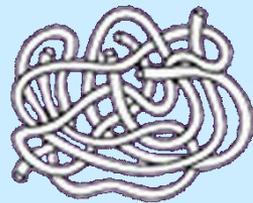
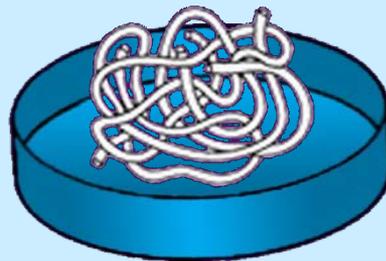


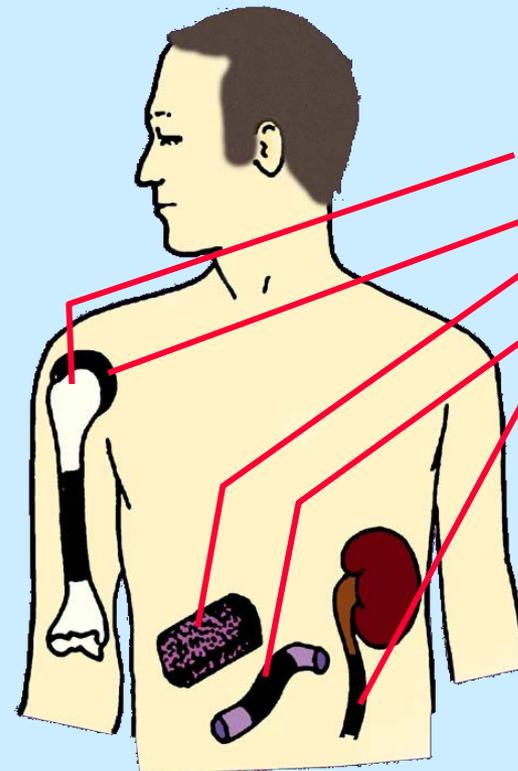
Biodegradable
polymer scaffold



In vitro tissue culture



In vivo implantation



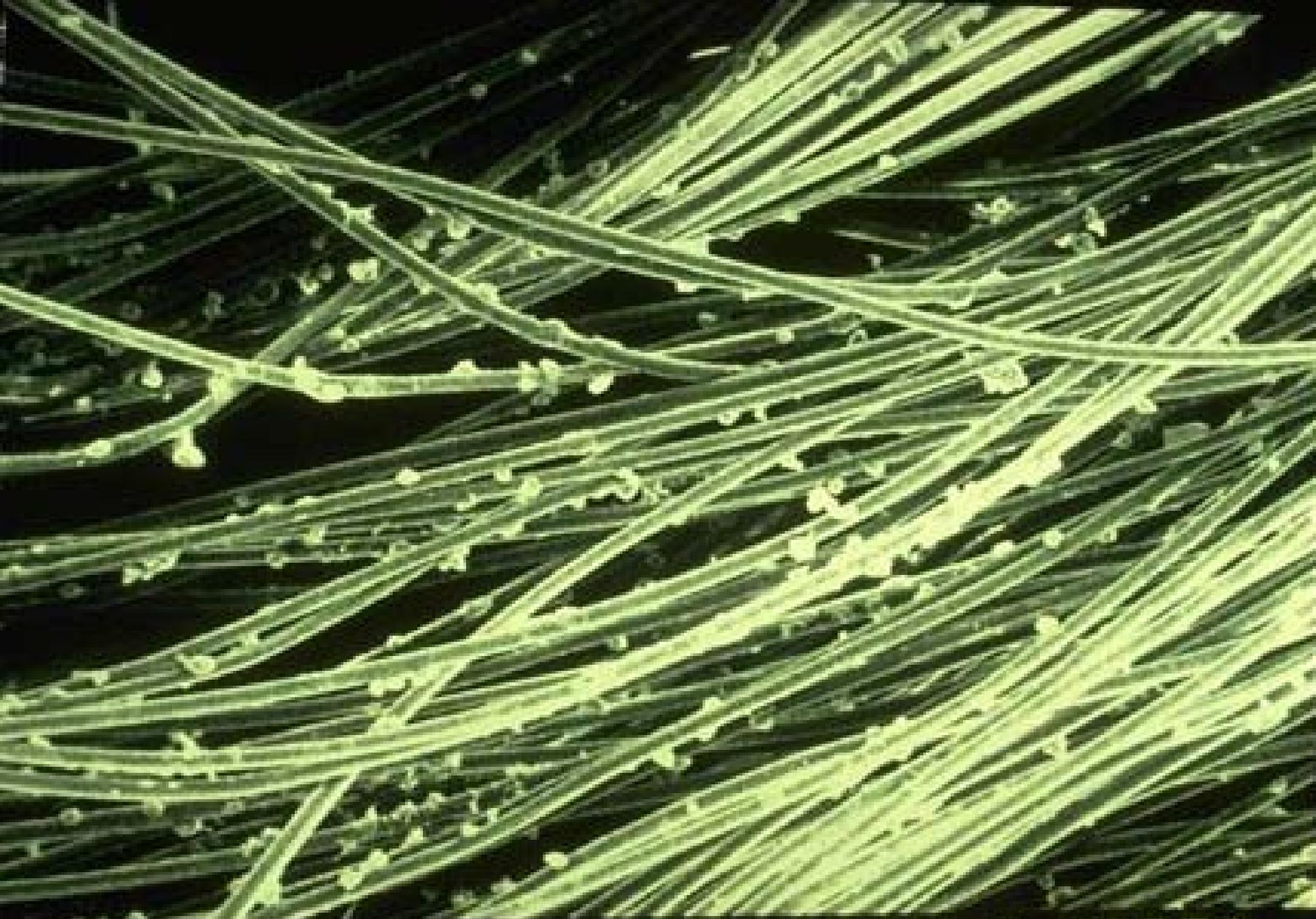
New

- Bone
- Cartilage
- Liver
- Intestine
- Ureter

Cells

- Osteoblasts
- Chondrocytes
- Hepatocytes
- Enterocytes
- Urothelial cells





20KV

121X

100U

1873

V-HEPTA

Cartilage tissue engineering



BEFORE
cell seeding

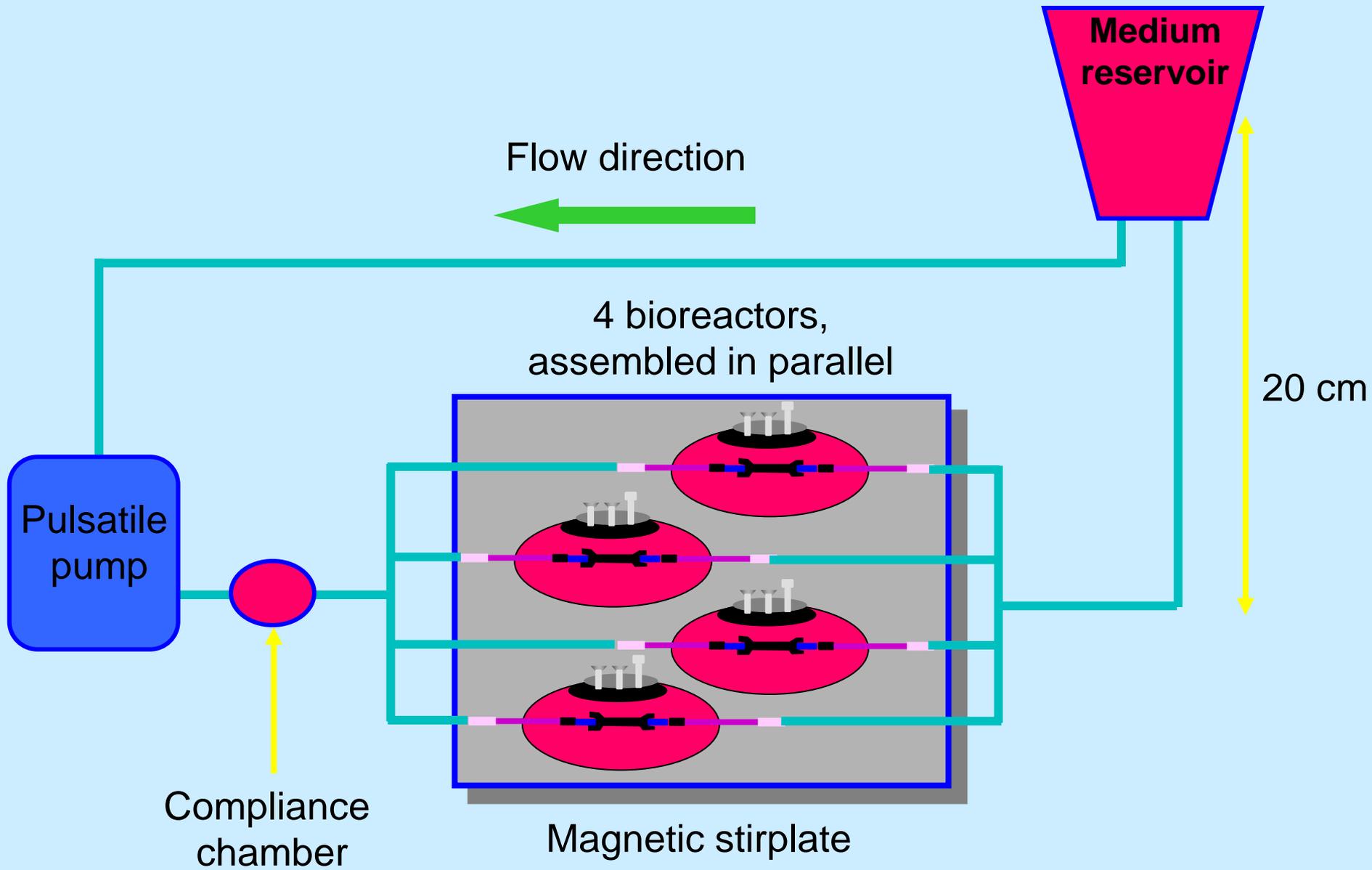


AFTER
2 weeks in culture



System

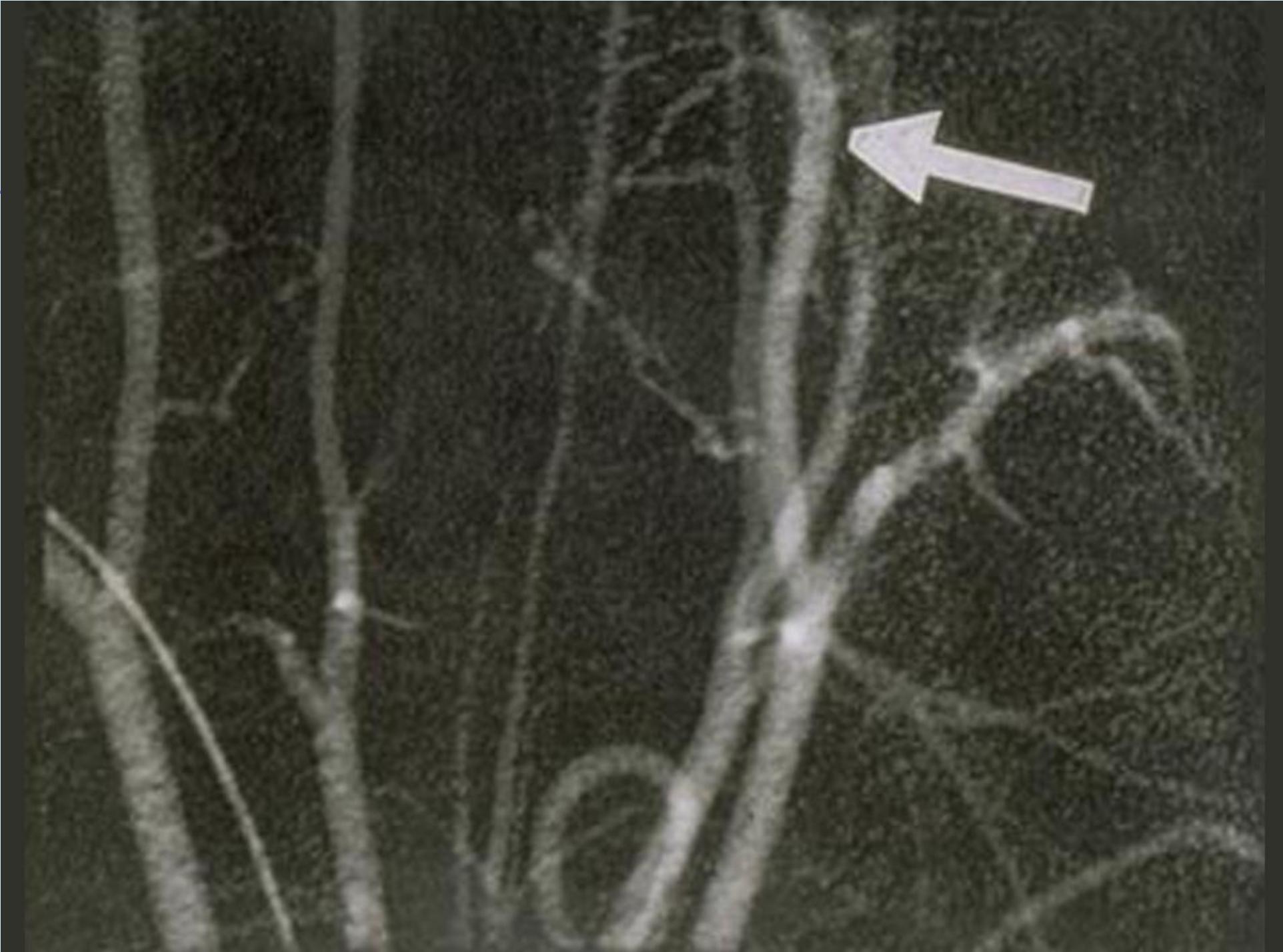
- Modified poly glycolic acid (PGA) tubes
- 8 weeks SMC culture, then EC
- Bio-Reactors – Pulsatile radial stress

