In vitro characterization of cardiovascular constructs: vascular grafts as a model system

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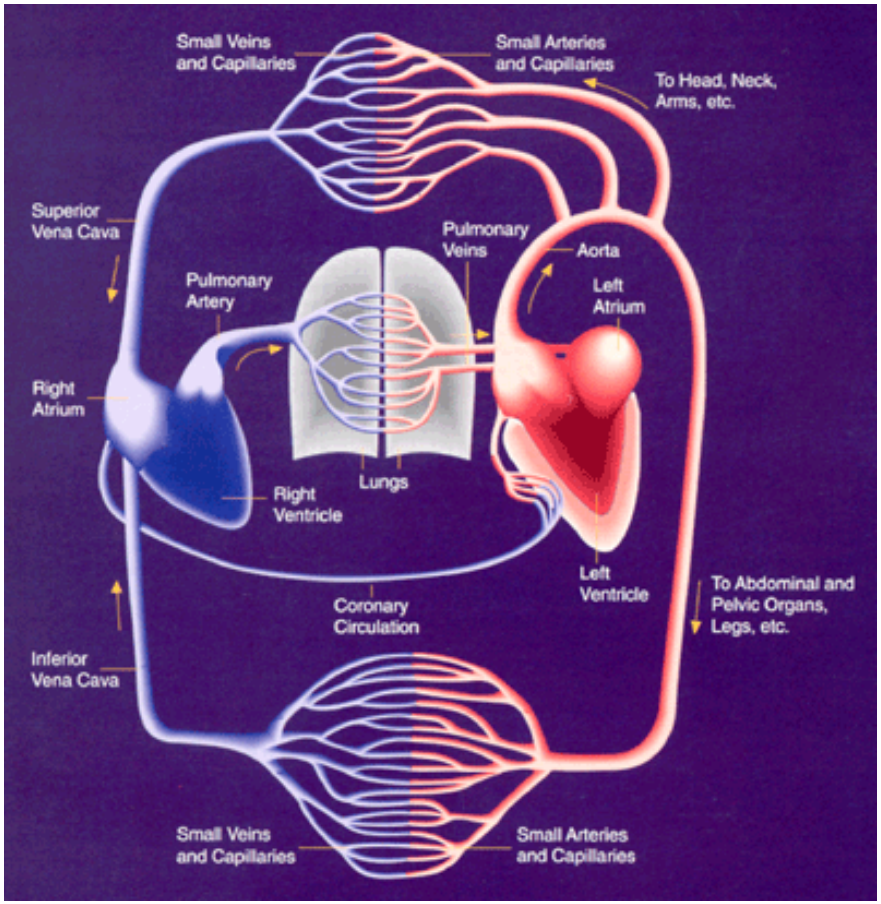
Engineered Cardiovascular Tissues

- Cardio
  - Heart valves
  - Heart patches
- Vascular
  - Microvascular
  - Conduit vessels
    - CABG
    - Peripheral Circulation
    - Pulmonary Circulation
    - A/V Fistulas for hemadialysis



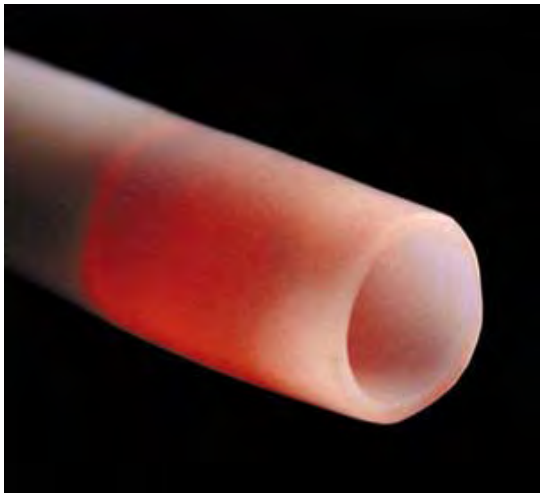
http://www.niaaa.nih.gov/Resources/GraphicsGallery/CardiovascularSystem/269f1.htm

Approaches for Engineering Conduit Vessels

- Cells + synthetic scaffold
- Cells + processed ECM
- Cells + cell-derived ECM
- Ex vivo remodeling of intact vessels

- Implant template and allow formation in vivo.

A tube



Is a given tube a “good” TE blood vessel?

- Good enough to publish and merit animal studies?
- Good enough to merit clinical studies?
  - What questions should be asked when evaluating cell/scaffold products in preparation for the first human studies.

Is a given tube a “good” TE blood vessel?

- What properties are required for good in vivo performance?
- How can these properties be assessed?
  - What test methods are available / should be developed to assess the products.

Two main goals of the workshop:

- What questions should be asked when evaluating cell/scaffold products in preparation for the first human studies; and
- What test methods are available / should be developed to assess the products.

What properties are required for good in vivo performance?

Essential functions:

- Contain blood
- Carry blood

Non-essential functions:

