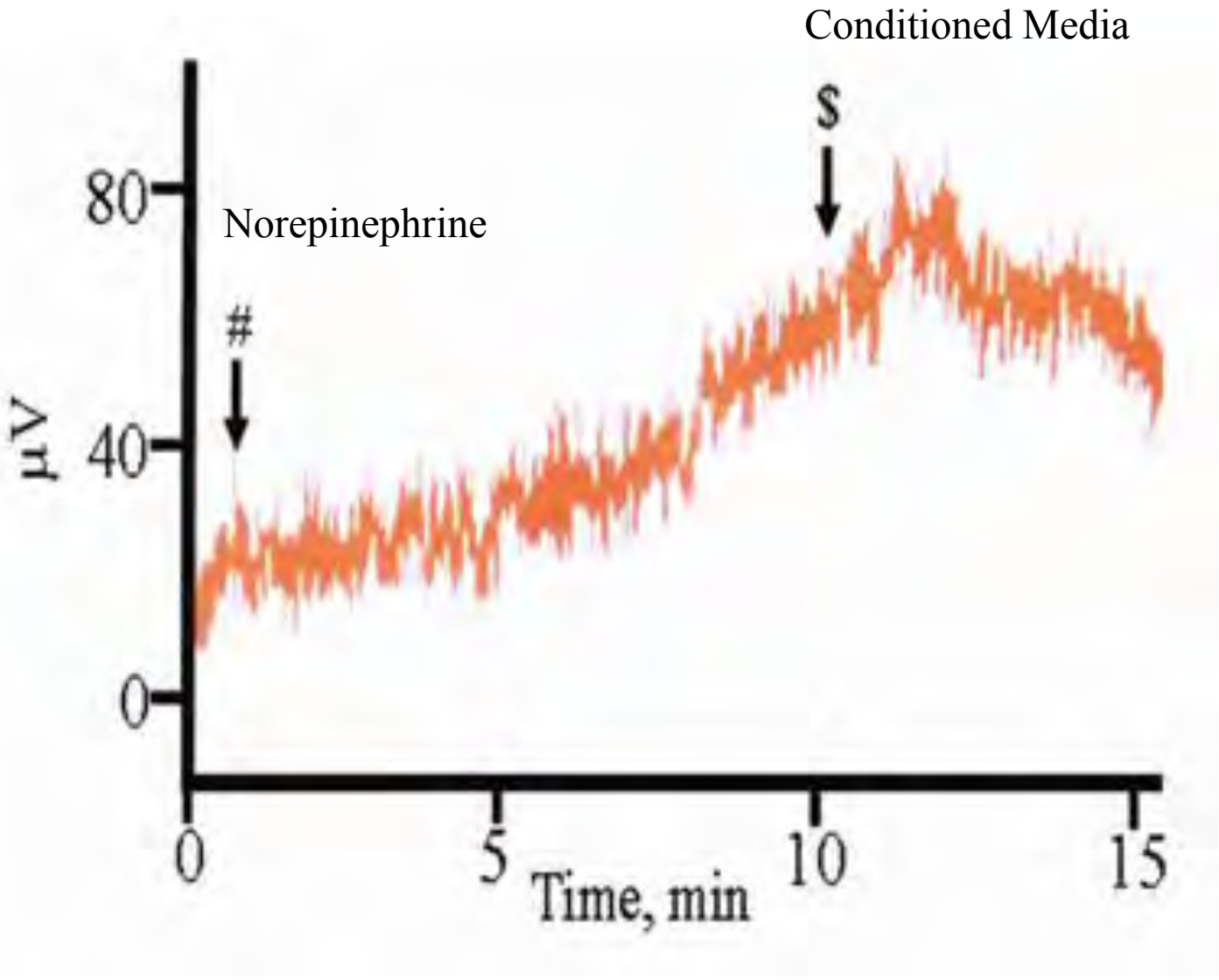


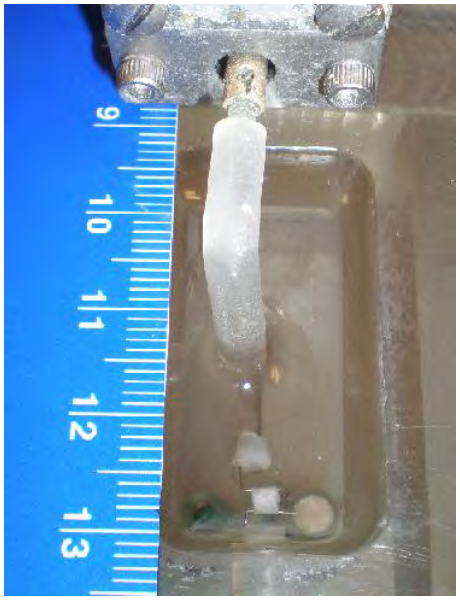


Transfected ASC produce NO

