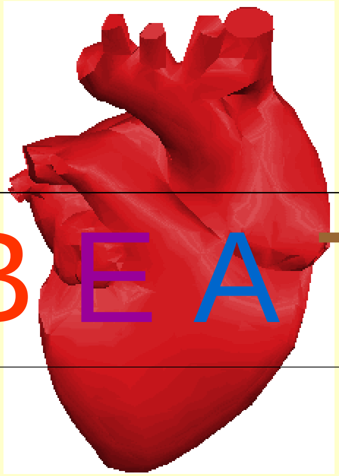
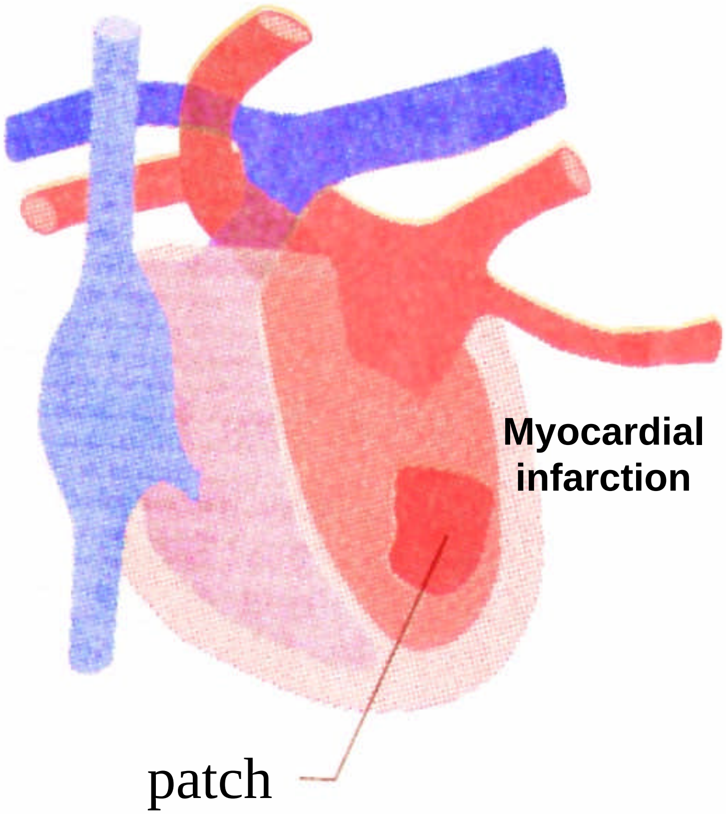


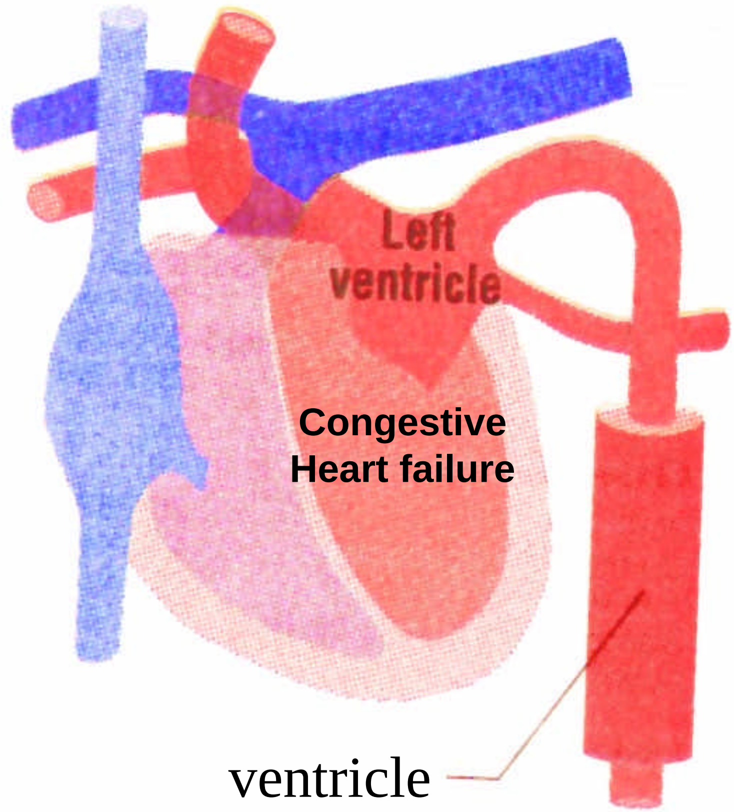
BEAT

Cardiovascular disease is the number one cause of death in developed countries.

An NIH Bioengineering Research Partnership grant

B E A T (BioEngineered Allograft/Autograft Tissue)





Goal: Years 1-5 - tissue engineer a chunk of living heart muscle

Goal: Years 6-10 - tissue engineer a living ventricle

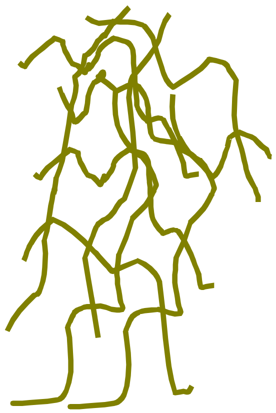
First, a description of tissue engineering…

Two related ideas:

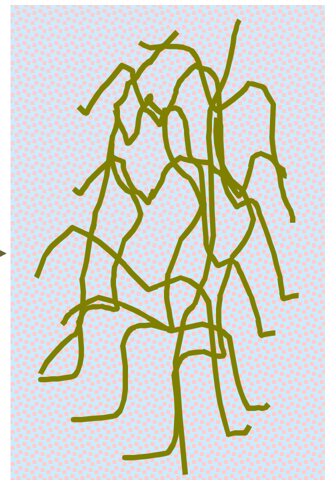
tissue engineering - an engineering/biology enterprise

regenerative medicine - mostly biology & and medicine

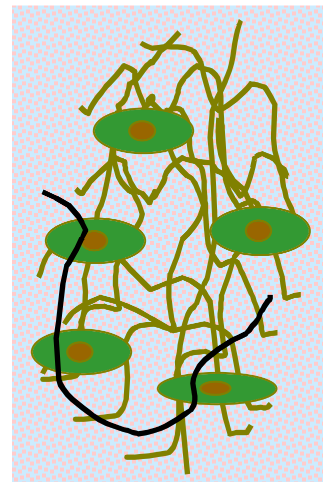
Tissue Engineering



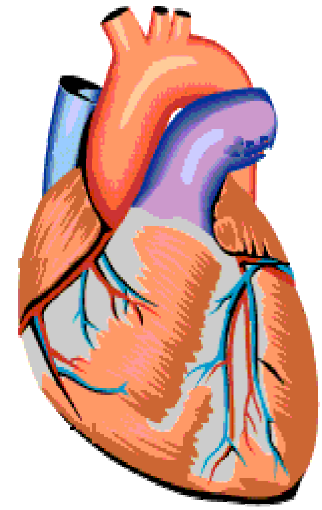
Start with a **porous matrix**



Mold to the **shape** of a tissue or organ



Seed with cells (autologous or allogeneic)



Culture the cells and **grow a tissue** or organ

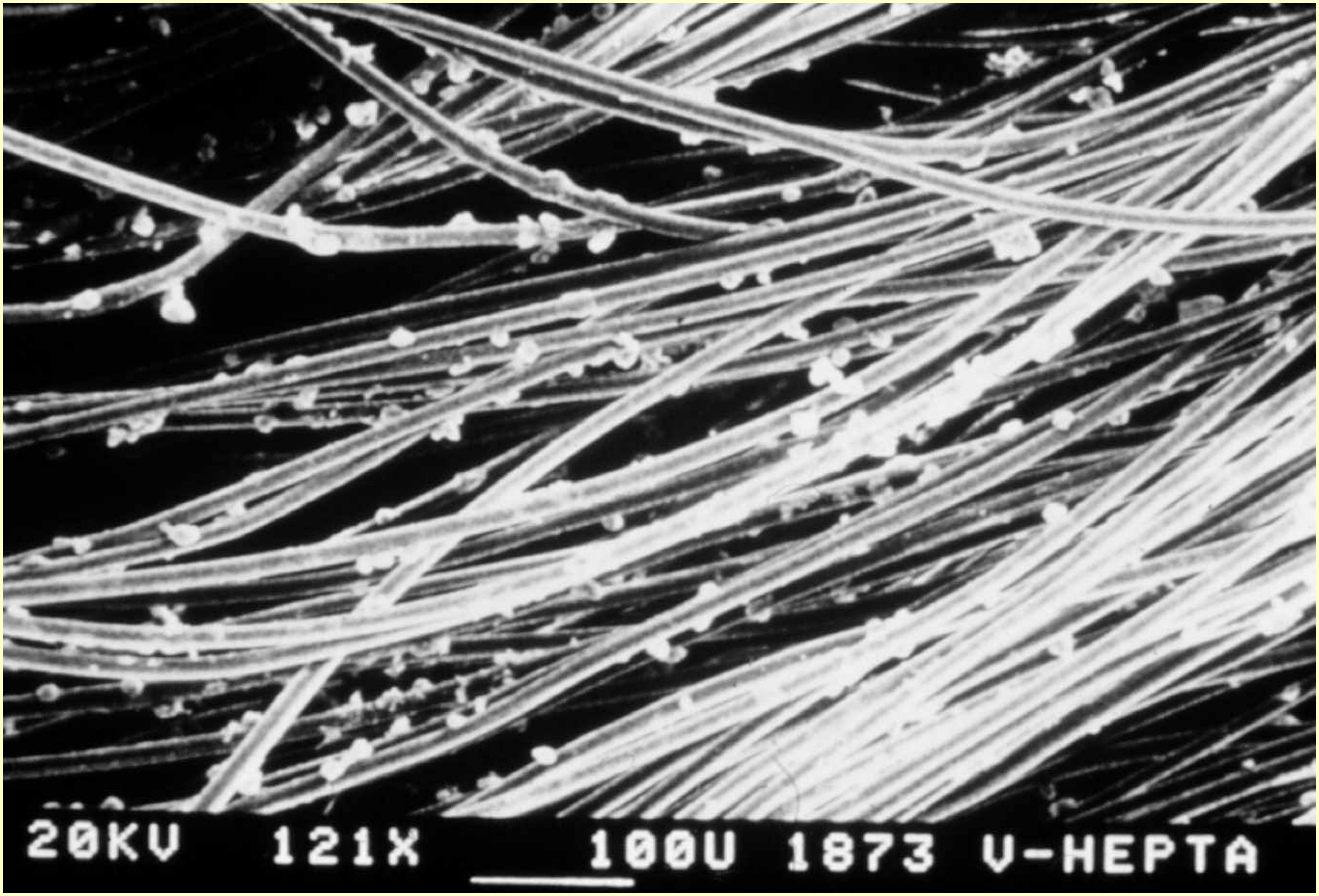
Poly(lactic acid)
Poly(glycolic acid)
PGLA
Collagen

→

Chondrocytes
Hepatocytes
Cardiomyocytes
Osteoblasts

→

Cartilage
Liver
Heart
Bone



Cells seed on poly(lactic acid) fibers

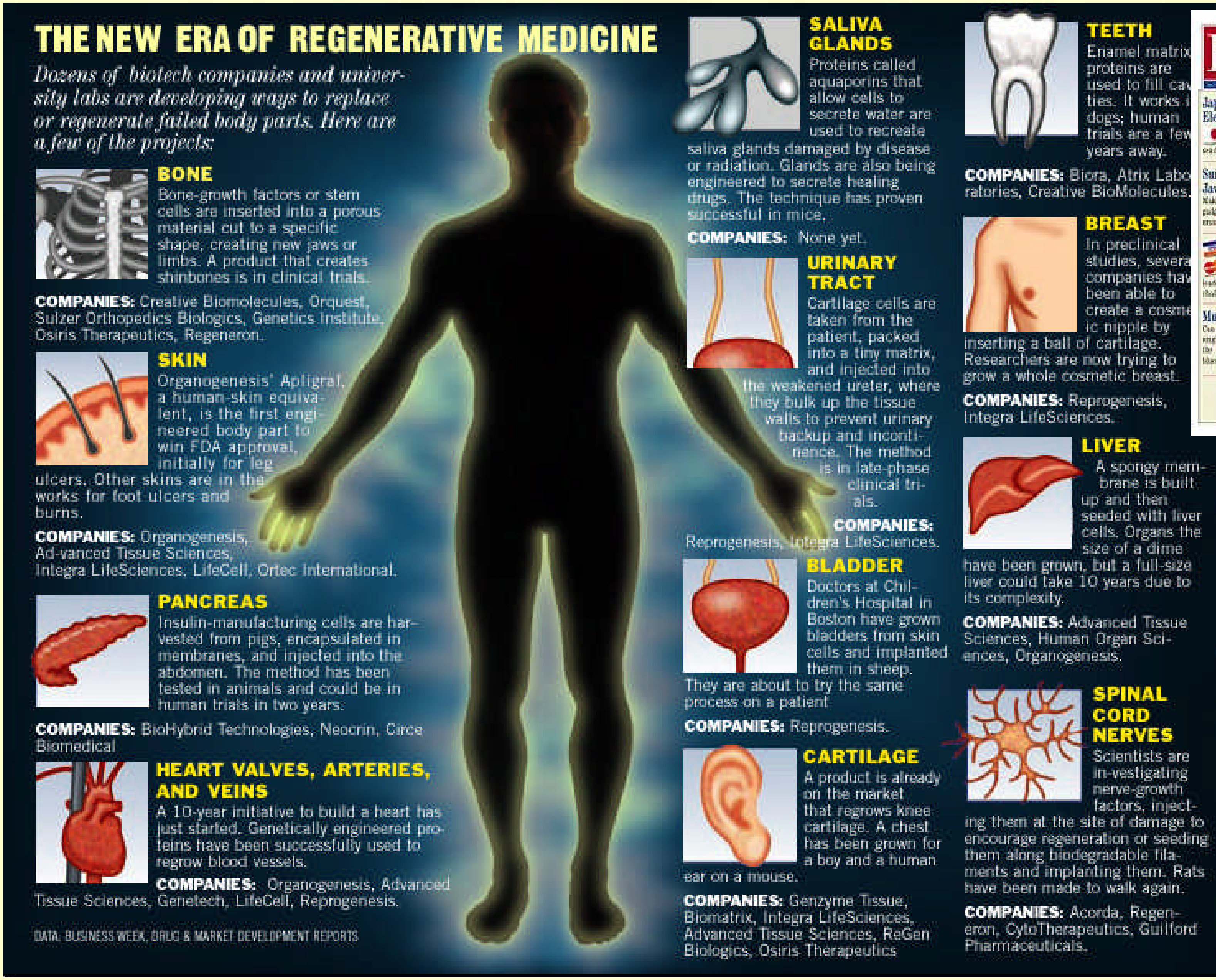
R. Langer, et al.

R. Langer, et al.

A contemporary thinker and his double.

From "Engineer's Toolkit" C. Mitcham and R. Duvall

http://www.cen.uiuc.edu/~vincens/peo_hydrogels.html



THE NEW ERA OF REGENERATIVE MEDICINE

Dozens of biotech companies and university labs are developing ways to replace or regenerate failed body parts. Here are a few of the projects:

BONE

Bone-growth factors or stem cells are inserted into a porous material cut to a specific shape, creating new jaws or limbs. A product that creates shinbones is in clinical trials.

COMPANIES: Creative Biomolecules, Orquest, Sulzer Orthopedics Biologics, Genetics Institute, Osiris Therapeutics, Regeneron.

SKIN

Organogenesis' Apligraf, a human-skin equivalent, is the first engineered body part to win FDA approval, initially for leg ulcers. Other skins are in the works for foot ulcers and burns.

COMPANIES: Organogenesis, Ad-vanced Tissue Sciences, Integra LifeSciences, LifeCell, Ortec International.

PANCREAS

Insulin-manufacturing cells are harvested from pigs, encapsulated in membranes, and injected into the abdomen. The method has been tested in animals and could be in human trials in two years.

COMPANIES: BioHybrid Technologies, Neocrin, Circe Biomedical.

HEART VALVES, ARTERIES, AND VEINS

A 10-year initiative to build a heart has just started. Genetically engineered proteins have been successfully used to regrow blood vessels.

COMPANIES: Organogenesis, Advanced Tissue Sciences, Genetech, LifeCell, Reprogenesis.

DATA: BUSINESS WEEK, DRUG & MARKET DEVELOPMENT REPORTS

SALIVA GLANDS

Proteins called aquaporins that allow cells to secrete water are used to recreate saliva glands damaged by disease or radiation. Glands are also being engineered to secrete healing drugs. The technique has proven successful in mice.

COMPANIES: None yet.

URINARY TRACT

Cartilage cells are taken from the patient, packed into a tiny matrix, and injected into the weakened ureter, where they bulk up the tissue walls to prevent urinary backup and incontinence. The method is in late-phase clinical trials.

COMPANIES: Reprogenesis, Integra LifeSciences.

BLADDER

Doctors at Children's Hospital in Boston have grown bladders from skin cells and implanted them in sheep. They are about to try the same process on a patient.

COMPANIES: Reprogenesis.

CARTILAGE

A product is already on the market that regrows knee cartilage. A chest has been grown for a boy and a human ear on a mouse.

COMPANIES: Genzyme Tissue, Biomatrix, Integra LifeSciences, Advanced Tissue Sciences, ReGen Biologics, Osiris Therapeutics

TEETH

Enamel matrix proteins are used to fill cav ties. It works i dogs; human trials are a few years away.

COMPANIES: Biora, Atrix Labo ratories, Creative BioMolecules.

BREAST

In preclinical studies, severa companies hav been able to create a cosme ic nipple by inserting a ball of cartilage. Researchers are now trying to grow a whole cosmetic breast.

COMPANIES: Reprogenesis, Integra LifeSciences.

LIVER

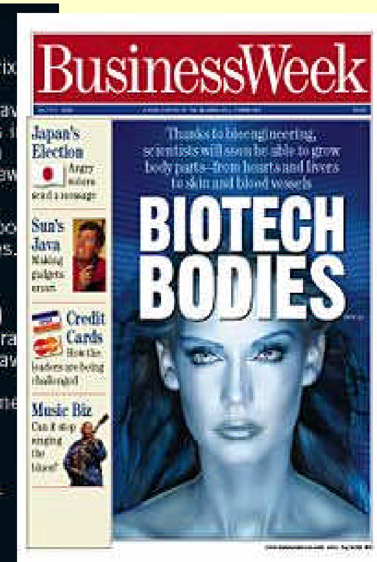
A spongy membrane is built up and then seeded with liver cells. Organs the size of a dime have been grown, but a full-size liver could take 10 years due to its complexity.

COMPANIES: Advanced Tissue Sciences, Human Organ Sciences, Organogenesis.

SPINAL CORD NERVES

Scientists are in-vestigating nerve-growth factors, injecting them at the site of damage to encourage regeneration or seeding them along biodegradable filaments and implanting them. Rats have been made to walk again.

COMPANIES: Acorda, Regeneron, CytoTherapeutics, Guilford Pharmaceuticals.



Tissue Engineering sounds great!