- Vasoregulation
- Ability to remodel

Standard Functions

- Immunocompatible
- Biocompatable
- Not prone to infection

Essential functions

- Contain blood → adequate mechanical strength

Acute evaluation

Burst strength

Suture strength

Caveat →

http://www.cytograft.com/pic_ePTFE_pu.html

Table 1 Mechanical properties of age- and risk-matched human TEBVs

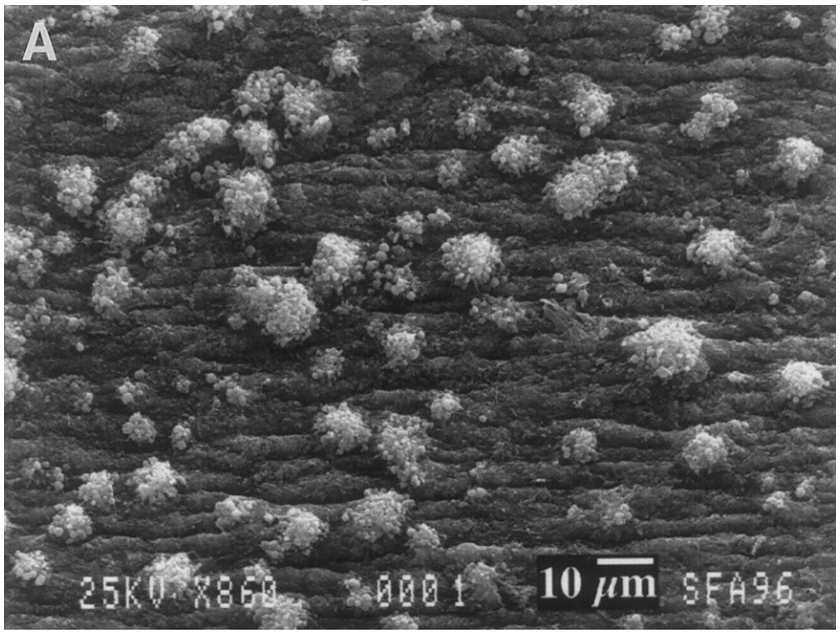
Vessel type	Burst pressure (mmHg)	Suture retention (gf)
TEBV (4.5 mm internal diameter)	3,468 ± 500 (n = 5)	162 ± 15 (n = 9)
TEBV (1.5 mm internal diameter)	3,688 ± 1865 (n = 9)	ND
Human saphenous vein	1,680–2,273 refs. 6,18	196 ± 2 (n = 7)
Human artery	2,031–4,225 (n = 13)	200 ± 119 (n = 9)

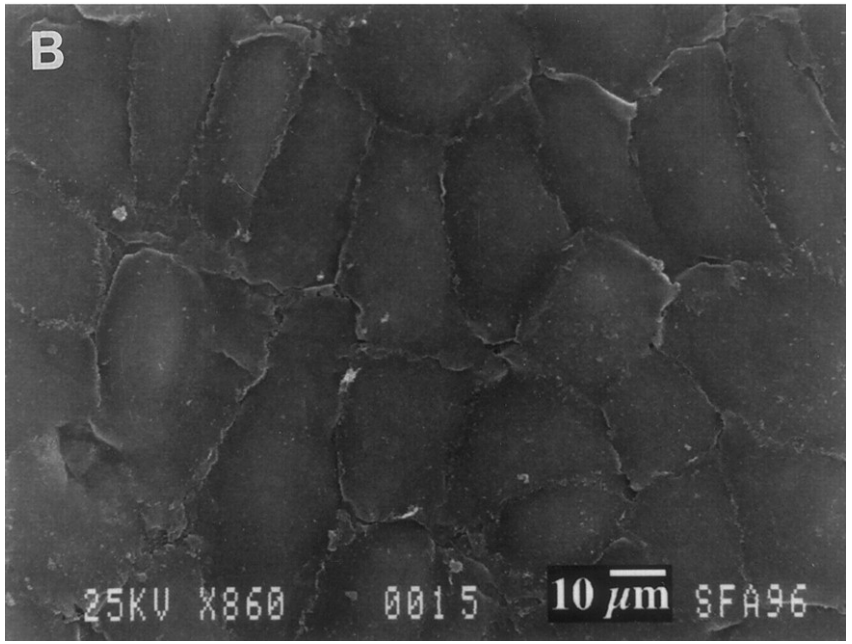
[a] Calculated for a pressure change from 80 to 120 mmHg. ND, not determined.

Essential functions

Carry blood → nonthrombogenic

- In vitro platelet adhesion





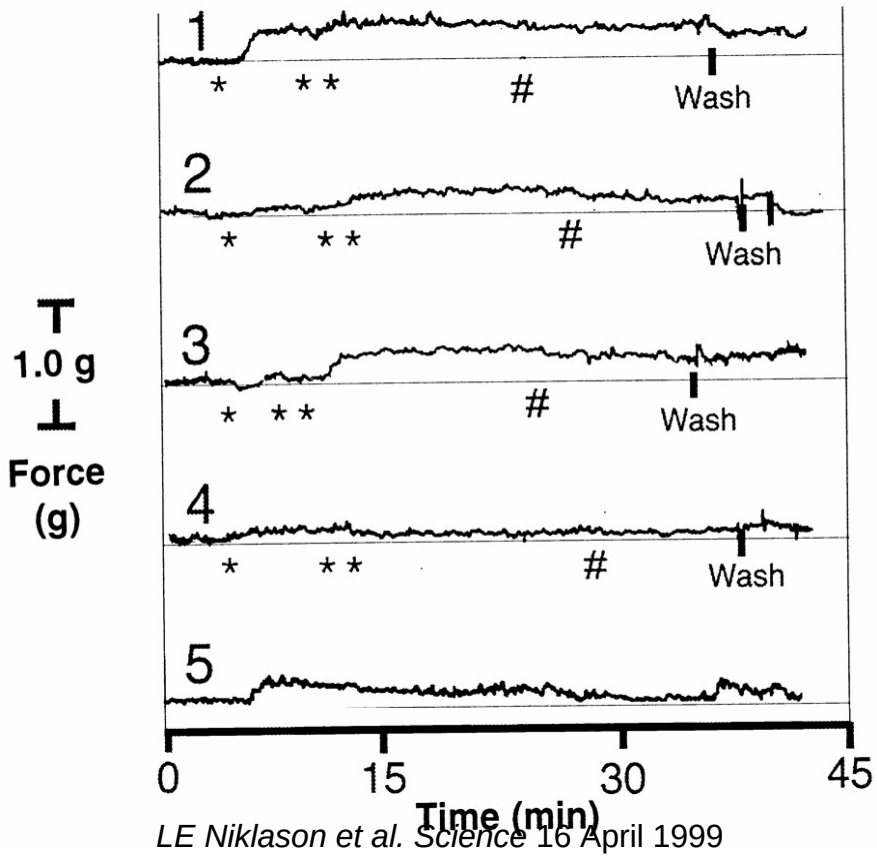
L'Heureux et at. FASEB J. 1998 Jan;12(1):47-56.