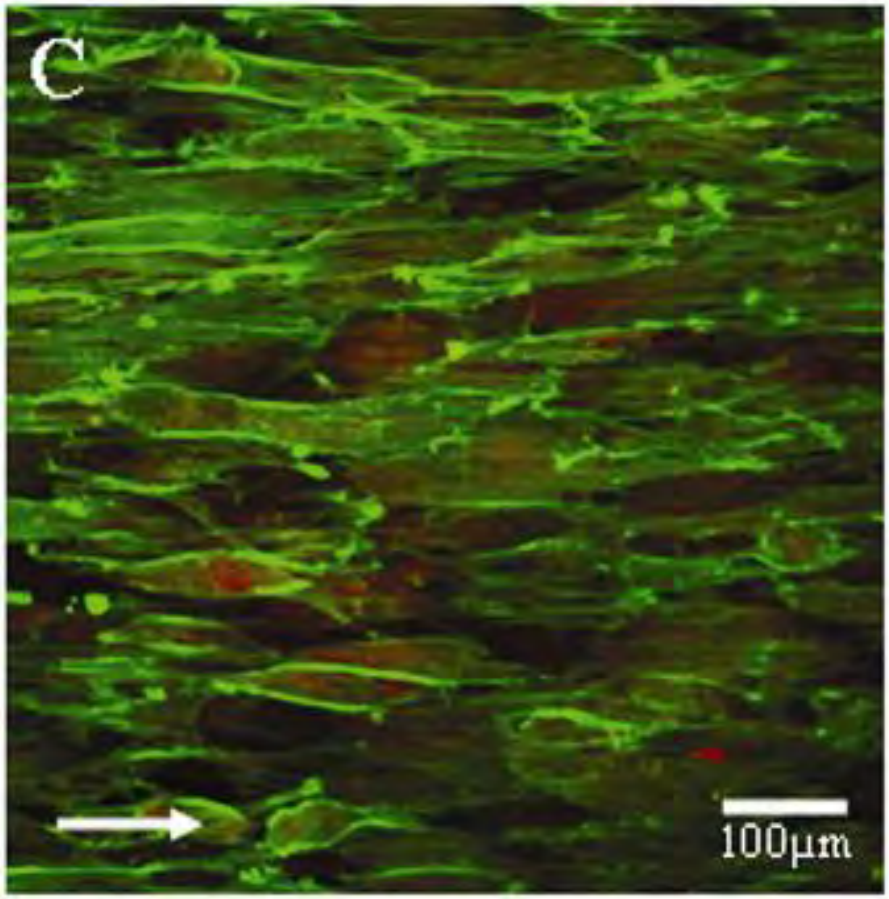


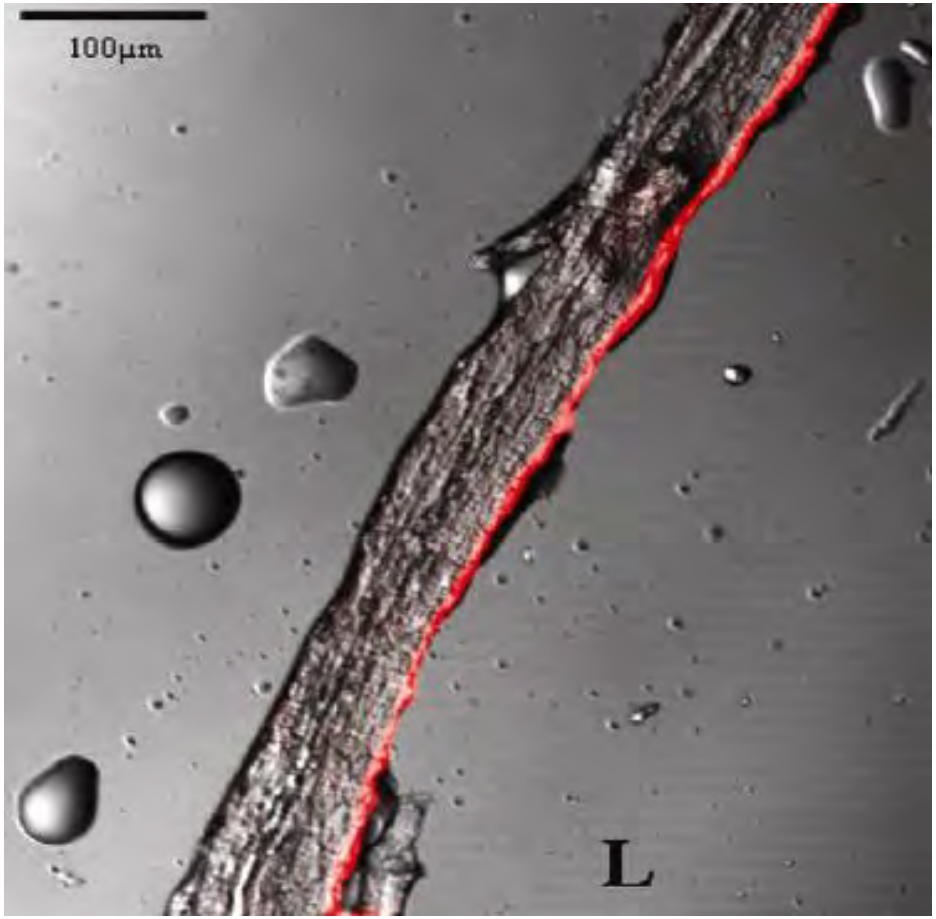
NO from transfected ASC stimulates vasodilation