Are there challenges remaining?

The hard problems in tissue engineering:

Angiogenesis (blood vessel formation)

Cell Differentiation (on and off)

Multiple cell types

Inflammation

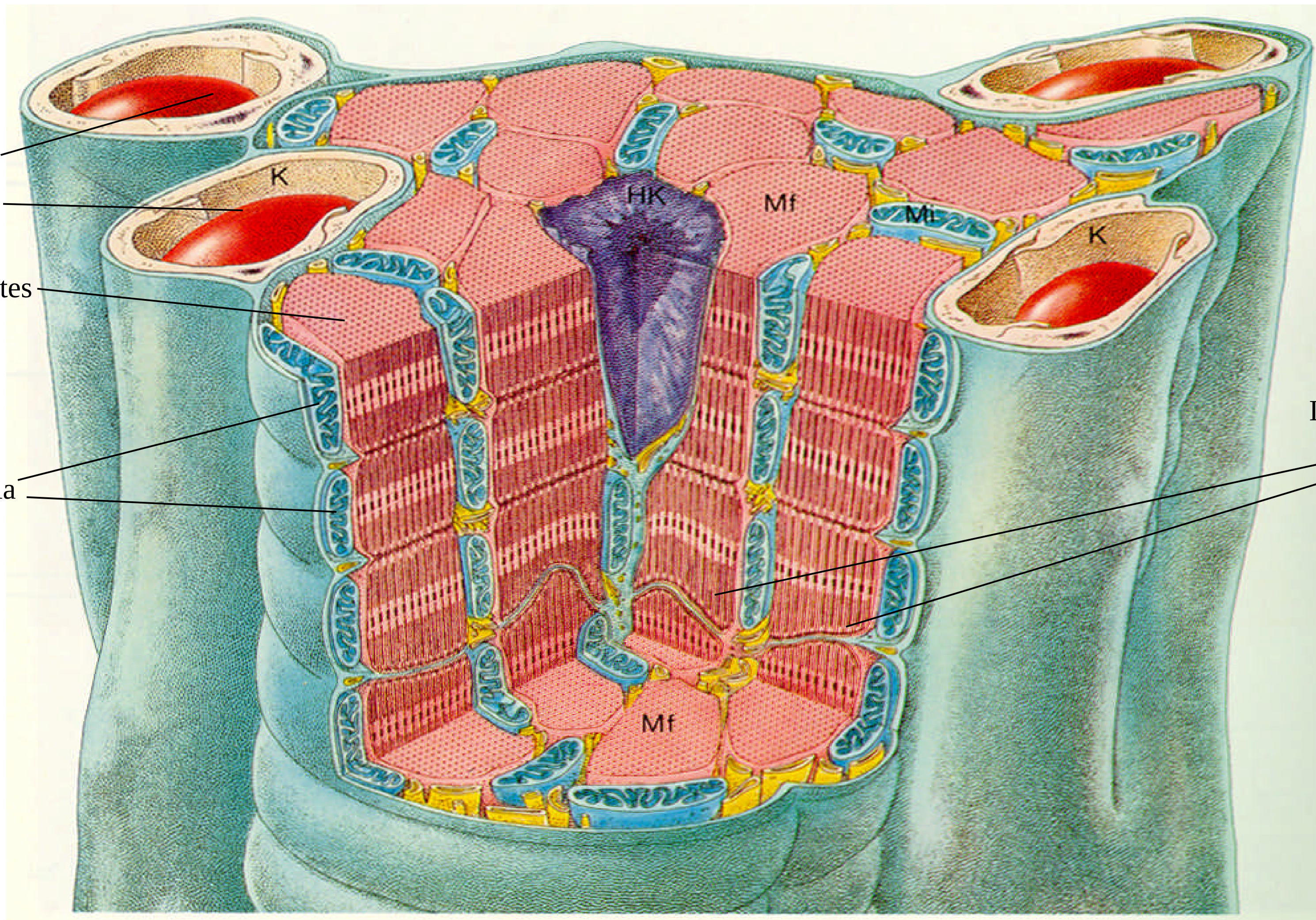
Immunology

Mechanics

Sterilization

Packaging

Myocardial Tissue



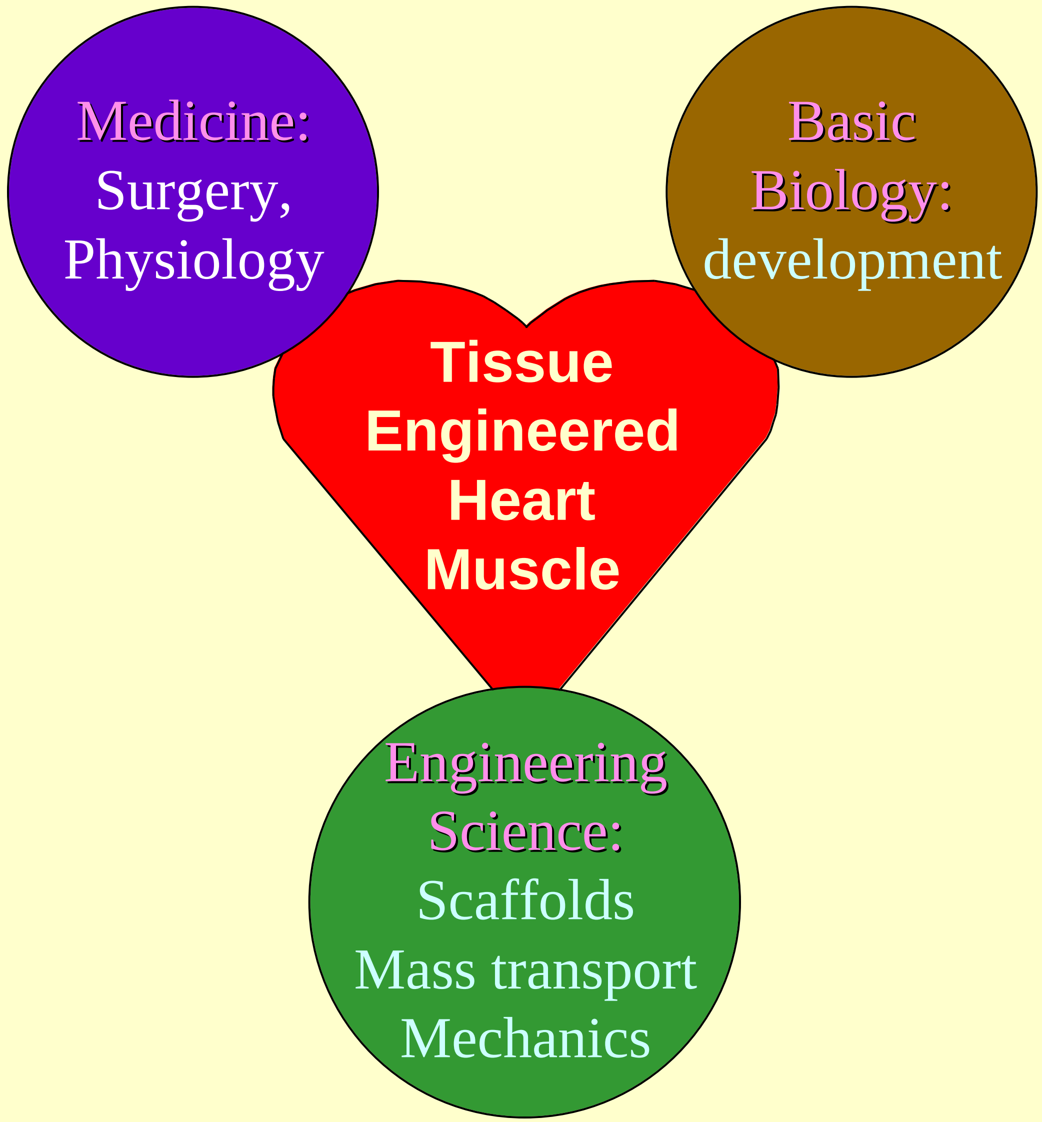
from: Myocardium, Vessels and Calcium, Lossnitzer, Pfennigsdorf, & Bräuer, 1983.

Heart Muscle Tissue Engineering

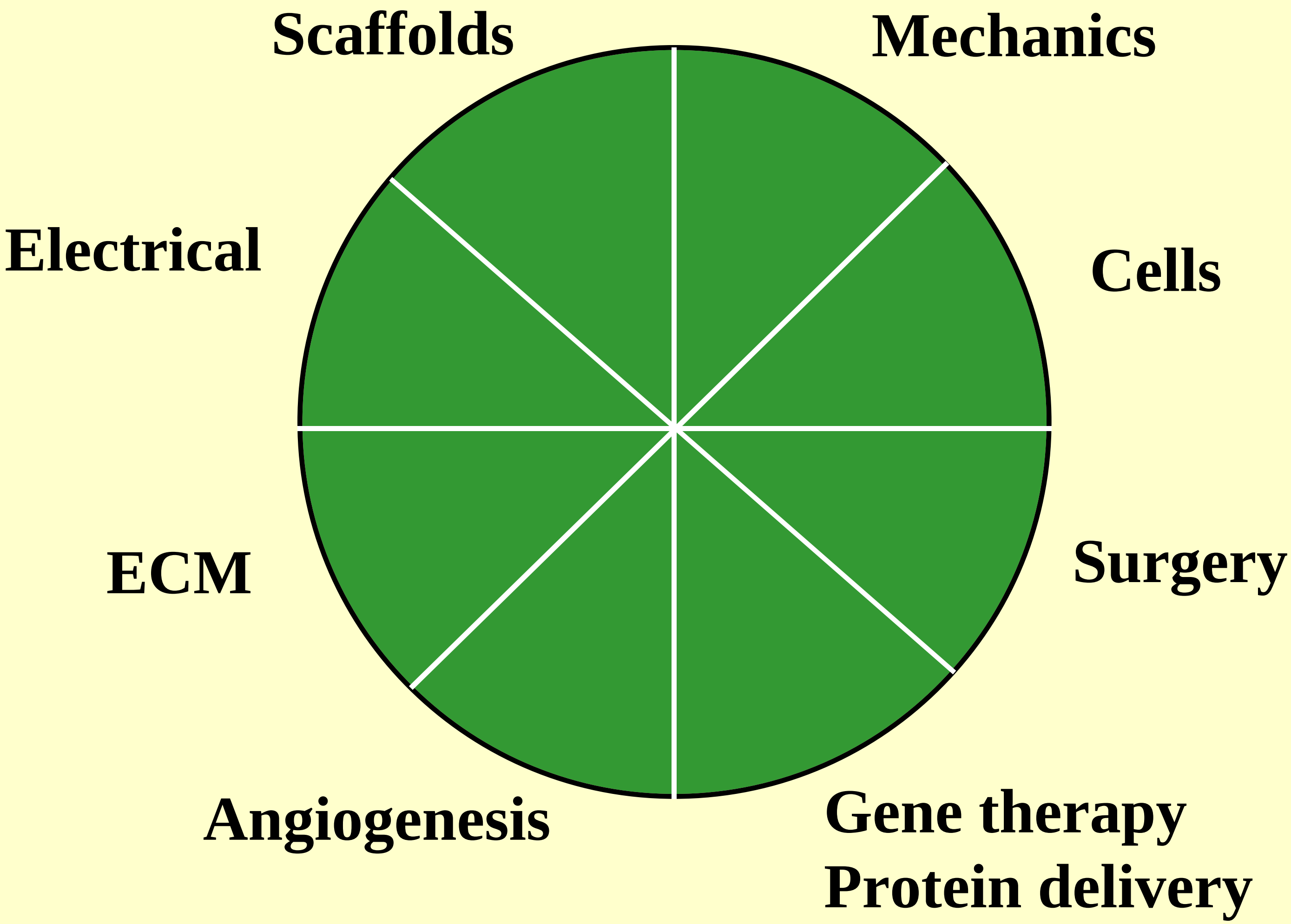
Issues that need to be addressed for cardiac muscle tissue engineering :

1. slow growing (or non-growing) cardiomyocytes
2. tissue engineering scaffolds that are elastomeric,
3. aligning/orienting cardiomyocytes
4. vascularizing the growing cell mass
5. innervating the tissue
6. incorporating strong and flexible ECM in the tissue,
7. inhibiting the inflammatory response (fibrosis) upon implantation,
8. preventing tissue rejection and
9. fusing the engineered heart muscle with existing heart tissue.

Elements that must coalesce to reach our goal: heart muscle



BEAT


Scaffolds
Mechanics
Electrical
Cells
ECM
Surgery
Angiogenesis
Gene therapy
Protein delivery

BEAT investigators

Ratner / Vogel / Nair
- **porous polymers (hydrogel, PLA), aphrons**

Sanders
- **fibrous materials / biomechanical considerations**

Woodhouse (U. Toronto)
- **novel biodegradable polymers**

Heller (Advanced Polymer Technologies, Inc.)
- **novel biodegradable polymers**

Hauschka/Angello
- **muscle cells**

Murry
- **healing in the heart**

Stayton / Hoffman
- **cell orientation/fusion / gene delivery**

Bornstein
- **ECM**

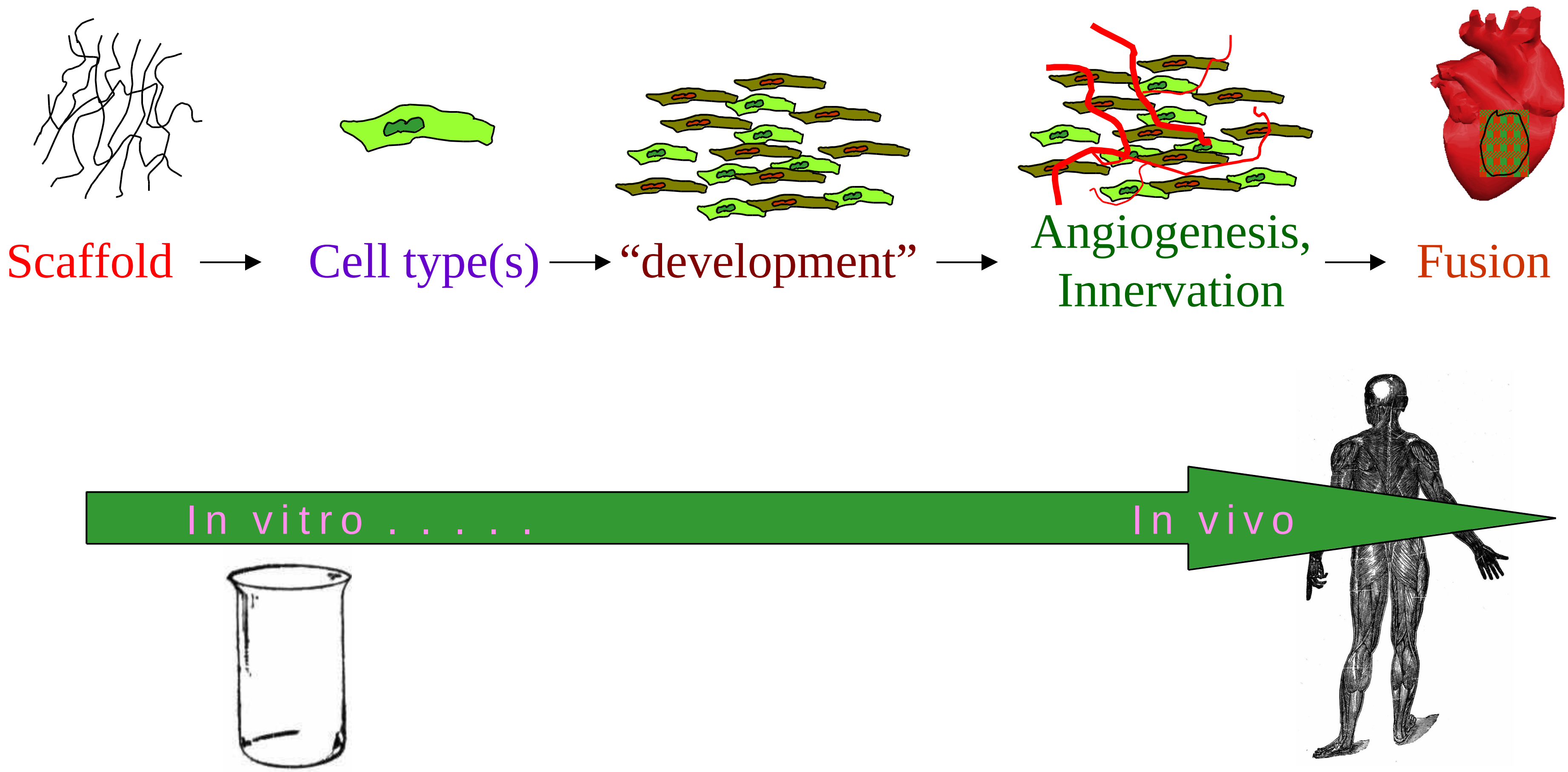
Allen
- **Endothelium, surgical aspects**

