In Vitro Evaluation of Electrospun Silk Fibroin Scaffolds for Vascular Cell Growth

Xiaohui Zhanga, Cassandra B. Baughman, and David L. Kaplan

Departments of Chemical and Biological Engineering and Biomedical Engineering, Tufts University and Tufts University School of Medicine, Sackler School of Graduate Biomedical Science, Department of Anatomy and Cell Biology

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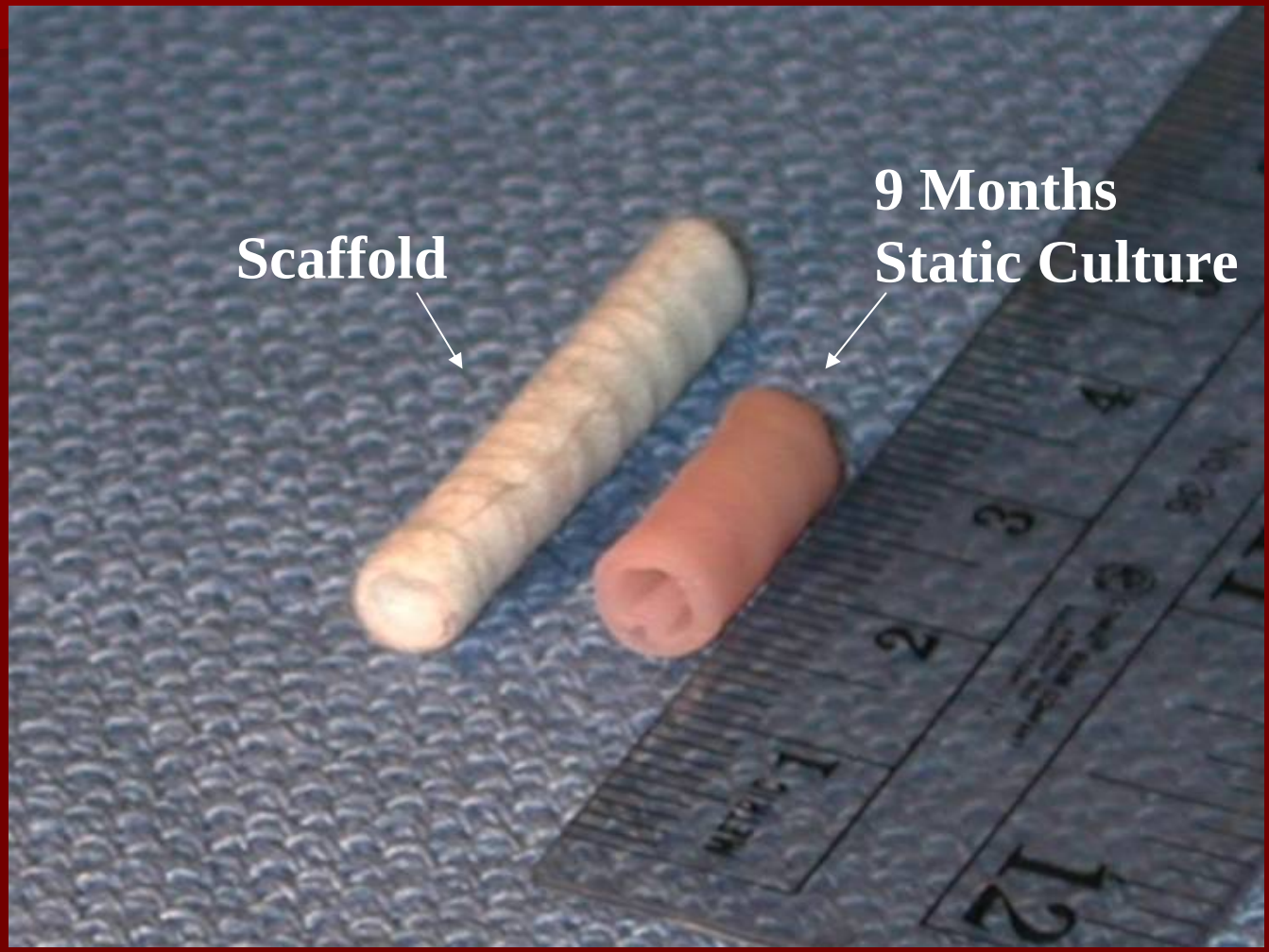
Virginia Commonwealth University BBSI

Scaffold and Tissue Engineered Blood Vessel

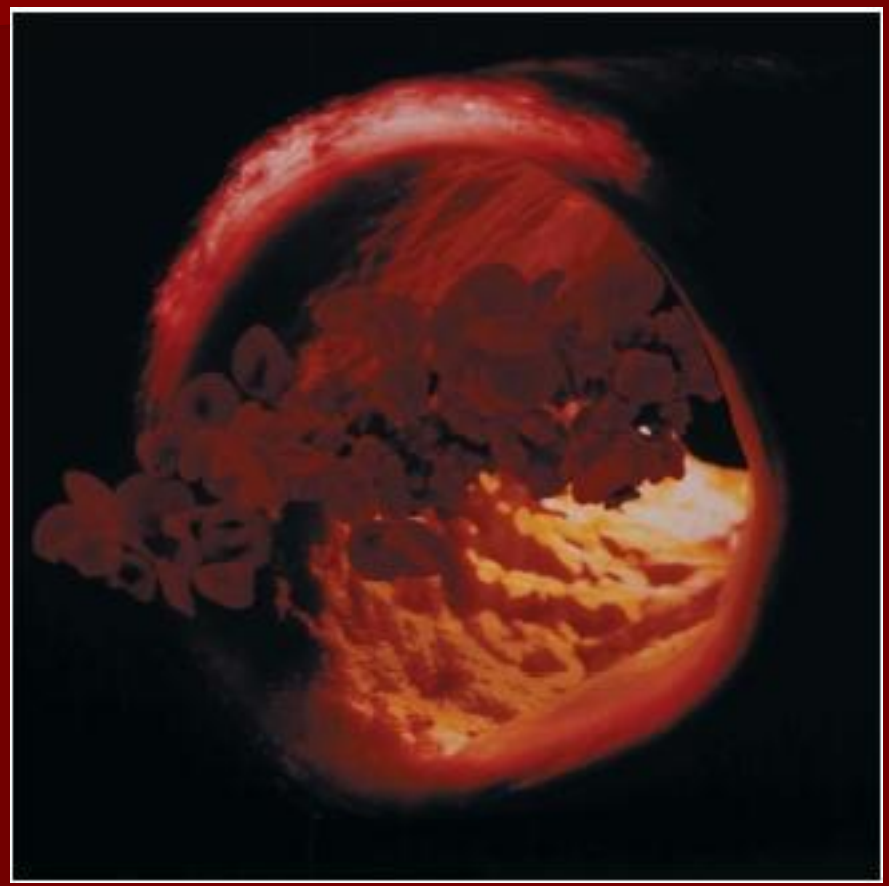


Dr. Gary Bowlin VCU Tissue Engineering Laboratory

Current Prosthetic Vascular Grafts

- < 6 mm small diameter
- Post surgical implantation leads to low patency
  - Acute thrombogenicity
  - Anastomotic intimal hyperplasia
  - Aneurysm formation
  - Infection
  - Progression of atherosclerotic disease