- Secretion of antithrombogenic agents

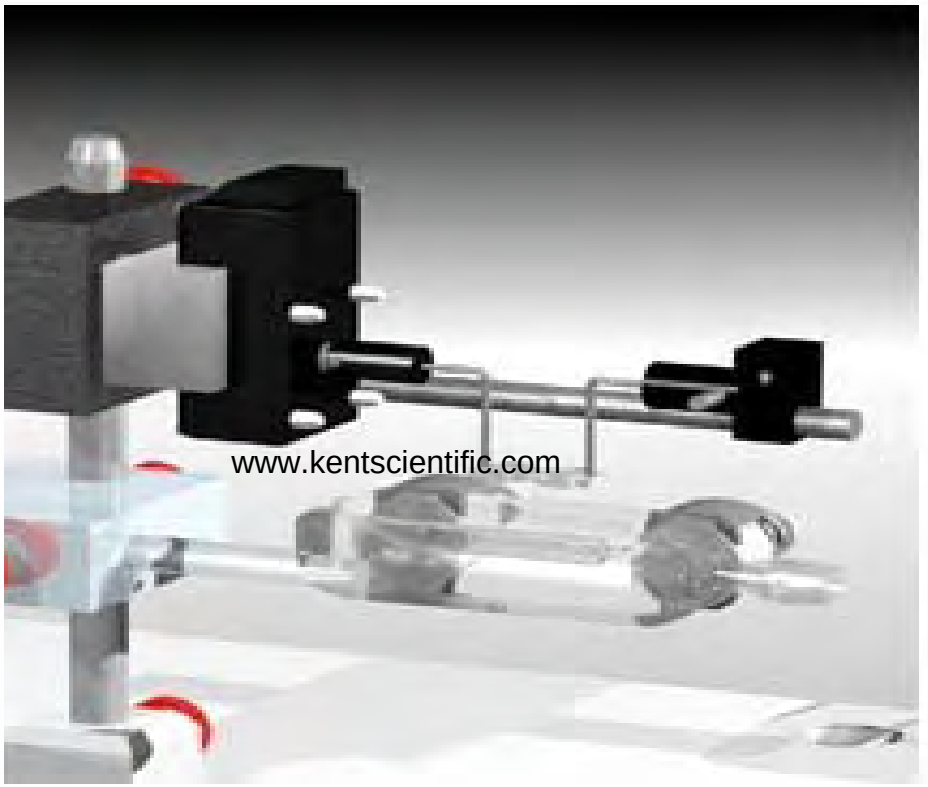
Nature Medicine 7, 1035 - 1040 (2001)

Non-essential functions

Vasoactivity

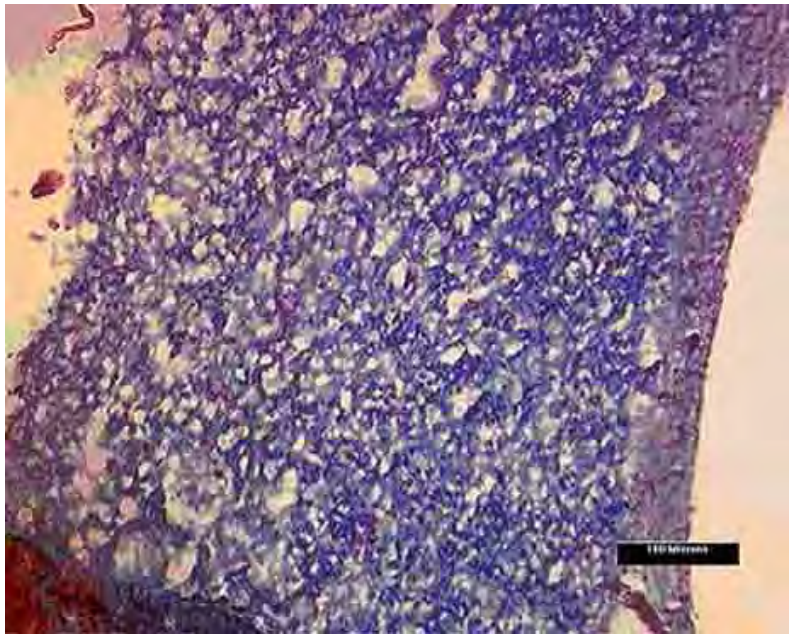


LE Niklason et al. Science 16 April 1999



Function ↔ Structure

- Biochemical assays
  - Collagen
  - Elastin
  - DNA
- Histology
- Immunohistochemistry
  - Cell phenotype
  - Markers of function



Frontiers in Bioscience 9, 1422-1432, May 1, 2004

Is a given tube a “good” TE blood vessel?

- ✓Good enough to publish and merit animal studies?
- Good enough to merit clinical studies?
  - Shin’oka T et al. (pulmonary circulation)
    - *N Engl J Med 2001* **344:**532–533
    - J Thorac Cardiovasc Surg. 2005 129(6):1330-8.
  - L’Heureux N *et al. (A/V fistulas for hemadialysis access)*
    - *N Engl J Med 2007* **357:1451-3**

Good enough to merit clinical studies?

- Safety
- Efficacy
- Consistency

(Safety, efficacy, and consistency)

- Animal studies absolutely essential.
  - Allografts
    - The engineered tissue is in their native environment
    - Not the species of interests
      - Engineered vessels
      - Restenosis
  - Human vessels in animals
    - The engineered tissue is in a foreign environment
    - The species of interest

In vitro characterization of safety

- **Essential functions:**
- Contain blood → Burst strength, suture strength, chronic?
- Carry blood