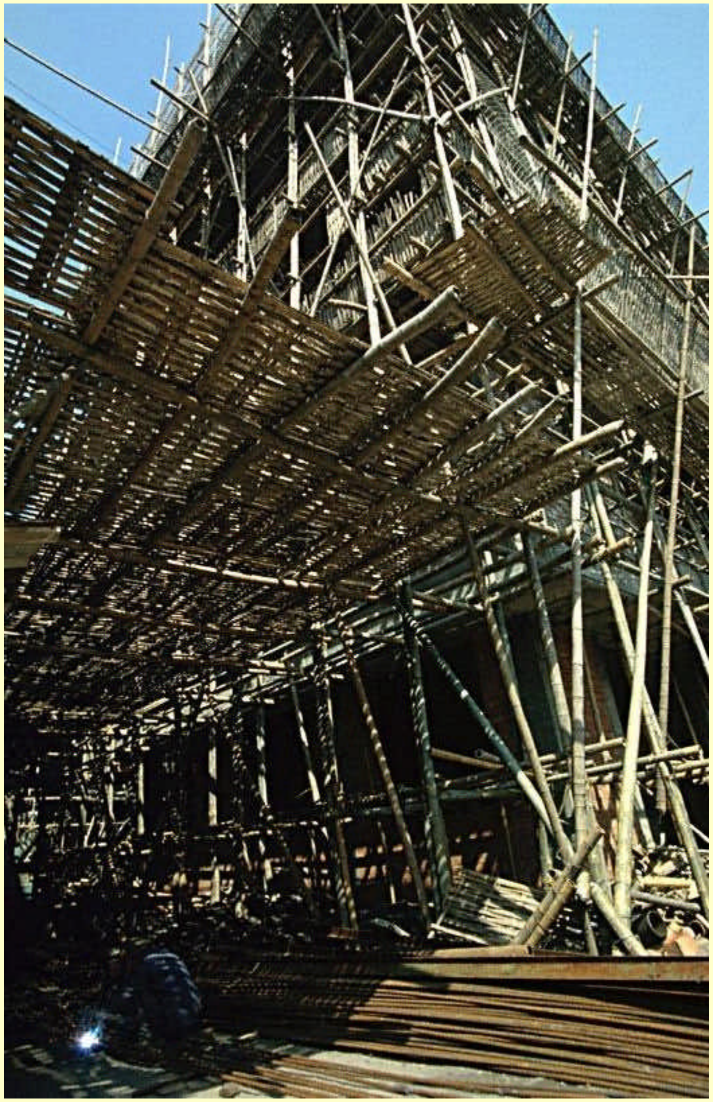
Vernon/Sage
- **Angiogenesis**

Mansbridge/Ratcliff (ATS, Inc.)
- **Stem cells / bioreactors & packaging**

Hauch
- **Microscopy, coordination**

The strategy to grow heart muscle…



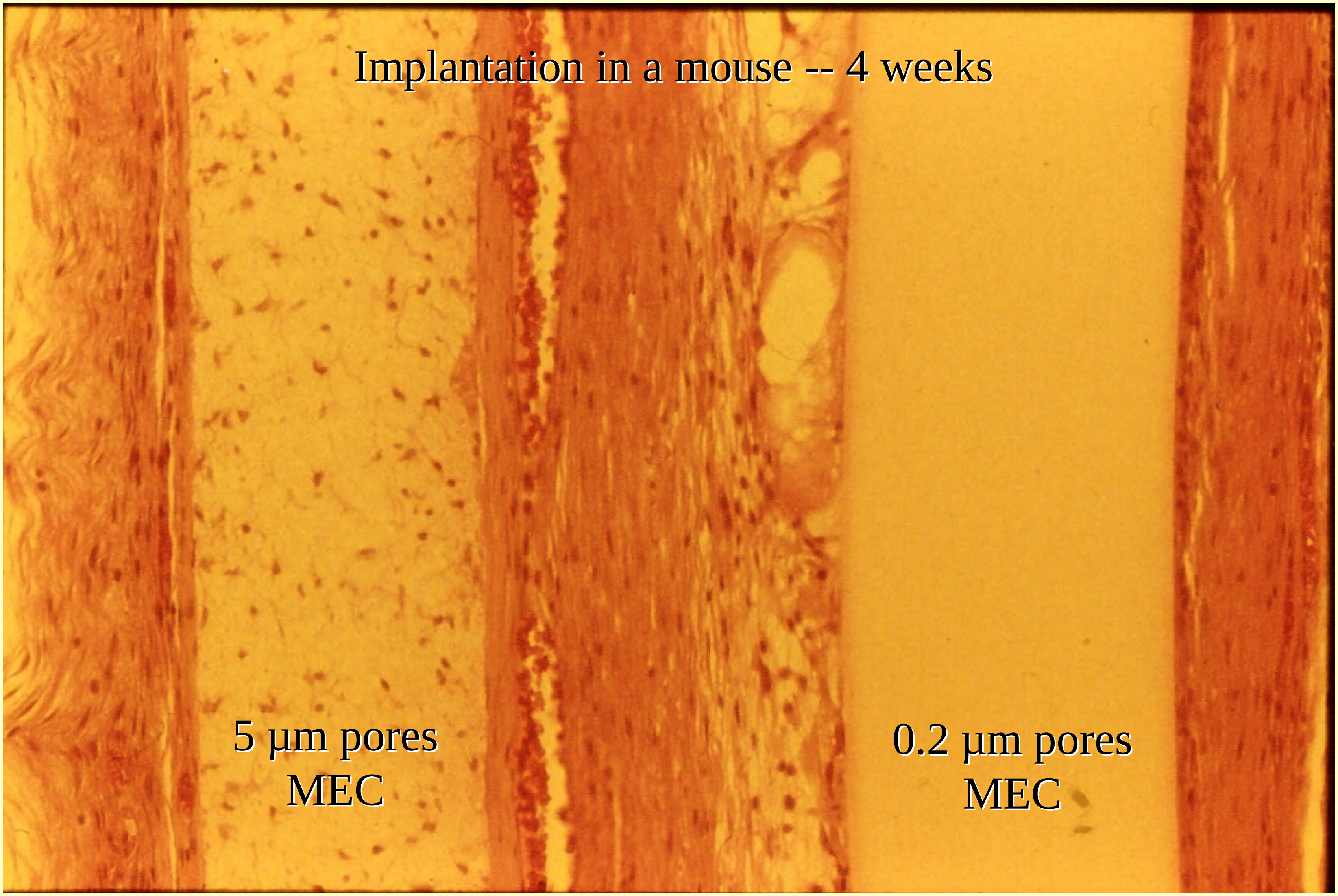


Let's start with scaffolds…

Pores, textures, roughnesses have a large effect on cells and living organisms!

Criteria and Considerations for Scaffolds for Heart Tissue Engineering

Biodegradable
Elastomeric
Control of pore size & distributions
A high void fraction (mostly air)
Oriented pores for muscle
Random networks for angiogenesis
Support cell attachment
how to get cells into the scaffold?
Biocompatible / low inflammation
Deliver drugs, cytokines, genes
Sectionable / stainable
Sterilizable
Manufacturable



For further information, see:

Brauker,JH; Carr-Brendel,VE; Martinson,LA; Crudele,J; Johnston,WD; Johnson,RC (1995): Neovascularization of synthetic membranes directed by membrane microarchitecture. J. Biomed. Mater. Res. 29, 1517-1524.

Approaches to Porous Matrices

Microsphere template gels

Controlled release from templated gels

Electrospun fibrous matrices and materials (Sanders)

Salt leachates

Chitosan matrices (Sandy Chian)

Aphron foams (Prabha Nair with Georgia Tech)

Parallel channels in materials

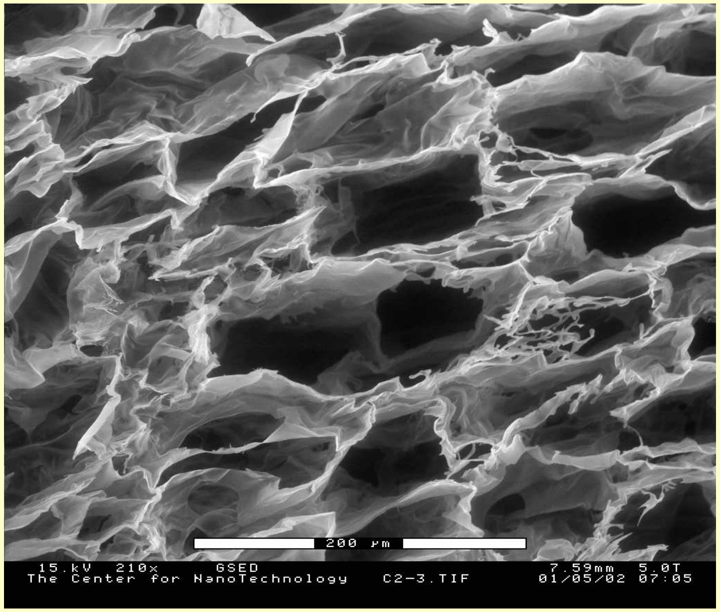
Laminin printed lines (McDevitt, Stayton)

Unique polymers

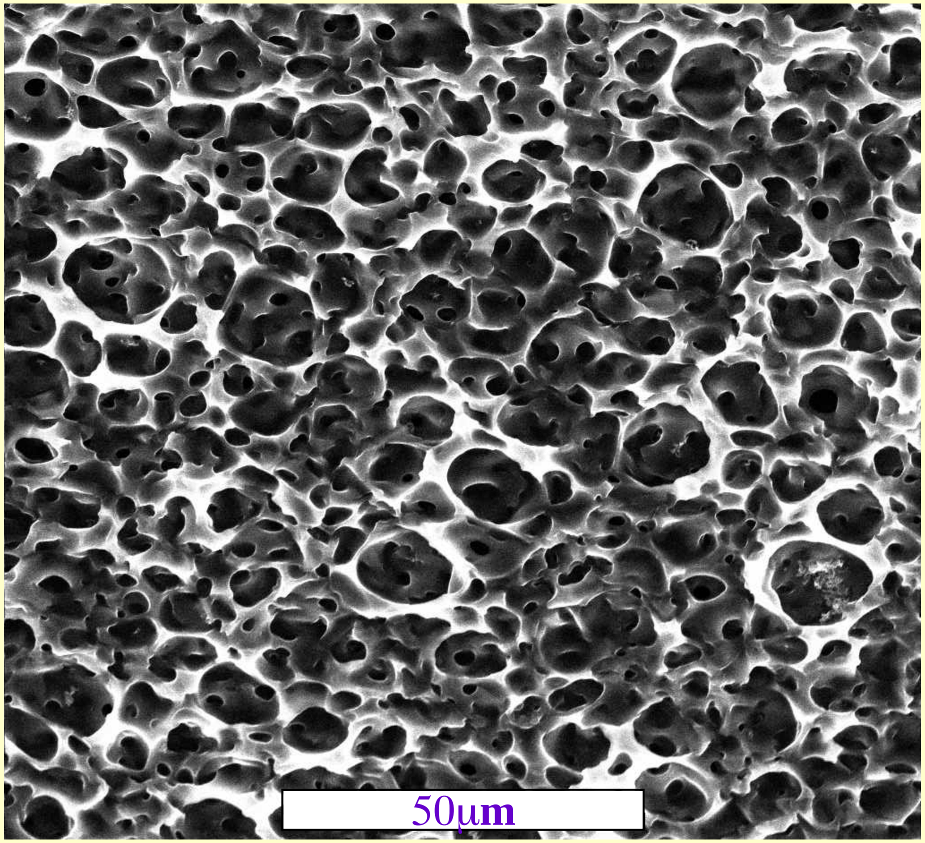
- Biodegradable polyurethanes (Kim Woodhouse)
- Elastins (Kim Woodhouse)
- Extracted tissue (Kim Woodhouse)
- Poly(ortho esters) (Jorge Heller)
- Biodegradable methacrylates (Andrew Marshall)

Two approaches:

Alginate-Amino Acid Aphron



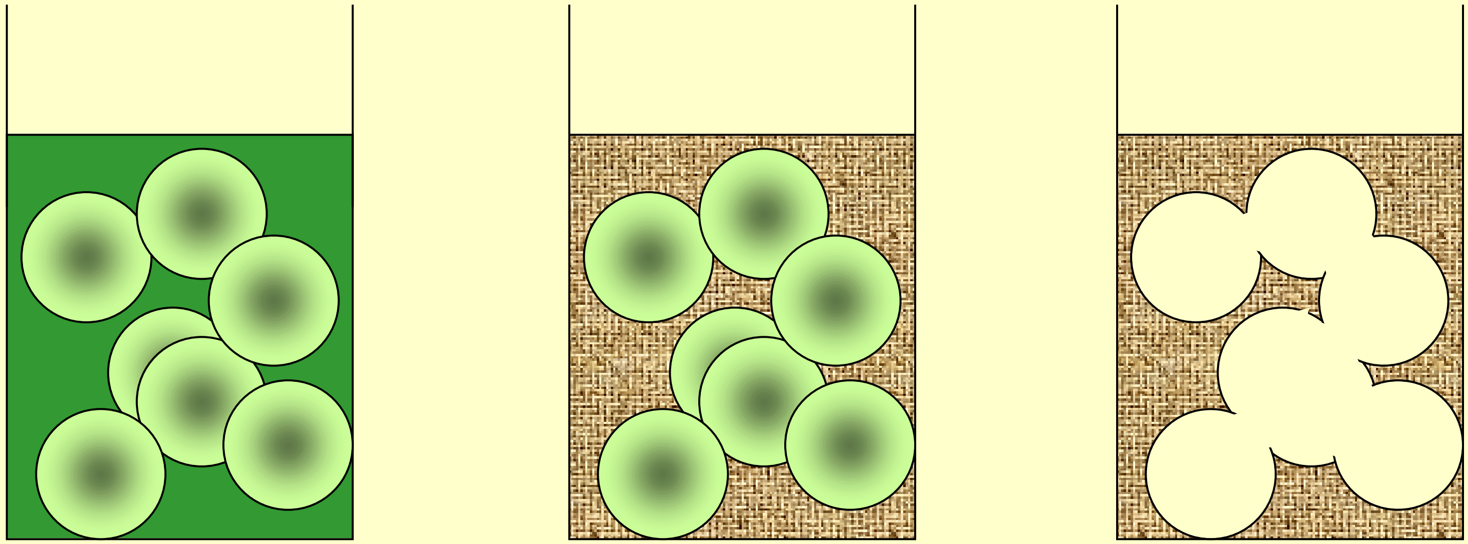
Microsphere templated hydrogel
5 µm pores



Andrew Marshall

Microsphere template gels

Preparation



Microspheres in monomer solution

Gel (crosslink) the monomer

Extract out the microspheres

Darcy's Law

Henry Philibert Gaspard Darcy, (1803-1858)

The rate of flow of liquids through porous media

$$Q = kS \frac{H + e}{e}$$

where

Q = volume of liquid/unit time,

S = porous bed area,

e = porous bed thickness,

H = height of the liquid on the bed

k = coefficient (nature of the bed, etc.)

http://biosystems.okstate.edu/darcy/

How do we measure interconnectivity?

- **We can use a correlation to determine the critical throat radius from measurable properties.***

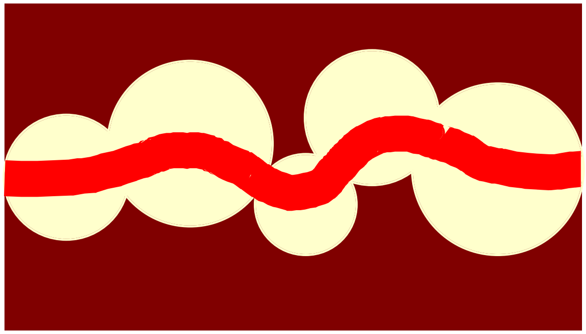
r_c = critical throat radius (~1.4 μm)

k = hydraulic permeability (~1.3 × 10^{-11} cm^2)

α = tortuosity (~1.2)

φ = porosity (%68)

$$r_c = \sqrt{\frac{226 k \alpha}{\phi}}$$



*Katz, A.J. and Thompson, A.H., *Phys. Rev. B*, **34**, 8179 (1986)

Quantitative Characterization of Porous Matrices

Porosity (68%)

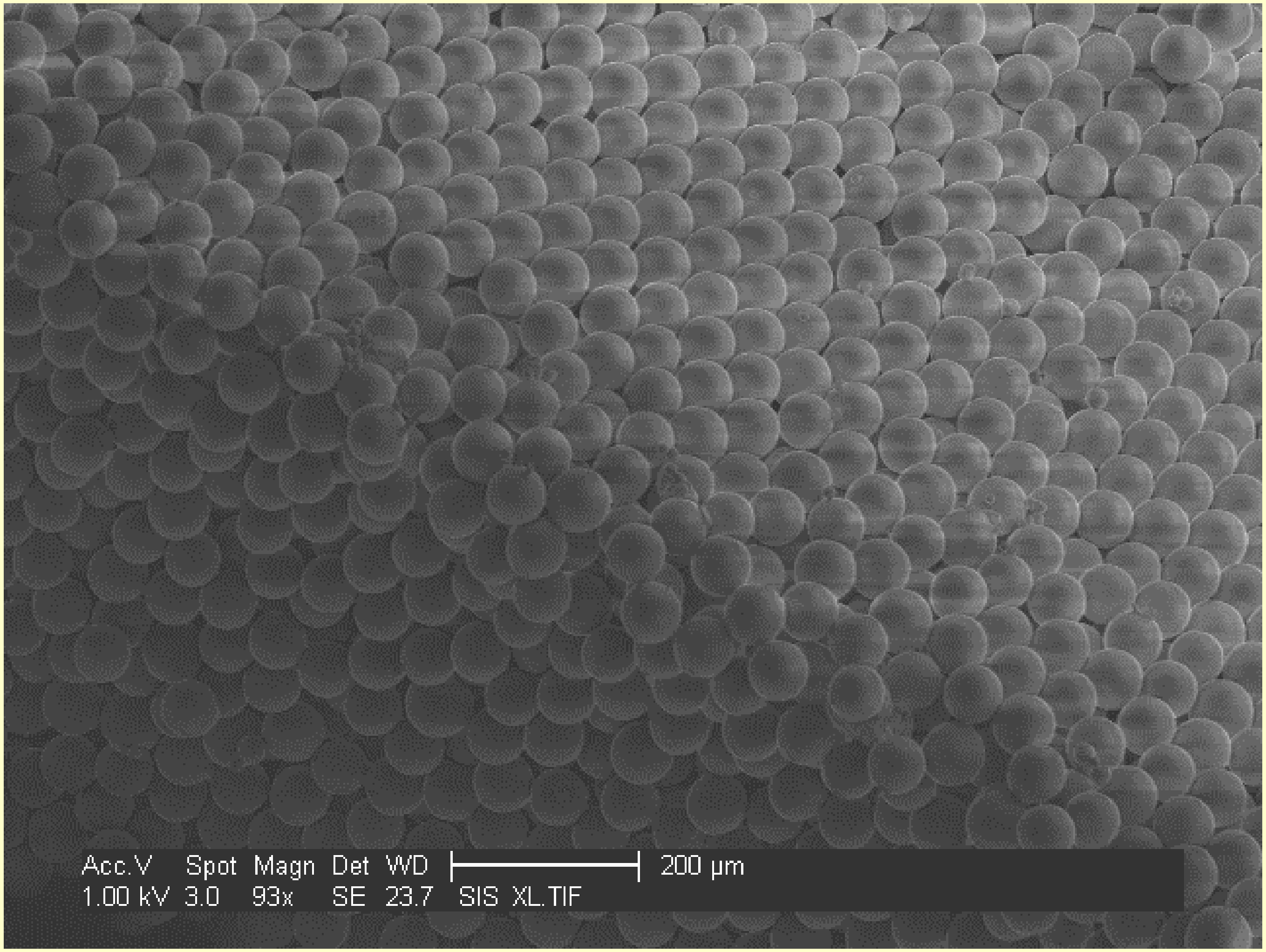
Pore size (~5 μm diam.)

Pore throat size (~1.8 μm diam.)