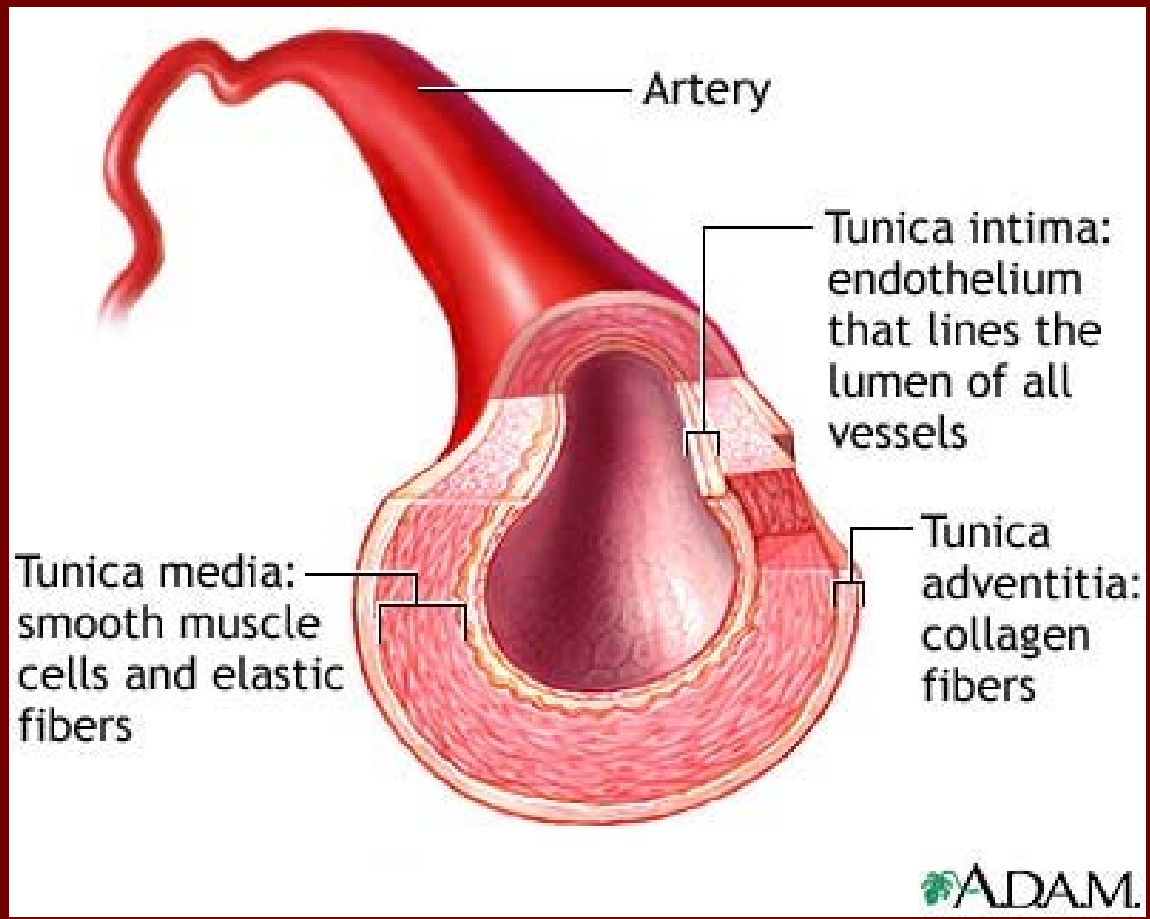
Blood Vessel Engineering



http://www.scienceclarified.com/images/uesc_02_img0092.jpg

New Design Method: Tissue Engineering

- Non-thrombogenic interfaces
- Vasoactivity
- Mechanical Properties



Current Tissue Engineering Vascular Scaffold Results

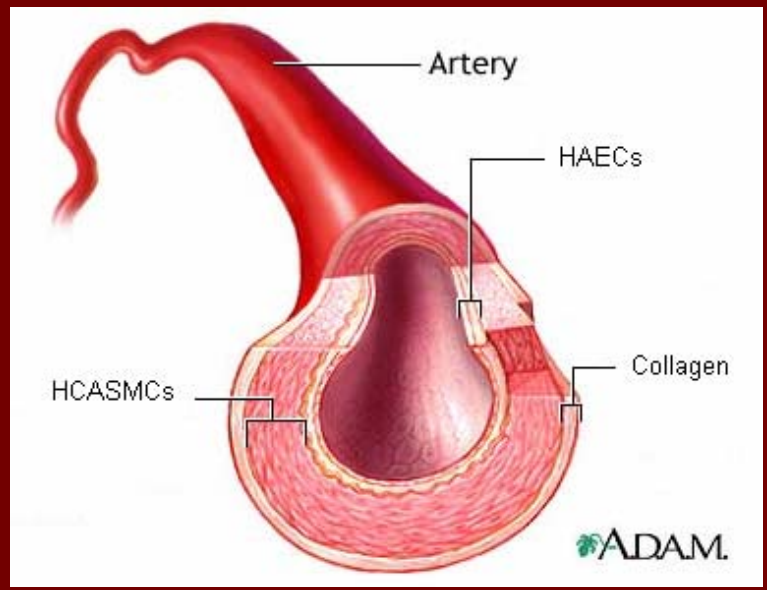
- Synthetic polymer tissue engineering scaffolds (Ex. PGA, PLLA)
  - Loss of mechanical strength before extracellular matrix (ECM) produced due to degradable nature of polymers
- Hybrid synthetic scaffold (PGA/PCL and PLA)
  - Worked under relatively low mechanical blood pressures ≈20–30 mm Hg during systole; needs to work at ≈100–140 mm Hg during systole
- Natural polymer tissue engineering scaffolds (Ex. collagen, fibrin)
  - ECM produced, high mechanical strength and flexibility
  - Even with Dacron mesh reinforcement, the burst strength of these constructs was lower than the burst strength of human saphenous veins (1680 ± 307 mm Hg) taken from the leg

Another Possible Natural Polymer: Silk Fibroin

- Unique mechanical properties in various structural alignments
- Biocompatibility
- Controlled degradability
- Versatile processability
- Electrospun silk fibroin tubular scaffolds have mechanical properties similar to native blood vessels

Purpose of Article

- Characterize the potential of these nanofiber silk matrices
  - Support vascular smooth muscle cell functions
    - Proliferate human coronary artery smooth muscle cells (HCASMCs)
  - Support endothelial cell functions
    - Proliferate human aortic endothelial cells (HAECs)
- Look for changes in HCASMCs and HAECs
  - Cell morphology
  - Viability
  - Proliferation
  - Phenotype