- **Non-essential functions:**
- Vasoregulation
- Ability to remodel

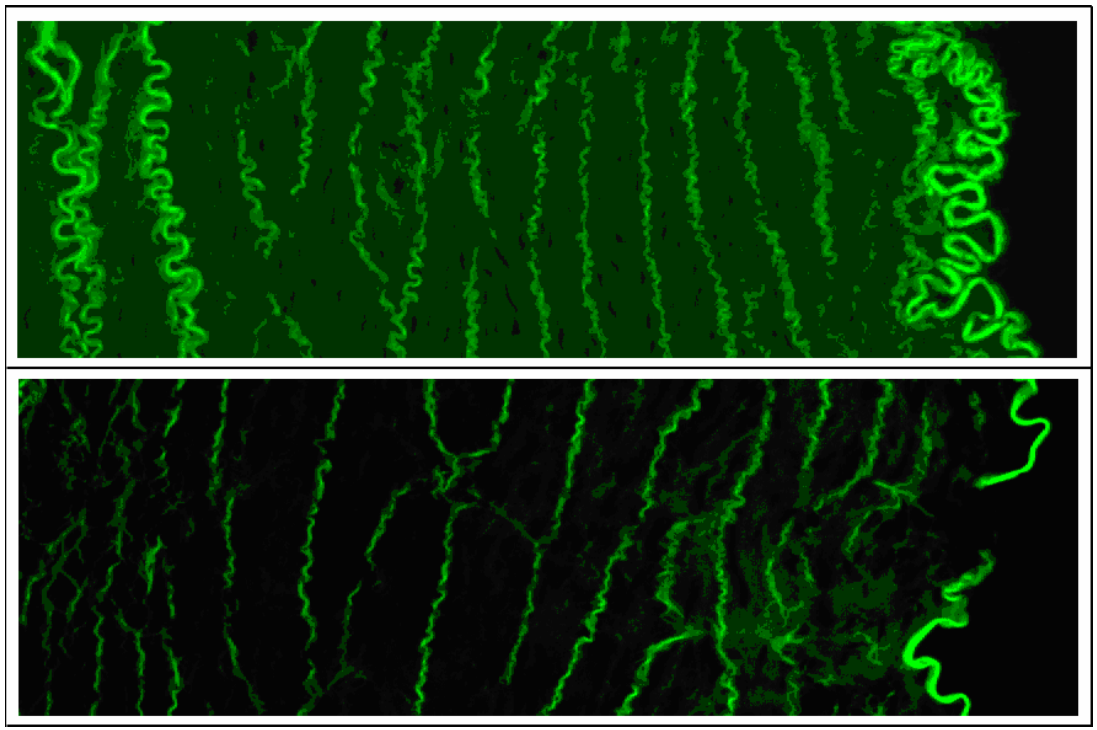
- **Standard Functions**
- Immunocompatible
- Biocompatable
- Not prone to infection
- “Sterility” / free from contamination