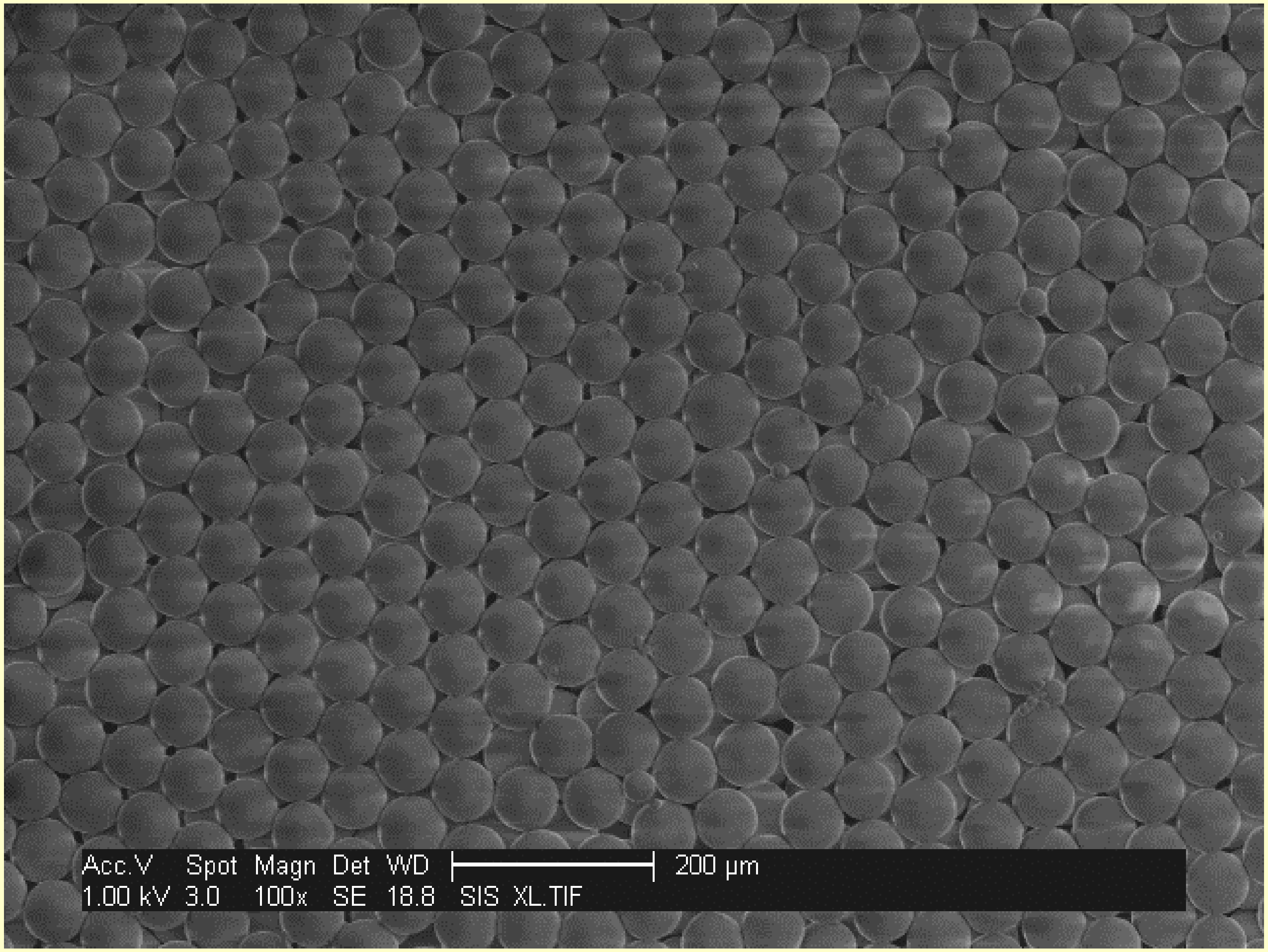
Throat/Pore ratio (~3.8)

Tortuosity (~1.2)

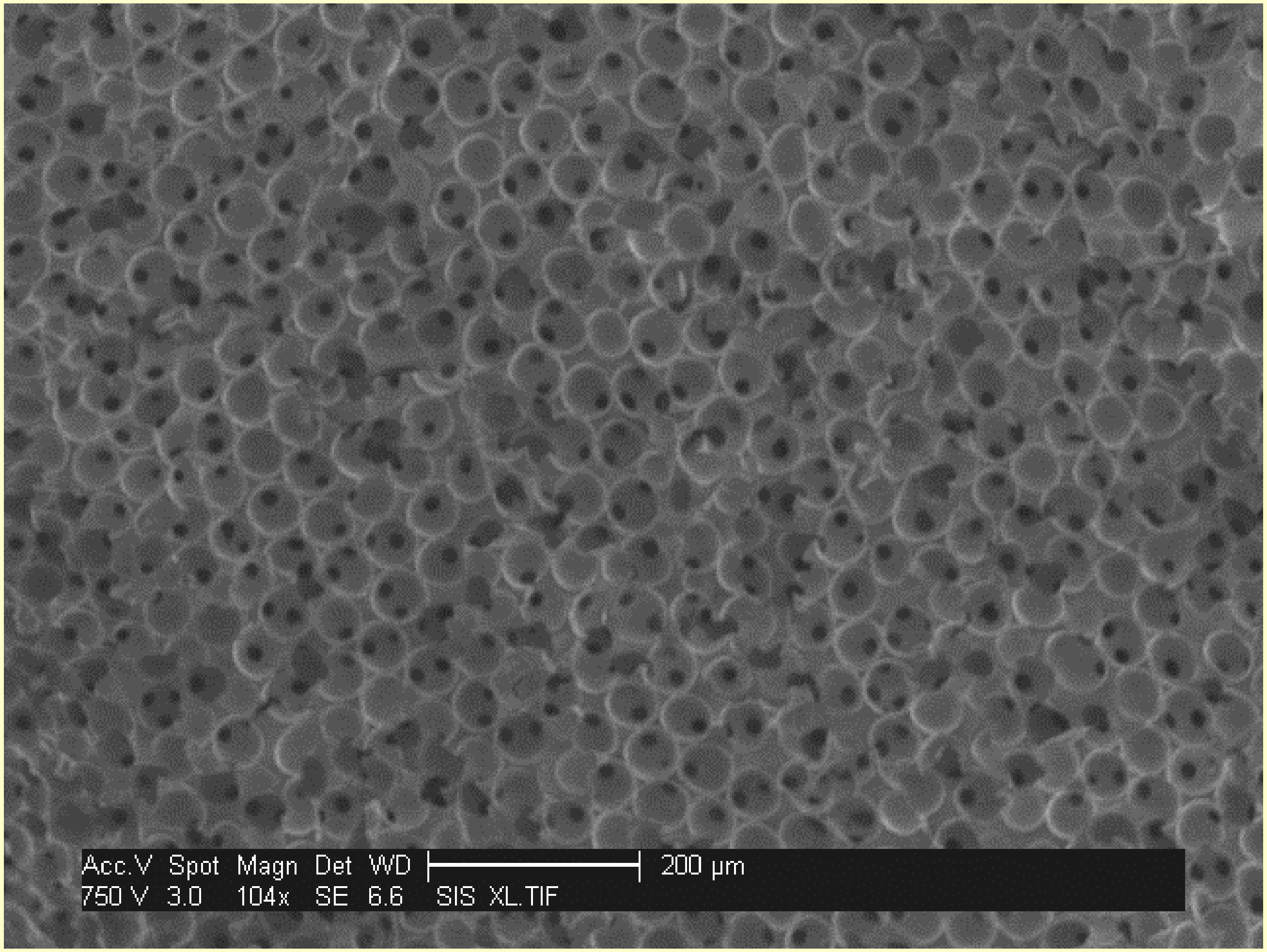
3-D crystalline array of fused 60µm PMMA beads



Crystalline surface of packed 60µm beads



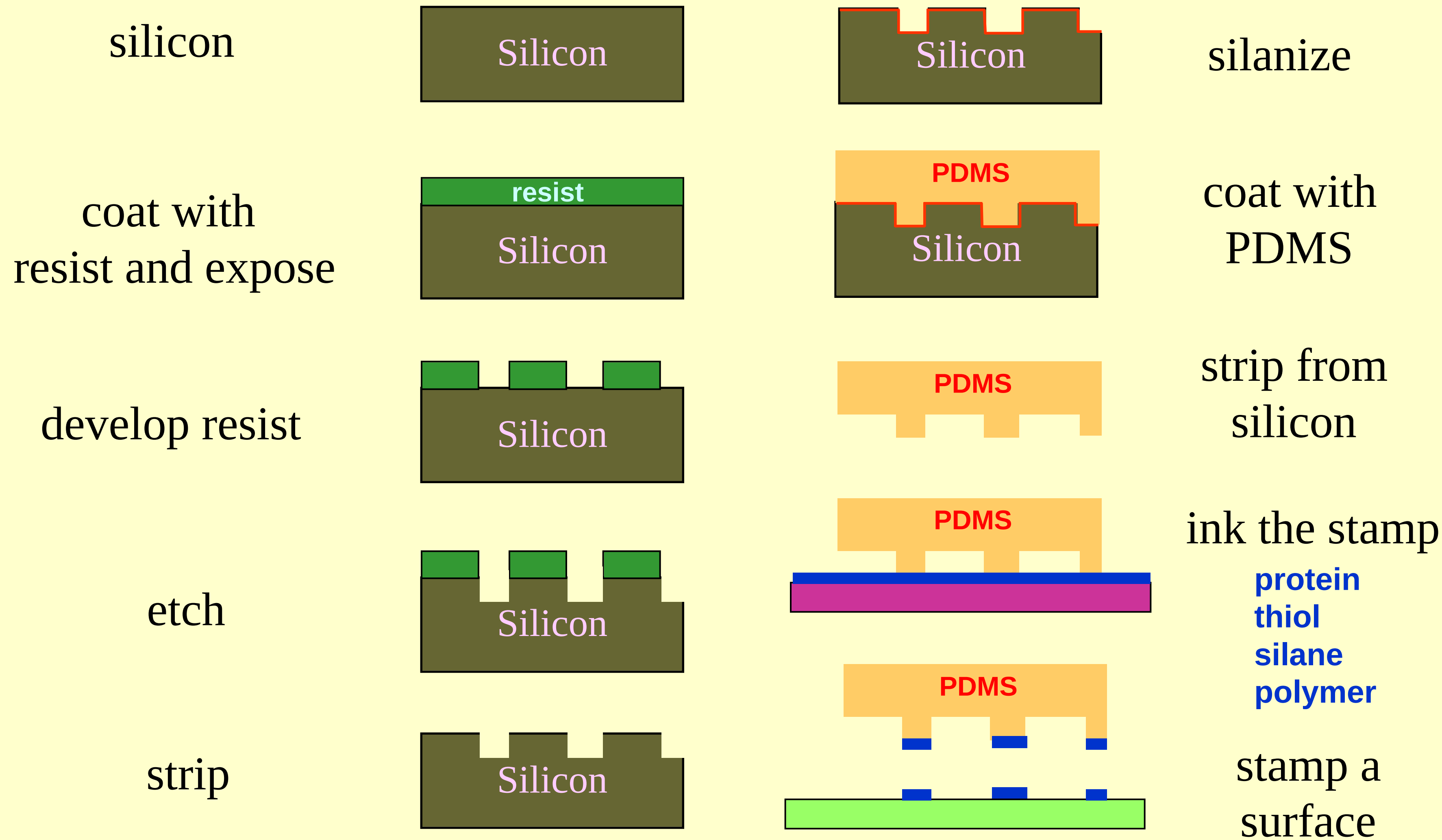
Porous pHEMA templated with crystalline array of 60µm beads



Engineering control of the texture and porosity

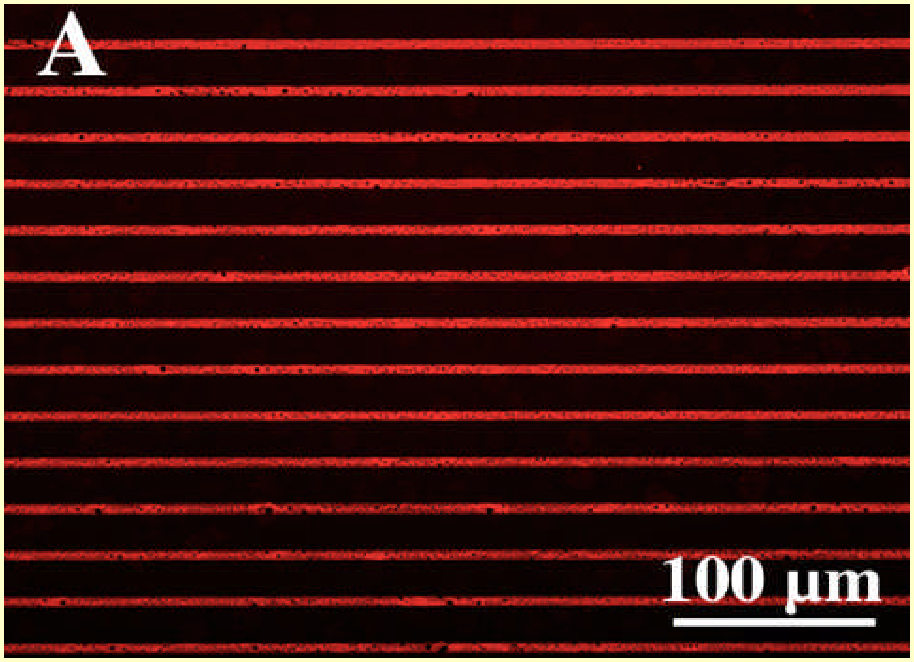
Another approach to achieve orientation/alignment:

Microcontact printing (µCP)