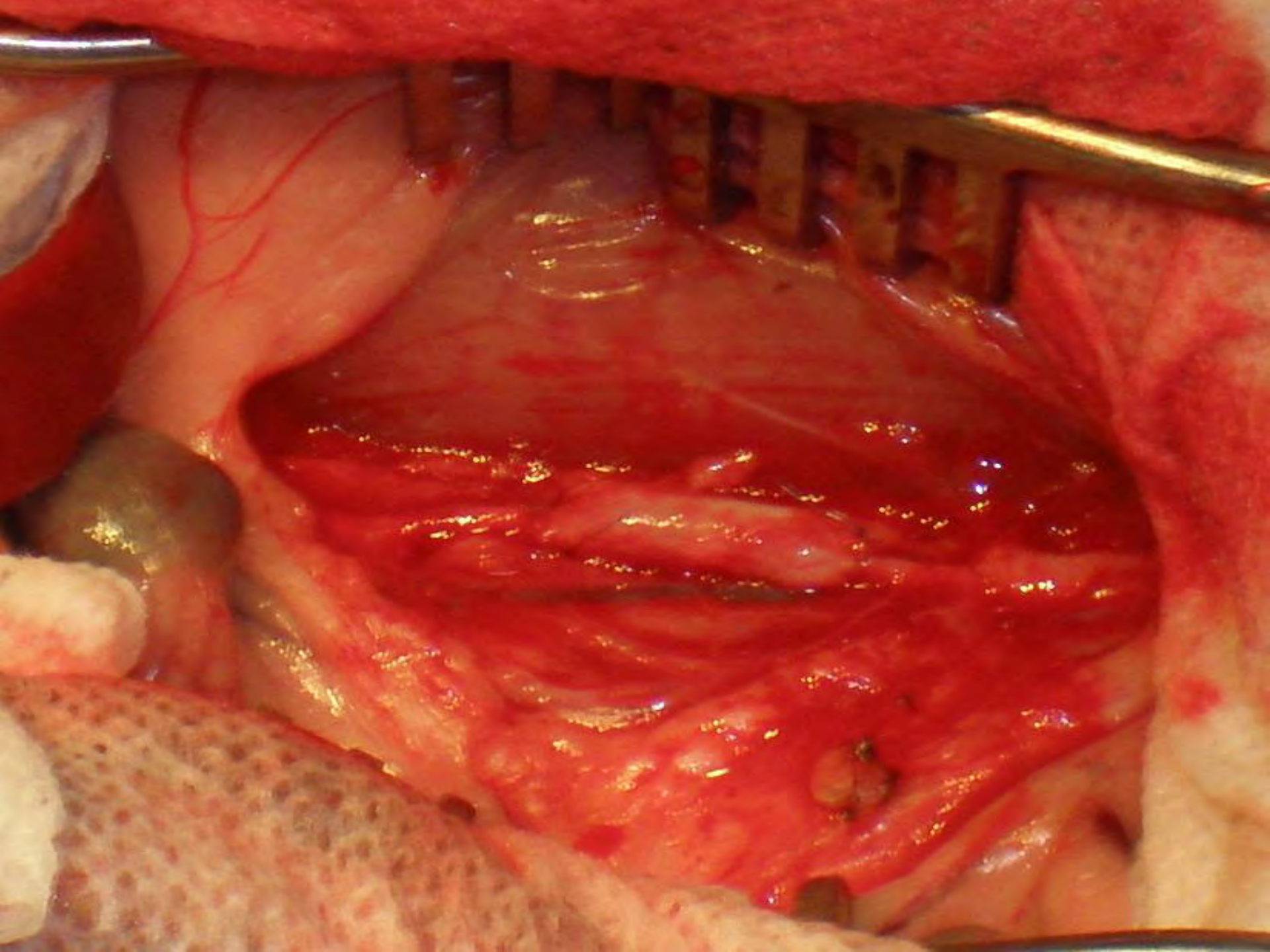
Transfected ASC are retained on decellularized vein @ >15 dyne