Essential functions

- Contain blood → adequate mechanical strength

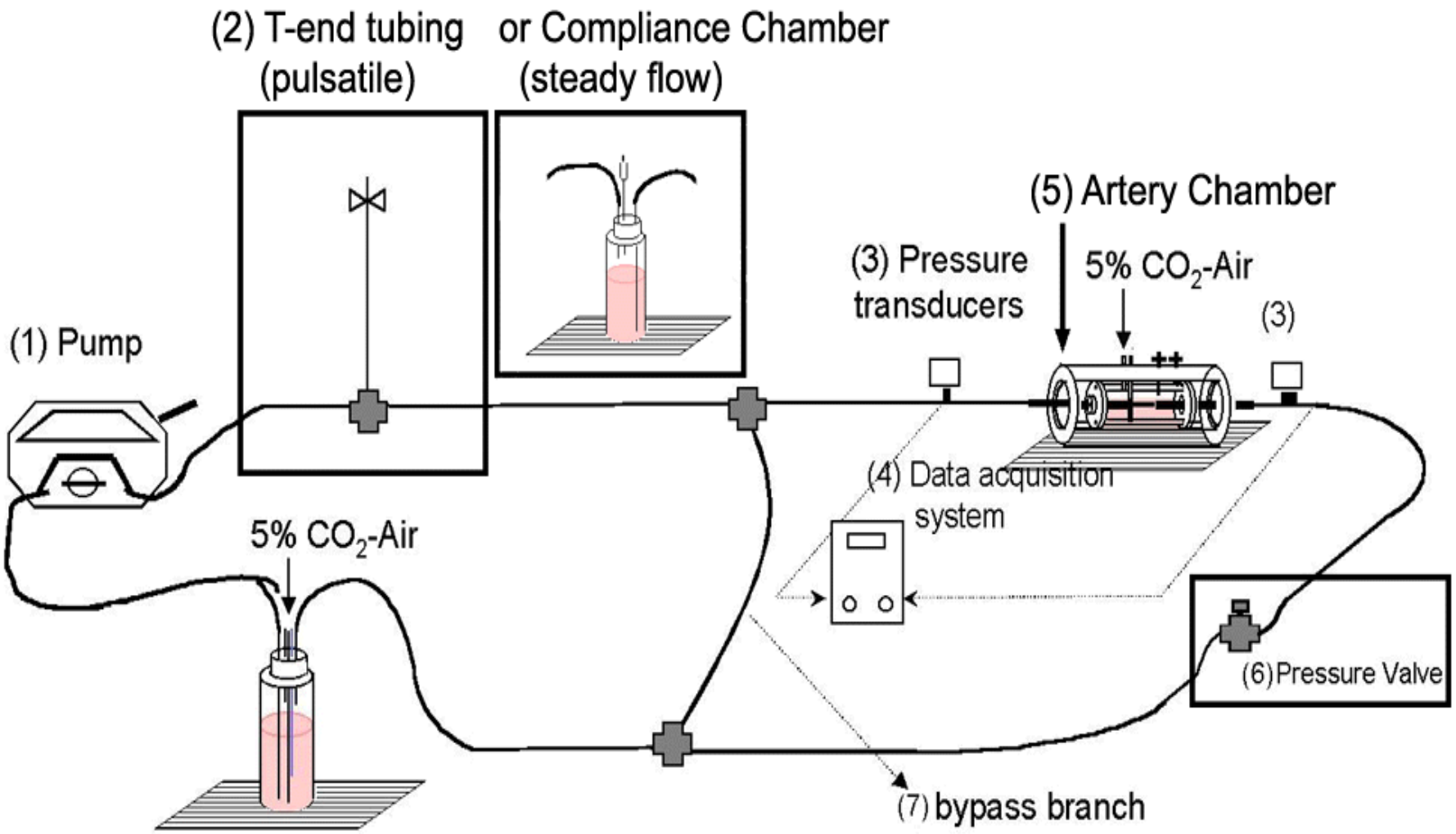
Acute evaluation

Chronic evaluation

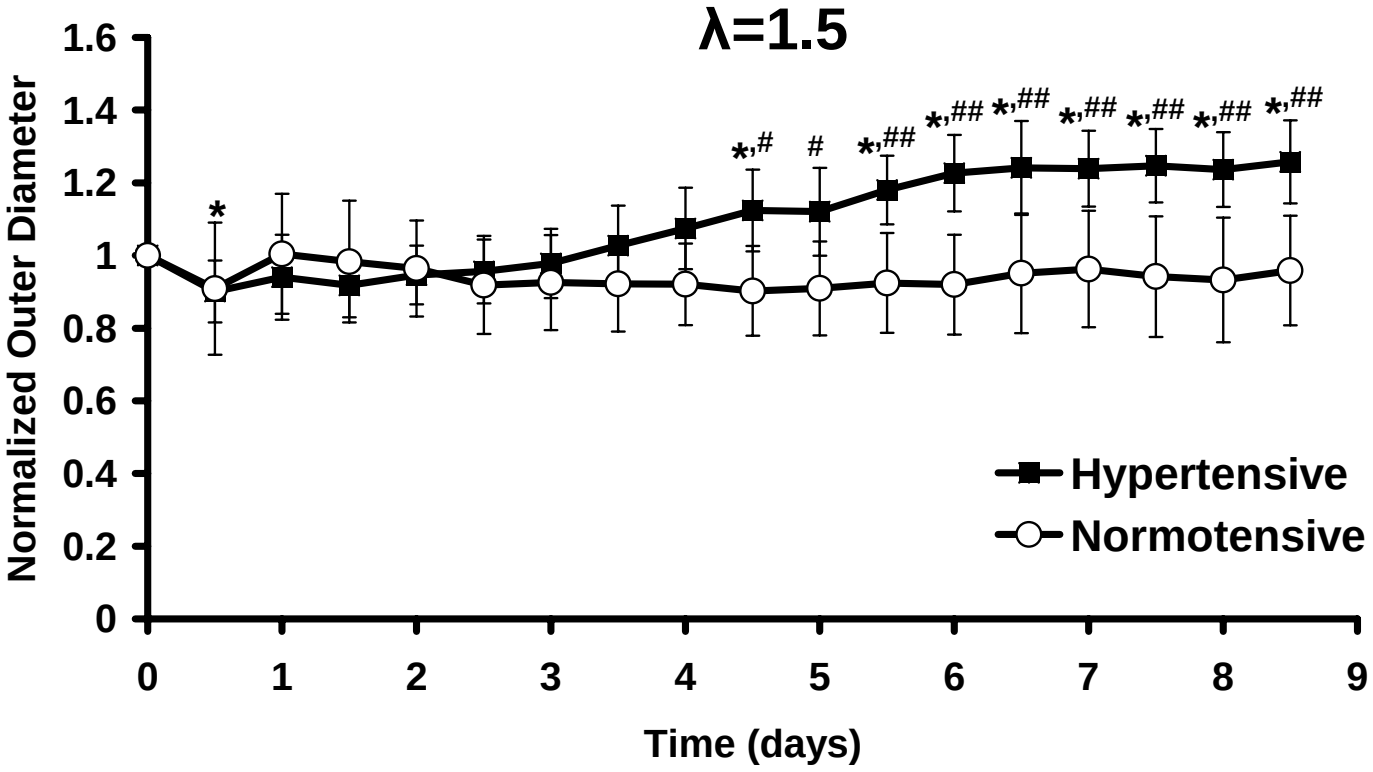


J. Nichol and K. Gooch (unpublished)

Ex vivo perfusion system



Porcine Carotid Artery



A Lawrence and K. Gooch (unpublished)

In vitro characterization of efficacy

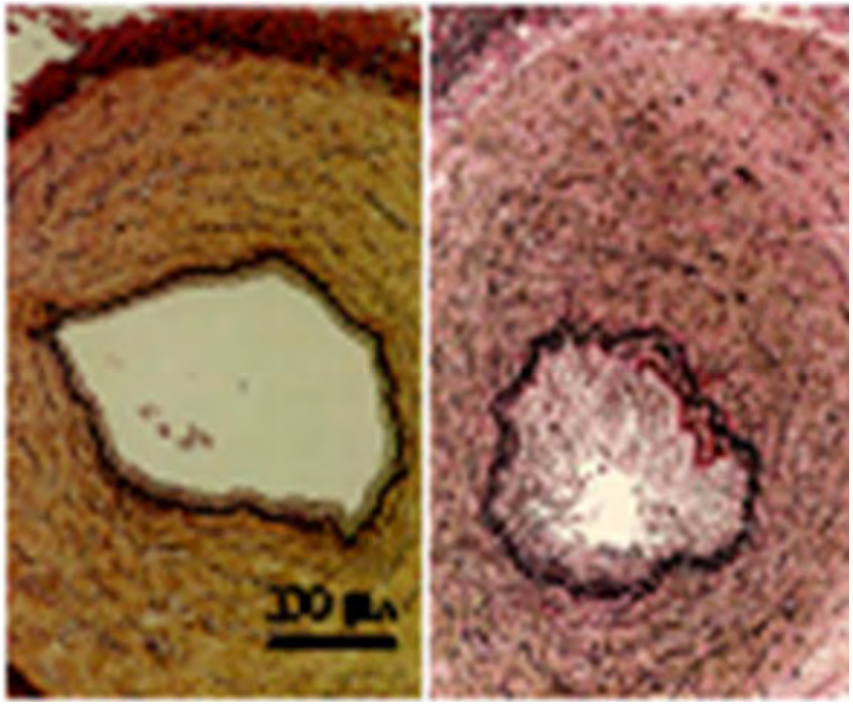
- **Essential functions:**
  - Contain blood
  - Carry blood (thrombosis and stenosis)
- Probably can detect poor potential

  (good enough to publish or merit animal studies)
- Probably cannot discriminate various levels of good
  - 5 yr saphenous vein patency ~50-70%
  - 5 yr internal mammary artery patency ~90%

    Why? IEL, NO production, ROS, compliance…

In vitro models of stenosis

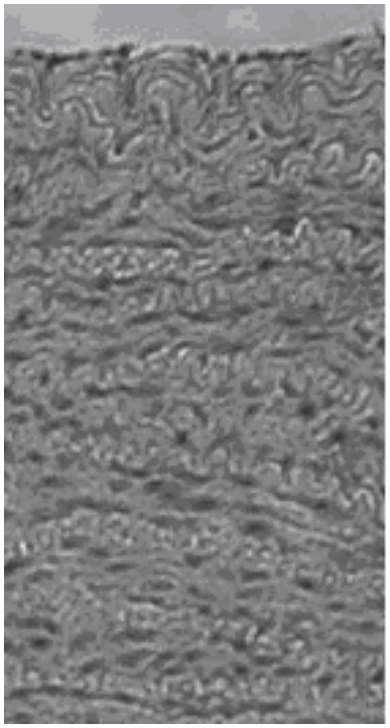
Cultured saphenous veins



Fresh Hypertrophic

+Eutrophic

Cultured carotid artery



R Gusic et al. J Biomech. 2005 Sep;38(9):1770-9.