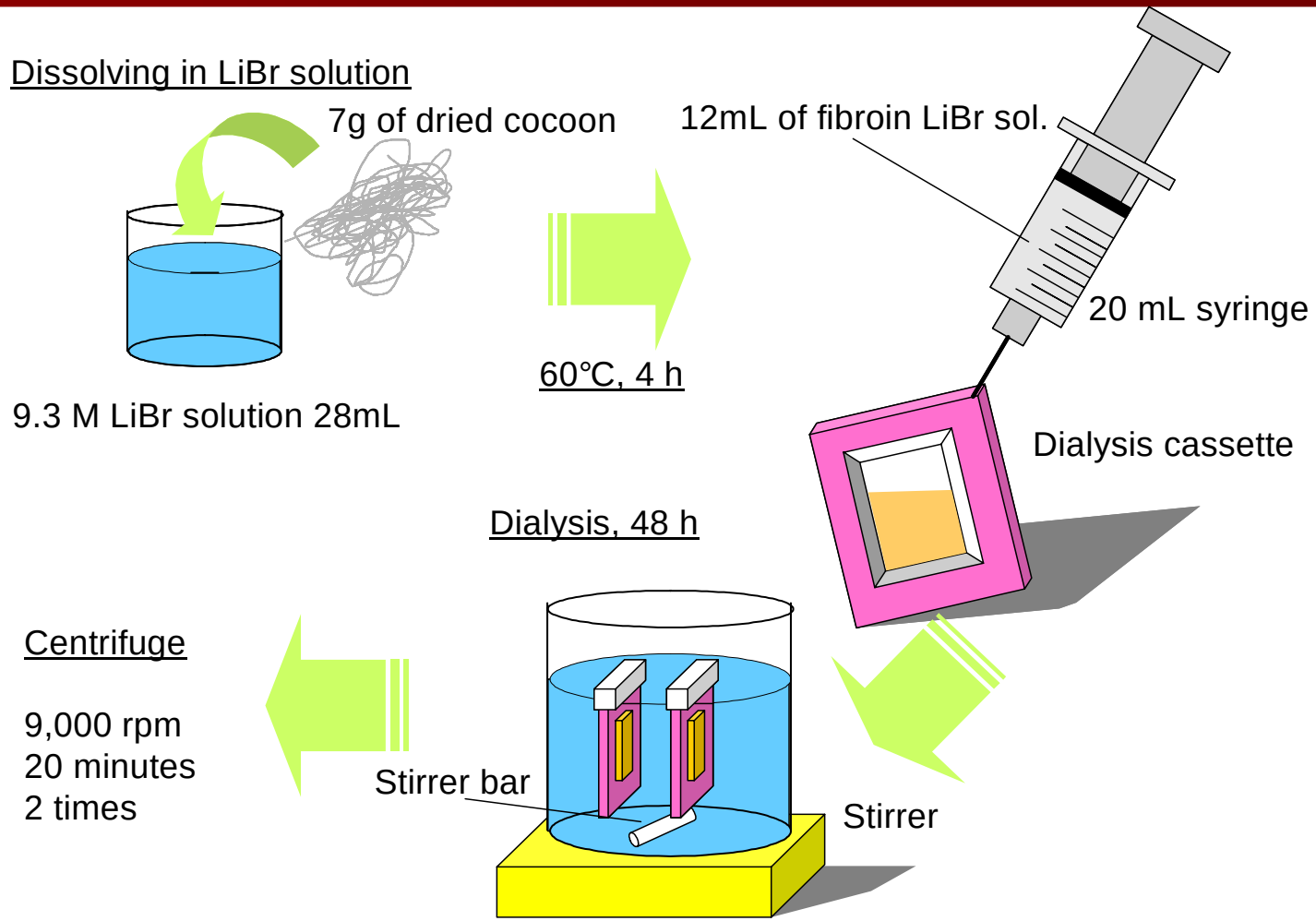
Bombyx Mori Silkworm Cocoon

http://www.dkimages.com/discover/previews/975/85004290.JPG

Silk Preparation Before Electrospinning

Tufts University Department of Biomedical Engineering

Silk Preparation Before Electrospinning



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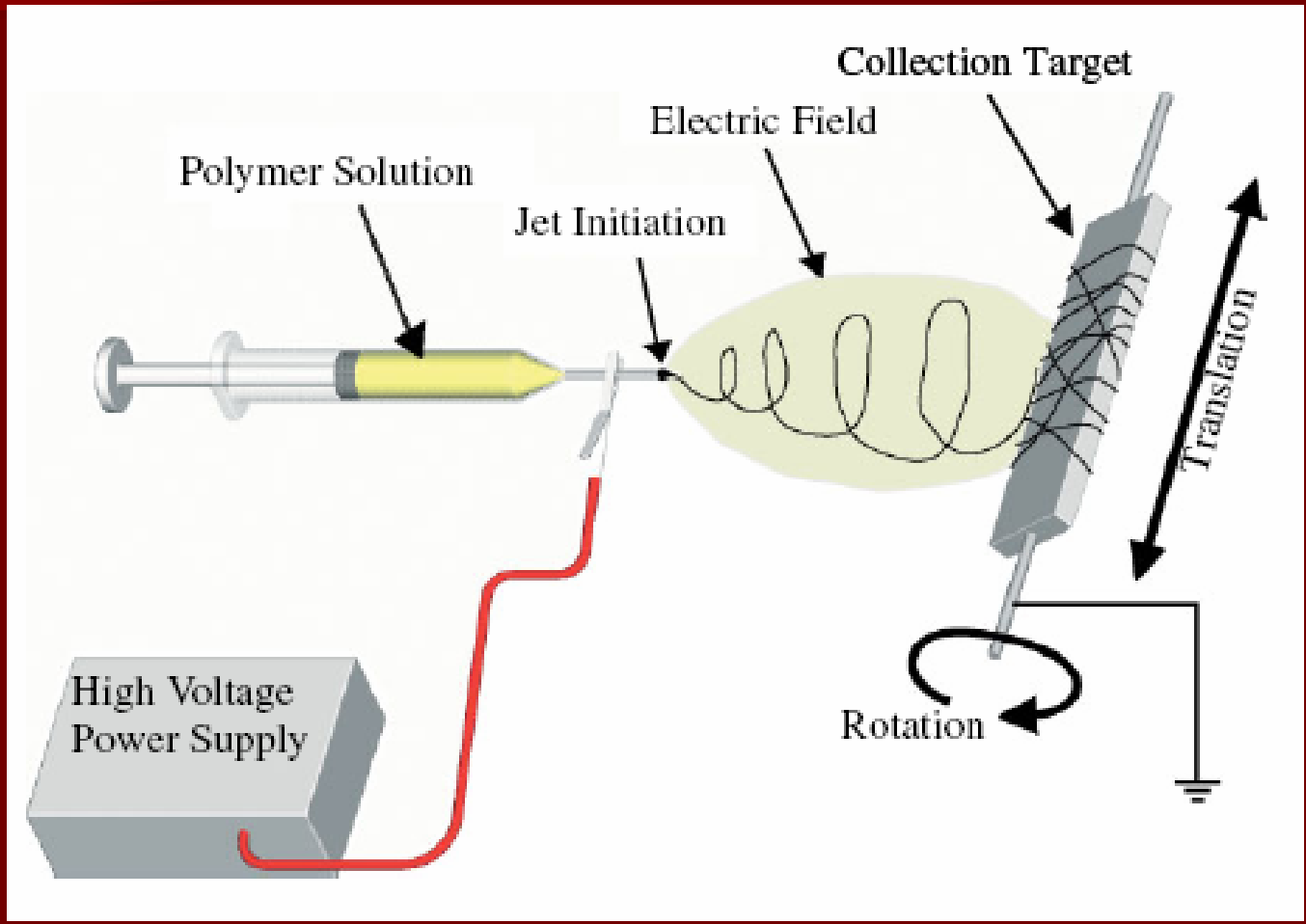
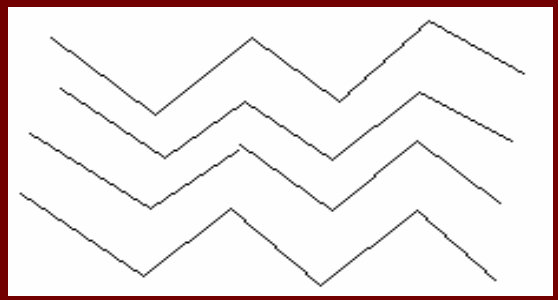
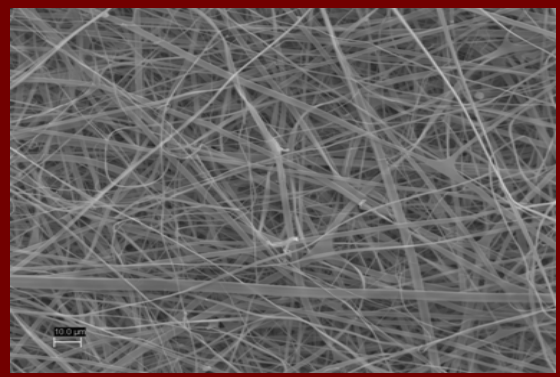
Electrospinning Nanofiber Matrices to Mimic the ECM



Electrospinning

- Silk/PEO solution prepared in H_2O for increased viscosity and surface tension (no organic solvents!)
- Electrospun co-polymer was treated with 100% methanol to form β-sheets -> water insoluble