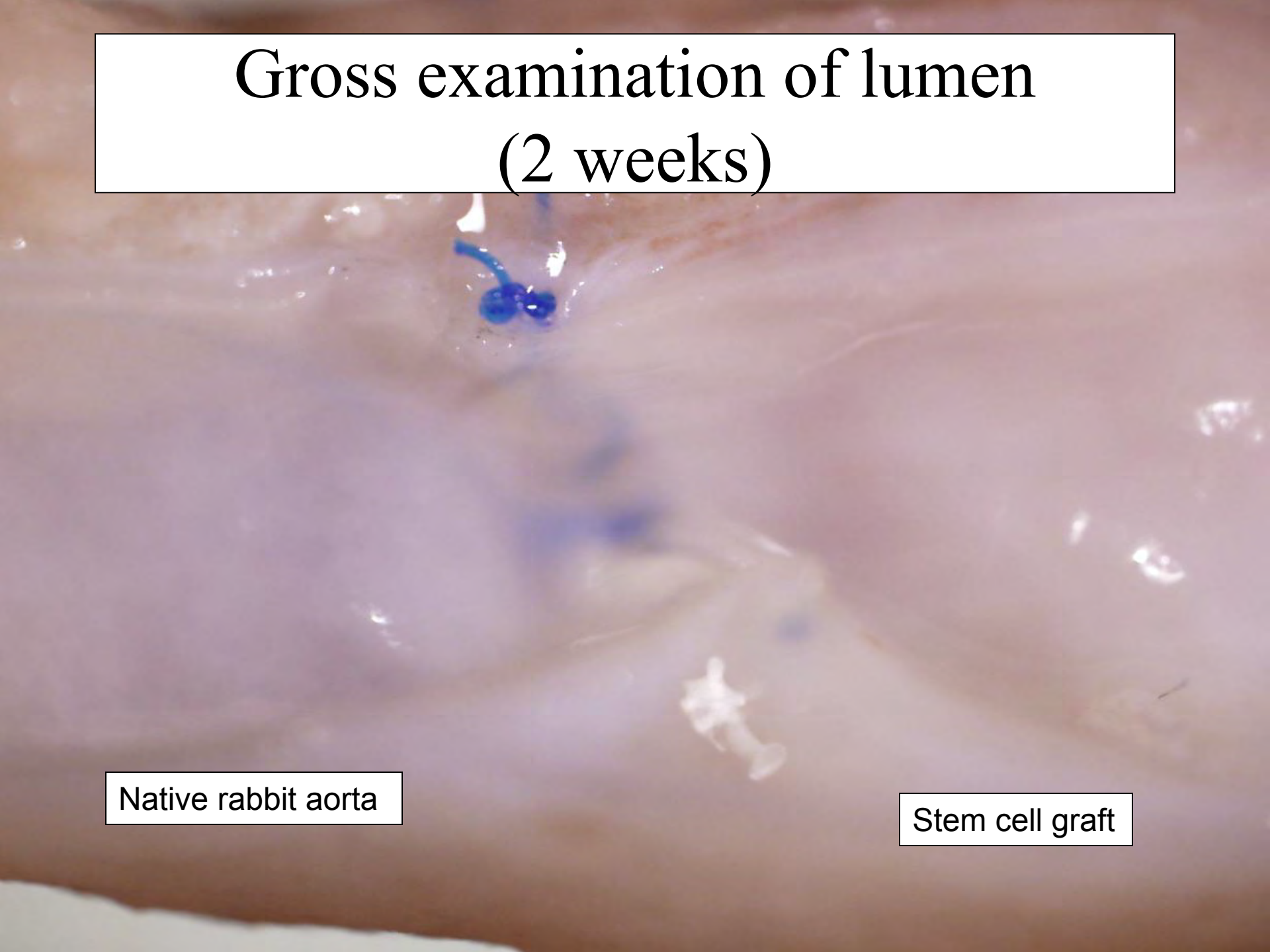


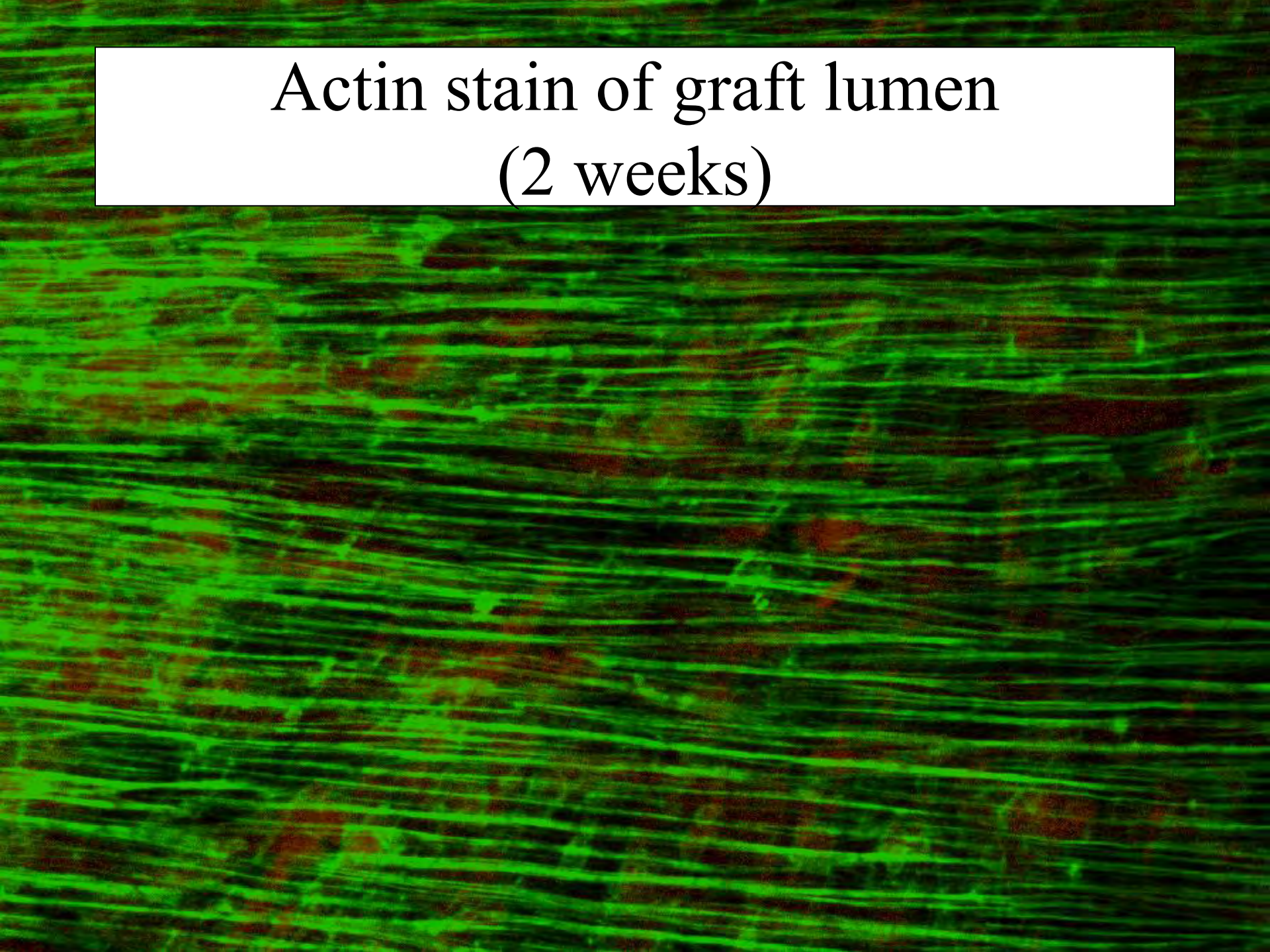
In vivo evaluation (graft production time ~ 2 weeks)

- Decellularized vein graft (human)
- Seeded with autologous rabbit stem cells
  - Differentiated into EC
  - Flow conditioned
  - Transfected with eNOS
- Implanted into the rabbit aorta
- 2 weeks and 2 months