In vitro characterization of consistency

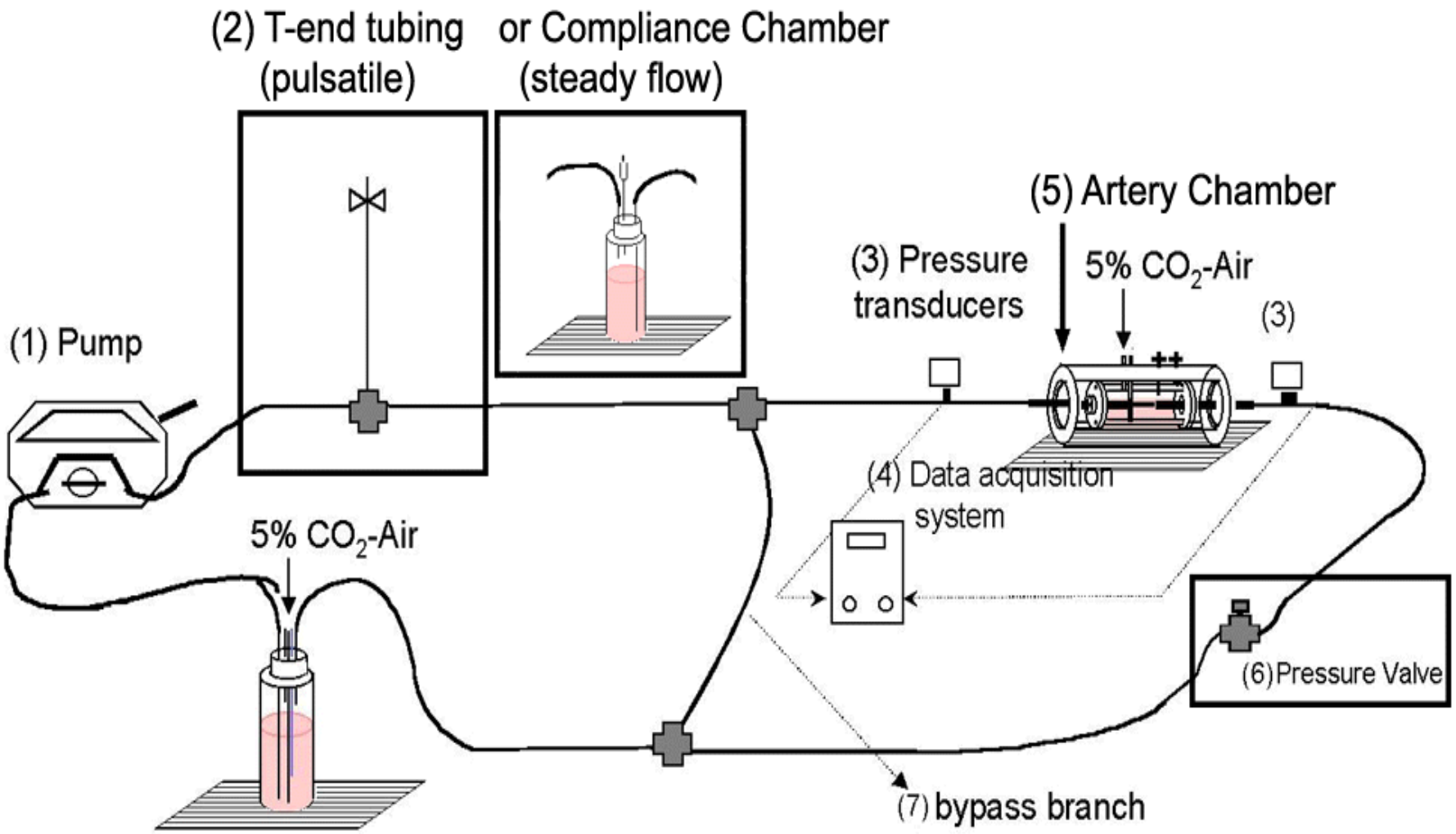
Biochemical composition

Cellular viability, proliferation, or phenotype

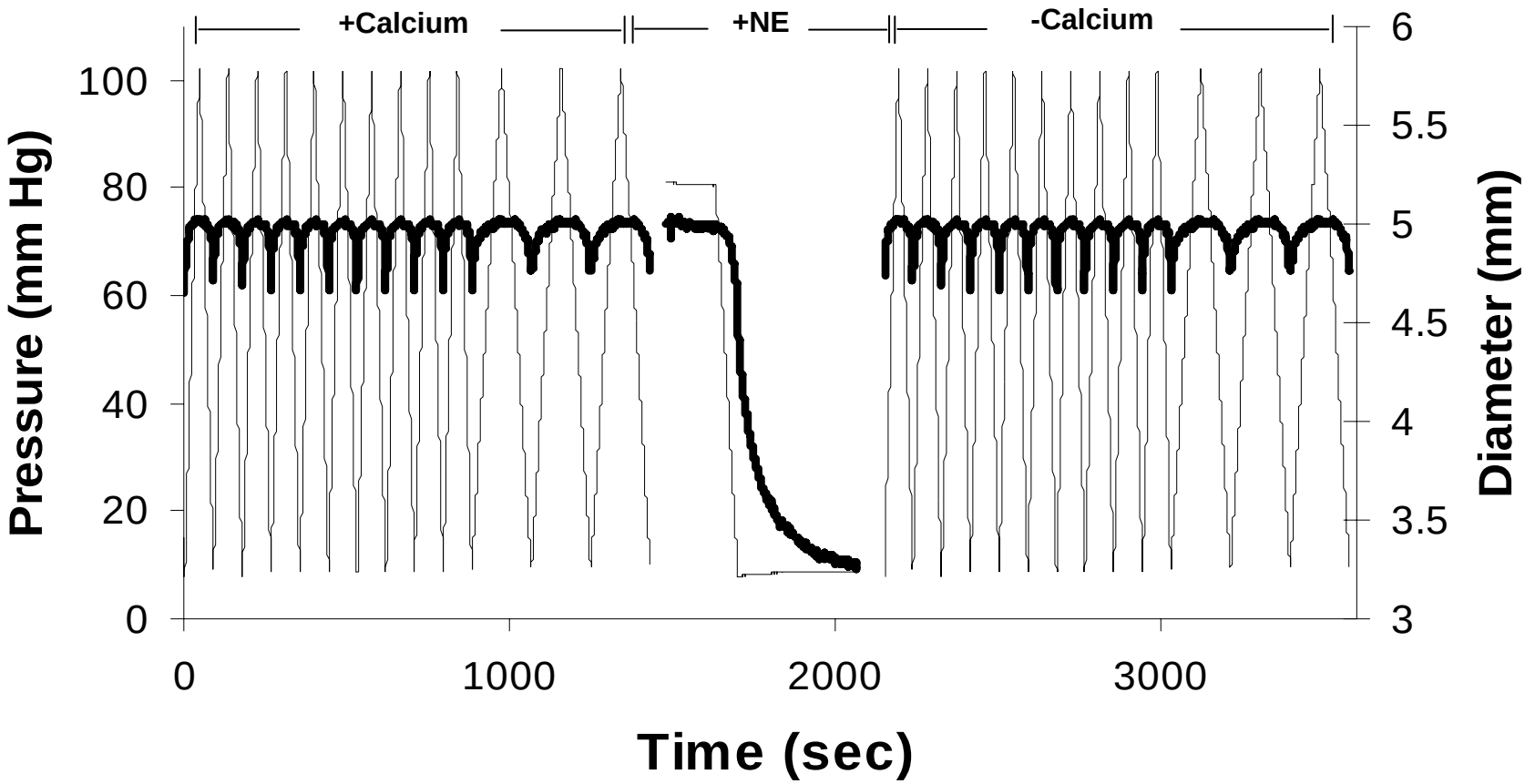
Dimensions, organization, structure