- PEO removed with distilled H_2O to provide a pure silk matrix

Cells for Seeding

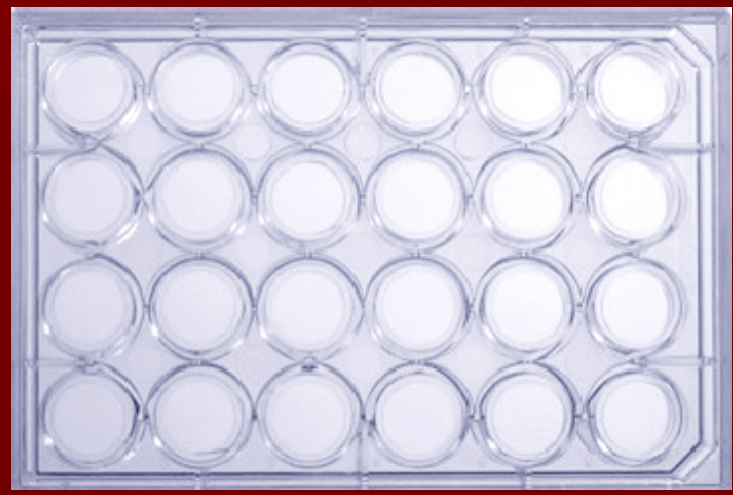
- HAECs and HCASMCs
  - Donated from 24 year old w/ no vascular disease
  - Cultured in endothelial growth medium-2 (EGM-2) supplemented with 10% fetal bovine serum (FBS) and 1 % smooth muscle cell medium (SMCM) with a 10% FBS and 1% SMCM and 1% penicillin/streptomycin supplements
  - Medium replaced every 2 days
  - Cells in incubator at 37° C and 5% CO_2

In Vitro Culture of Vascular Cells

Electrospun Scaffold

1.43 cm diameter

X 24 →



http://www.glass-bottomdishes.com/24well1-1.JPG

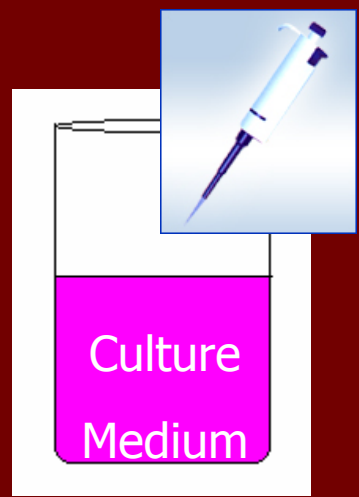
HCASMCs

Density = 7.5×10^4 cells/cm^2

HAECs

Density = 2.5×10^5 cells/cm^2

+



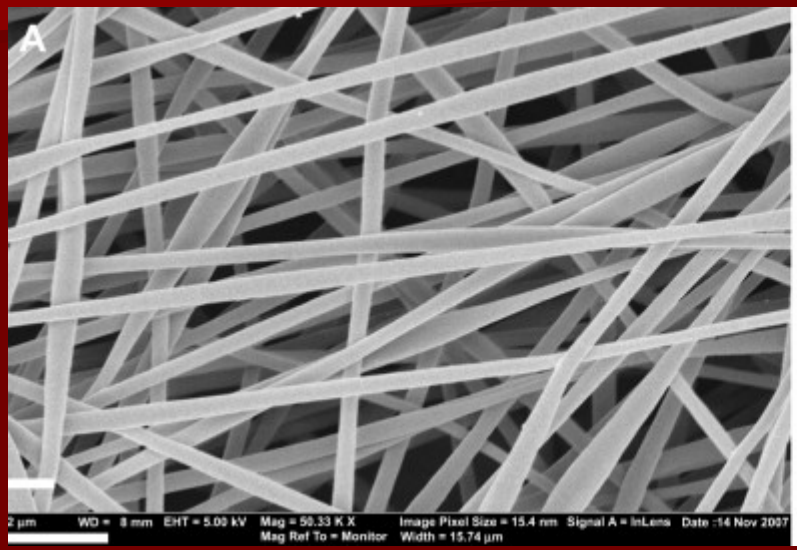
http://cellapplications.com/images/humanimages

http://www.scientific-laboratory-instruments.com

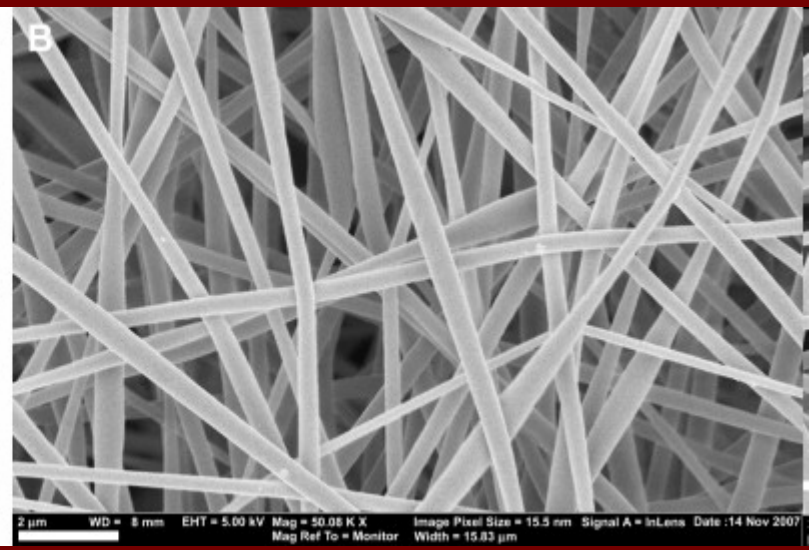
Tests Performed

- Scanning electron microscopy (SEM)
  - Cell morphology
- DNA content assay
  - Cell proliferation
- Cell viability and metabolism activity assay
  - Cell viability
- Immunocytochemistry assay
  - Cell differentiation
- Real-time RT-PCR anaysis
  - Genotype analyses from RNA
- Statistical analysis
  - Two-tailed t test; statistically significant if $p < 0.05$