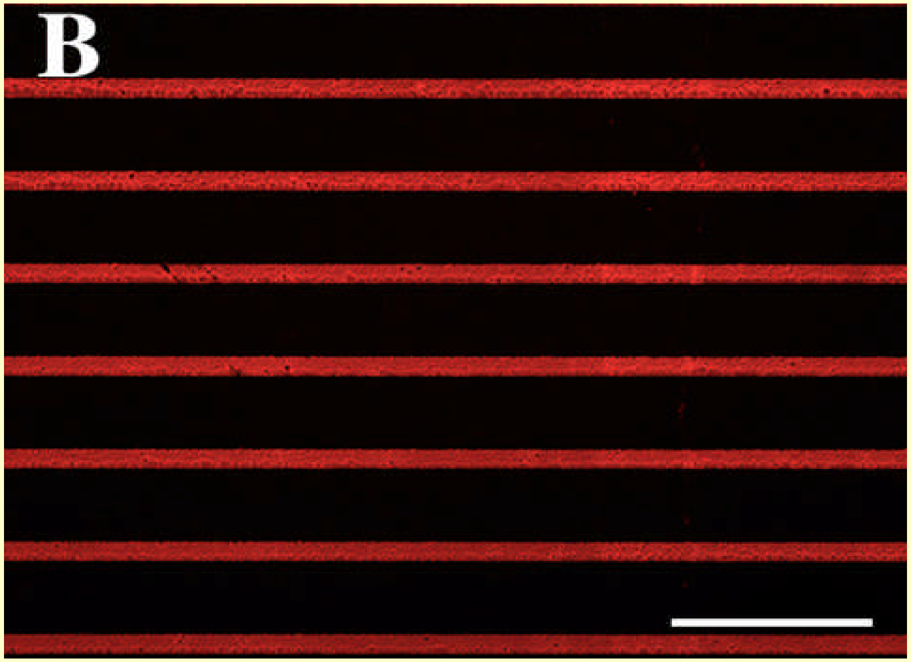


Laminin Patterning

5 x 20 μm



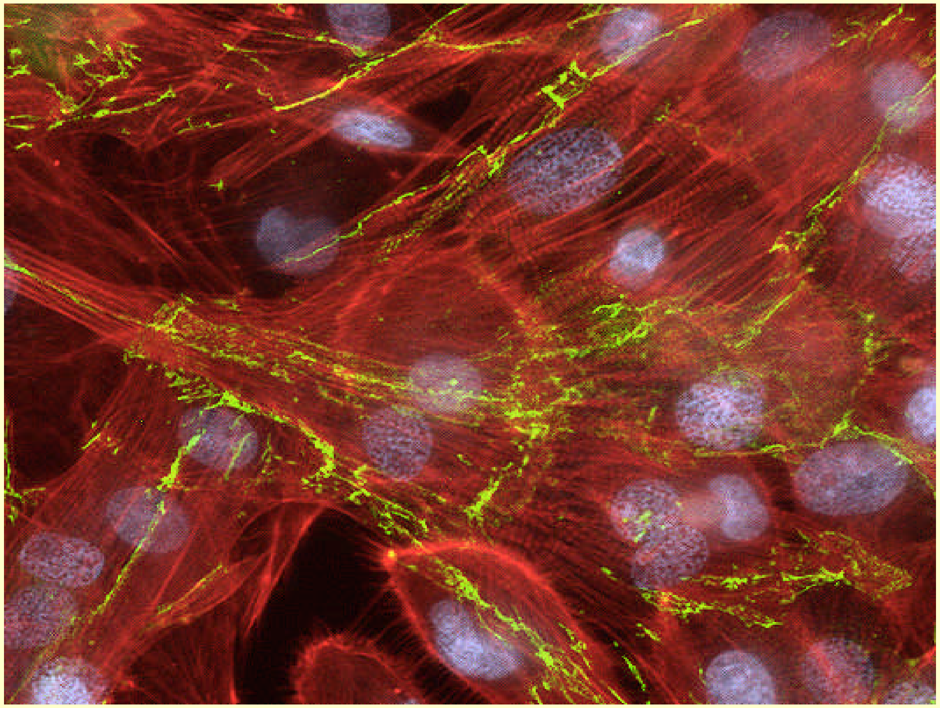
10 x 40 μm



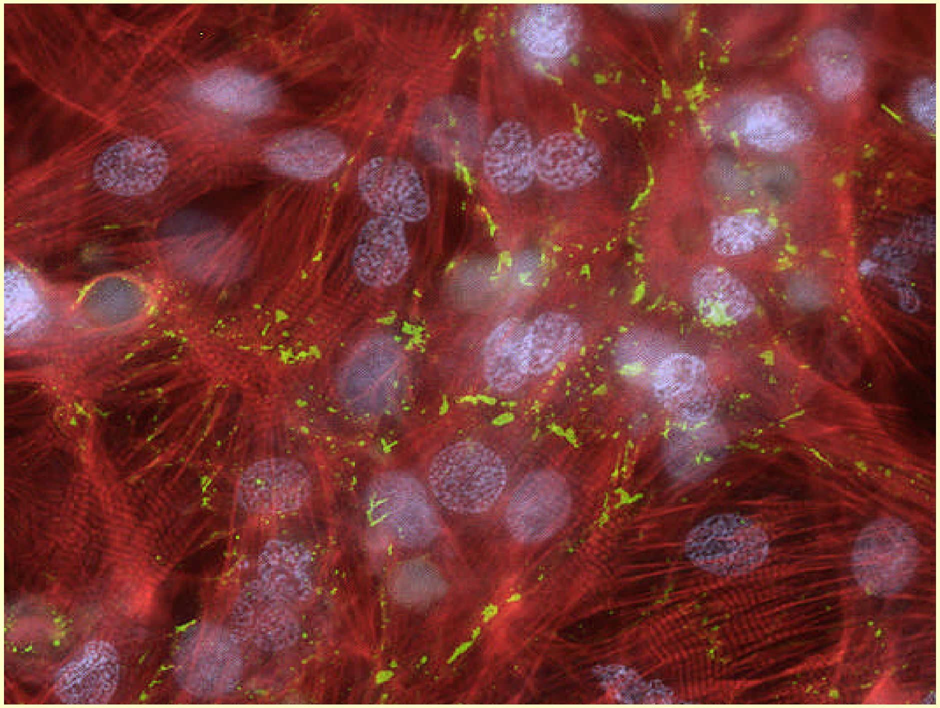
Todd McDevitt, P. Stayton

Junction Staining

N-cadherin



Connexin43



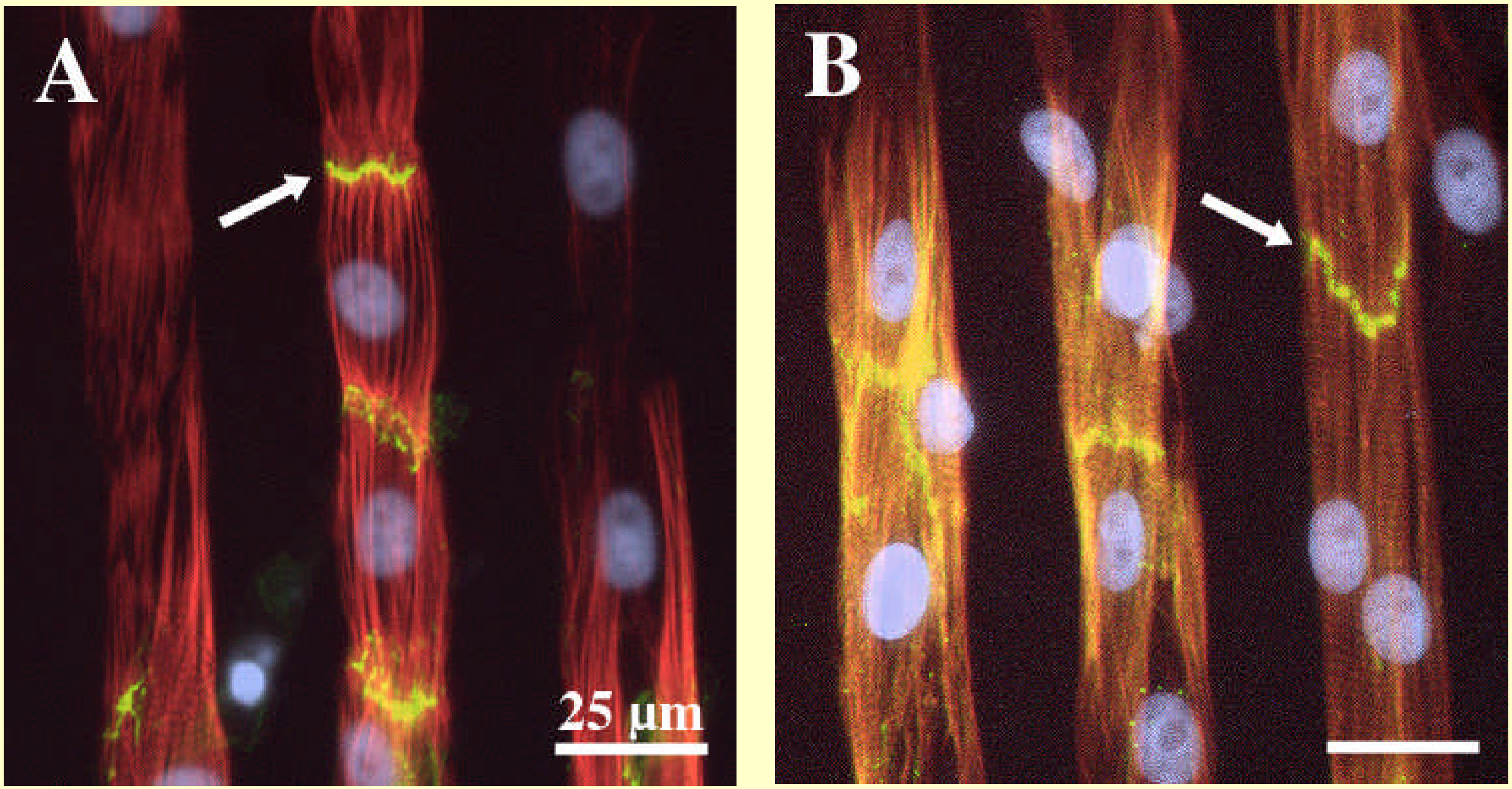
Neonatal rat cardiomyocytes, 7 days in culture

Nuclei - DAPI (blue) **Actin - Phalloidin (red)**

T. McDevitt, P. Stayton, C. Murry and S. Hauschka

Intercalated Disks

N-cadherin　　Connexin43



Neonatal rat cardiomyocytes, 4 days in culture

Nuclei - DAPI (blue)　　**Actin - Phalloidin (red)**

T. McDevitt, P. Stayton, C. Murry and S. Hauschka

Heart muscle is much more than cardiomyocytes!

Cardiomyocytes

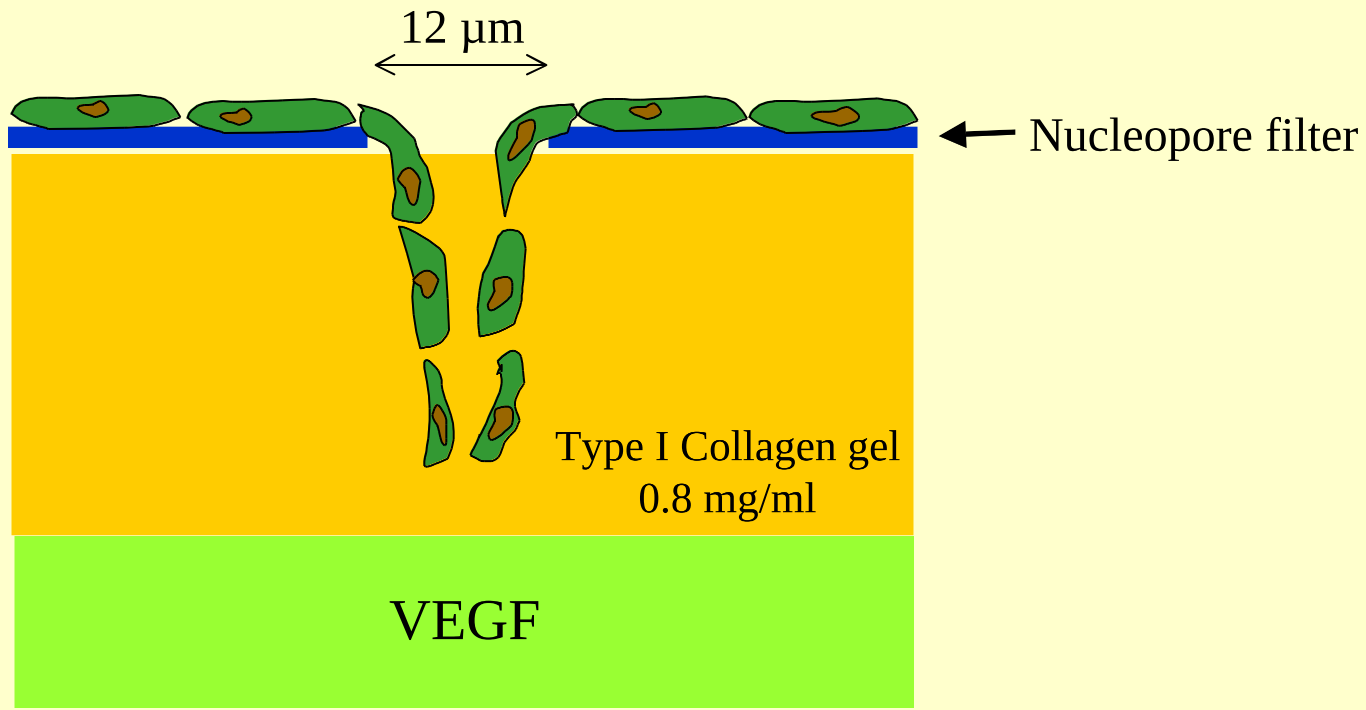
Endothelium

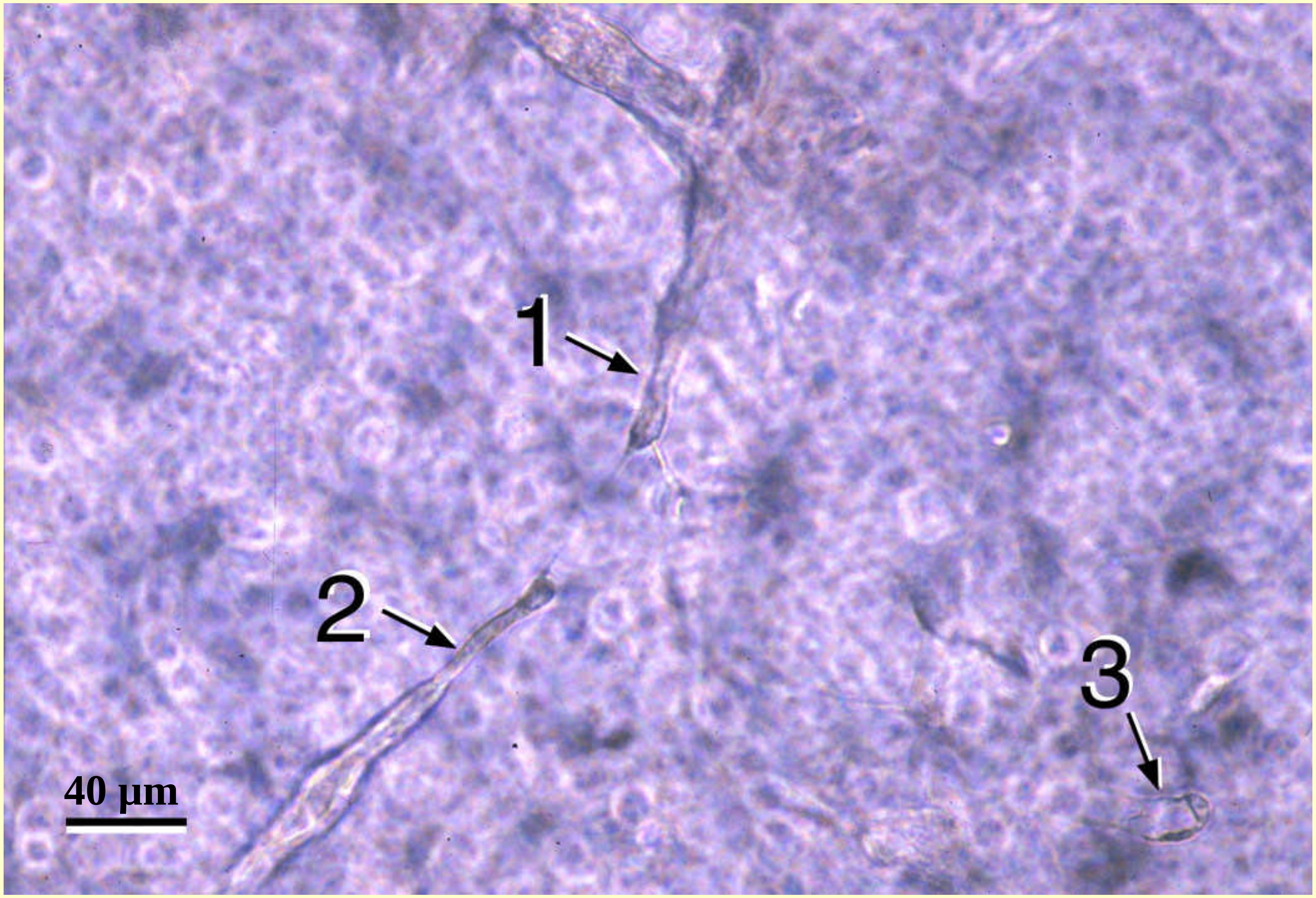
Nerve

ECM

Angiogenesis

(Dr. Robert Vernon, Dr. Helene Sage)



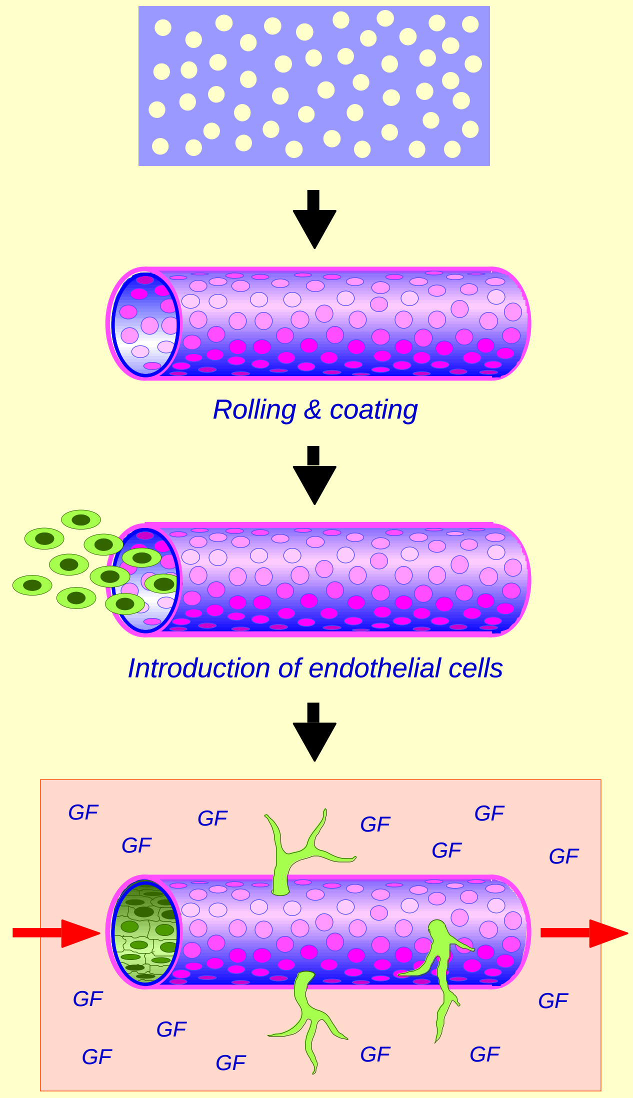


Endothelial sprouts through Nucleopore filter

1 and **2** appear to be growing toward each other -- there are filopodia extending from the tip of each sprout.
3 appears to be thin-walled and hollow.