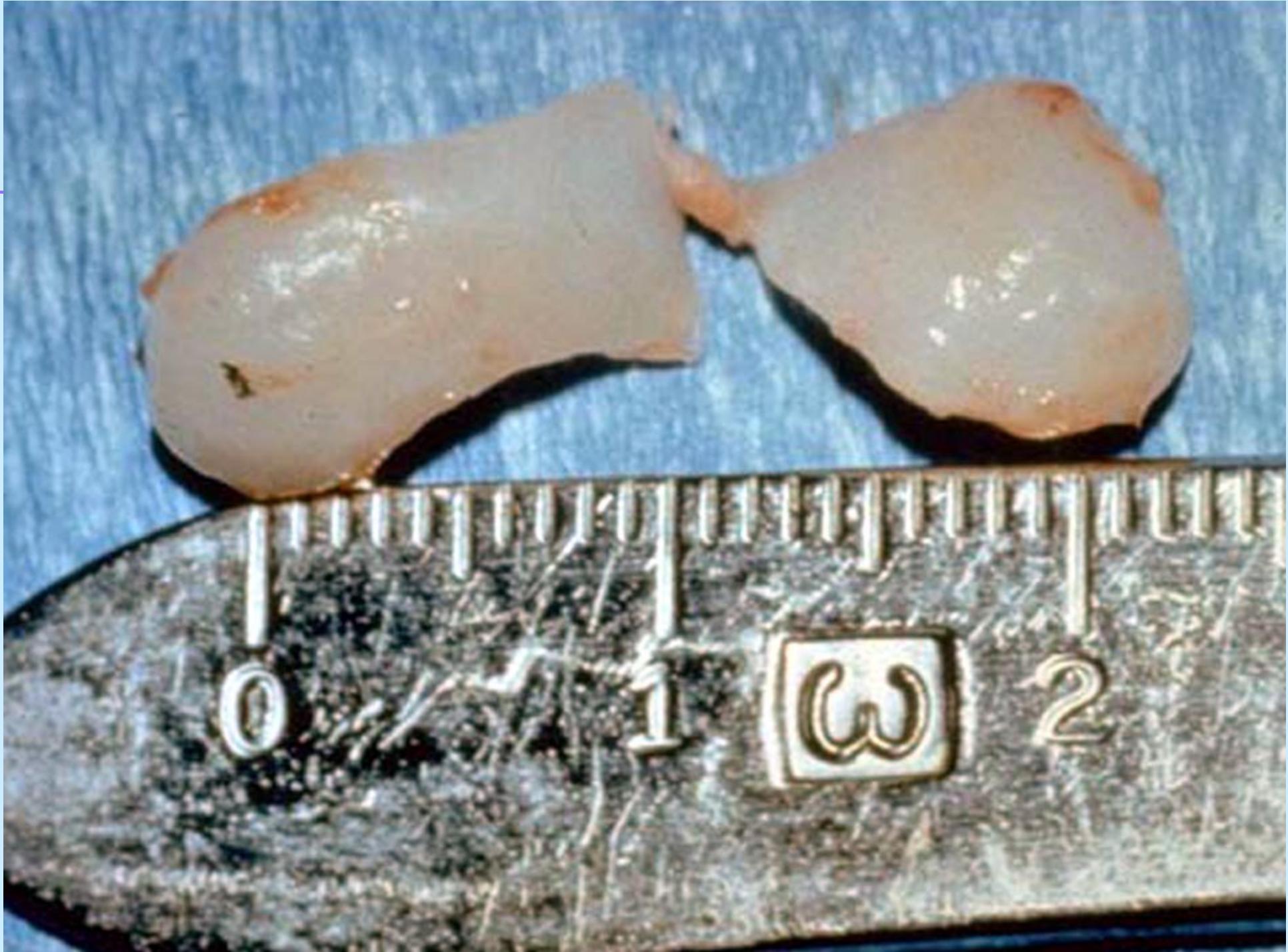




Characteristics

- 50% collagen
- Rupture strengths > 2000 mg Hg
- Suture retention – Strengths up to 90g
- Demonstrates contractile responses to serotonin, endothelin-1, and prostaglandin F₂α

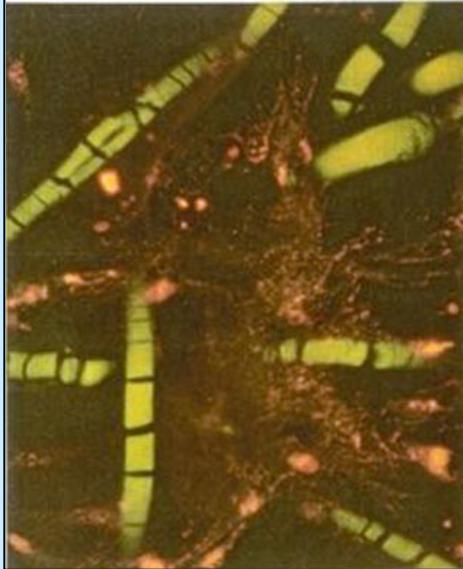




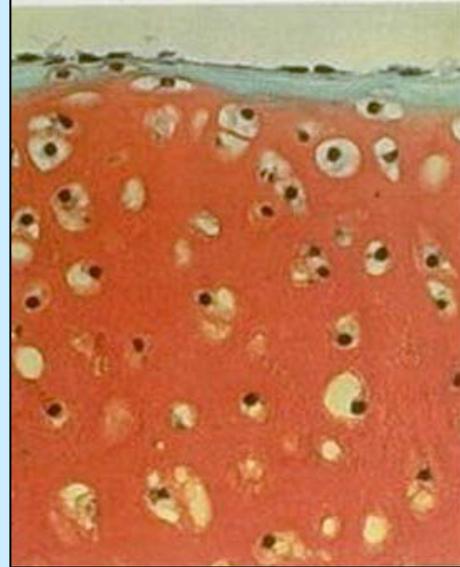
a) Glycosaminoglycan
(X 40, safranin-O)



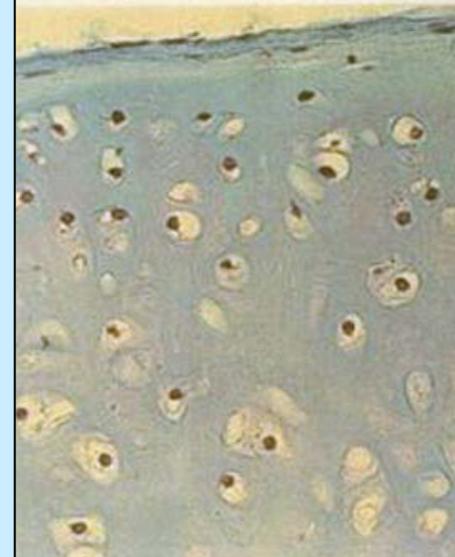
b) Collagen
(X 400, trichrome, fluorescent light)



c) Glycosaminoglycan
(X 400, safranin-O)



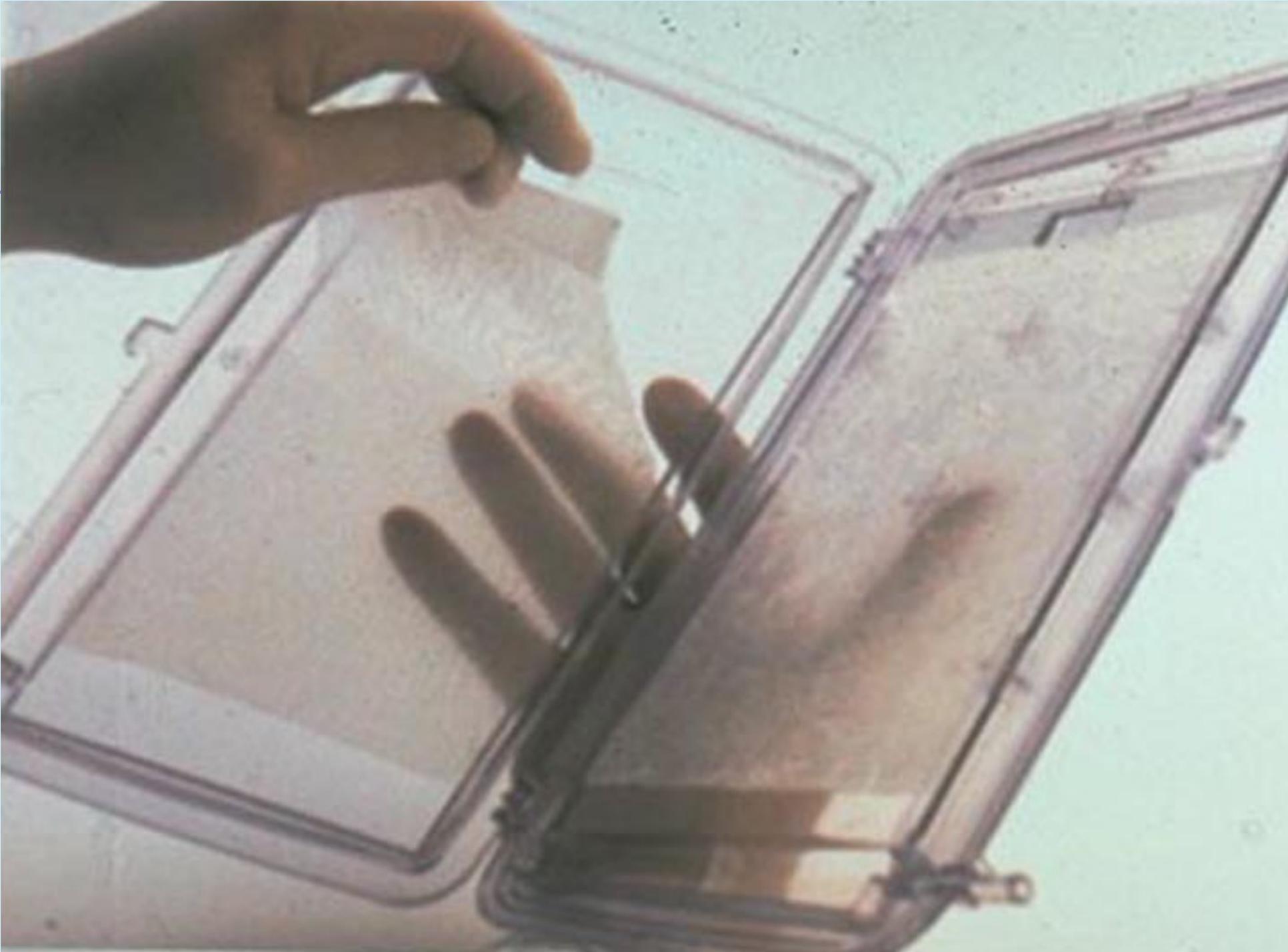
d) Chondroitin Sulfate
(X 400, alcian blue)

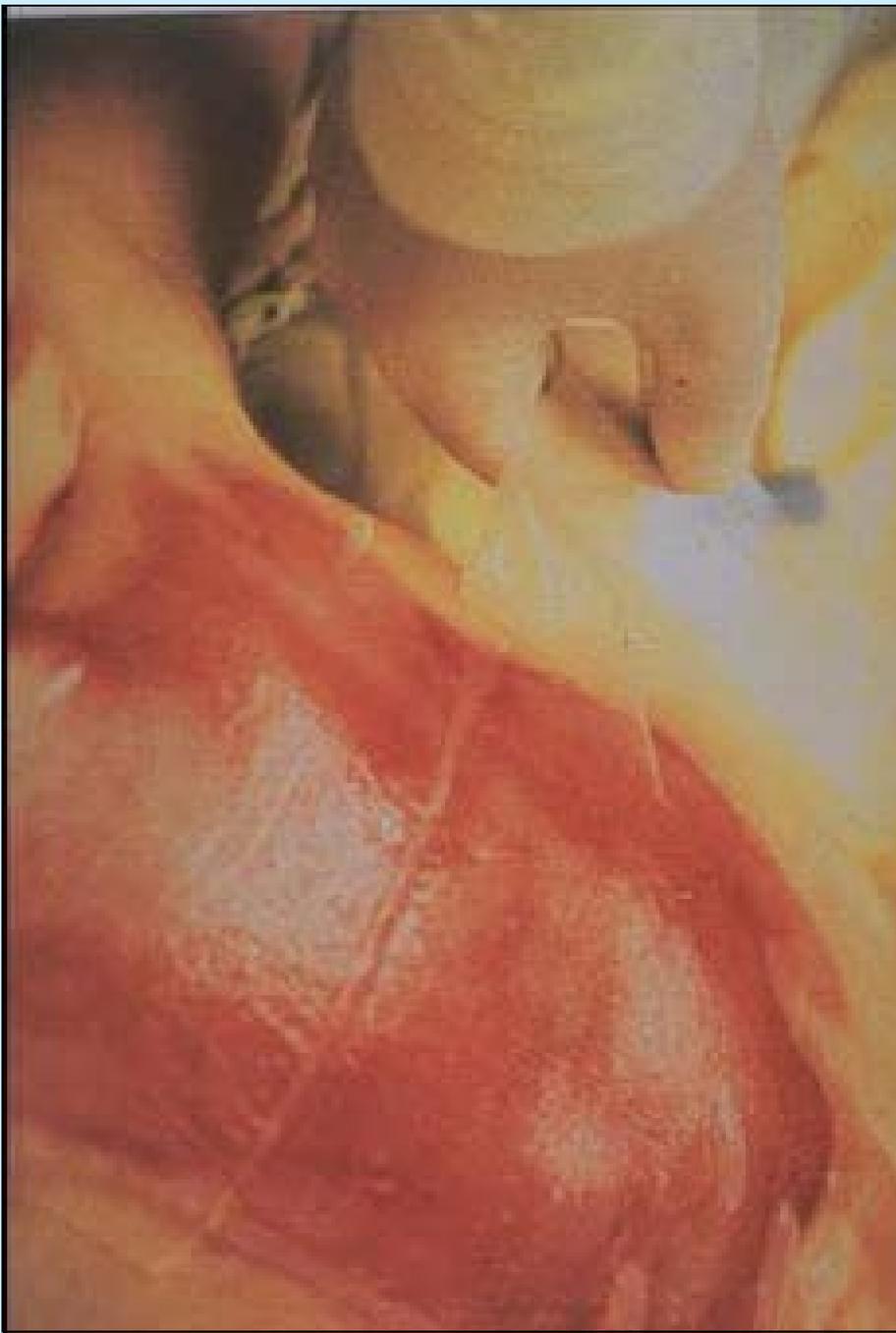






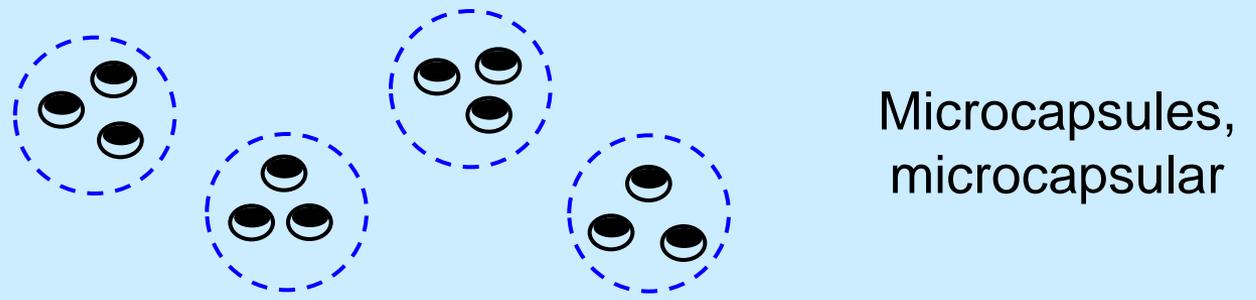
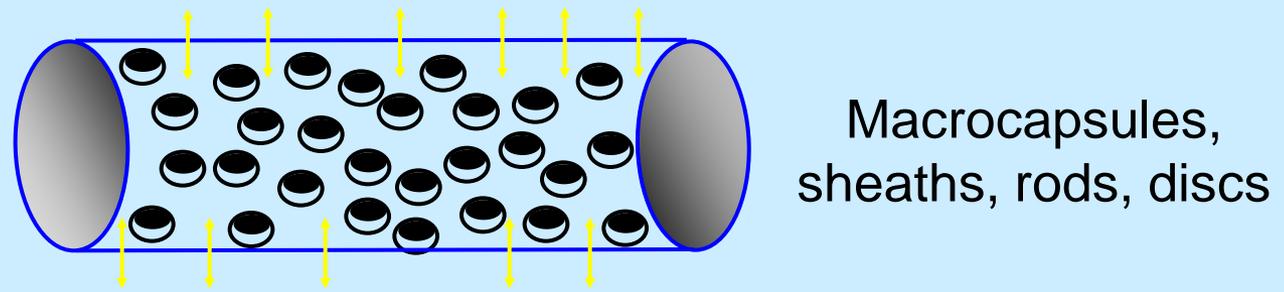
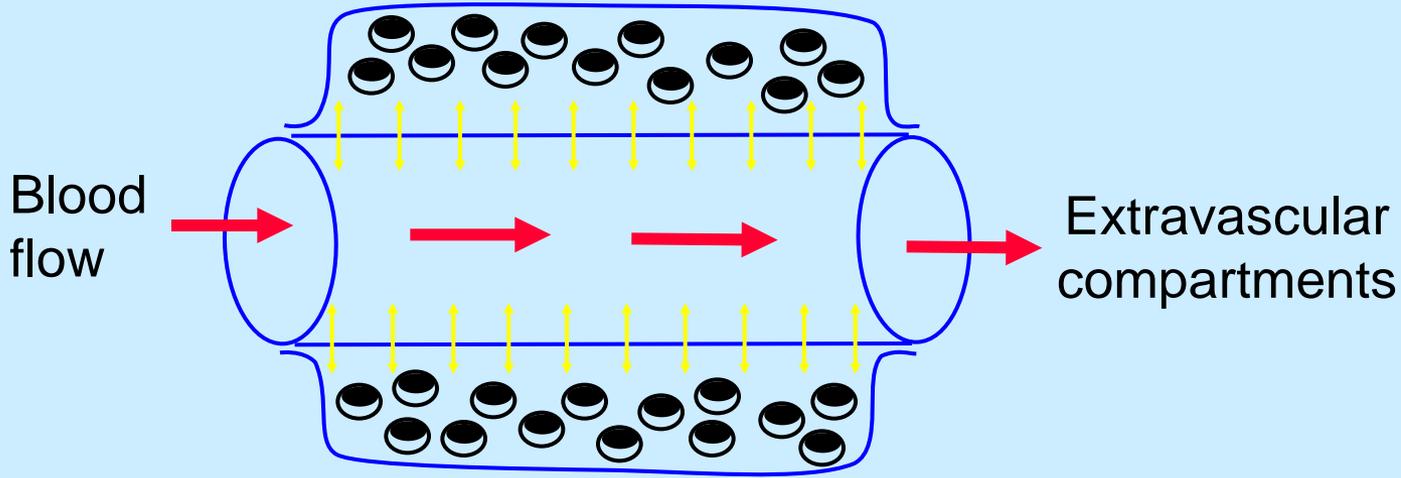