SEM Results



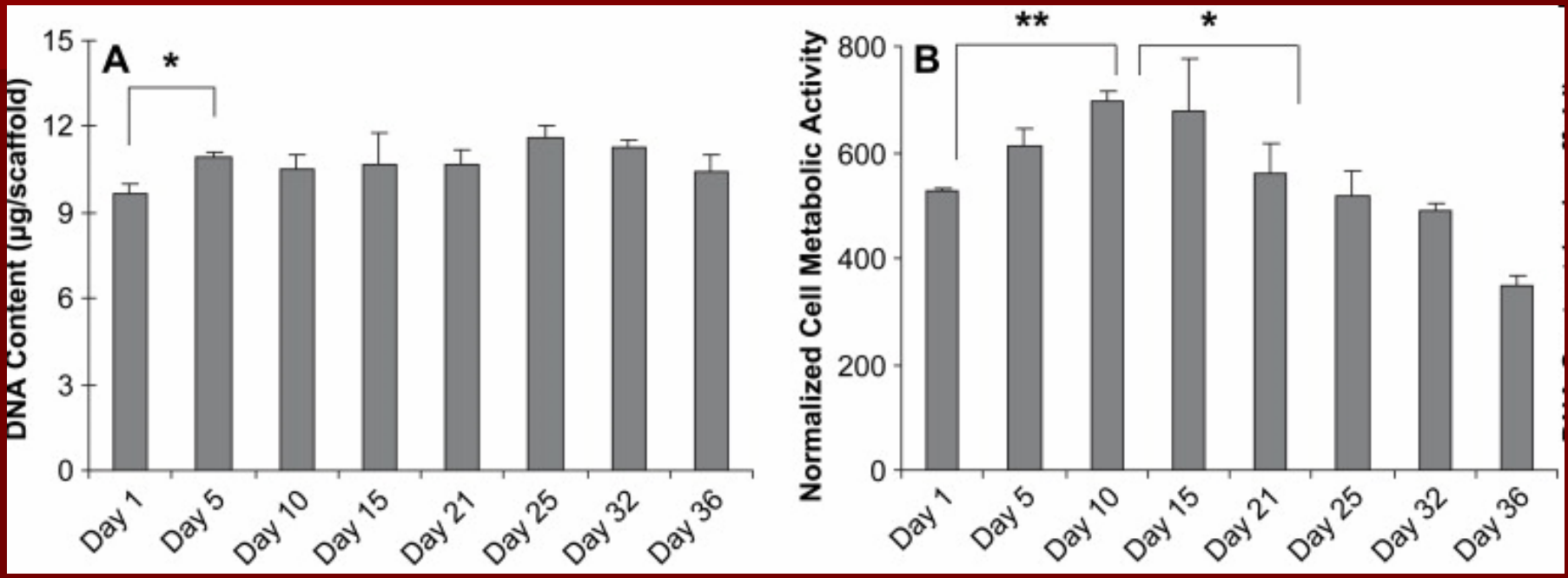
Electrospun Silk/PEO



Electrospun Silk after PEO extracted

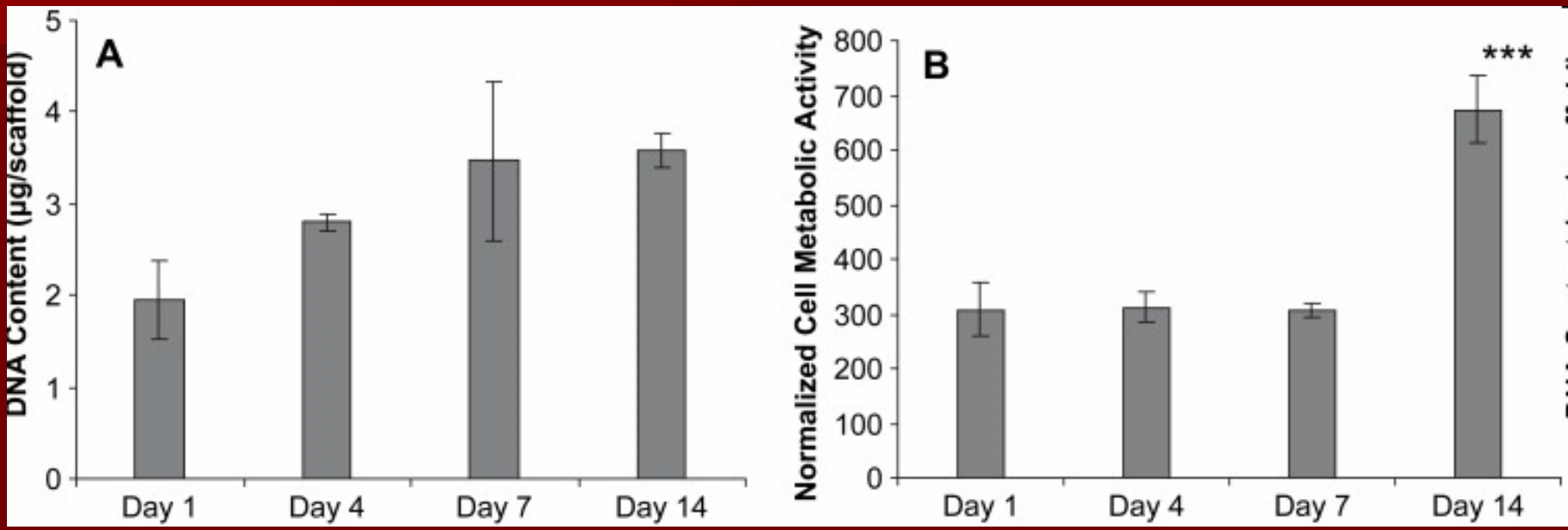
Morphology and diameter of electrospun fibers were not significantly affected once PEO was extracted.

DNA Content and Metabolism Activity Assay Results for HCASMCs



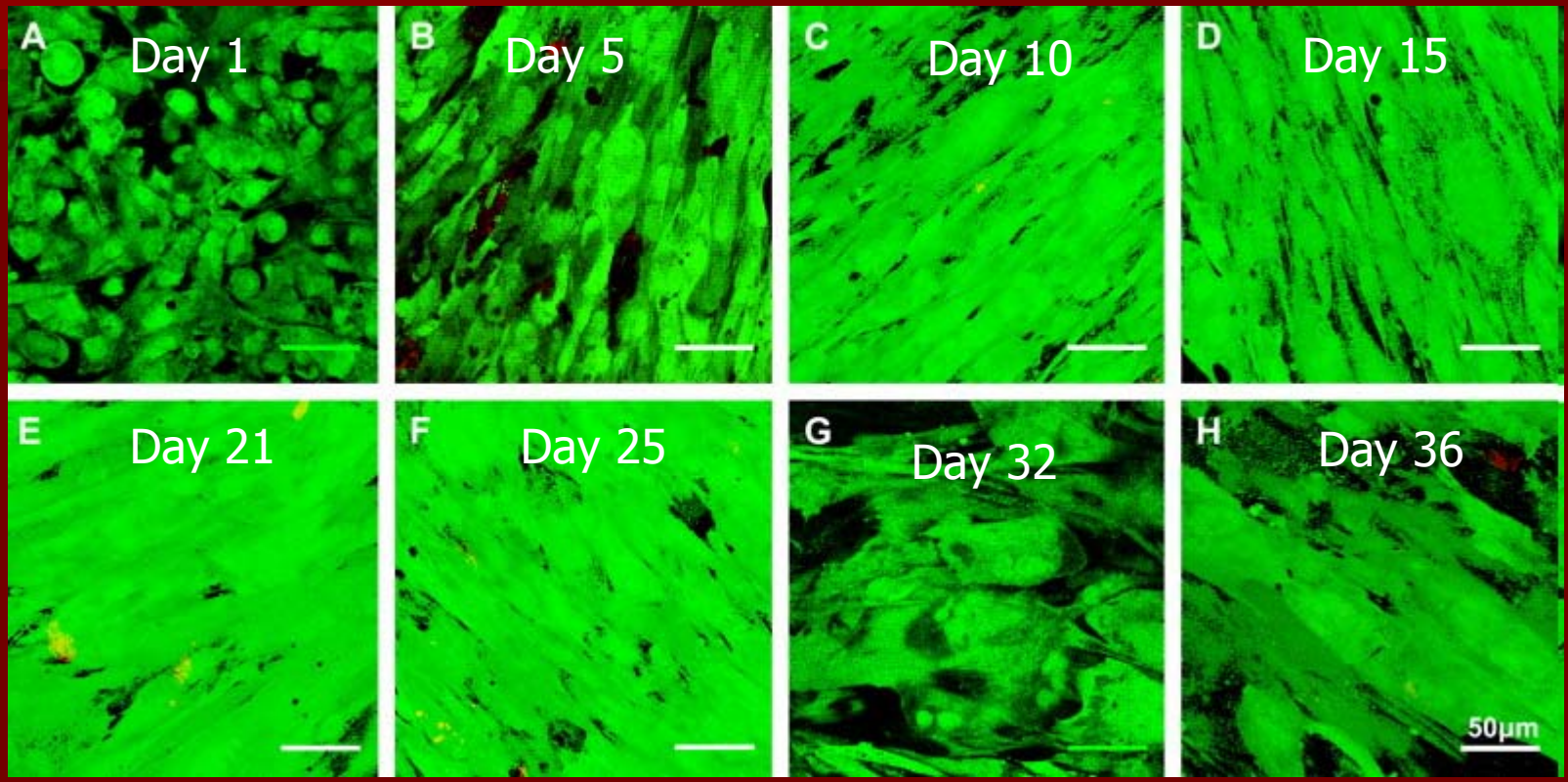
- Significant increase of DNA content was observed on day 5 (*$p<0.01$)
- Significant increase of metabolic activity was observed during the first 10 days (**$p<0.01$)
- Significant decrease of metabolic activity was observed after day 15 (*$p<0.05$)