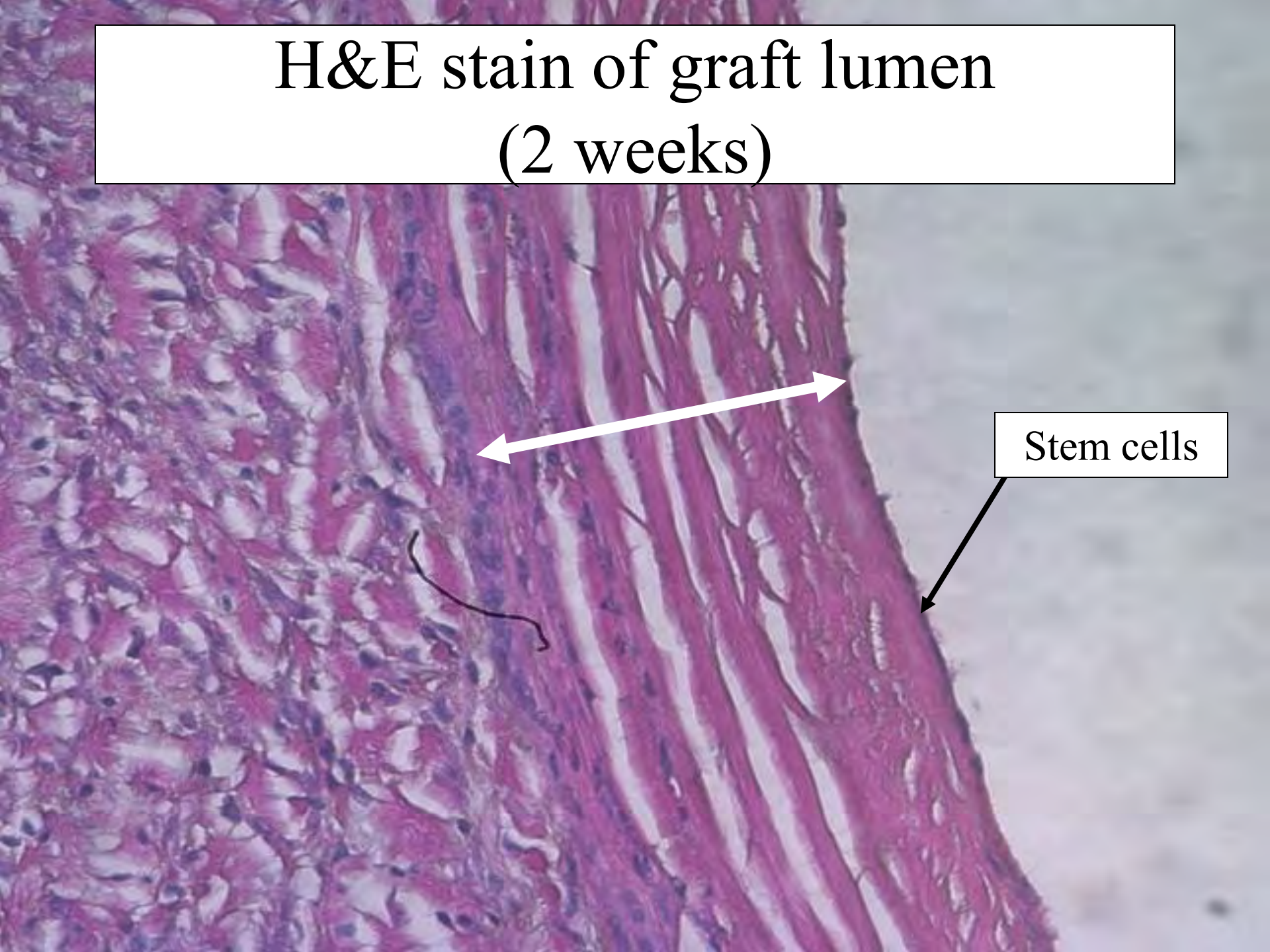


Gross examination of lumen (2 weeks)