Mechanical properties

Mechanical Testing: Non-essential functions

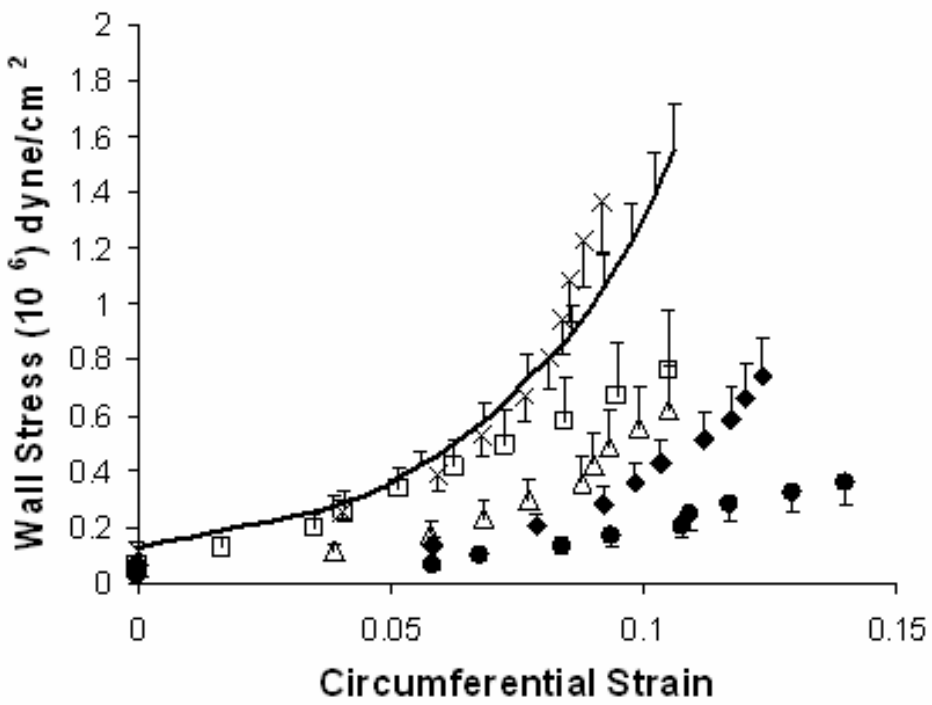


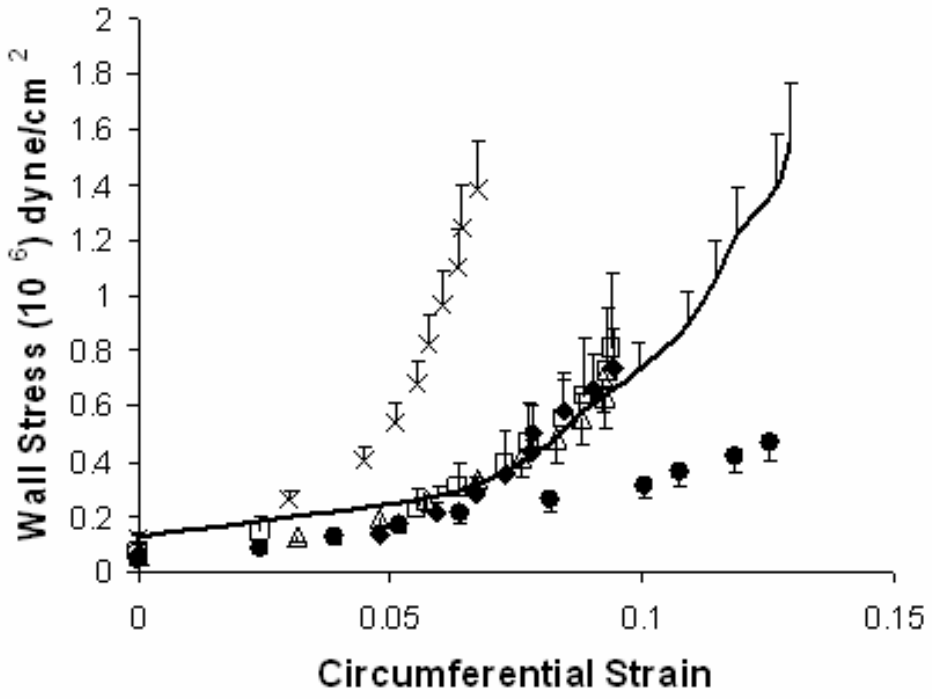
Mechanical Testing: Non-essential functions



R Gusic et al. J Biomech. 2005 Sep;38(9):1770-9.