Robert Vernon

Conversion of a Porous Membrane into a Testbed for Capillary Sprouting



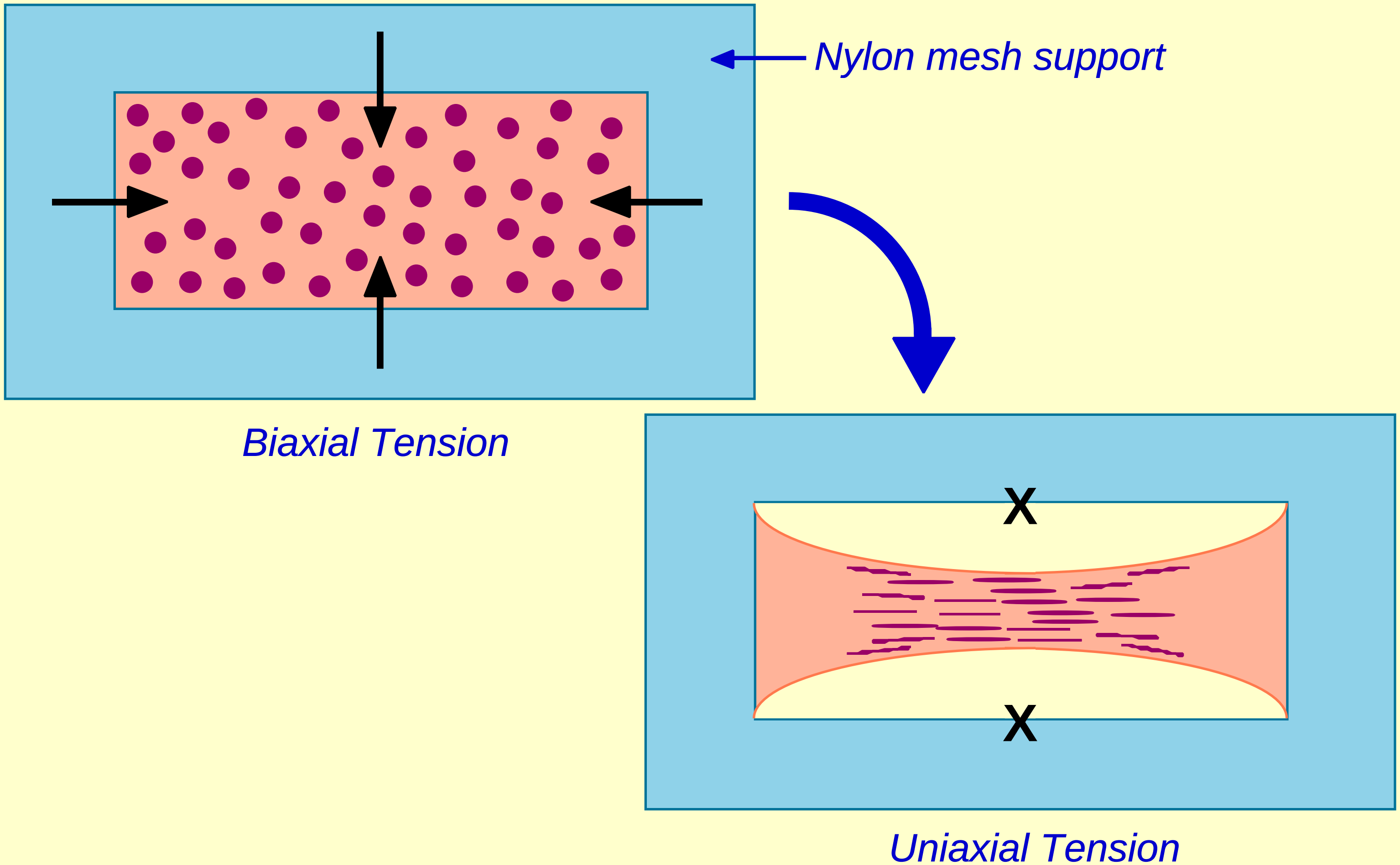
Rolling & coating

Introduction of endothelial cells

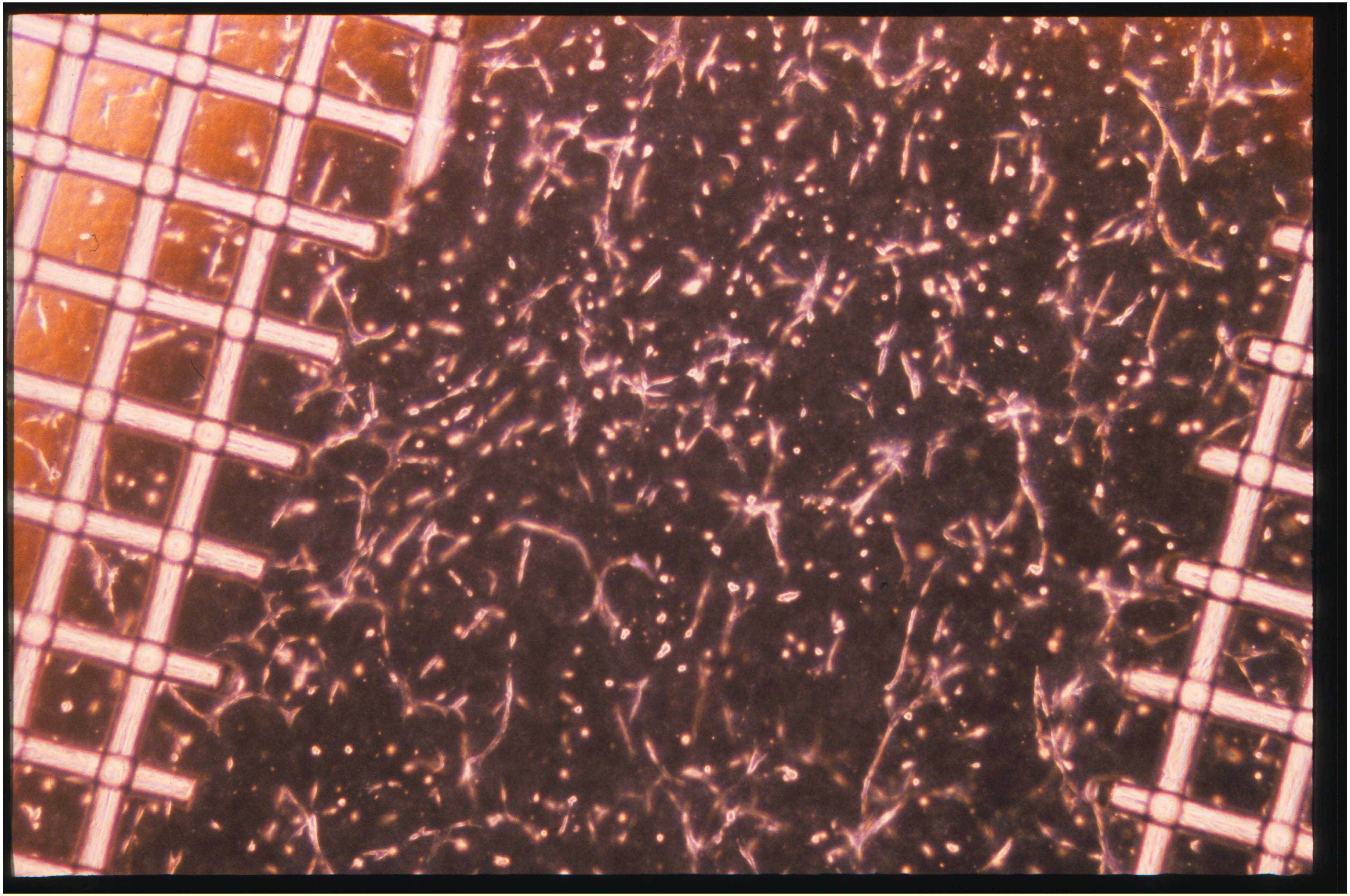
Embedment in collagen gel

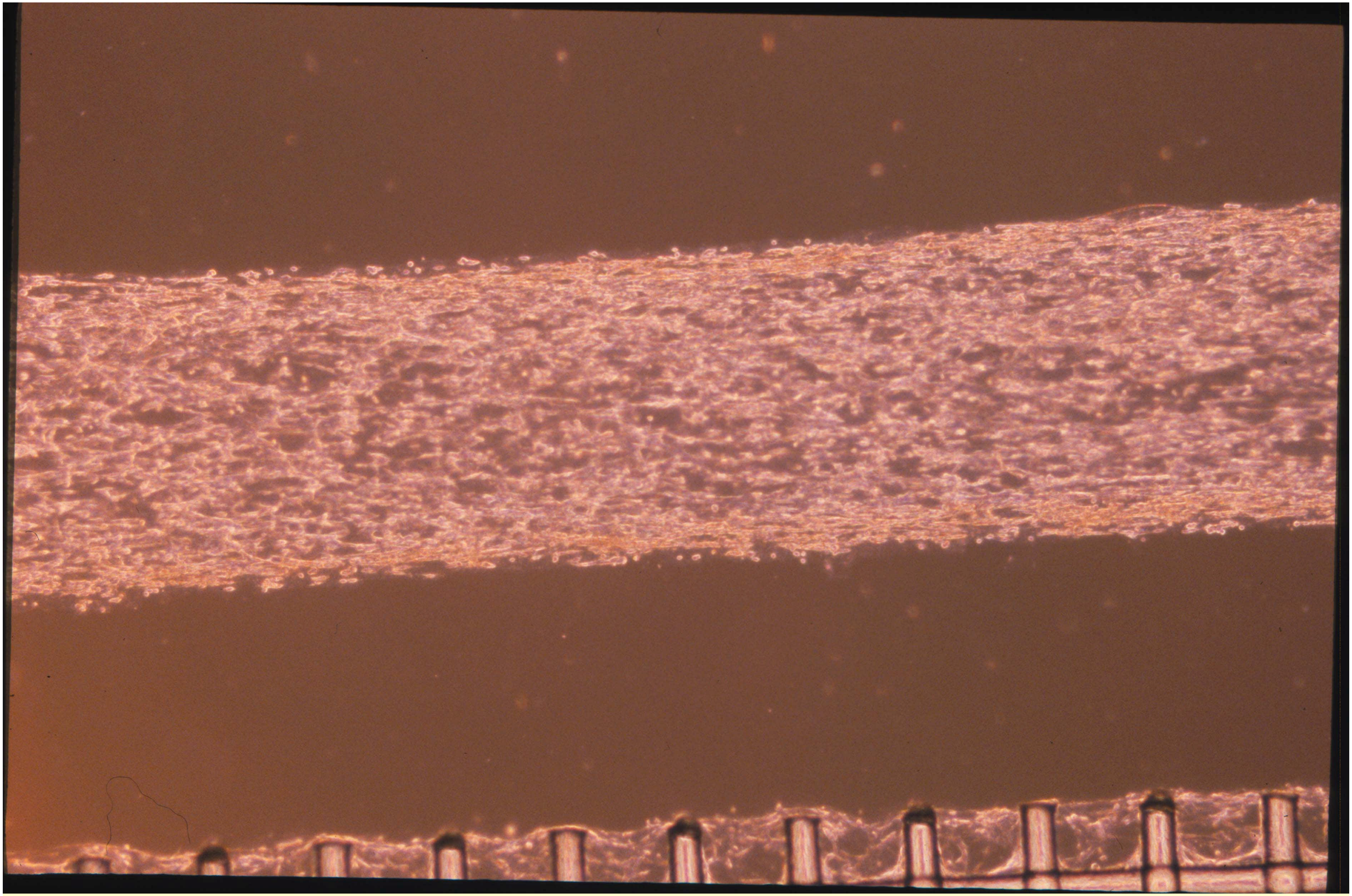
R. Vernon

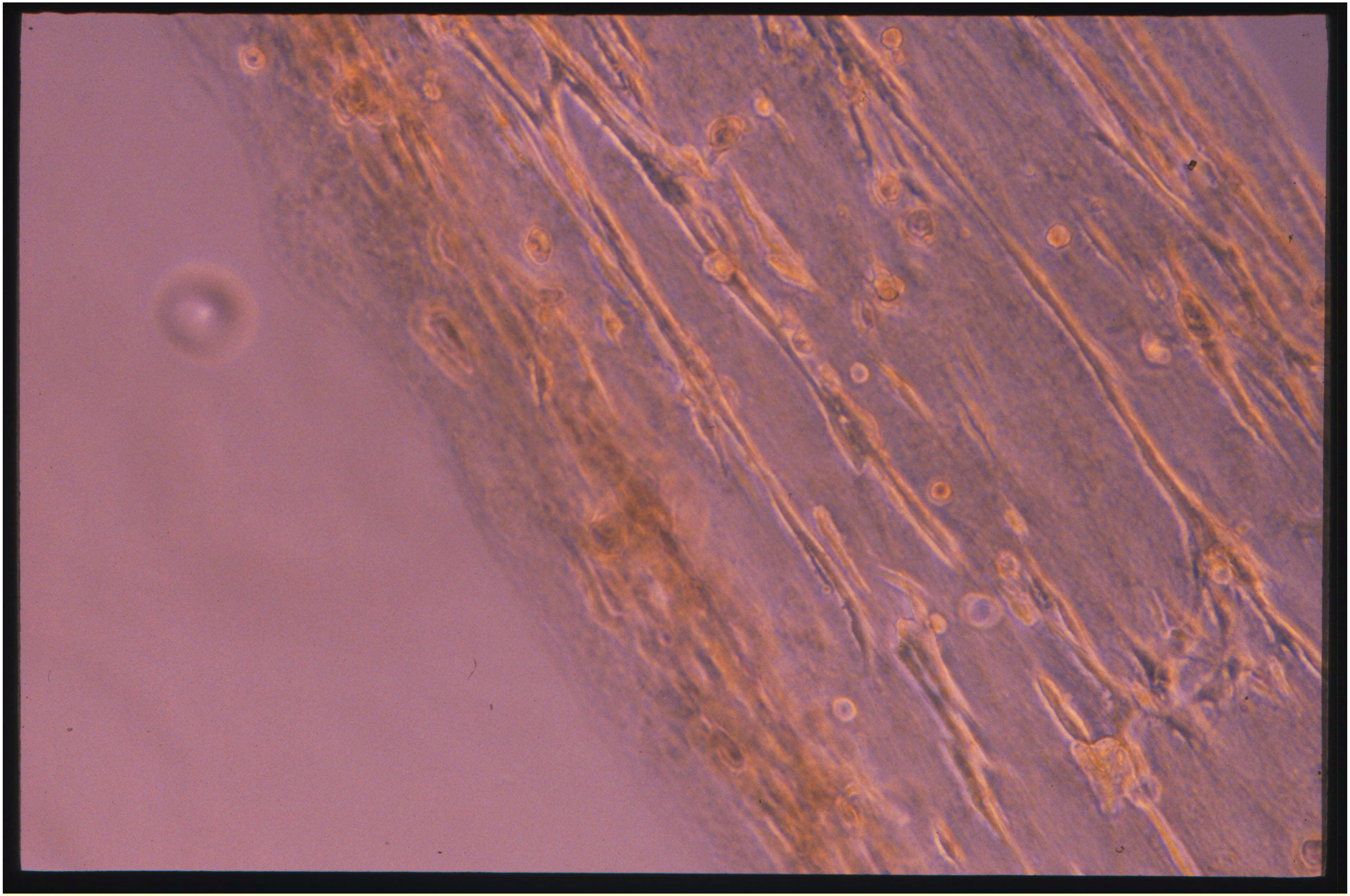
Traction-Mediated Uniaxial Organization of Cells & ECM in a Collagen Membrane



R. Vernon

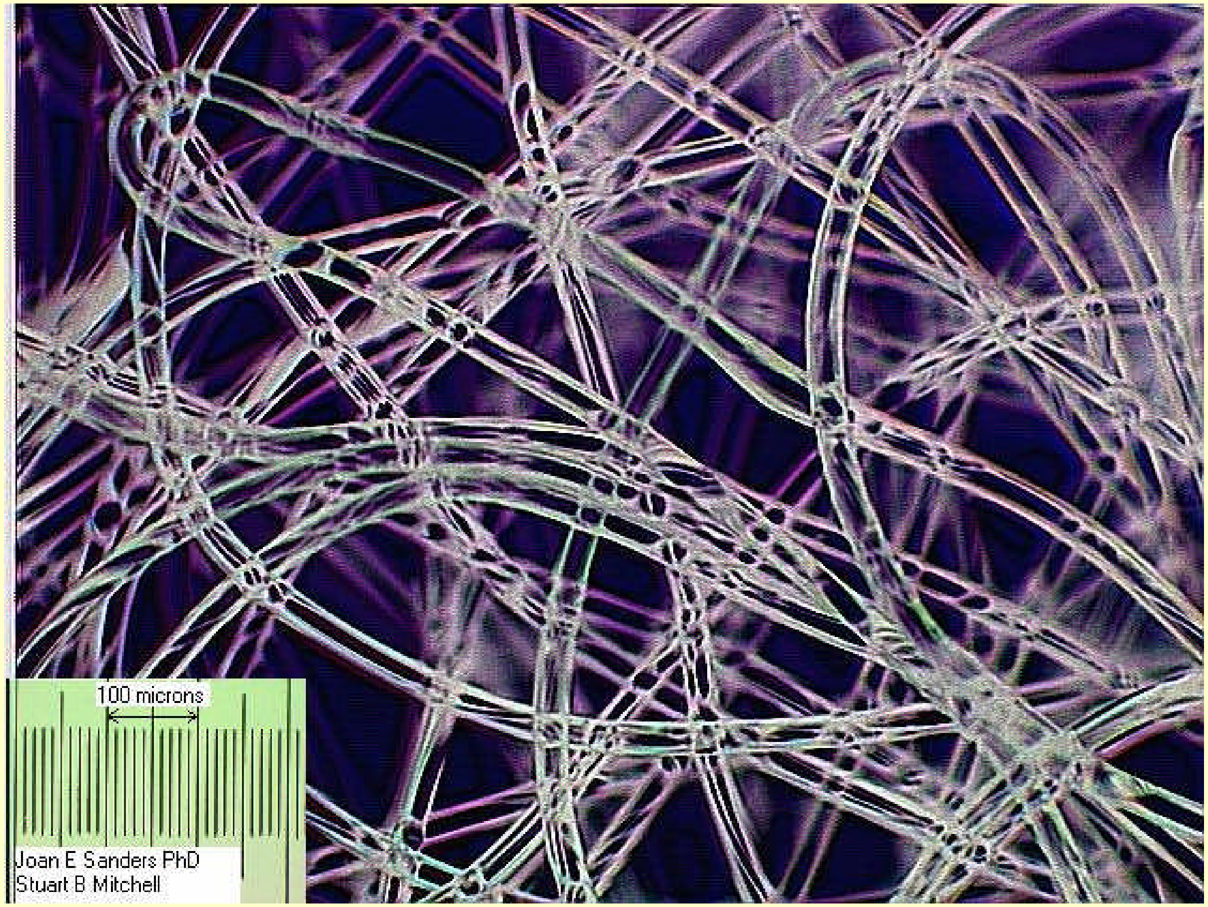






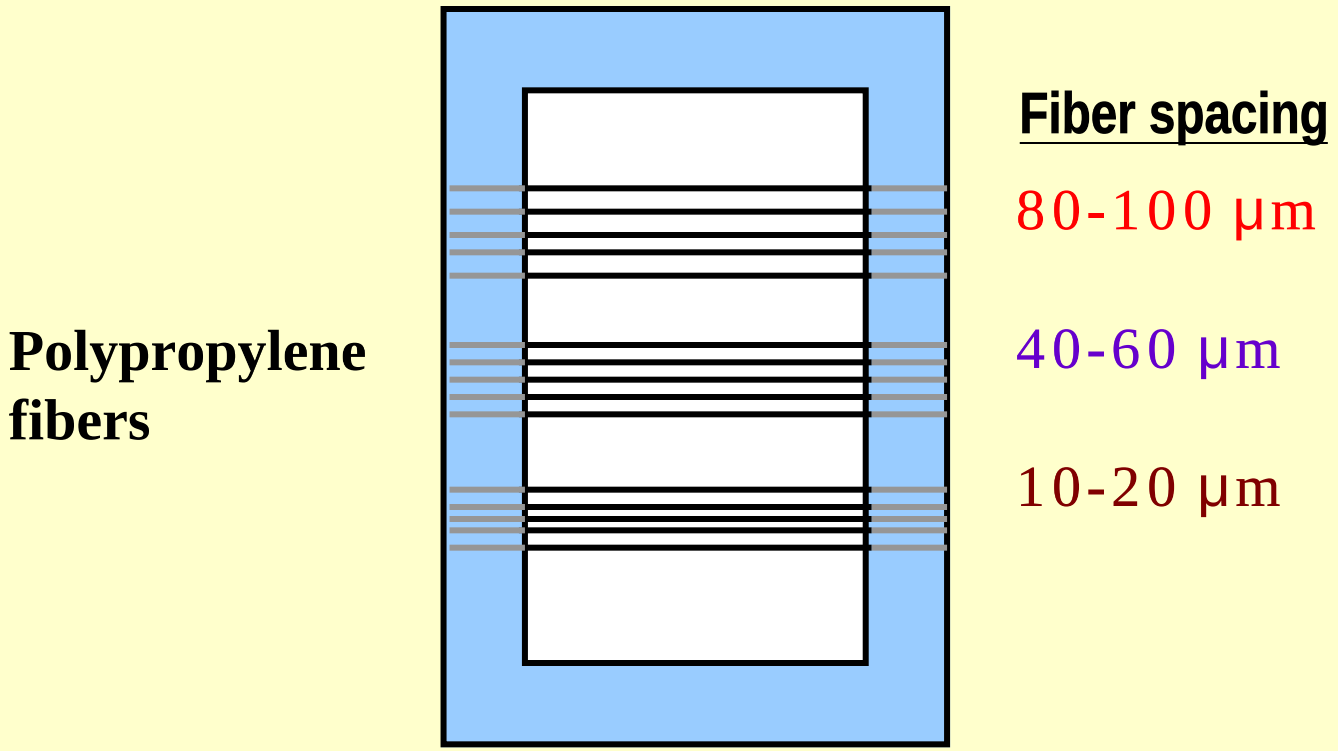
Electrospinning of fibro-porous materials

"skinny fibers"



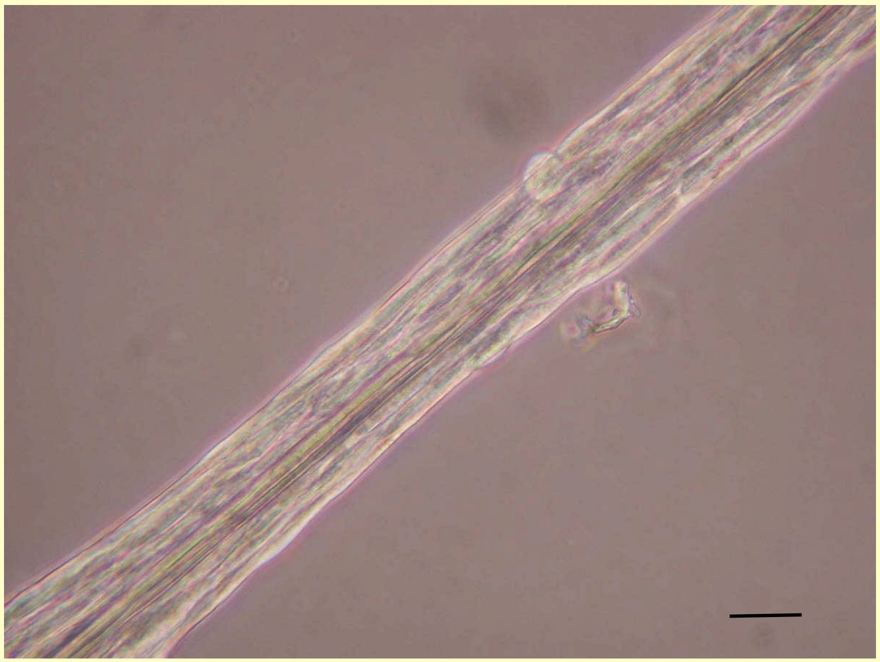
Joan Sanders, et al.