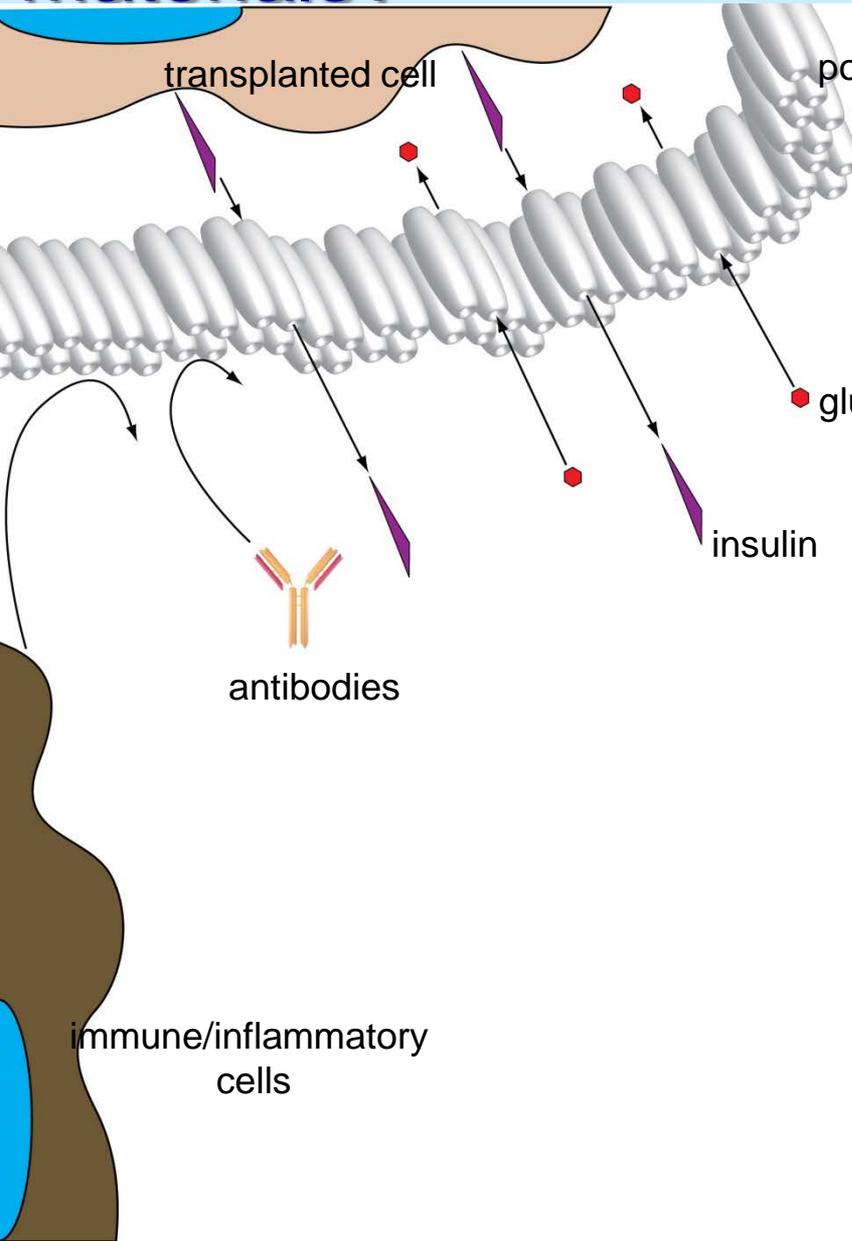








Cell encapsulation: Can cells be protected with materials?

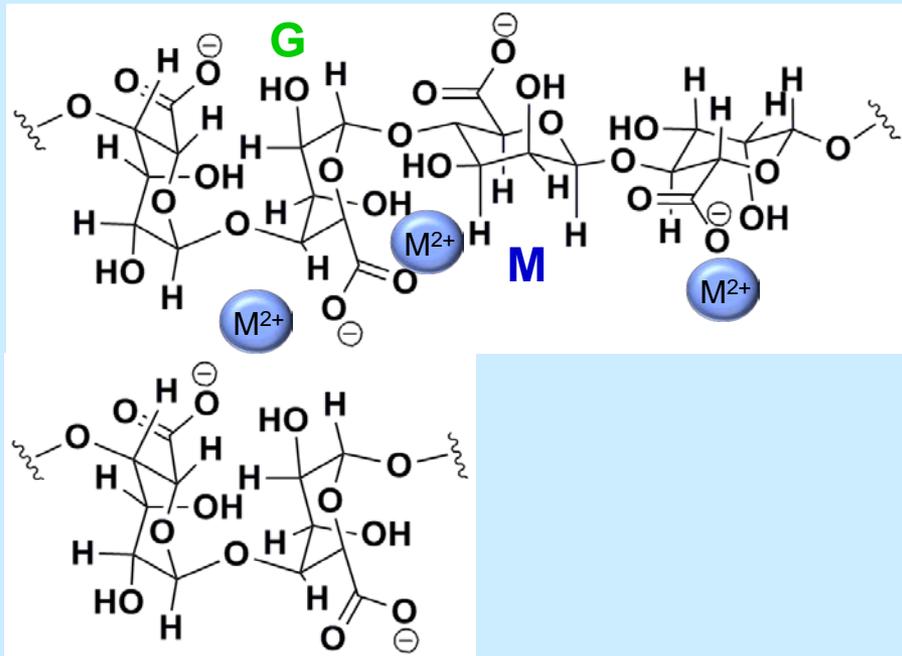


porous, semi-permeable barrier } size exclusion

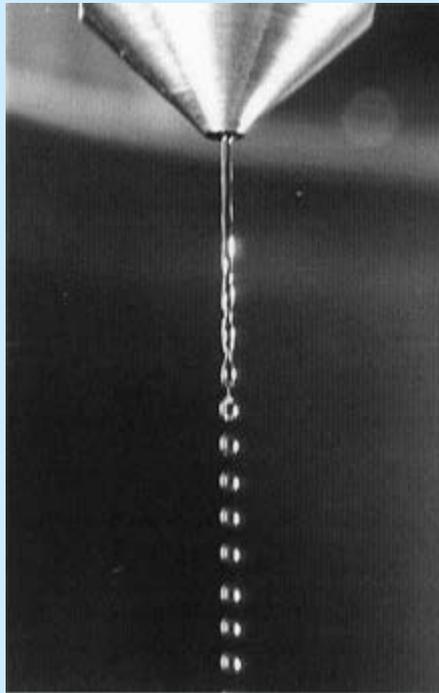
glucose }
insulin }
passive diffusion of
nutrients/therapeutic

Can a device be made to allow transplanted cells to live and function, but protect them from the immune system? What material will we use?

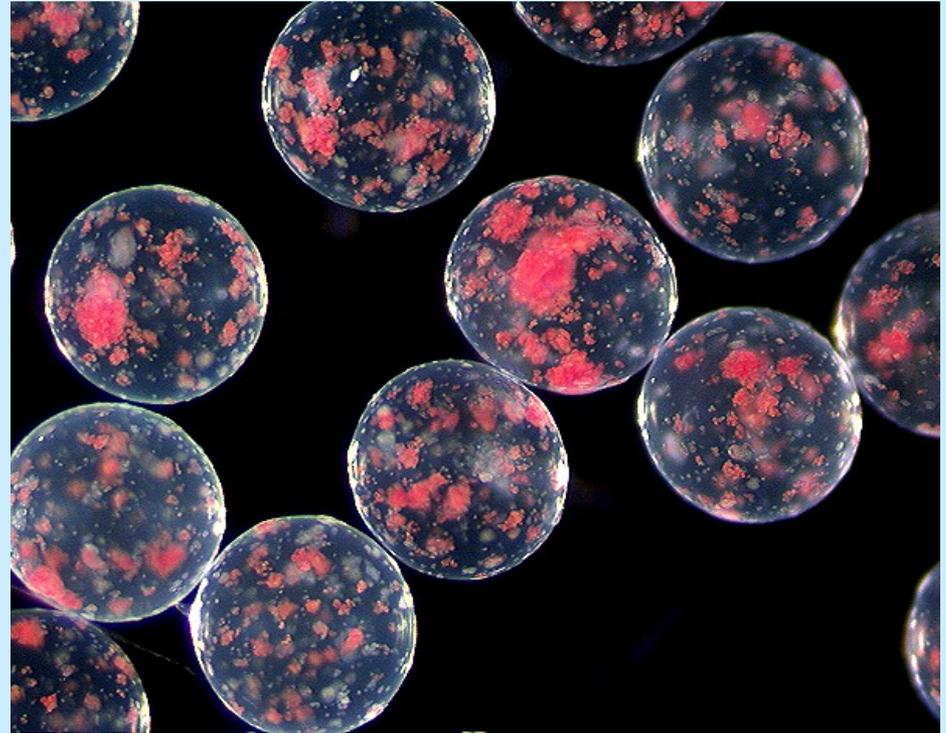
Only a few materials have been investigated – most work has been with alginate from seaweed



Islet encapsulation in alginate microbeads

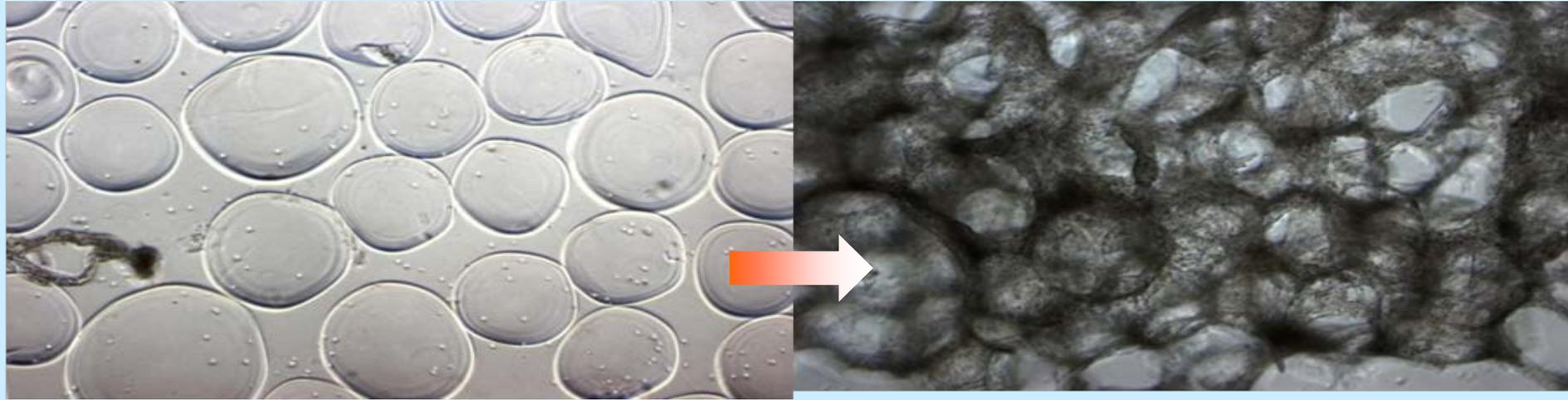


electrostatic droplet
generator



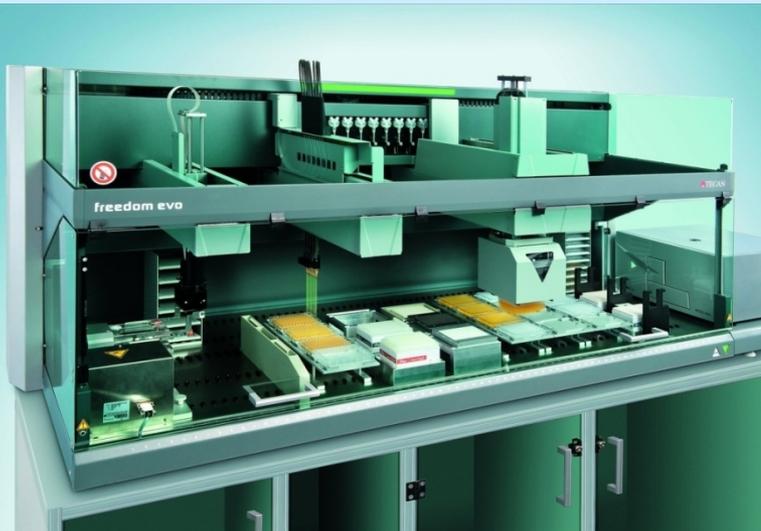
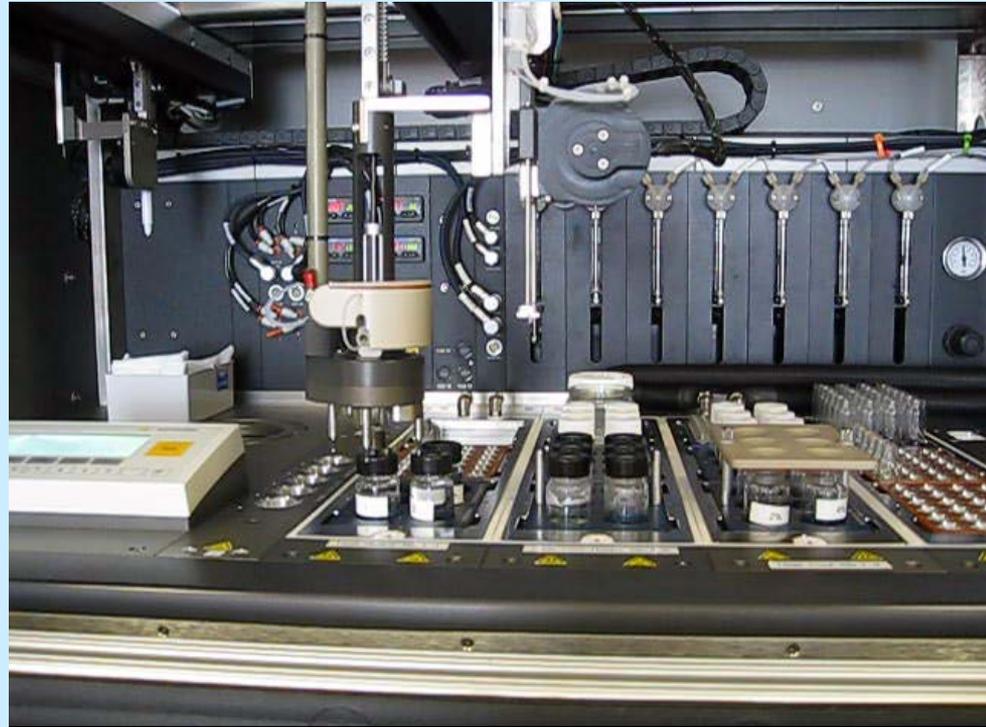
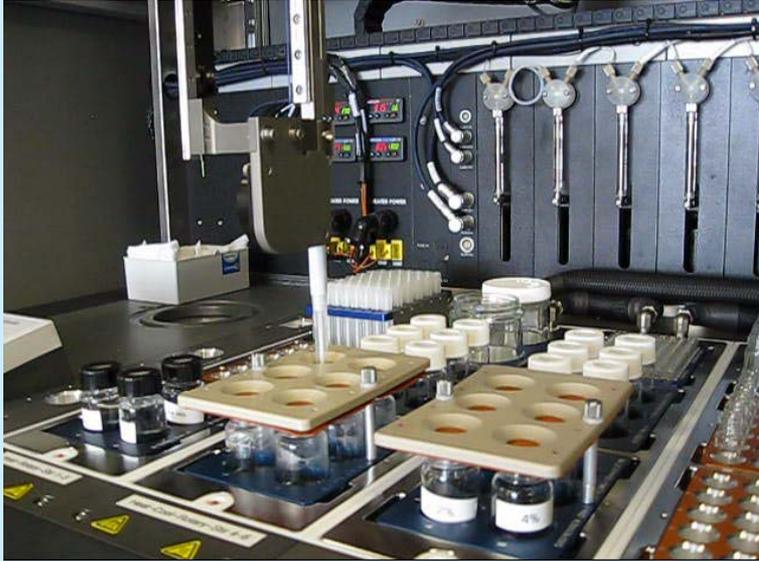
500 um