Active and Passive Mechanical Properties

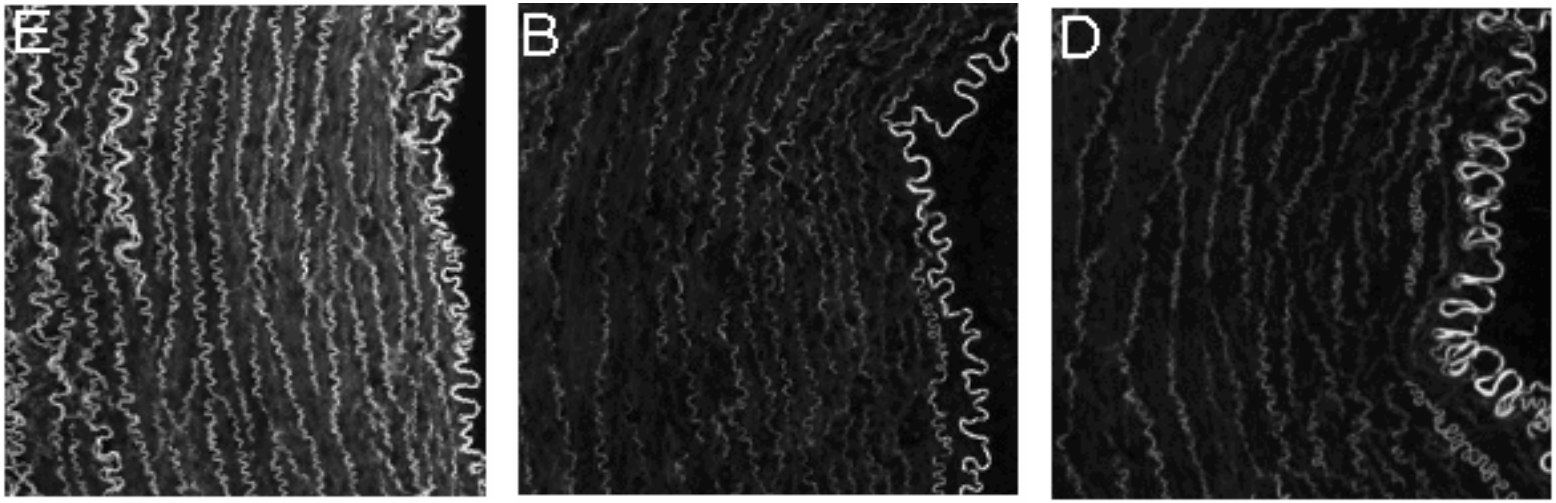


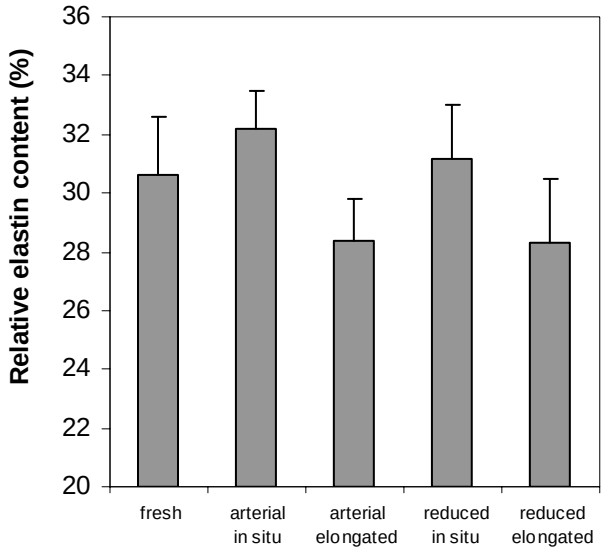


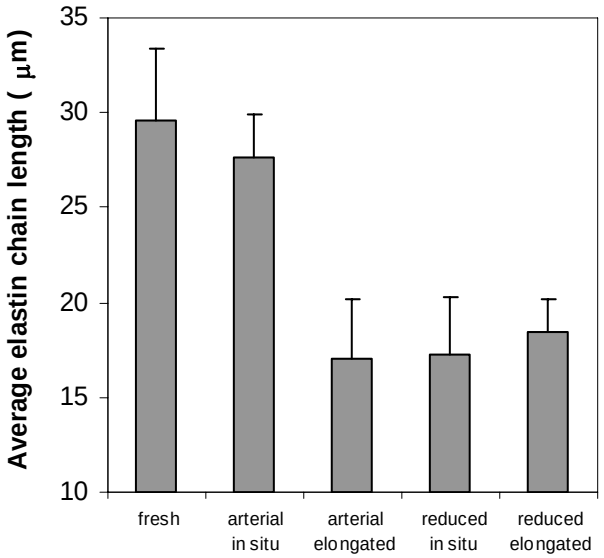
	Fresh Veins	Venous	Elevated Flow	Ramped Pressure	Controlled Shear Stress	Bypass Graft
Mean	23.55	30.29	23.37	28.83	27.19	51.99
Standard Error	3.49	8.91	6.98	4.83	2.19	16.37

R Gusic et al. J Biomech. 2005 Sep;38(9):1770-9.

Elastin


B
B
D


Relative elastin content (%)
36
34
32
30
28
26
24
22
20
fresh
arterial in situ
arterial elongated
reduced in situ
reduced elongated


Average elastin chain length (μm)
35
30
25
20
15
10
fresh
arterial in situ
arterial elongated
reduced in situ
reduced elongated

Consistency is easy to assess but potentially difficult to correlate with utility.

A consistent product is not necessary functional.

Consistency of an engineered tissue from autologous cells.

- Variation in native human saphenous vein grafts
- Expected variation in engineered tissue
  - Due to variation in cells
  - Due to variation in processing

- Should tissue engineered grafts be more, less, or as consistent as native graft material?

Consistency of an engineered tissue from non-autologous cells.

- Potential for single or a small number of cell donors (e.g., ATS TE-skin).
- Expected variation in engineered tissue
  - Due to variation in cells ↓
  - Due to variation in processing

In vitro characterization of engineered blood vessels

Essential functions:

- Contain blood
- Carry blood

Non-essential functions:

- Vasoregulation
- Ability to remodel

Standard Functions

- Immunocompatible
- Biocompatable
- Not prone to infection

Ultrastrutural analysis