DNA Content and Metabolism Activity Assay Results for HAECs



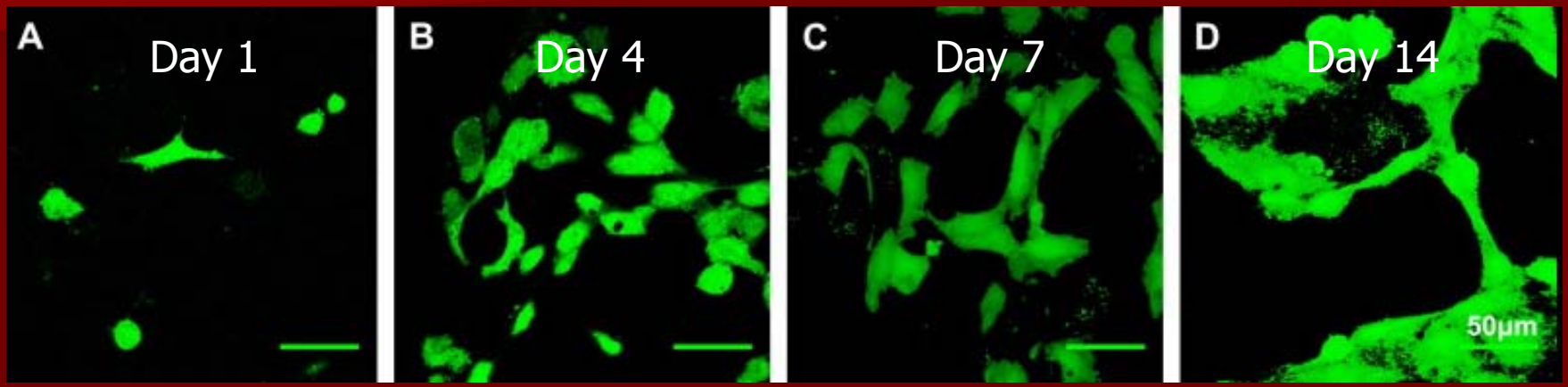
- No Significant increase in DNA observed
- Significant increase of metabolic activity was observed on day 14 (***$p<0.001$)

HCASMCs Viability Assay

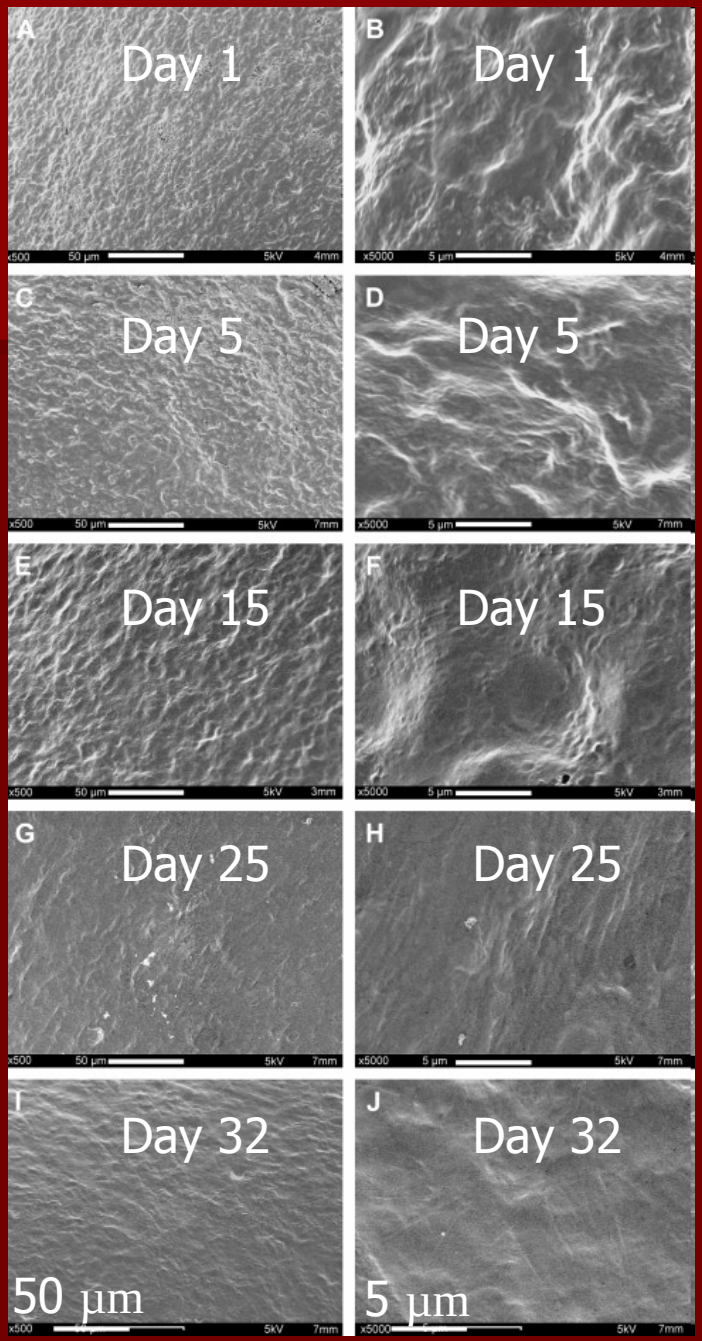


- High viability and cell alignment of HCASMCs observed

HAECs Viability Assay



- High viability of HAECs
- Short cord-like structures at Day 4
- Interconnecting network of capillary tubes at Day 7



SEM Micrographs of HCASMCs

Day 1 – random orientation, cells are spread

Day 5 – spindle shaped cells with parallel alignment

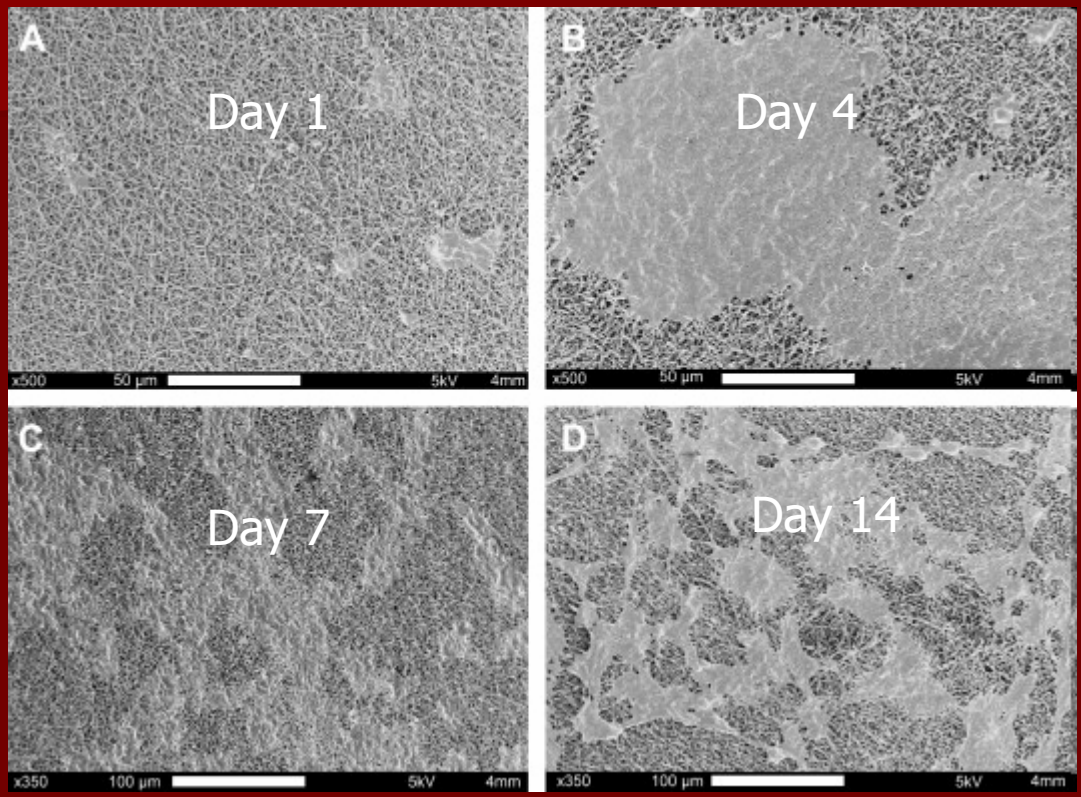
Day 10 – significant cell elongation

These results indicate alignment on randomly oriented non-woven fibrous scaffolds.