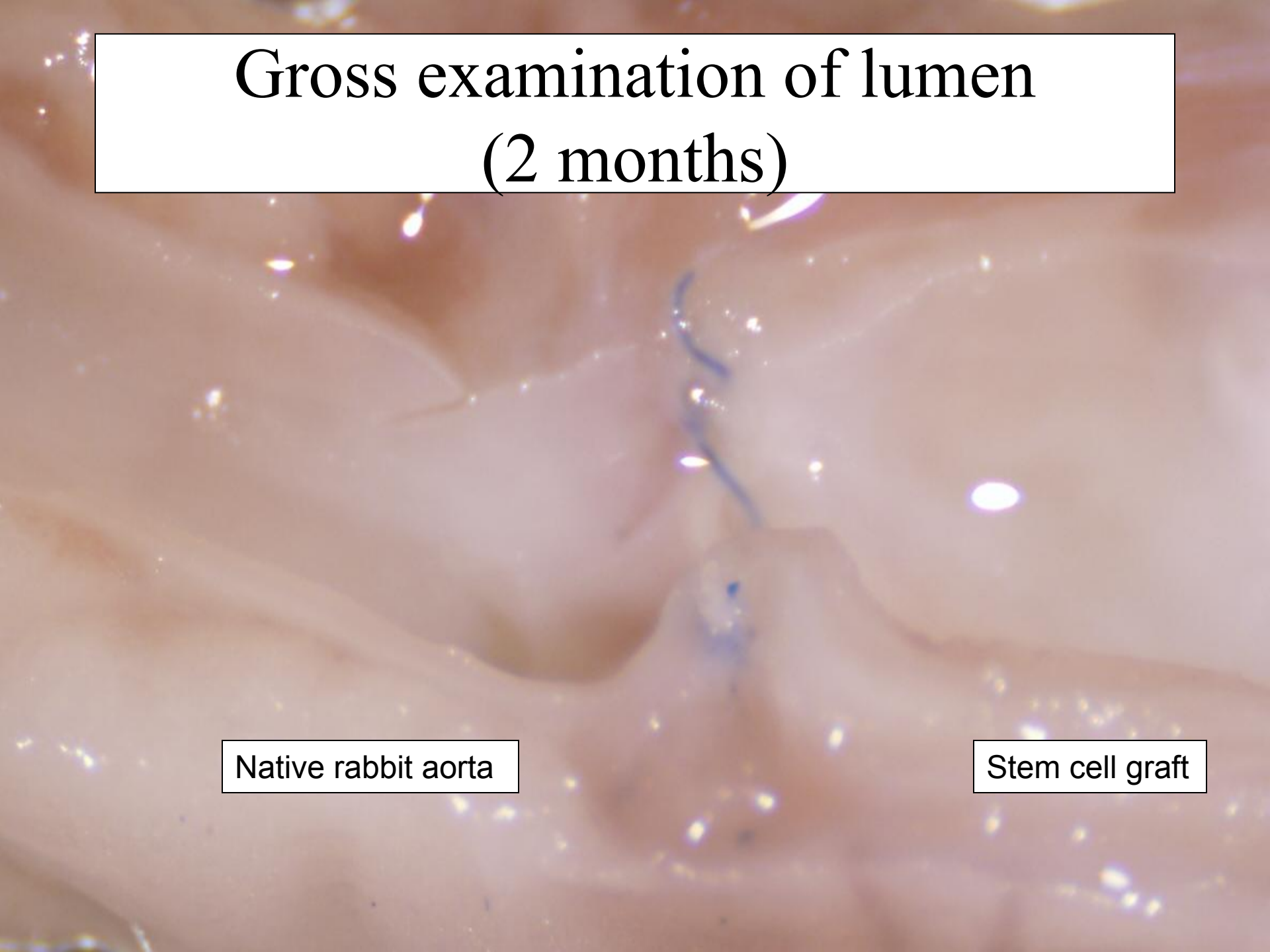



Actin stain of graft lumen
(2 weeks)

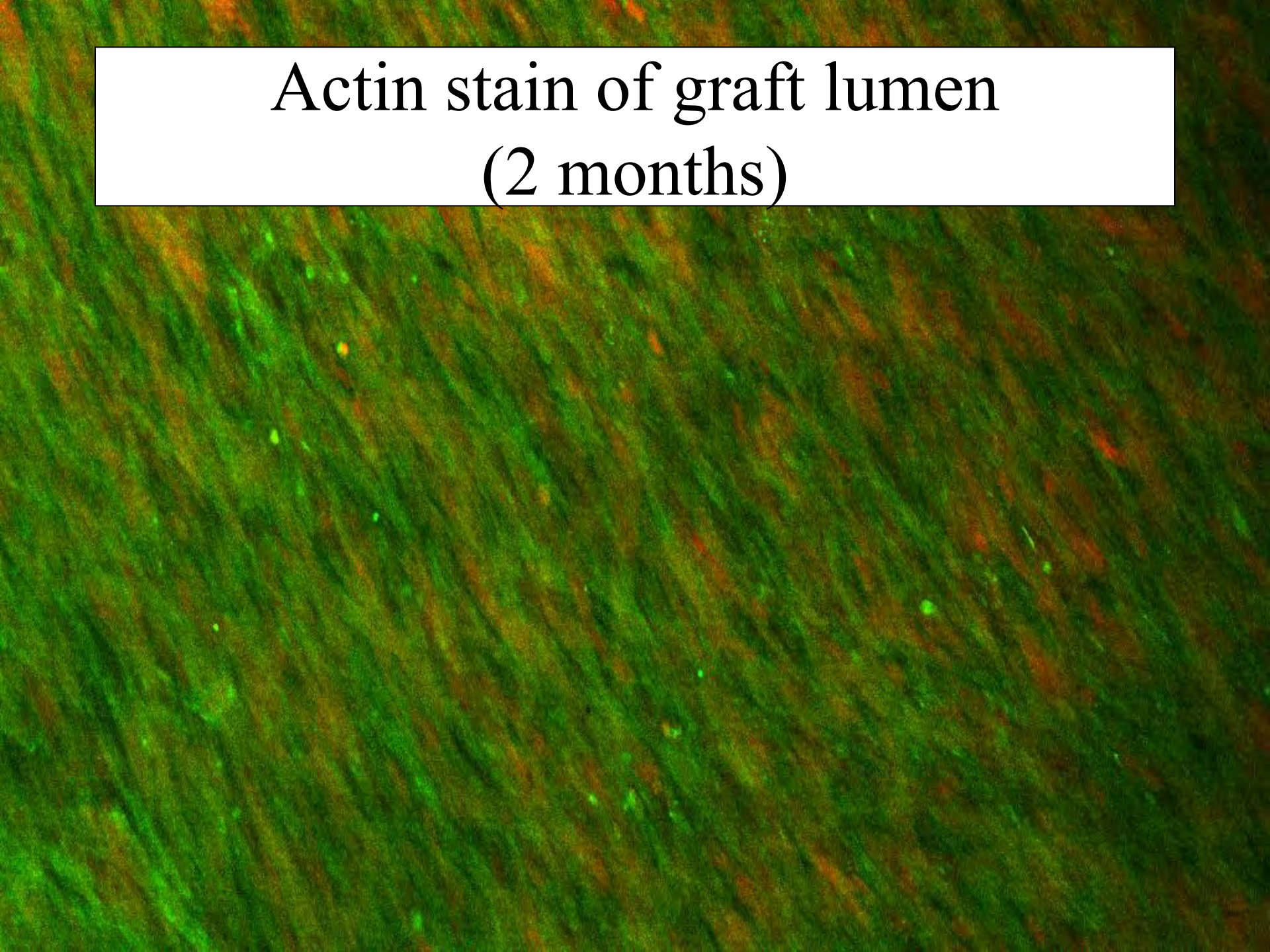
H&E stain of graft lumen (2 weeks)