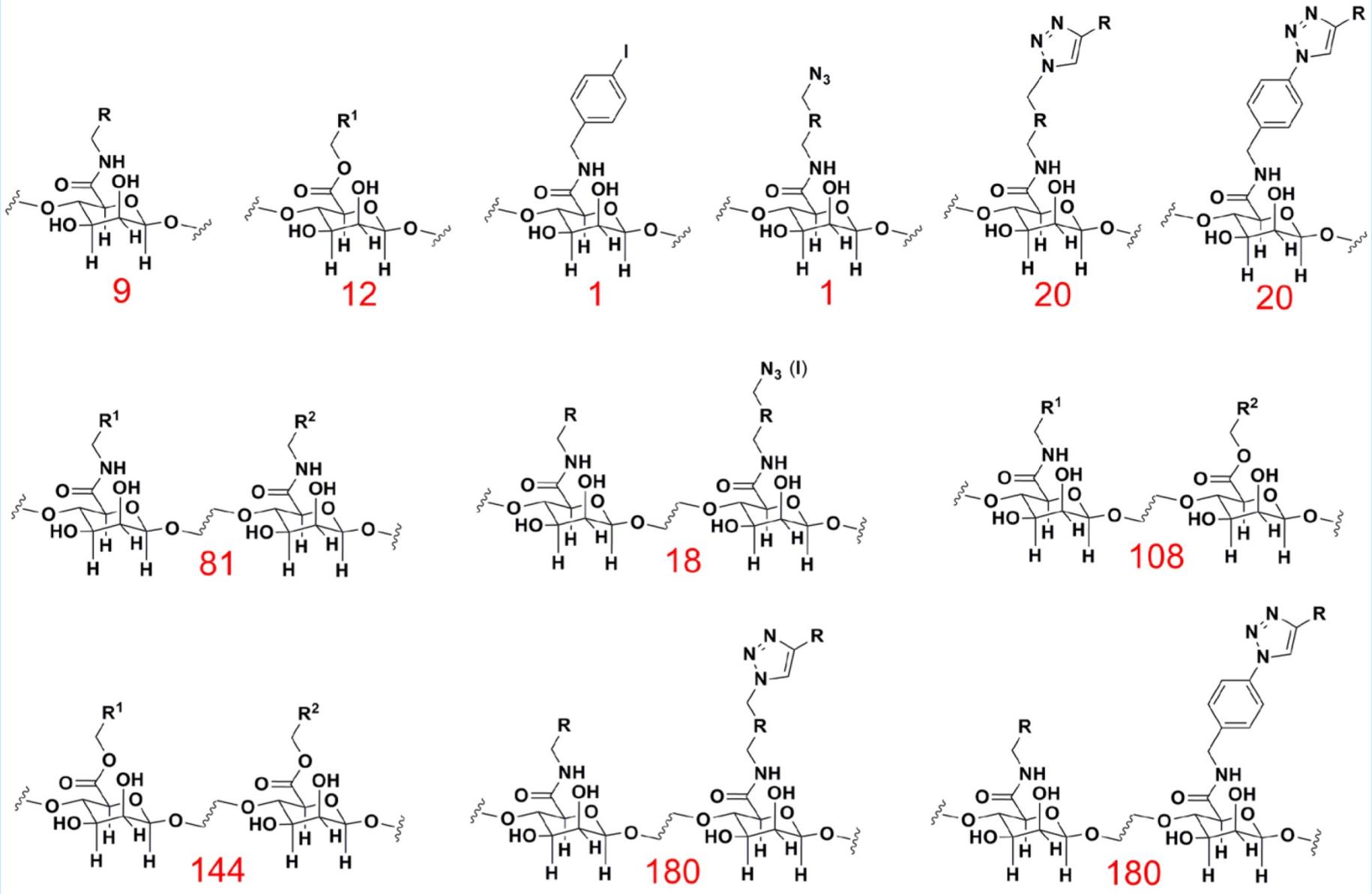
Transplanted, encapsulated islets become covered in scar tissue



- Alginate is not sufficiently biocompatible and is recognized as a foreign material
- Can we develop materials that keep cells alive and functioning but do not get covered in scar tissue?

Automated, high throughput polymer synthesis





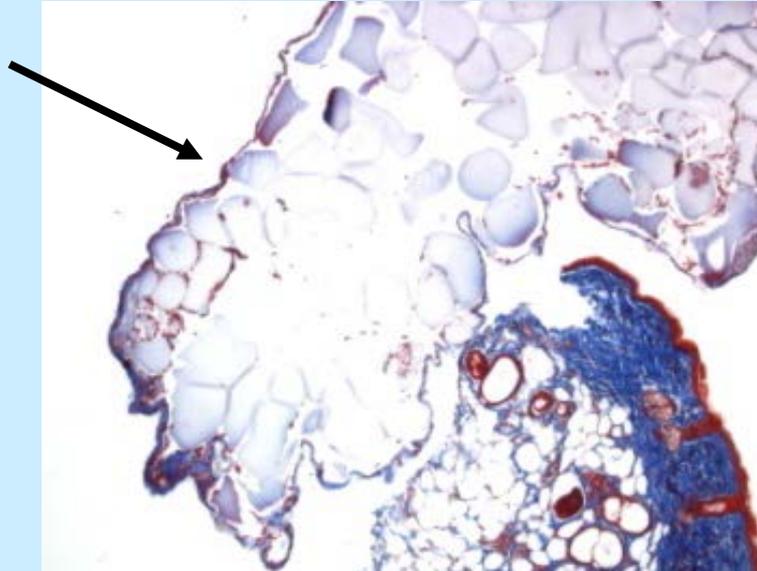
Current progress for the alginate modification library. Numbers indicate the number of unique, diverse alginates that correspond to each general structure.

Rapid evaluation of biocompatibility

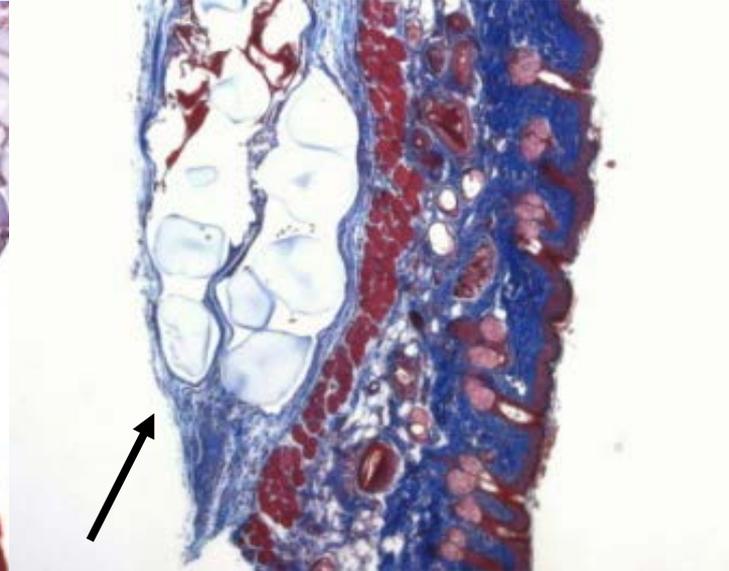
- How do we rapidly evaluate whether a material is superbiocompatible?
- Conventional biocompatibility analysis is slow and requires tissue histology.
- Can we evaluate the inflammation response rapidly?

Improved biocompatibility *in vivo*

263_C12



Unmodified Alginate



The modified alginate is covered with a thin fibrotic layer (blue, pointed with arrow) surrounding all of the capsules and with very little collagen infiltrating and surrounding individual particles. The fibrotic layer is approximately 1-2 layers thick which indicates a score of 1. Unmodified alginate has collagen penetrating the fibrotic capsule (arrow) along with a large amount of collagen clustering on the sides of the implant. The concentric fibrotic coverage indicates a score of 3 for unmodified alginate.