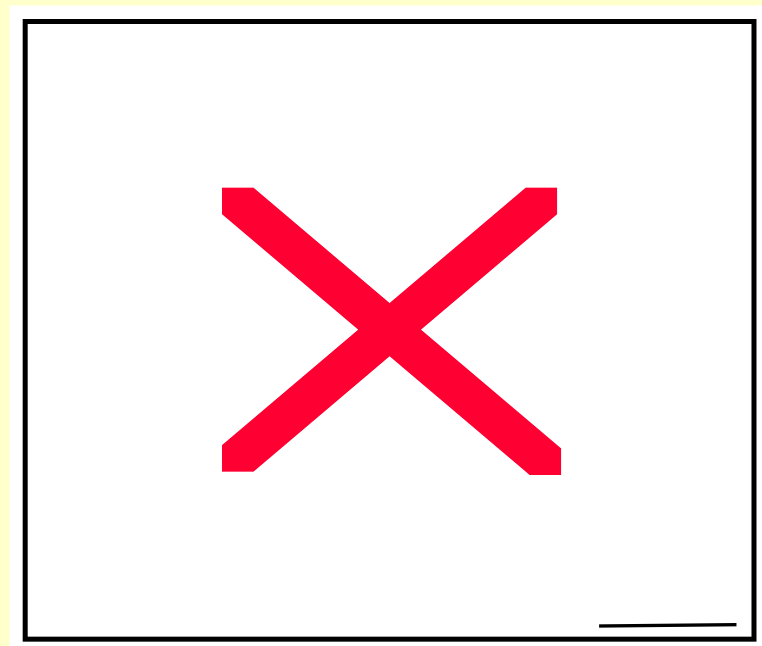
Studies with ultrafine fibers



Joan Sanders Lab

Aortic smooth muscle cells seeded on fibronectin-coated polypropylene fibers





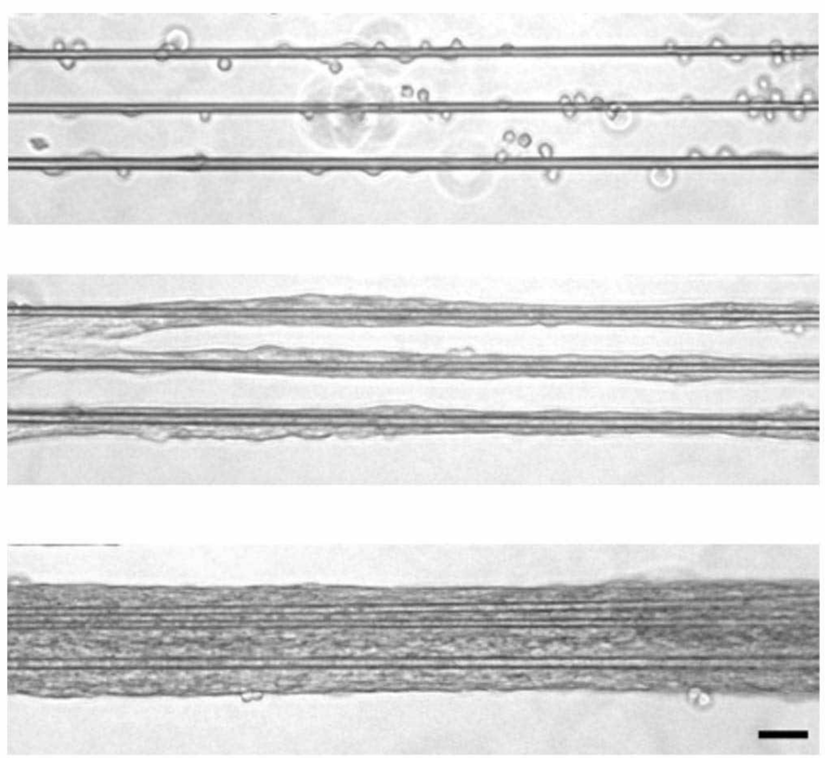
Bar = 20 µm

Joan Sanders Lab

Micro-fiber arrays

SMCs are seeded onto single, parallel aligned micro-fibers

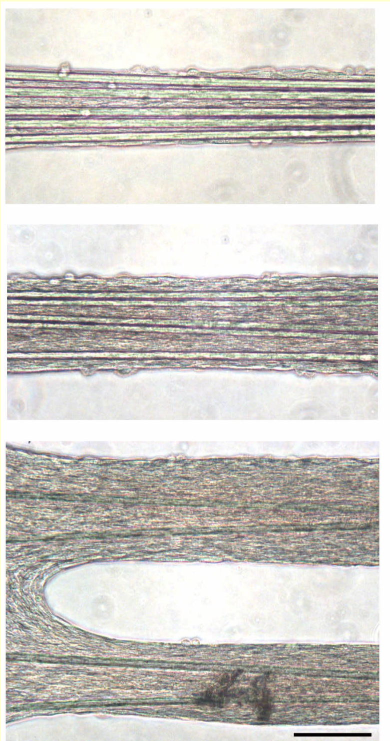
- **30 min: cells attach to the fibers**
- **2 days: cell layers form**
- **7 days: layers attach to form bridges between fibers**



bar = 50 μm

JE Sanders, et al

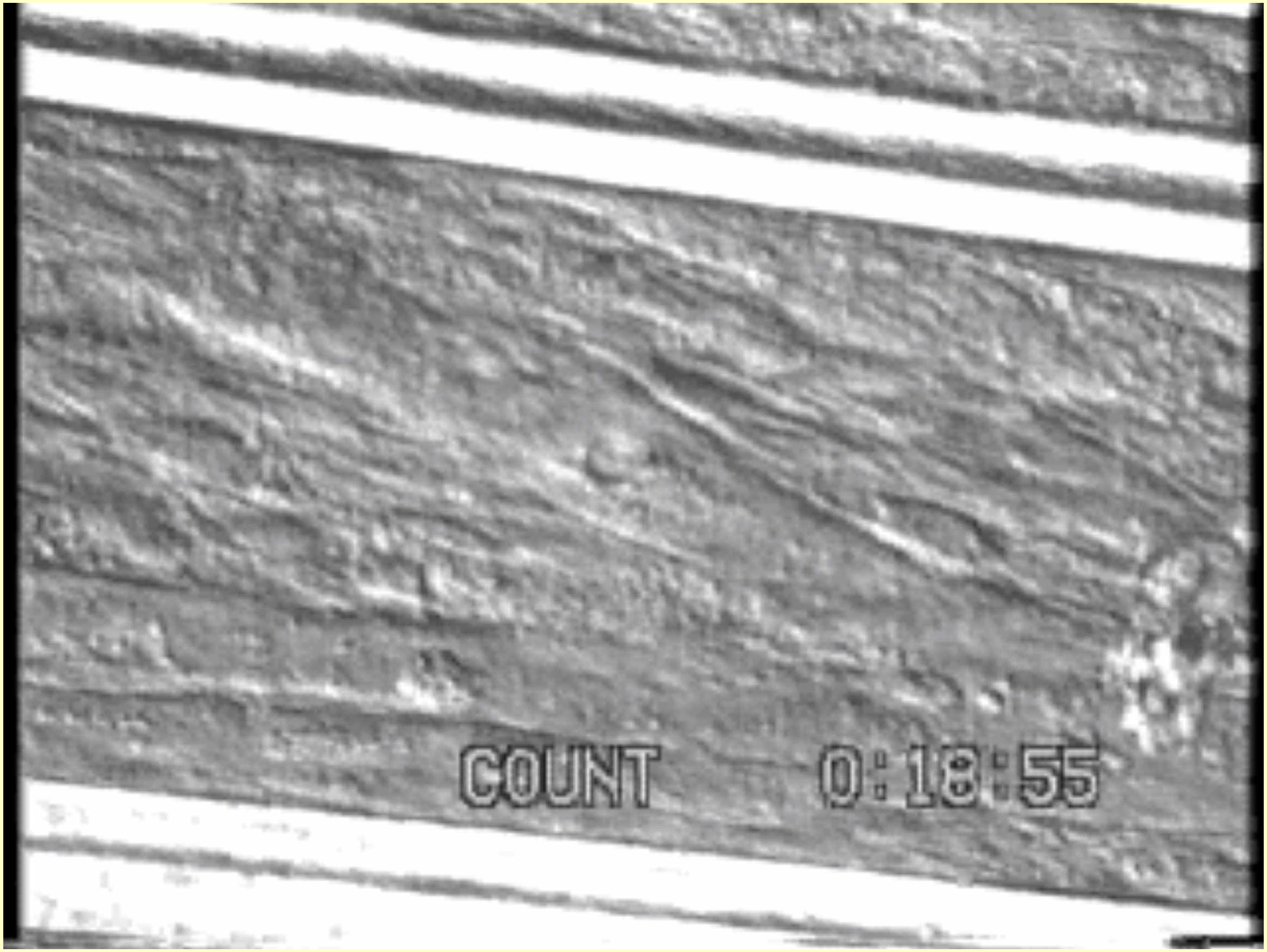
Dependence on micro-fiber spacing



Continuous cell sheets form at 10-20 µm (top) and 40-60 µm (middle) spacings; large holes form at 80-100 µm (bottom)

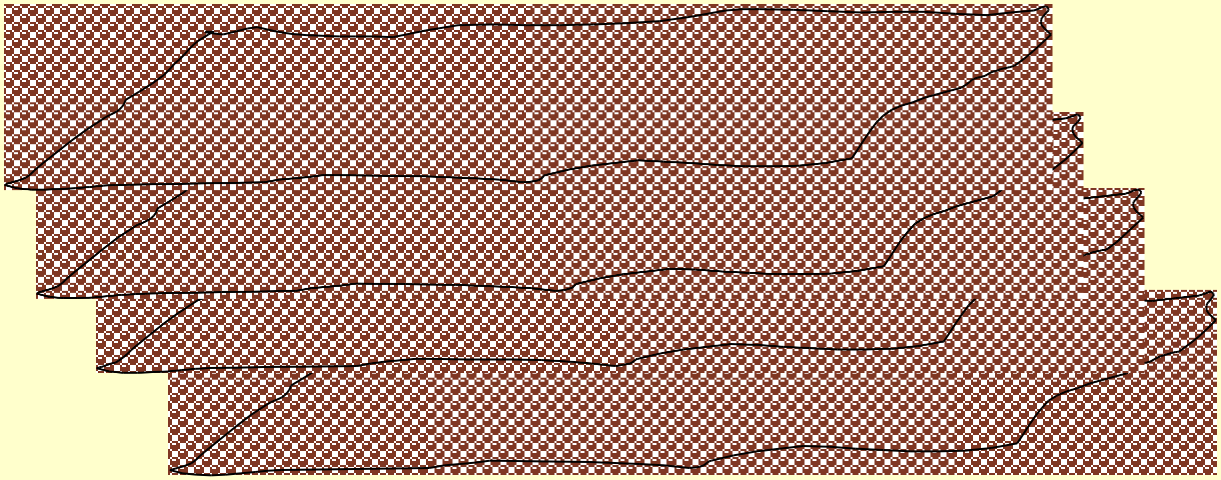
Fibers remain evenly separated with continuous cell sheets only at the 40-60 µm spacing

JE Sanders, et al


COUNT 0:18:55

Another idea:

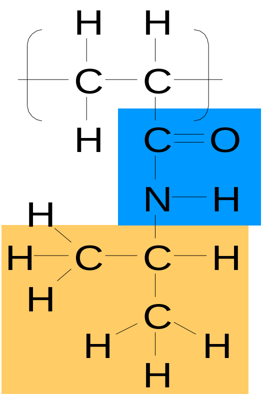
Build up tissues from cell layers



pNIPAM -- a Thermally Responsive Polymer

Room temperature:
A soft, swollen hydrogel

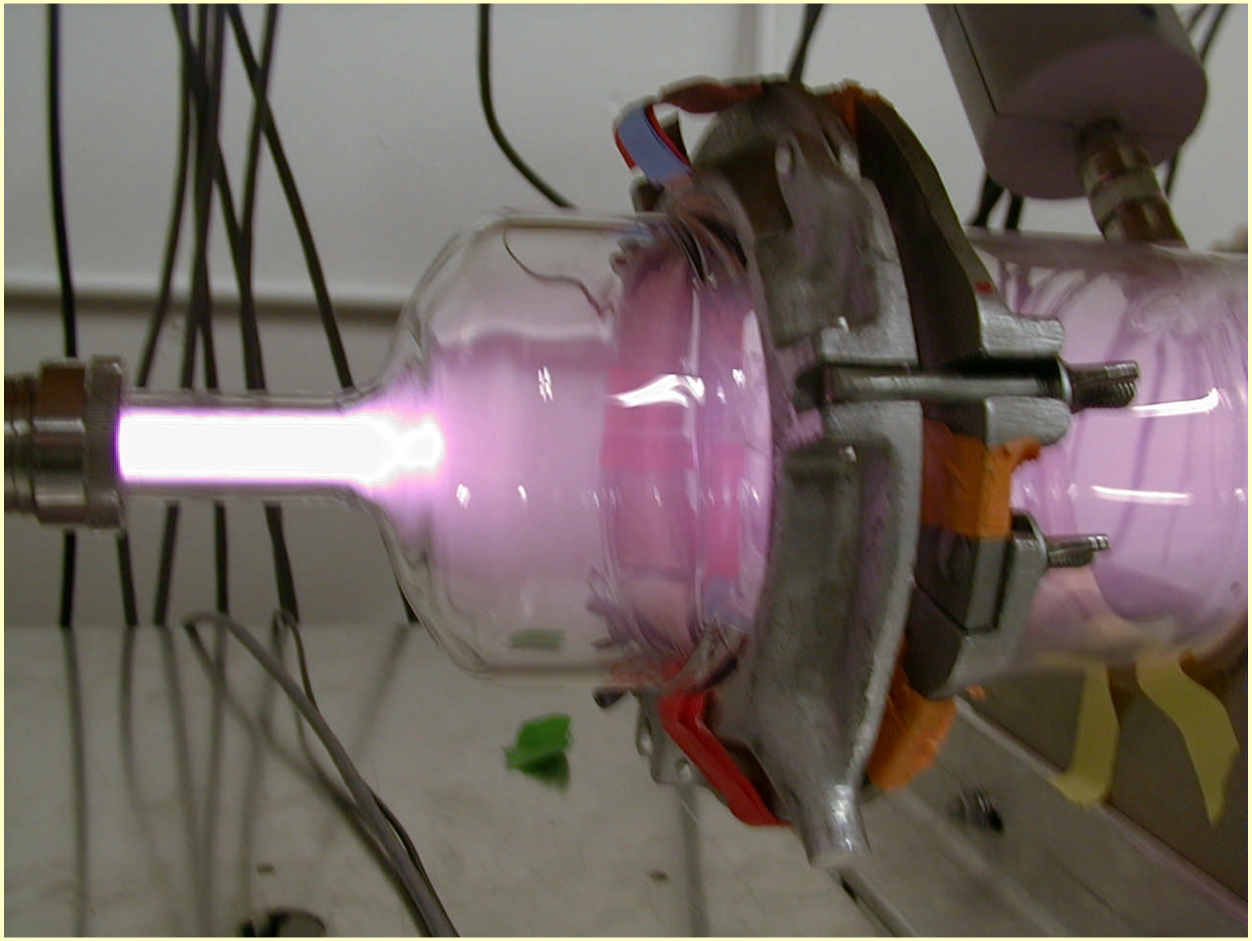
Body temperature:
A hard plastic



Poly(N-isopropyl acrylamide)

- M. Heskins & J. E. Guillet (1968)
- Allan Hoffman (1990+)

We coat surfaces with the NIPAM polymer in a glow discharge (ppNIPAM)

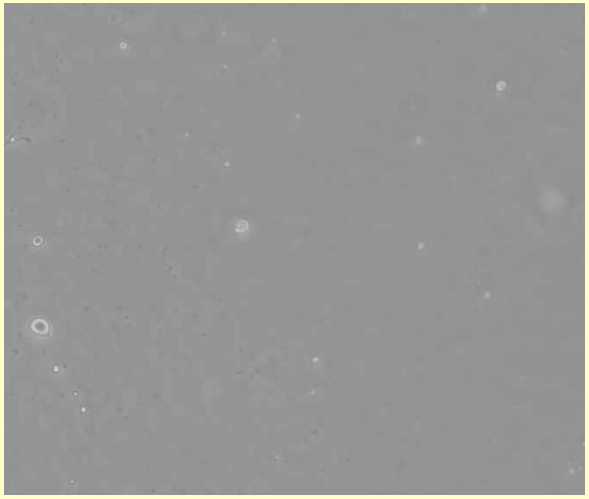


Smooth muscle cells on ppNIPAM for 3 Hours

DMEM/1.5% FBS

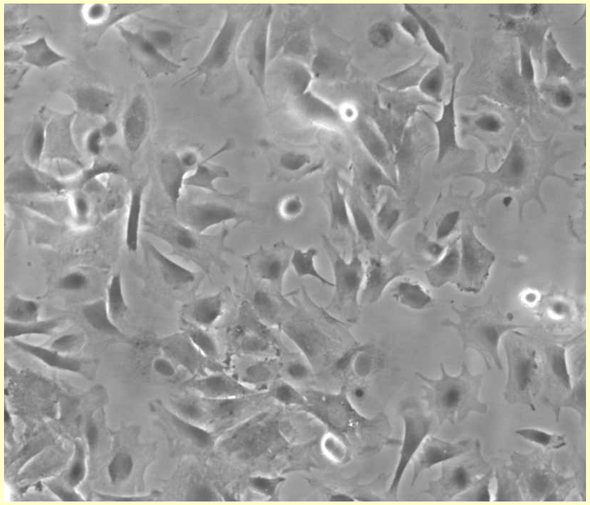
Room temperature

23ºC



Body temperature

37ºC



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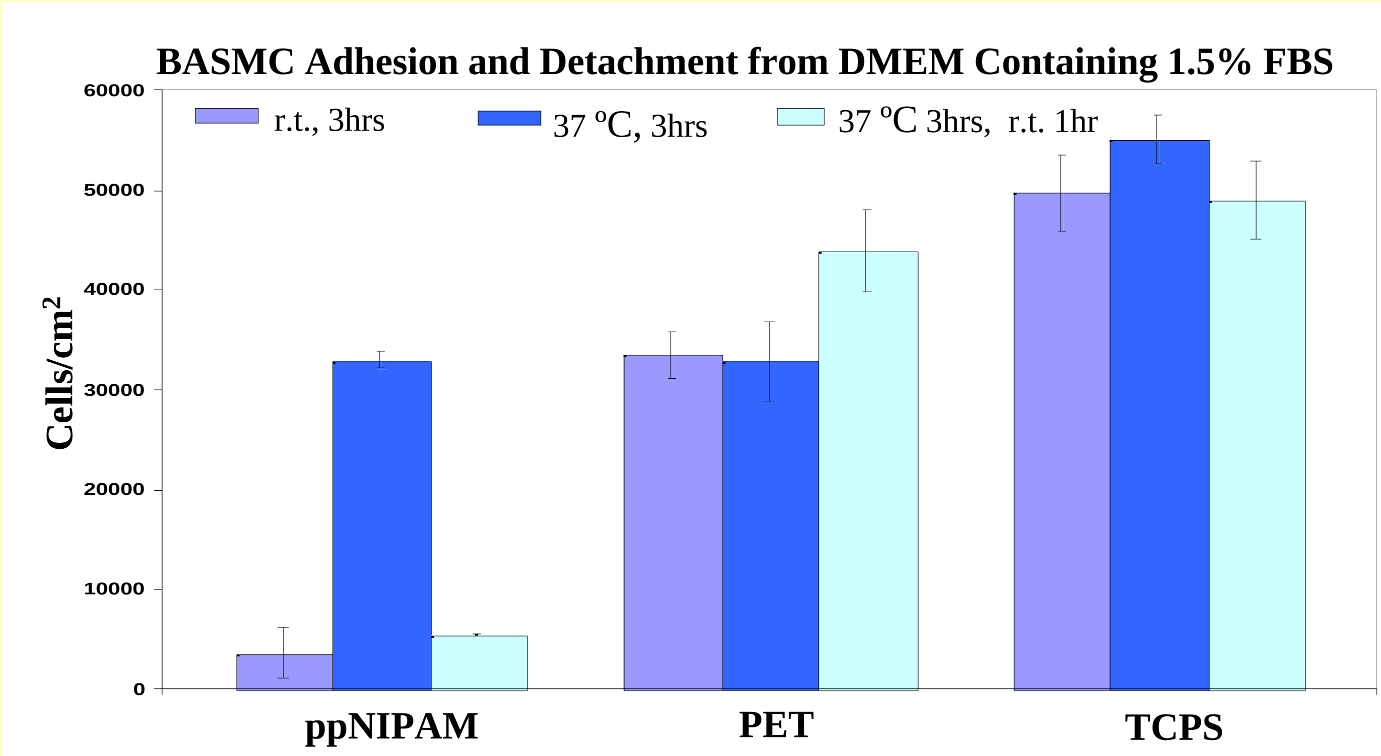
BASMC Adhesion from Serum Containing DMEM



- 10 times increase in cell adhesion on ppNIPAM at 37ºC compared to 23 ºC.
- Cell adhesion on ppNIPAM is reversible.