Characteristic of "hill and valley" distribution of the smooth muscle cells

Self-organizing smooth muscle cells on the scaffolds-reveals the importance of cell-matrix interactions for cell alignment

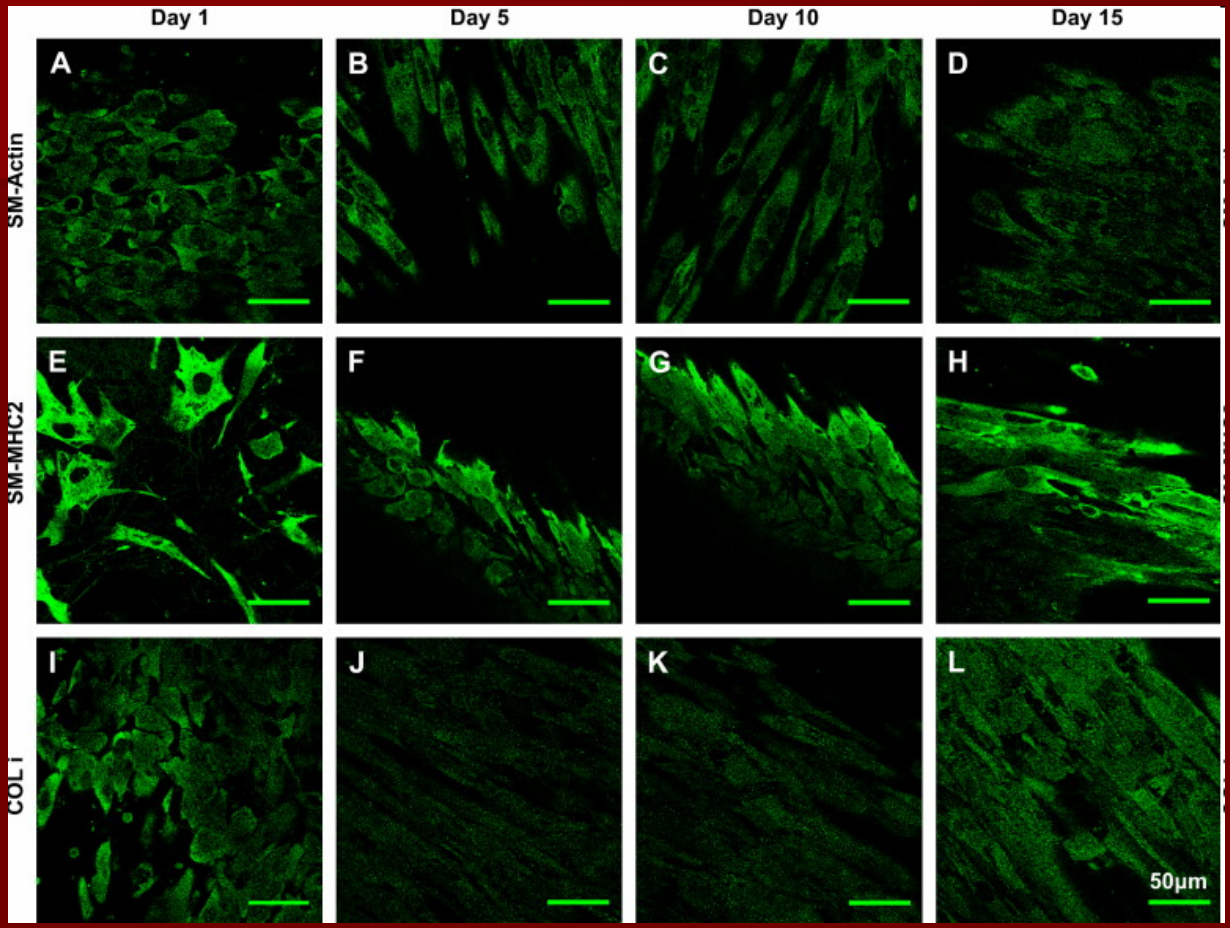
SEM Micrographs of HAECs



Angiogenesis occurred – migration and differentiation of endothelial cells in angiogenic pathways

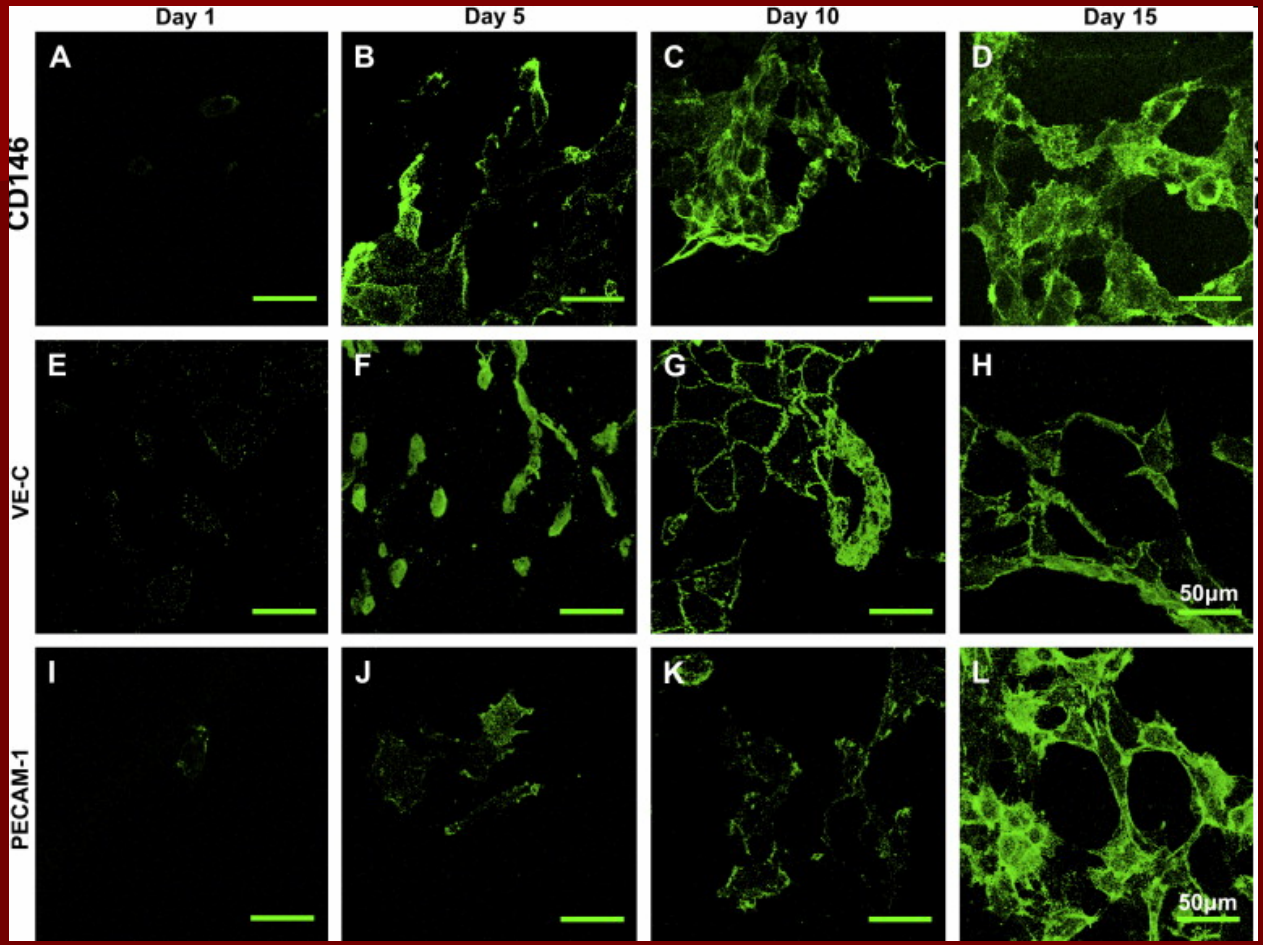
Day 7 – network formation of capillary-like tubes with lumen