**Can other
technologies help
tissue engineering?**

Slow release of active factors from polymer

Growth Factors

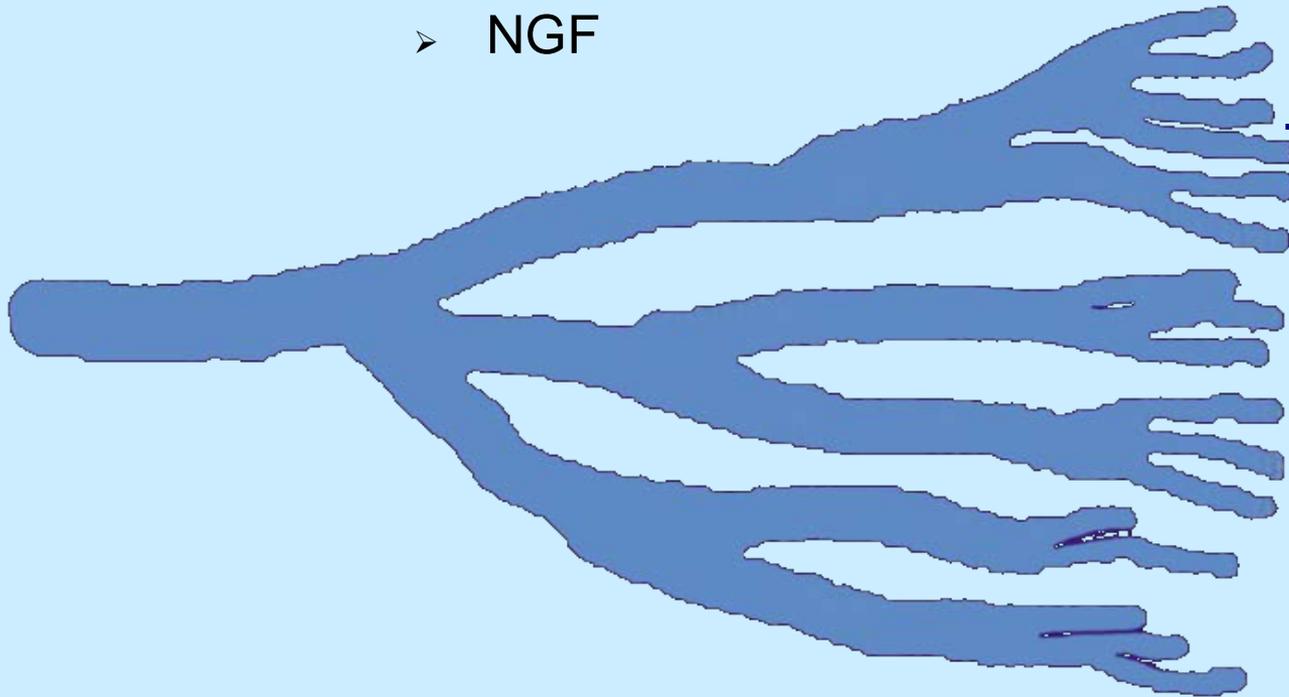
- Basic FGF
- EGF
- NGF

Growth Inhibitors

Differentiating Factors

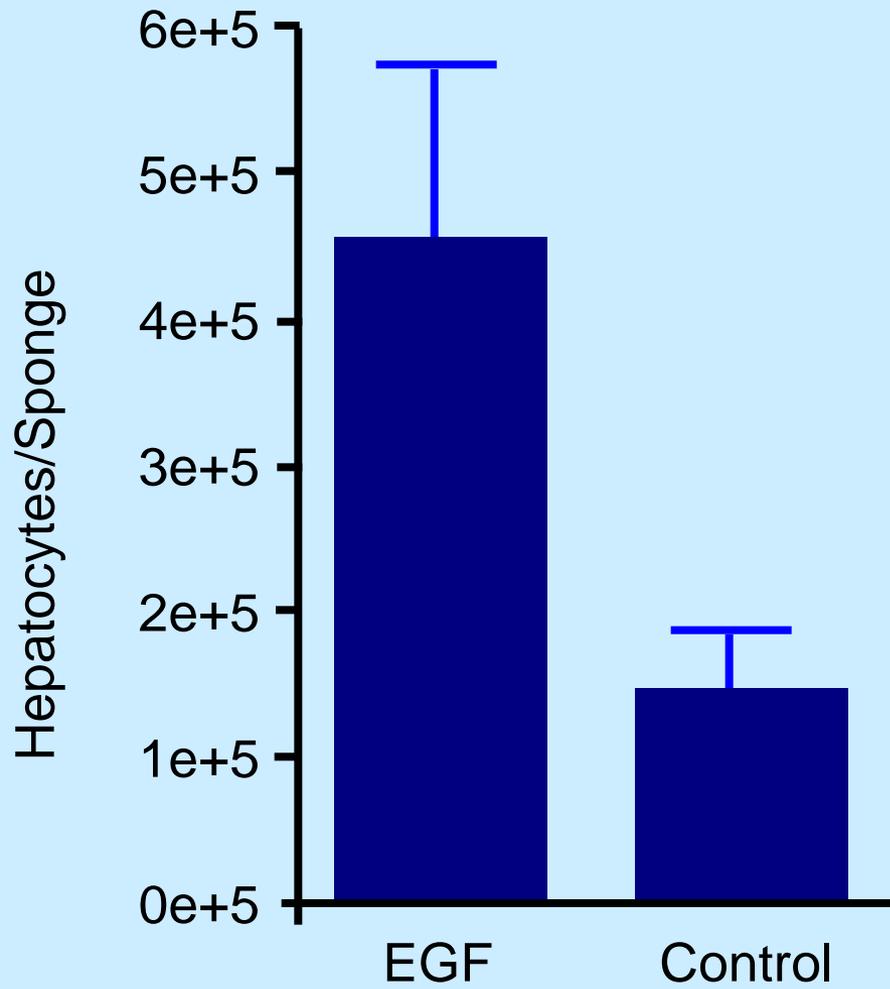
- The Retinoids

Anti-Inflammation Agents



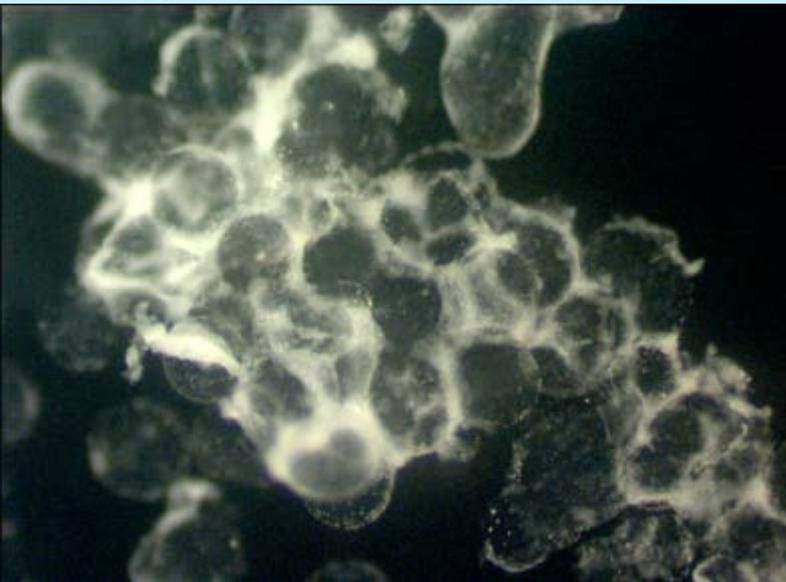




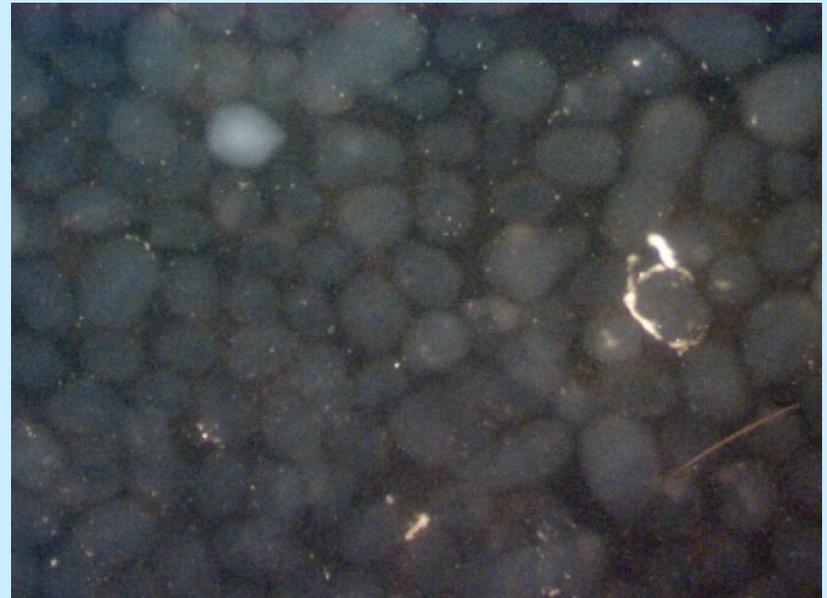


Microencapsulated islets release scar-blocking over an extended time after implantation

No drug in capsule



Drug releasing capsules after implantation



Drug releasing capsules capable of treating diabetic mice

The gene therapy bottleneck: Delivery

“There are only three problems in gene therapy: delivery, delivery, and delivery.”

—Inder Verma, 1999

Primary Concerns:

- Safety
- Efficiency

Viral Vectors

- Highly efficient
- Safety concerns

Synthetic Vectors

- Potentially safer
- Cheaper and easier to manufacture
- Currently less efficient

Goal:

Using simple robotic systems, develop high-throughput synthesis and screening methods

Synthesis:

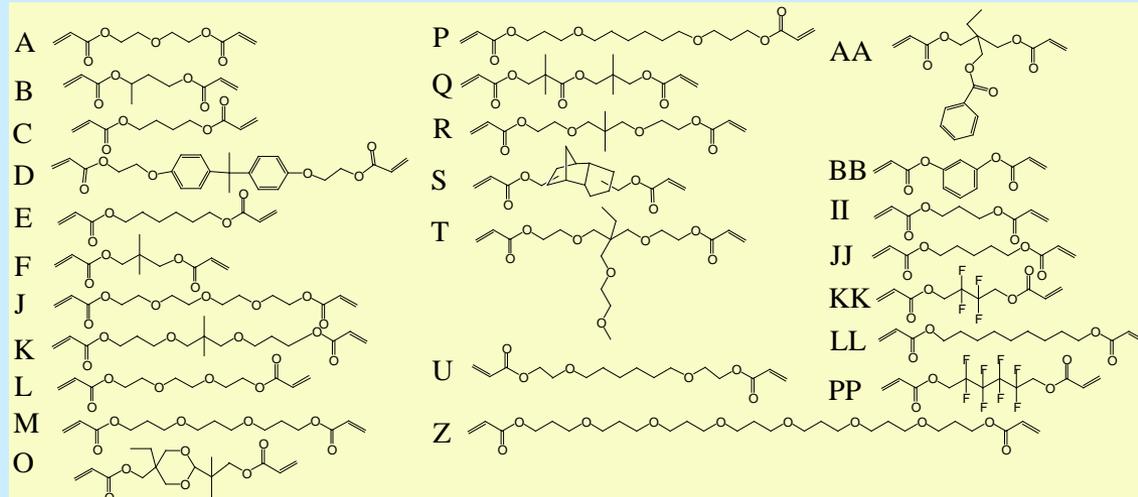
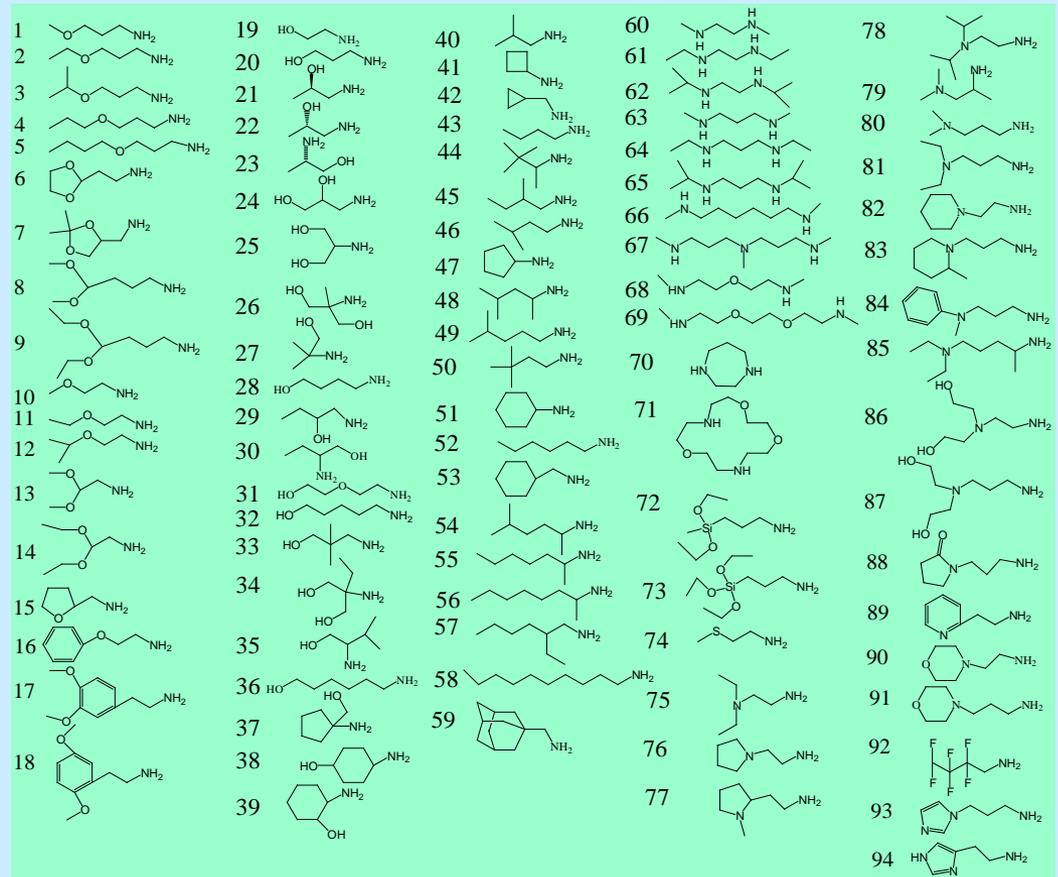
94 Amino monomers

X

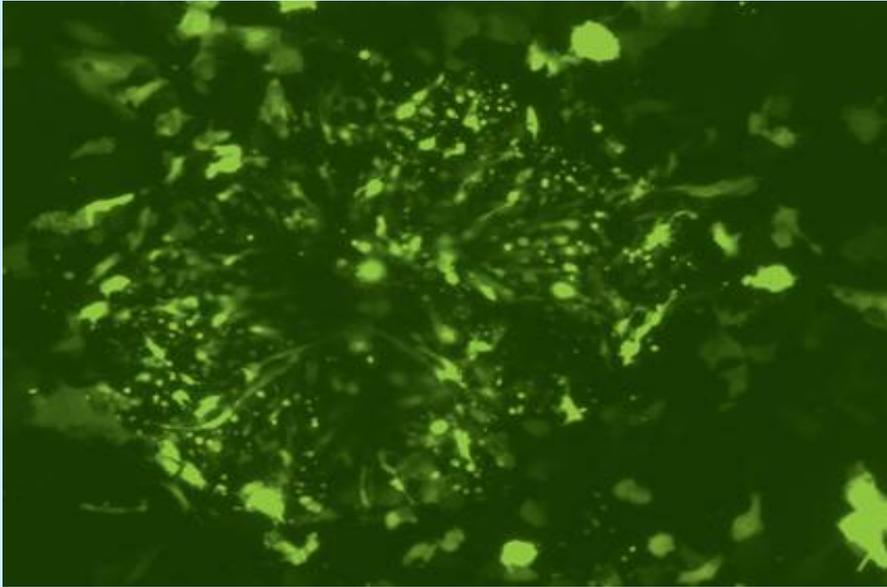
25 diacrylate monomers

=

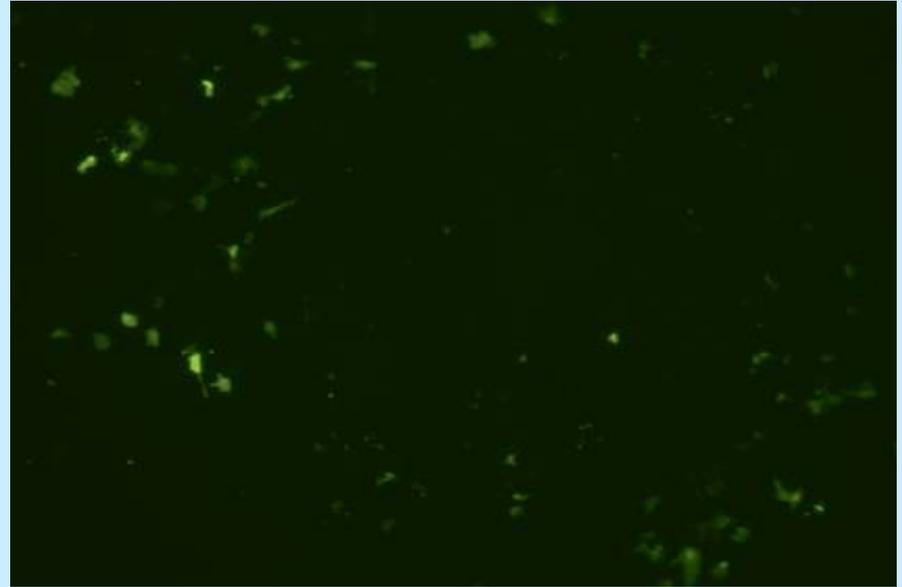
2350 Structurally diverse, degradable polymers