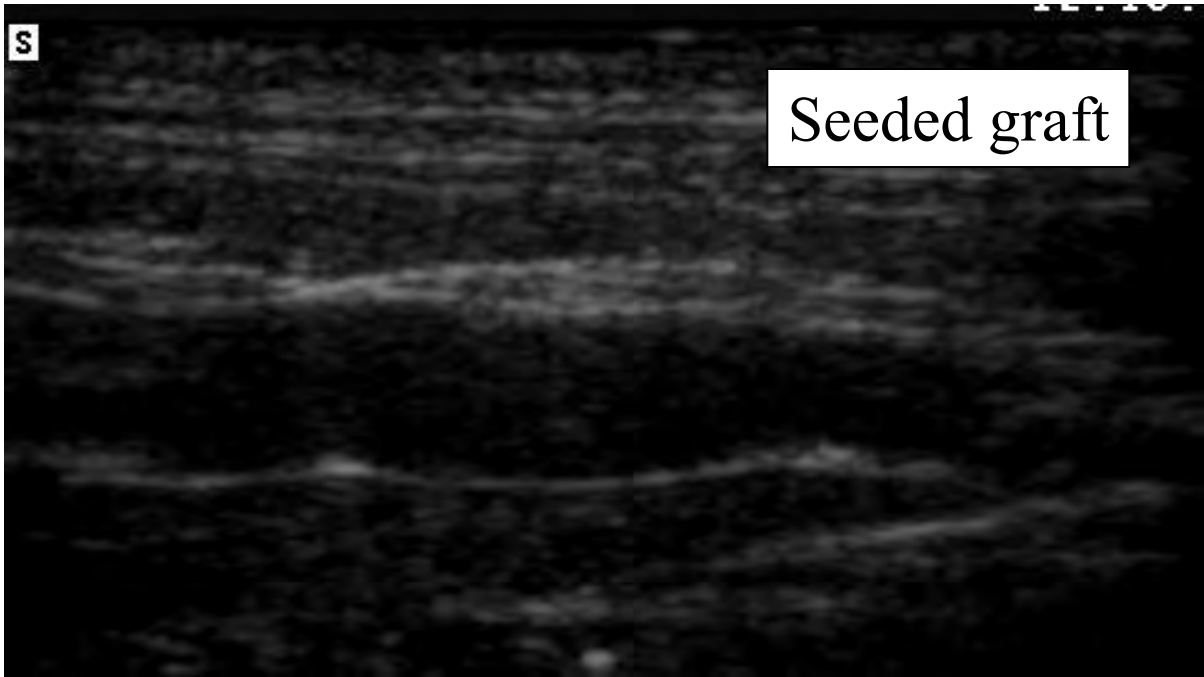
Gross examination of lumen (2 months)



Actin stain of graft lumen (2 months)



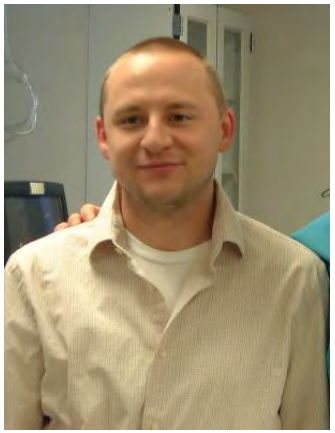
Duplex examination (2 weeks)



5. FUTURE DIRECTIONS

Future directions

1. Create clinically relevant bioreactor (25cm)
2. Improve eNOS transfection (nucleofection)
3. Explore role of ASC-SMC seeding
4. Human clinical trial: Dialysis graft creation

K08 Mentors	Research Associates	Pre-doctoral student	Vascular Fellow, Residents	Funding
Thomas Tulenko, PhD Irving Shapiro, PhD  	Ping Zhang, PhD Chris DiMatteo, BS  	Stephen McIlhenny, BS 	Gabor Bagameri, MD Lauren Fischer, MD Negar Golesorkhi, MD Dan Grabo, MD Nick Tarola, MD Eric Hager, MD Neil Moudgill, MD Jason Comeau, MD Sarah Fernandez, MD	National Institutes of Health American Vascular Association American Heart Association Pacific Vascular Research Foundation

Thank you


Differentiation of Adult Stem Cells into Smooth Muscle for Vascular Tissue Engineering
Lisa J. Harris MD, Hamid Abdollahi MD, Ping Zhang PhD, Stephen McInerney BS, Thomas Tulenko PhD, Paul Sibulo MD
Department of Surgery, Thomas Jefferson University, Philadelphia, PA
EXIT