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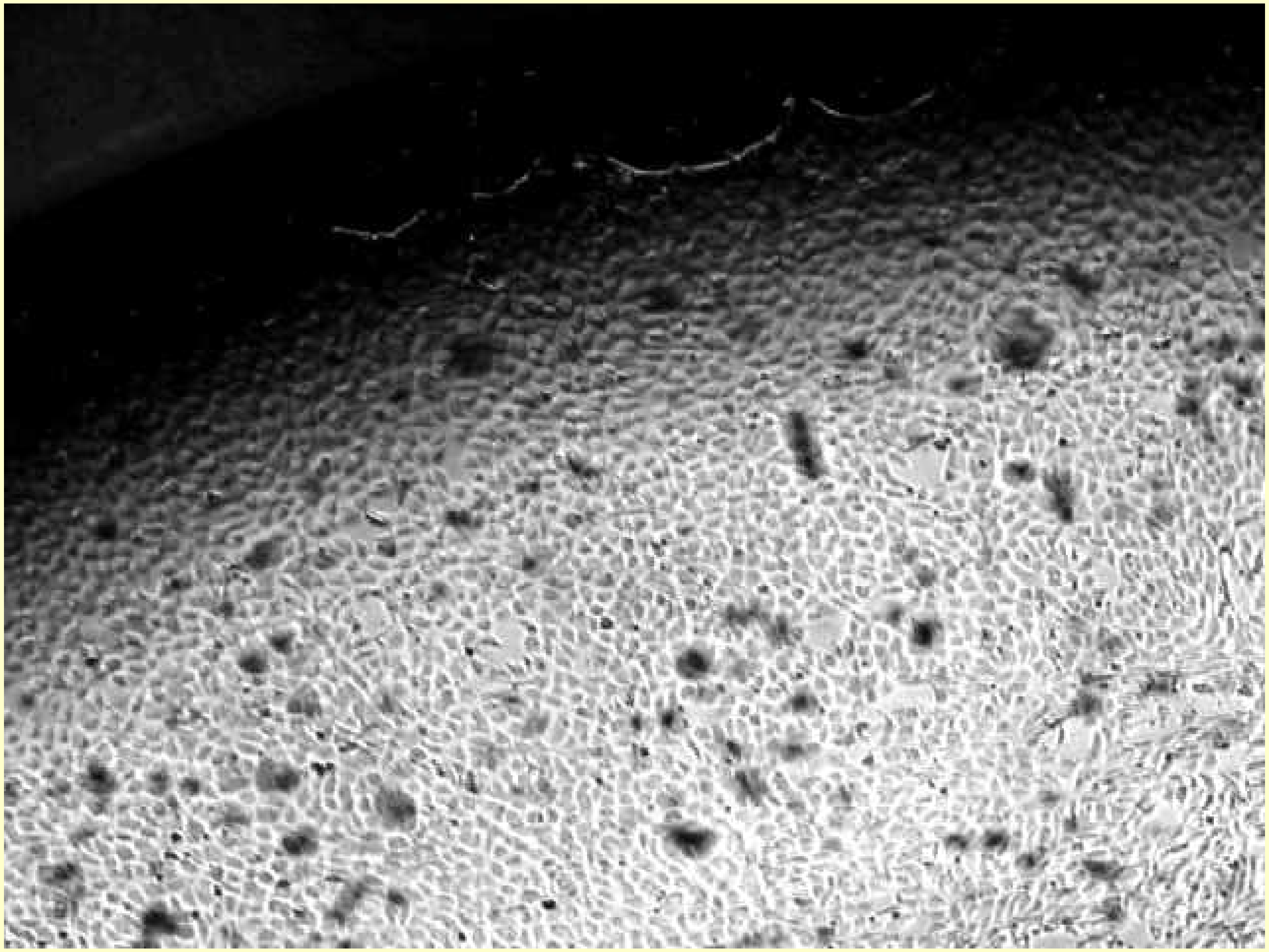
Detachment of Confluent BAEC Cell Sheet



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Cell sourcing:

Criteria for cells for tissue engineering

- robustness
- reproductive vitality
- differentiation potential
- phenotypic stability
- phenotypic functionality
- cell line purity
- freedom from viral contamination
- immunologic issues.

BEAT Cell Differentiation, Modification and Proliferation Experiments

Stem cells (mouse) differentiate to cardiac cells

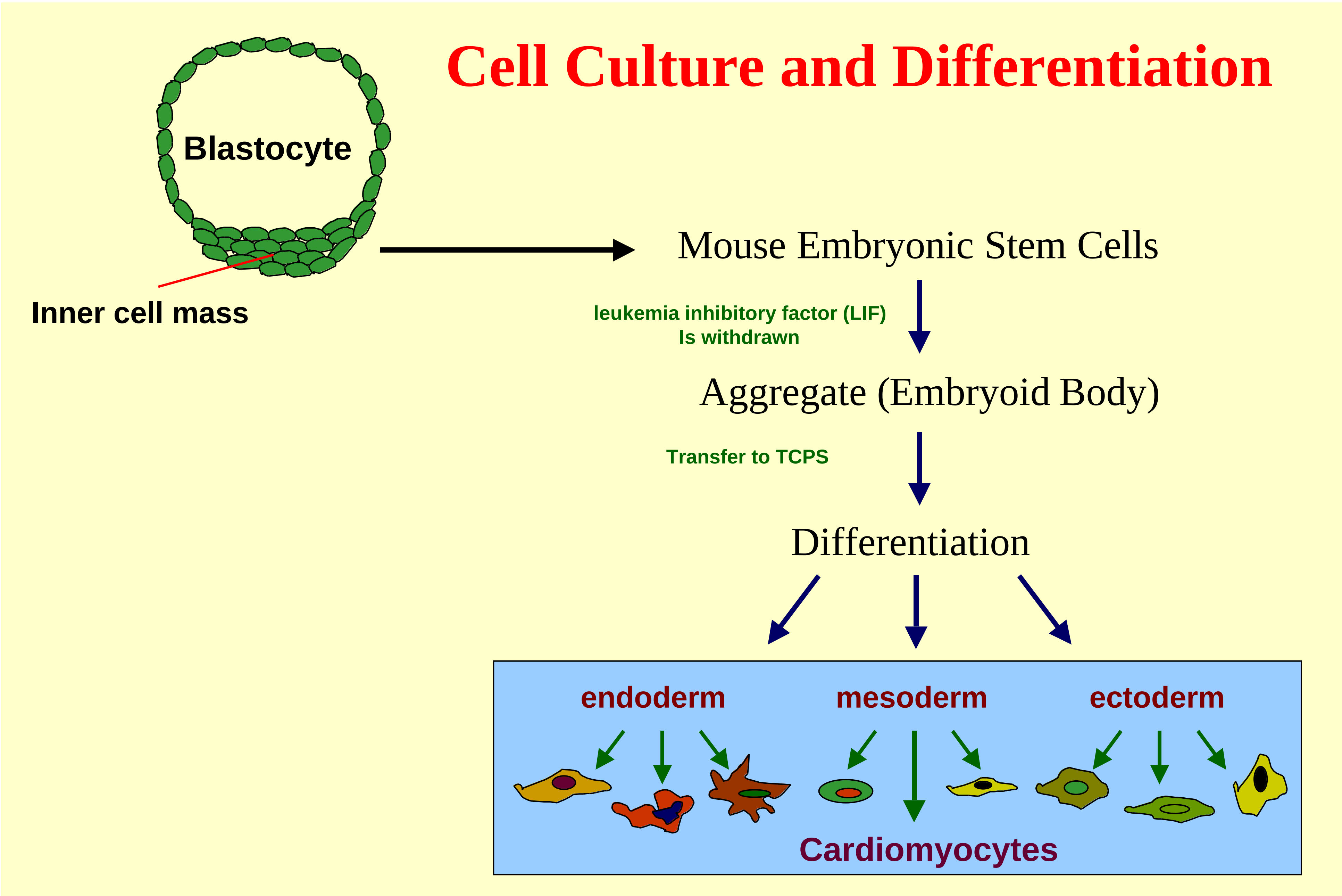
Patterned cardiac myocytes to Purkinje fibers

Stimulate cardiomyocyte proliferation

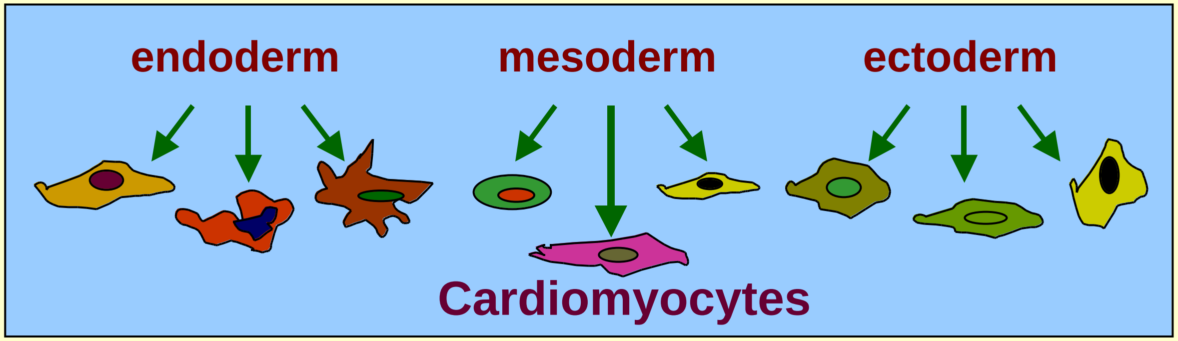
Stimulation of muscle cell graft growth *in vivo*

Co-culture of skeletal muscle and vascular cells

Enhancing the number of laminin receptors in cells

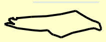

Cell Culture and Differentiation
Blastocyte
Inner cell mass
Mouse Embryonic Stem Cells
leukemia inhibitory factor (LIF)
Is withdrawn
Aggregate (Embryoid Body)
Transfer to TCPS
Differentiation
endoderm
mesoderm
ectoderm
Cardiomyocytes

Cell Separation scheme

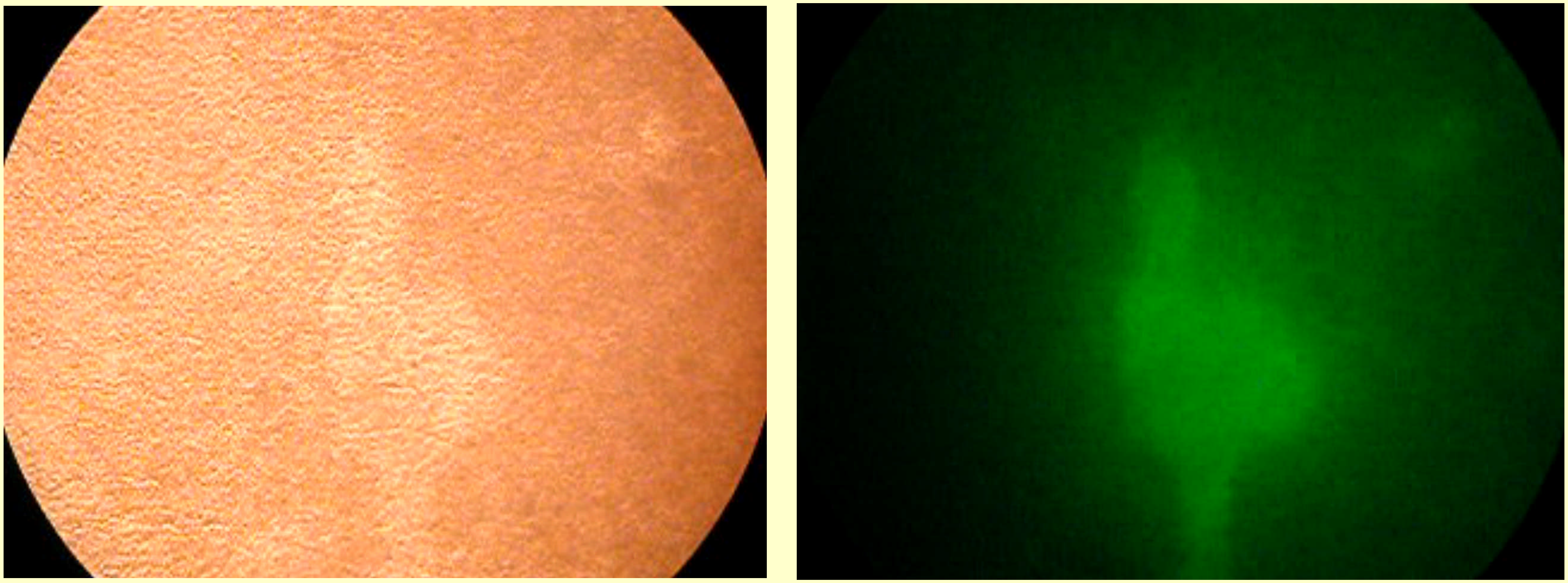


+

plasmid



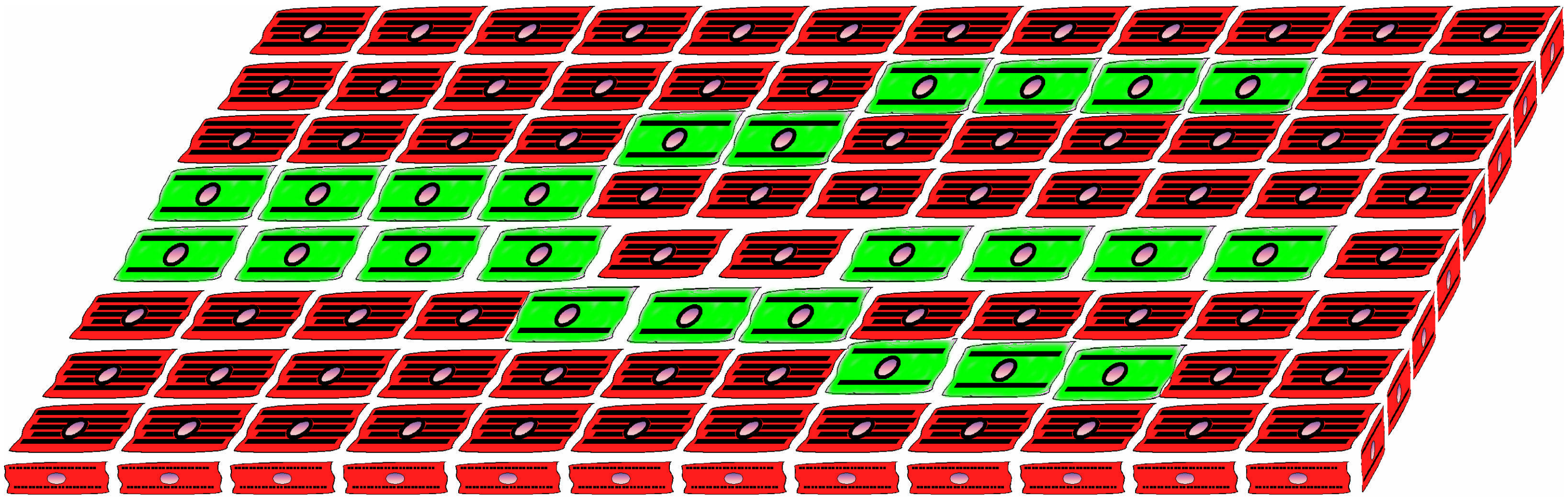
GFP transfection of cardiomyocytes



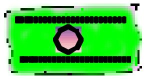
1 week after seeding

Charles Murry, Jeanette Nissbaum

A Bio-engineered Ventricular Patch Will Require A Purkinje Fiber Conduction System




Working Cardiac Myocyte


Purkinje Fiber Cell

S. Hauschka

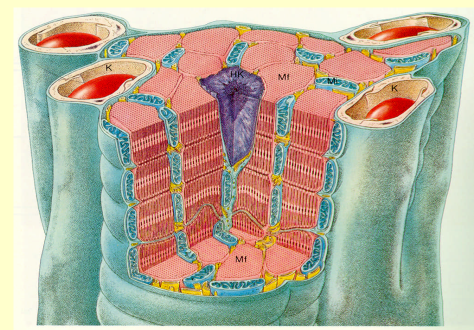
The BEAT Strategic Plan

Why?

- We have 3.5 years to accomplish a <u>very</u> difficult task
- 10 investigators need coordination to work <u>together</u>
- We are exploring many avenues -- are all productive forever?
- A highly goal-oriented program, rather than curiosity driven
- People are looking to us to succeed
- Resources are limited -- how to use them best?
- Flexibility is critical / creative problem solving essential

Strategic Plan

Polymer 1
Polymer 2
Porous 1
Porous 2
Porous 3
Porous 4
Printed laminin
Myoblasts
Stem cells
Cardiac muscle
Gene delivery
Angiogenesis
In vivo



2000 2001 2002 2003 2004 2005

Anti-life (2000)

Heart disease
Cancer
Diabetes
New diseases?
Stress
Environmental Pollution
Wearing out of “parts”

Pro-life (2000+)

Cancer cure?
Organ regeneration?
Telomers and aging
Alzheimer’s cure
Diabetes cure

1999 2001 2003 2005 2007 2009 2011 2013 +++

Prolong Life with Good Quality

Prosthetic Parts
Pharmaceutics Technology
Tissue Engineering?

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