Human embryonic stem cells

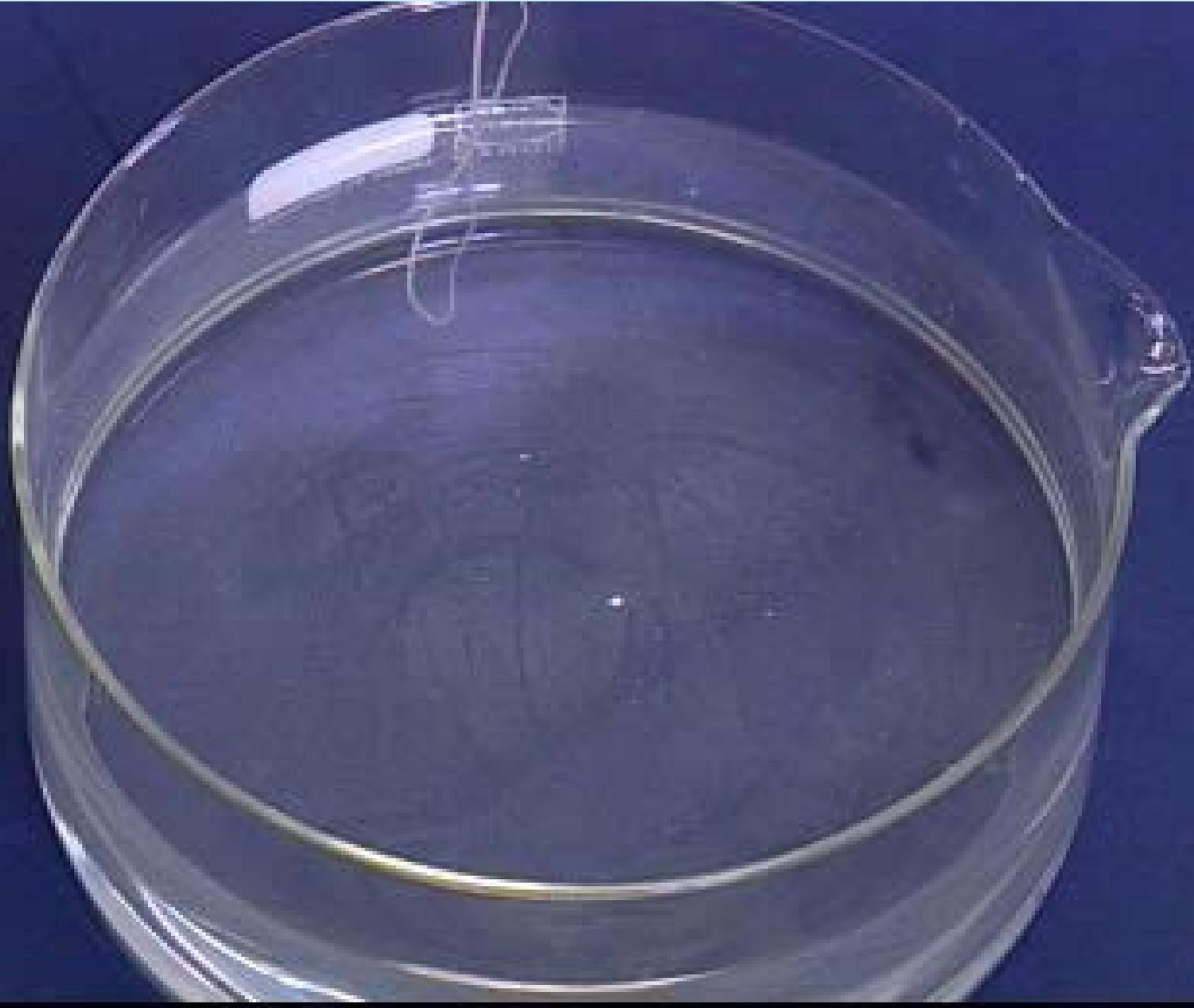


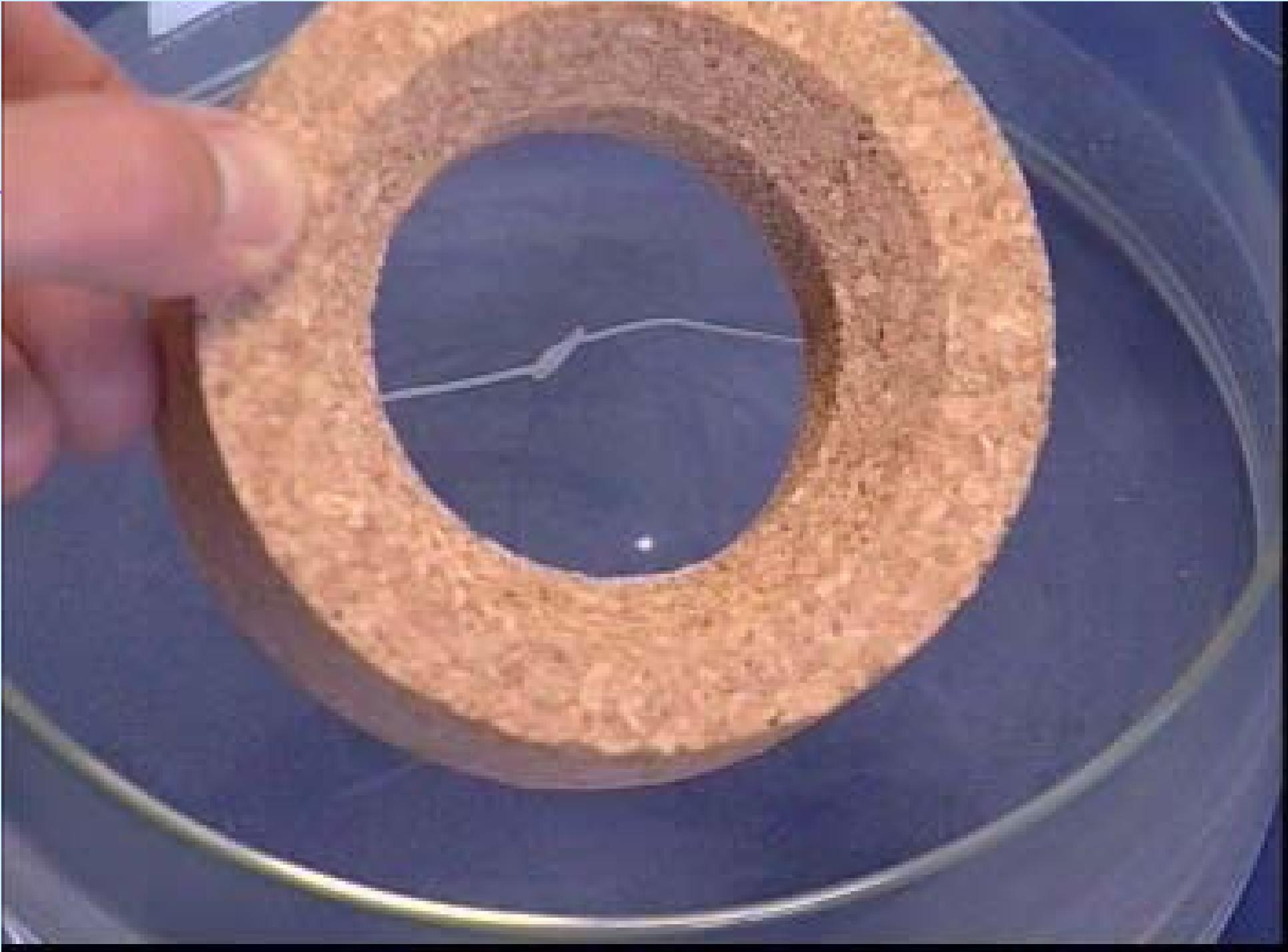
C32-118



Lipofectamine 2000

**Can new
materials help?**



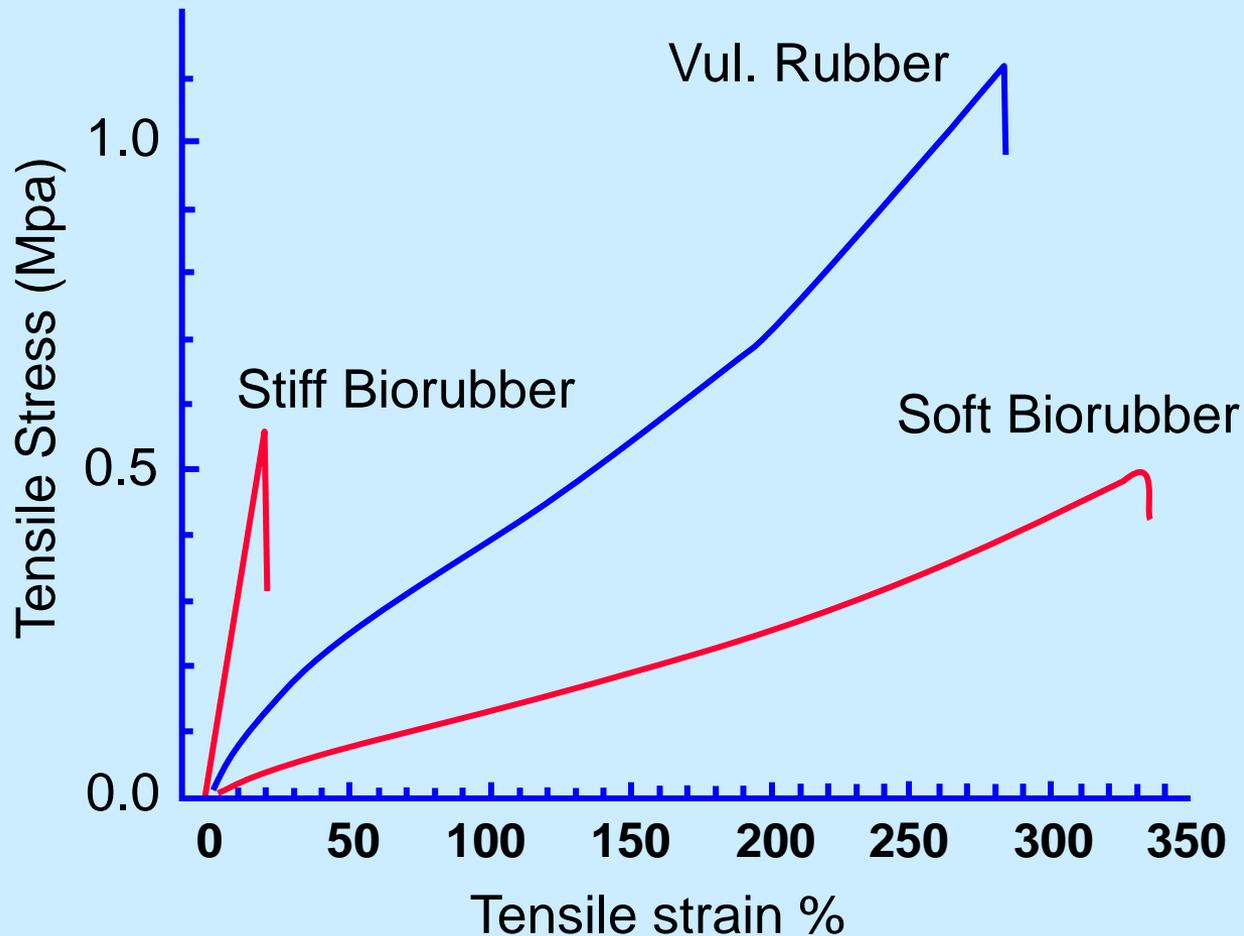


Cyclic elongation of biorubber



15 x 5 x 0.7 mm 500mm/min 100% strain 5 cycles

Mechanical properties - Elongation



E = 0.282 to 2.75 MPa

Dimension: 25x5x0.7 mm

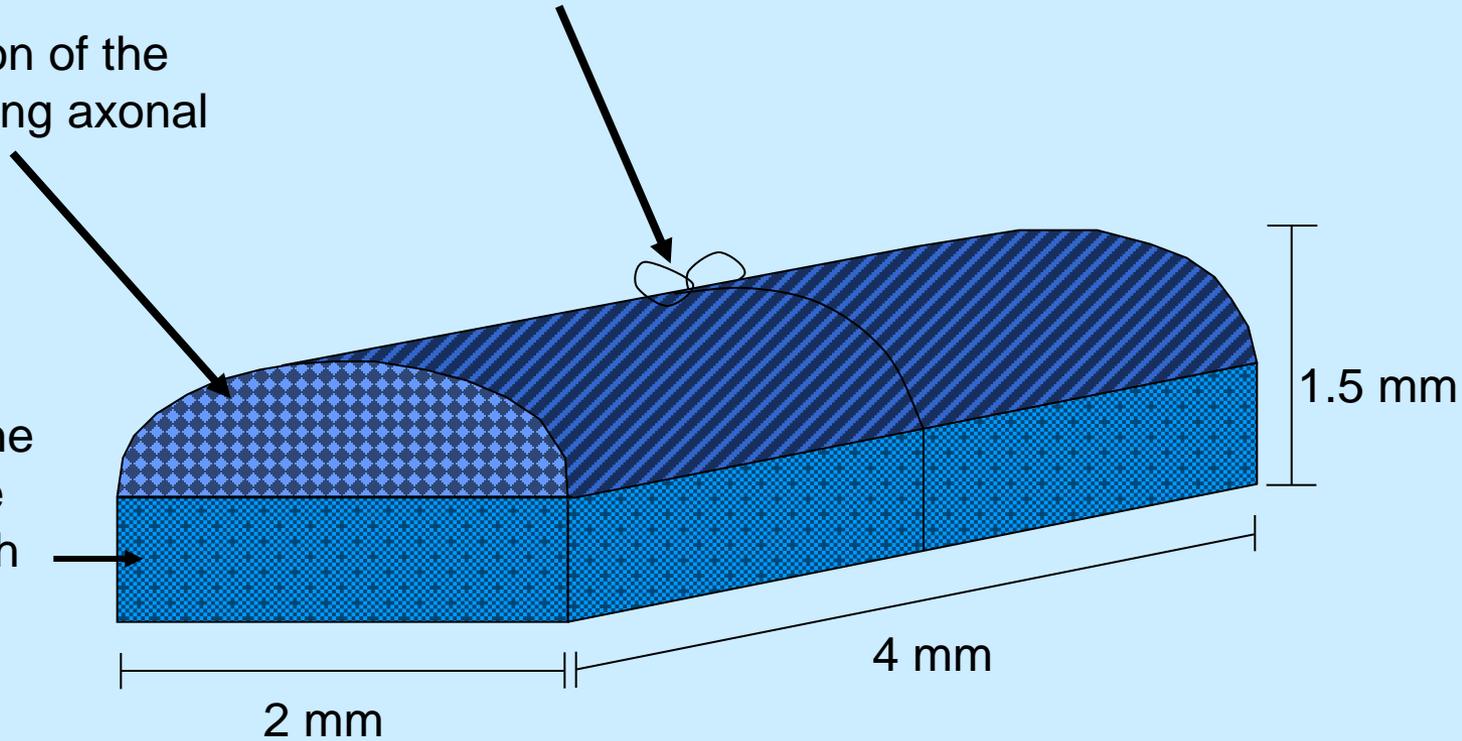
Deflection rate: 50 mm/min

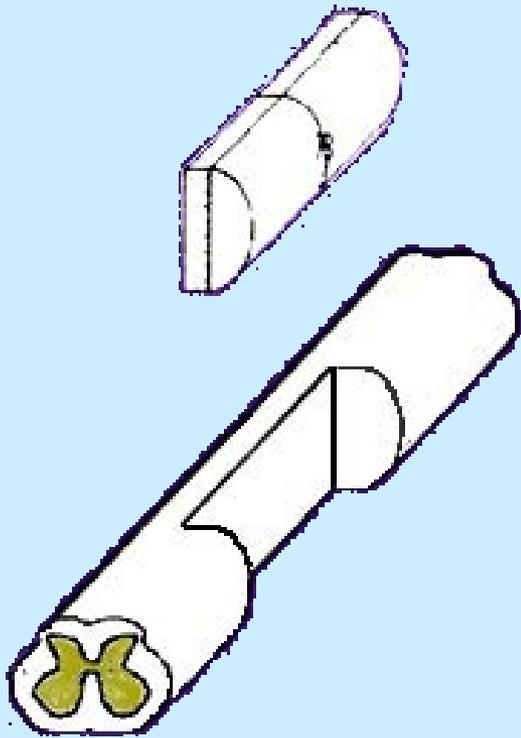
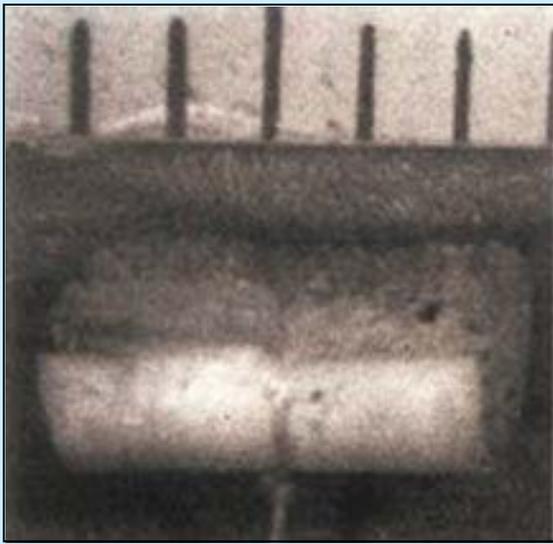
**Can polymer architecture
help?**

Degradable suture material tied to hold both parts of the implant together

Oriented portion of the implant providing axonal guidance

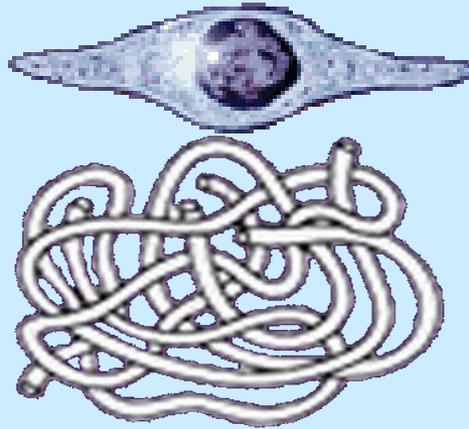
Inner portion of the implant with large pores seeded with neural stem cells