Immunocytochemistry Assay for HCASMCs



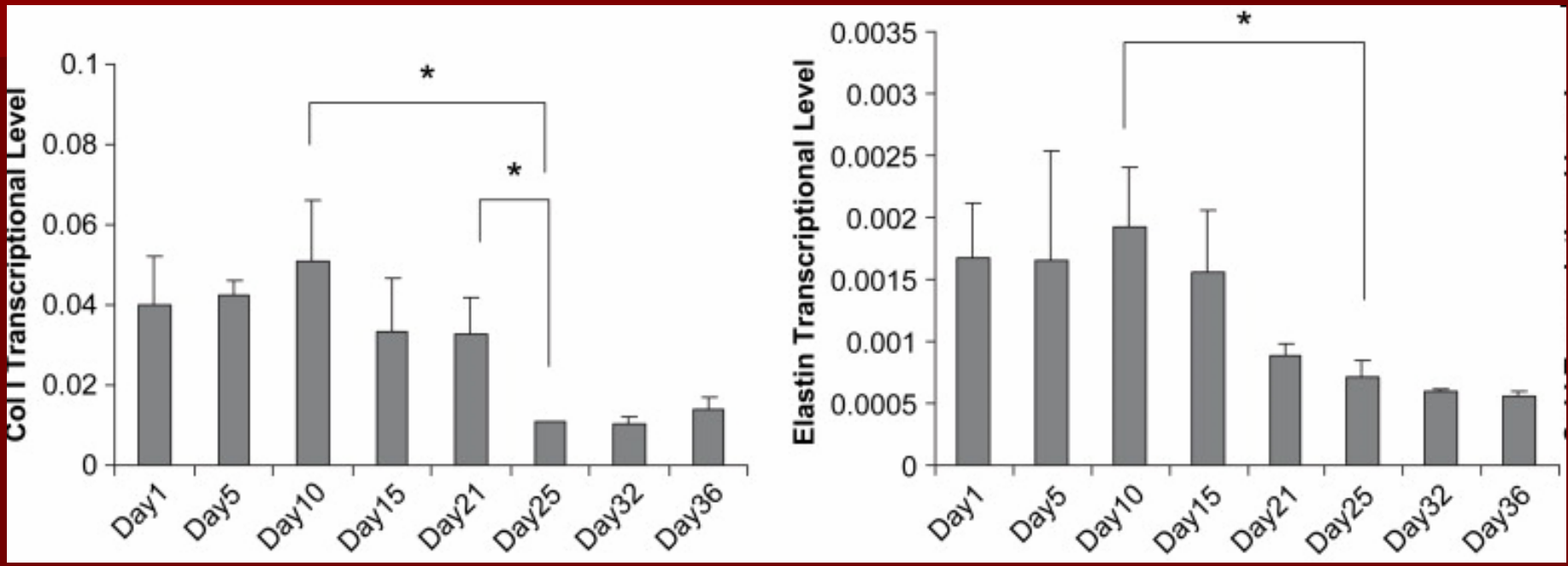
Scaffold supported smooth muscle cell contractile phenotype. Stained protein markers denote smooth muscle actin, smooth muscle myosin heavy chain, and aligned collagen type I.

Immunocytochemistry Assay for HAECs



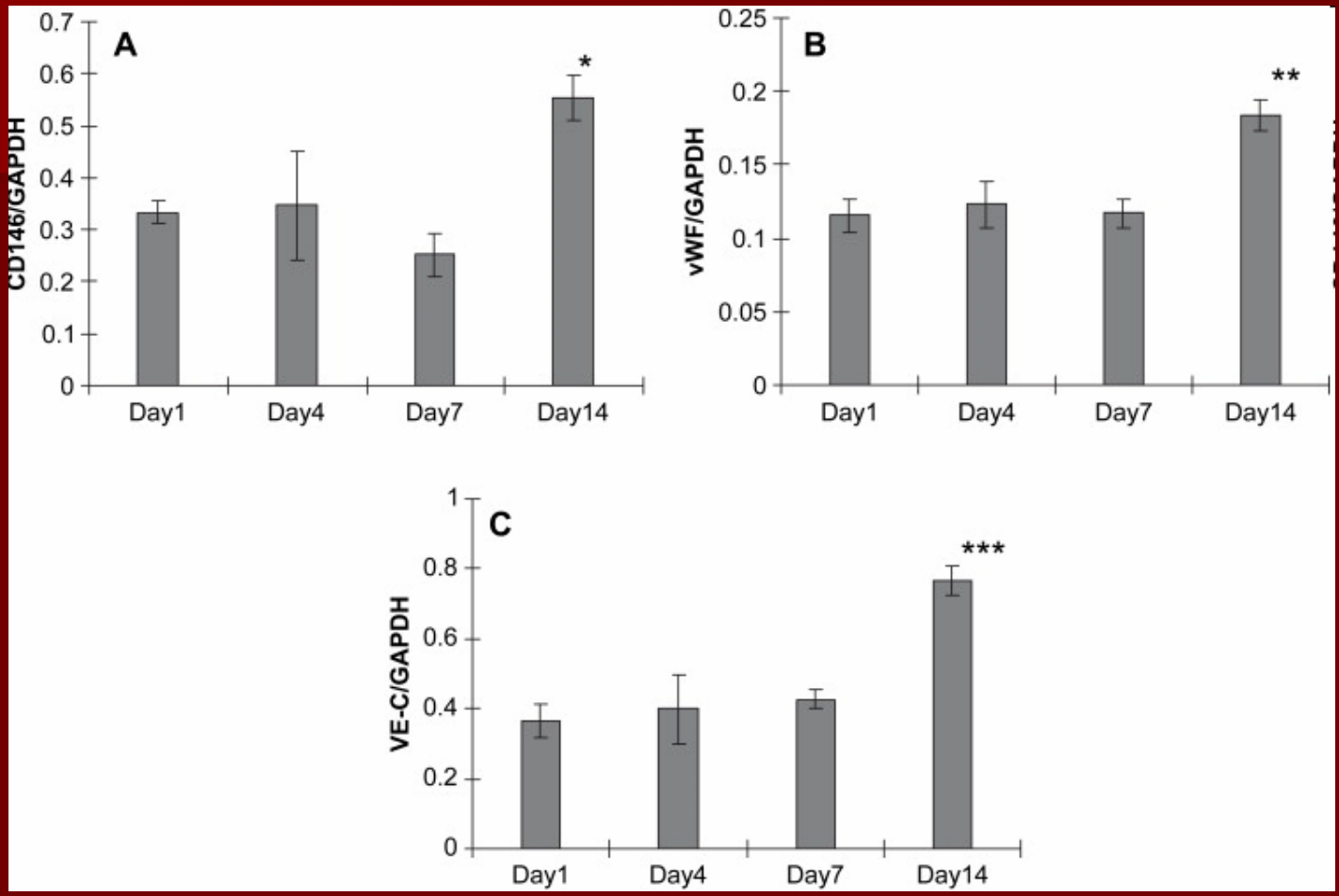
HAECs maintained characteristic endothelial cell phenotype on the scaffold. Stained protein markers for universal endothelial cells are shown.

Real-Time RT-PCR Anaysis for HCASMCs



Collagen type I and elastin markers in mRNA showed statistically significant decreases over time.

Real-Time RT-PCR Analysis for HAECs



Endothelial cell markers examined in mRNA showed statistically significant increases at Day 14 for each of the 3 markers.

Conclusions

- HAECs and HCASMCs seeded on electrospun silk fibroin scaffolds
  - Support vasular cell viability
  - Maintain cell phenotype
  - Promote cell growth, expansion, and reorganization
- Silk scaffolds should be researched further as a tissue engineered possibility for small diameter vascular grafts
  - Studies have already indicated that silk can be spun into vessel like tubes and support vascular pressures