Materials can affect cell behavior

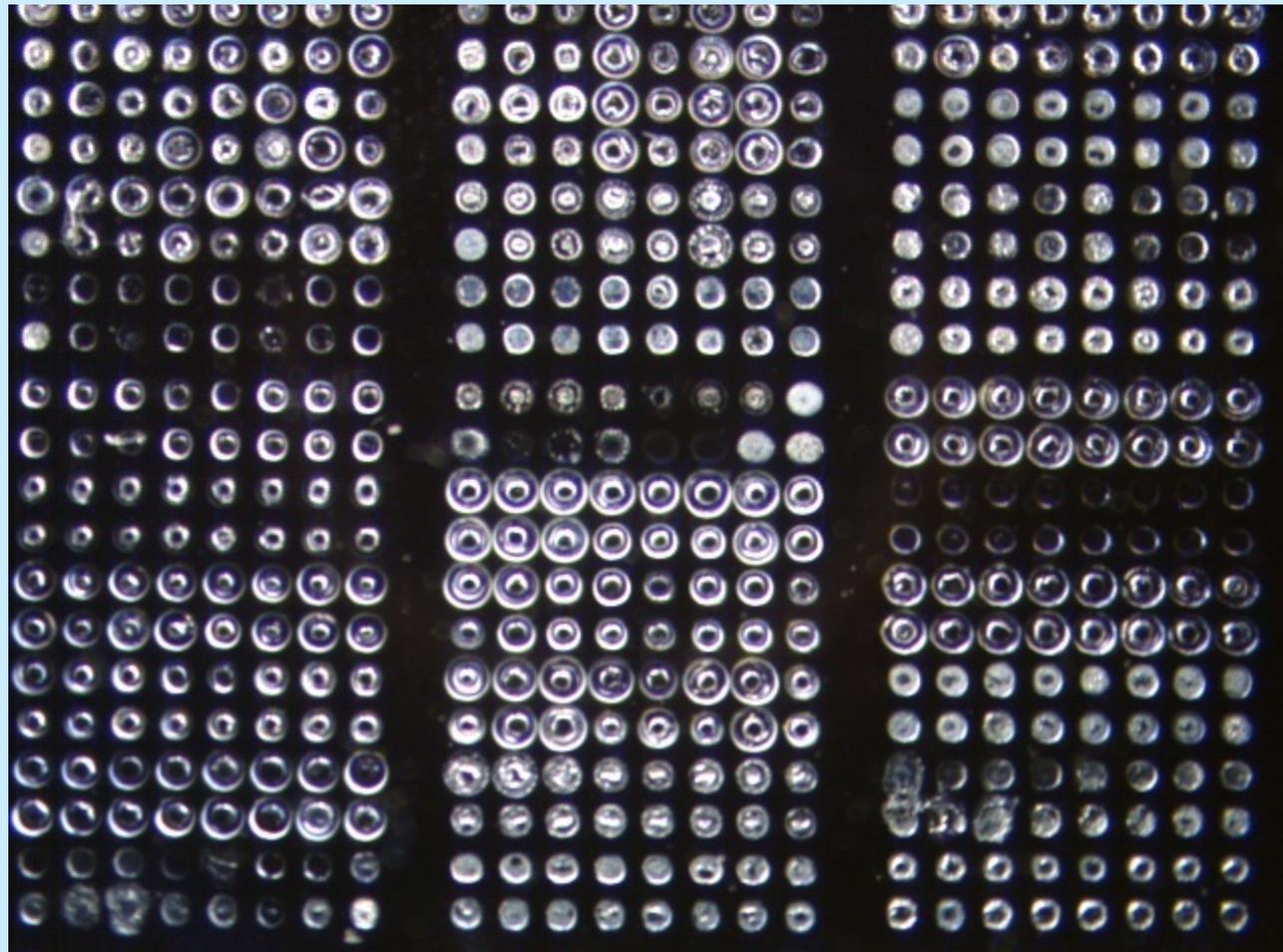
Soluble factors: Media, growth factors



Insoluble factors: ?

Can we identify polymers that can control cell behavior?

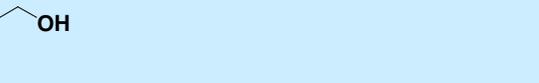
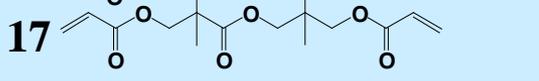
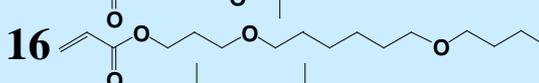
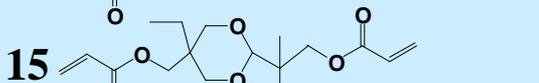
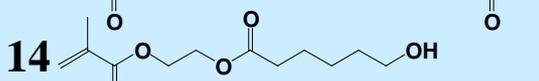
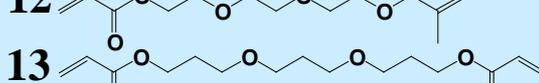
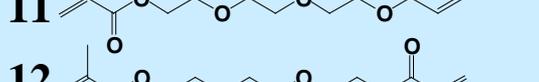
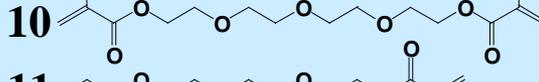
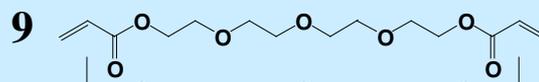
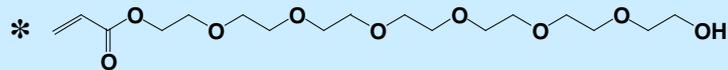
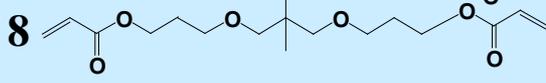
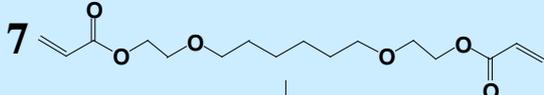
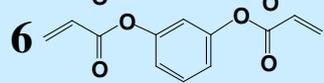
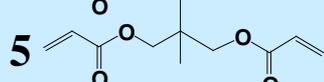
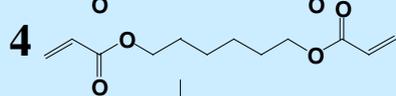
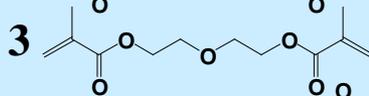
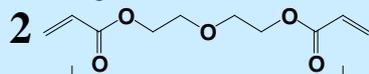
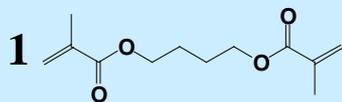
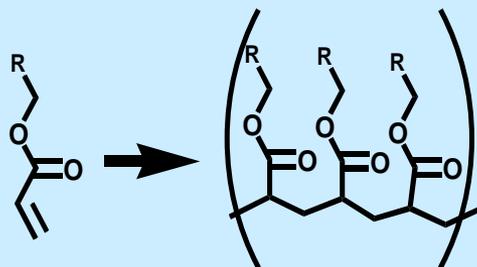
The solution: Microarrayed polymers



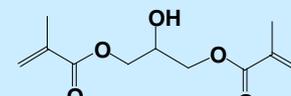
Design challenges for polymer microarrays:

- Synthesize large numbers of diverse materials in nanoliter volumes
- Attach materials to slide in a manner compatible with diverse materials and an aqueous environment
- Cell growth must be limited to polymer spots to be independent of neighbor effects
- Designed to allow simple, simultaneous assay of cellular markers

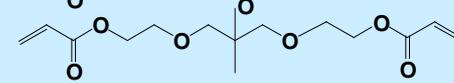
Chemical diversity through acrylate polymerization



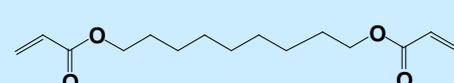
18



19



20



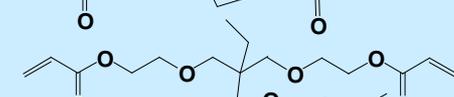
21



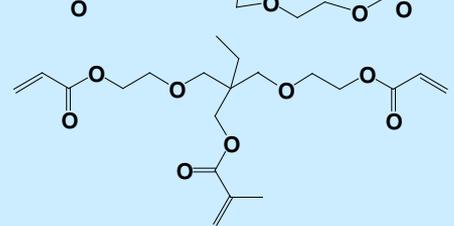
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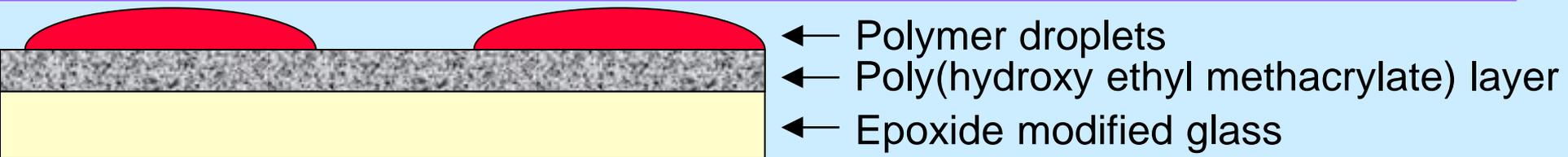
23



24

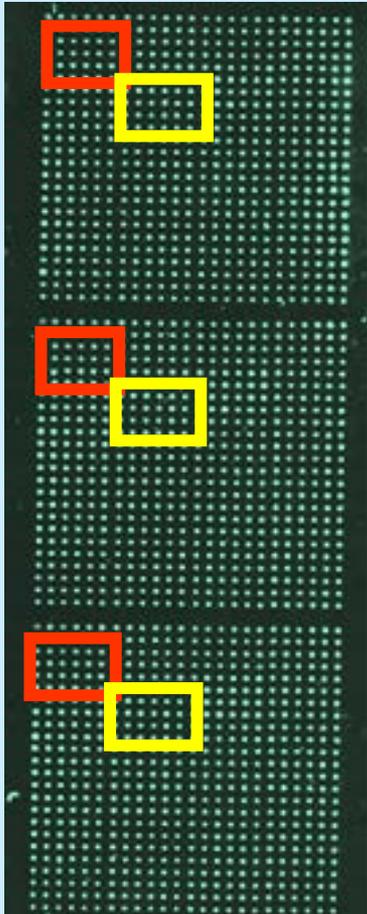


Design of a cell compatible microarray

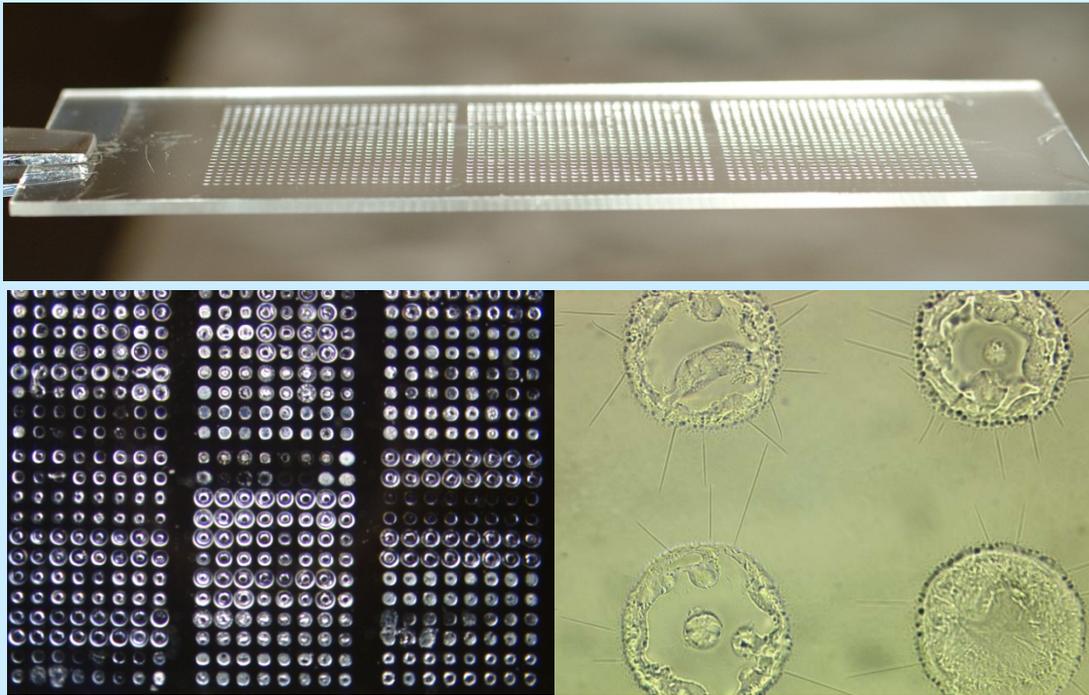


25 different monomers mixed pairwise at 70:30 v/v ratios

576 polymer spots in triplicate:
1728 individual polymer spots

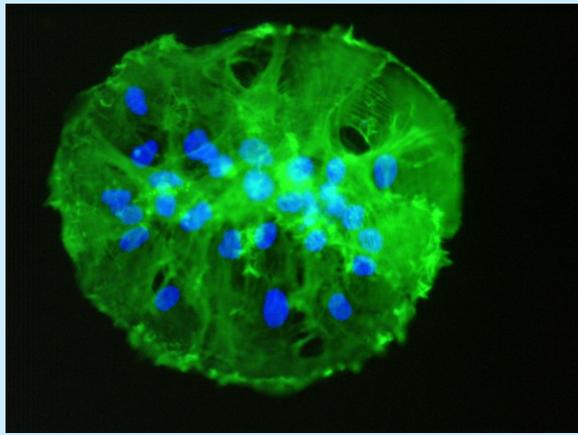


Cell compatible polymer micro arrays

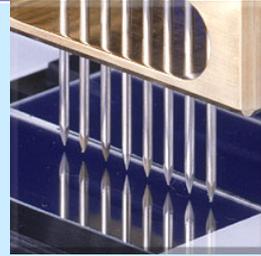
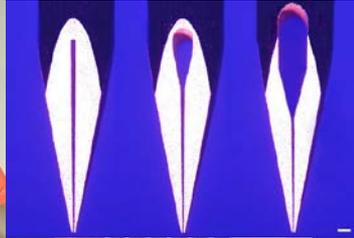


Print 1500-3500 individual polymer composites on a single slide in a cell compatible format

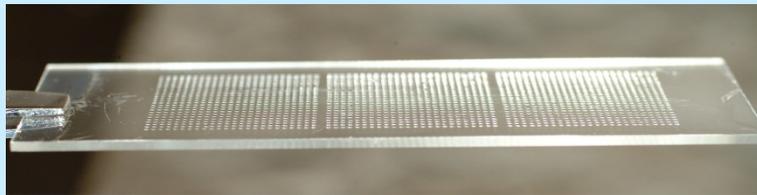
Cells can only grow on the polymers NOT in the spaces between them



Tens of thousands of experiments can be performed simultaneously



20 slides with 1500-3500 individual spots can be synthesized in a single day



+

media, growth factors, etc.

+

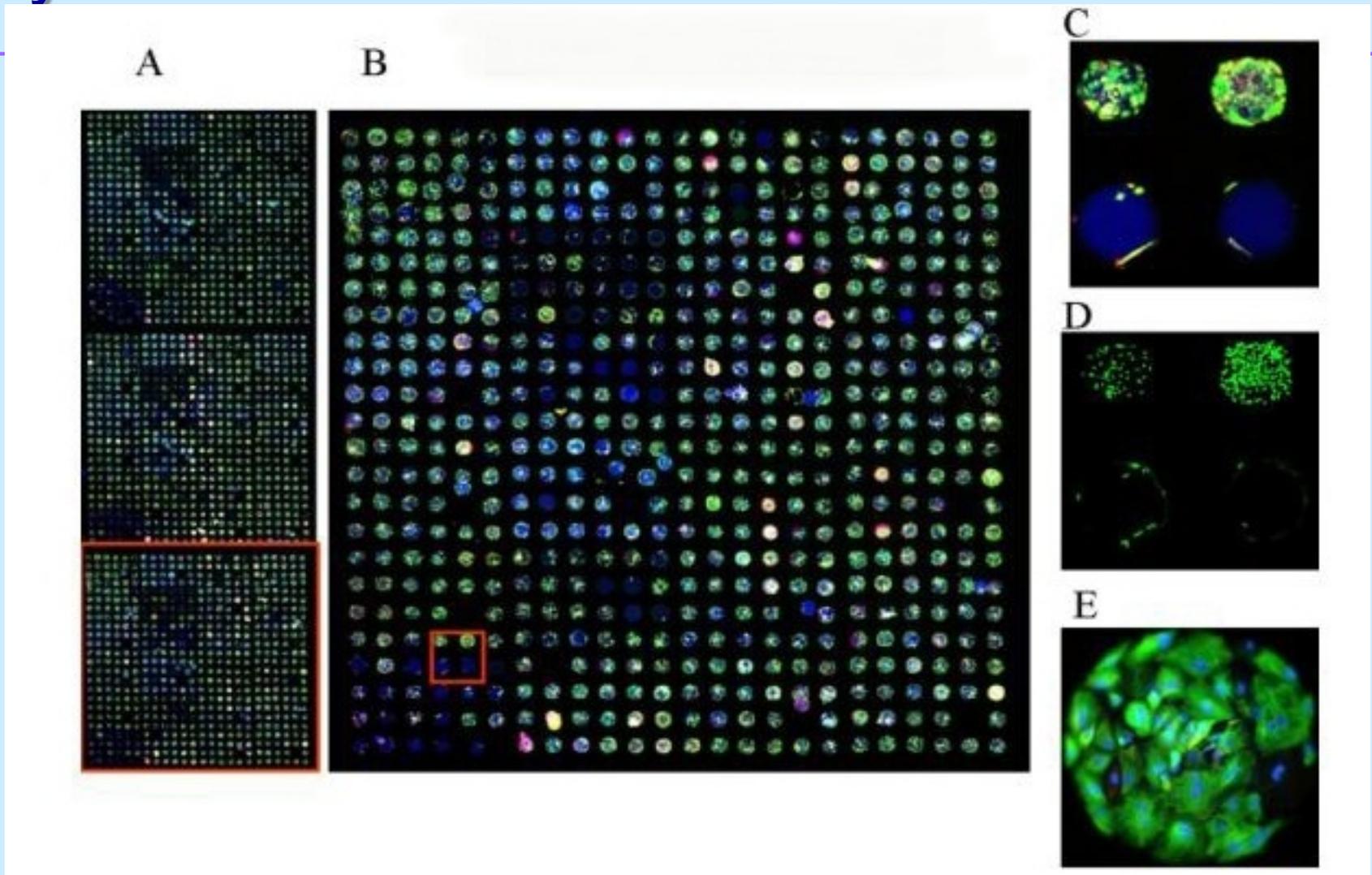
different time periods

All 20 slides (or more) can be seeded with cells and examined with different media, cells or at different time points

Example

Can we convert human embryonic stem cells to epithelial cells?

Rapid synthesis and characterization of cell-polymer interactions

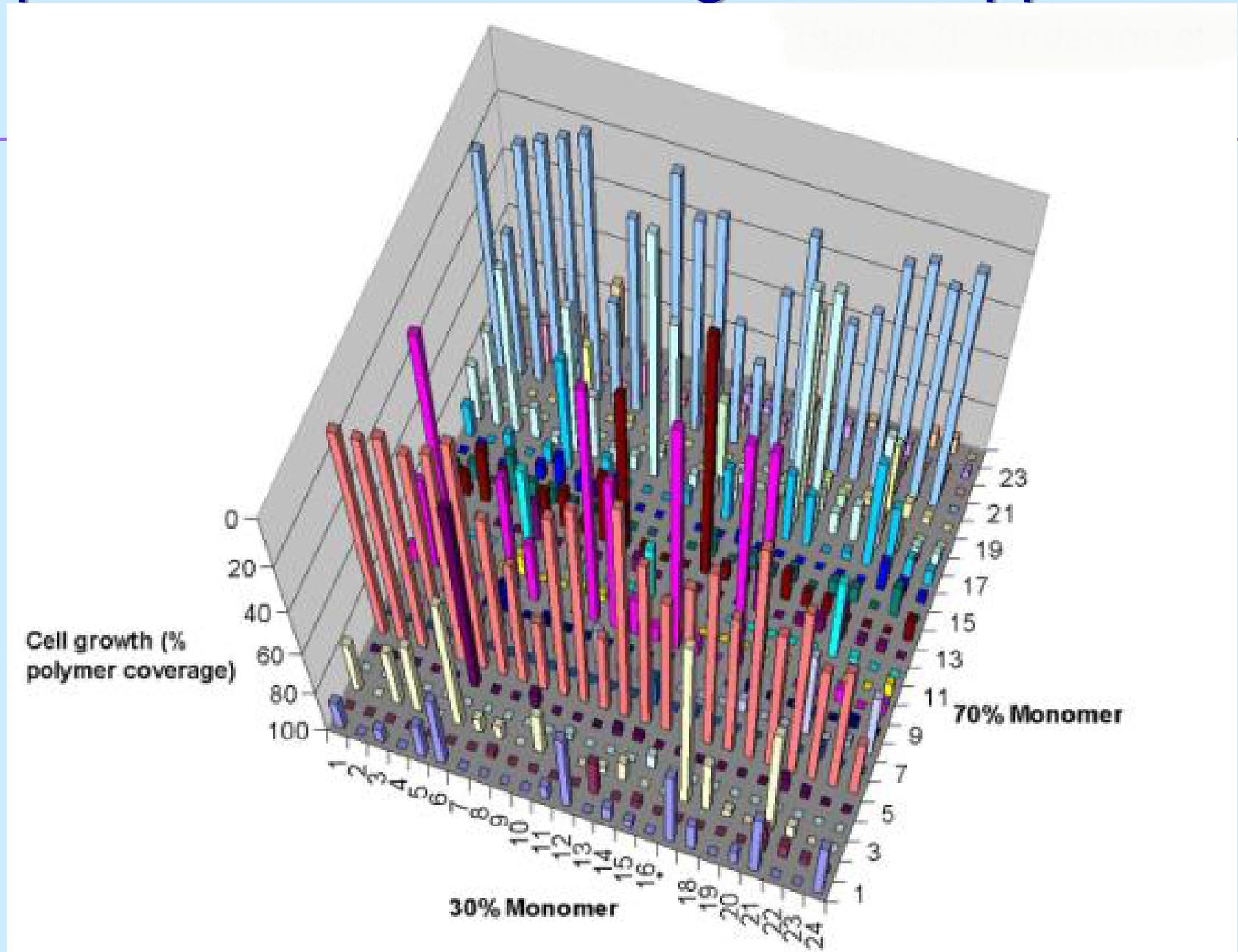


Green: Cytokeratin

Red/purple: Vimentin

Blue: Polymer

Rapid characterization of growth support



Multiple experiments in multiple conditions

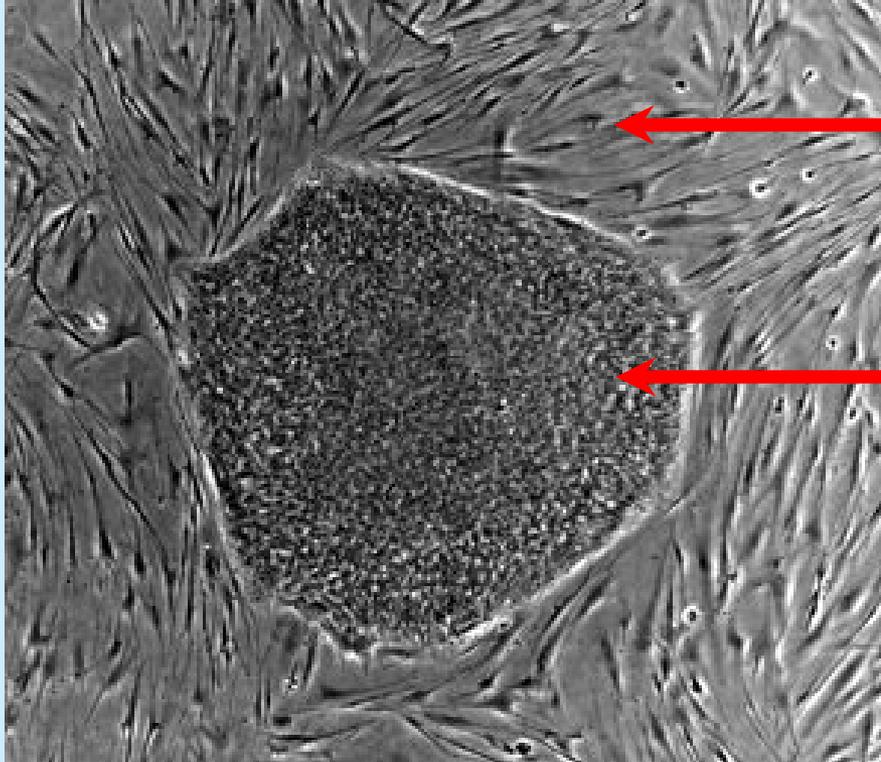
Monomer Composition	RA Day 6	Day 6 24hr RA Pulse	Day 6 No RA	RA Day 1	Day 1 No RA
100% 1					
70% 1, 30% *					
100% 3					
70% 3, 30% 1					
70% 3, 30% 18					
70% 3, 30% 21					
100% 6					
100% 13					
100% 7					
70% 7, 30% 4					
70% 7, 30% *					
100% 11					
70% 11, 30% 1					
70% 11, 30% 21					
100% 12					
70% 12, 30% 3					
70% 12, 30% 21					
100% 18					
70% 18, 30% *					
70% 18, 30% 13					
100% 21					
100% 23					
70% 23, 30% 1					
70% 23, 30% 21					

Polymers that support/inhibit growth of hES cells

Polymers that support growth only in certain media

Polymers that support growth of certain cell types

The present state-of-the-art: hPSCs cultured on MEFs



Mouse embryo
fibroblasts (MEFs)

hPSCs

hPSCs are currently passaged as small clumps of cells.

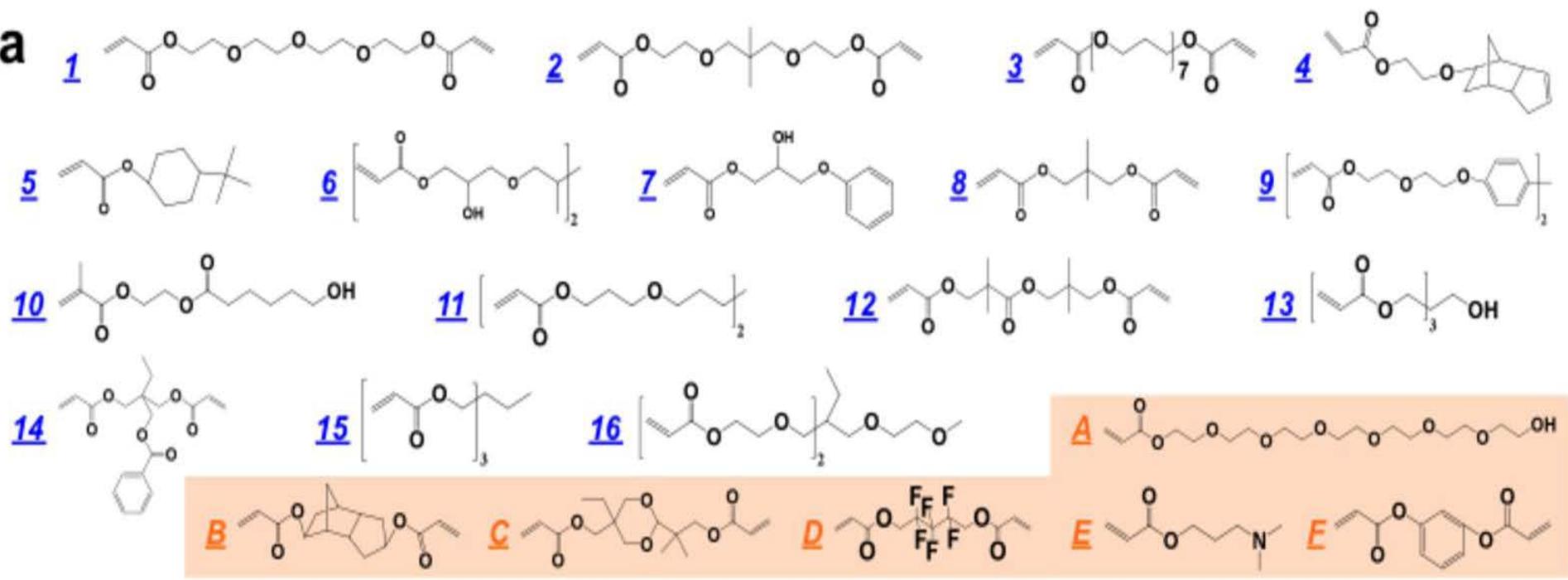
It can be challenging to genetically engineer hPSCs.

Maintenance on mouse embryo fibroblast feeder layers

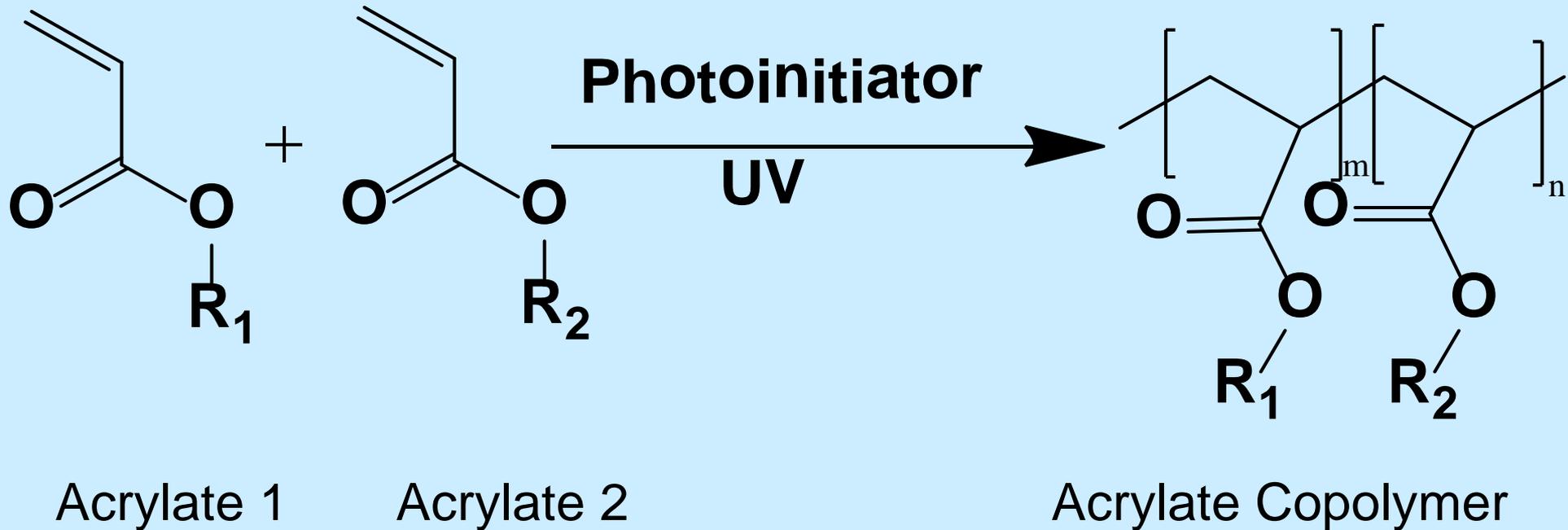
- Production of MEF is laborious and this limits large scale production of hESC's
- Animal pathogen and animal immunologic protein contamination

Feeder free substrates; (ECM)/serum proteins

- Don't support efficient growth (i.e., less than 10%) of hESC's from fully dissociated cells
- Don't support long term growth
- Don't support clonal growth of single human cells

a

Polymerization scheme



Copolymerization between acrylate monomers enables us to rapidly construct polymer libraries with diversified properties.

Hits

- Screened for SSEA4 and OCT4
- Monomer 9, copolymer with monomer A shows comparable efficiency to MEF
- Does not correlate with surface roughness (AFM), hydrophobicity, elastic modulus

Hits (cont.)

- Correlates with surface structures with hydrocarbon ions ($C_2H_3^+$, $C_3H_3^+$), oxygen containing ions from esters (CHO_2^- , $C_3H_3O^+$, $C_2H_3O^+$) and ions from cyclic structures (C_6H^- , C_4H^- , C_2H^-)
- After 10 passages – full pluripotent potential as judged by multiple hESC markers (Tlal-60, Nanog, Oct4, Sox2, SSEA4) karyotype, and gene expression (into all 3 germ lineages)
- Ultimate system is chemically defined, xeno-free, feeder free