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Lecture 22
Tissue Engineering

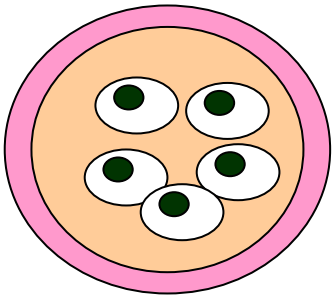
Tissue Engineering: a field that seeks to replace, repair or enhance biological function at the scale of a tissue or organ by manipulating cells via their extra-cellular environment

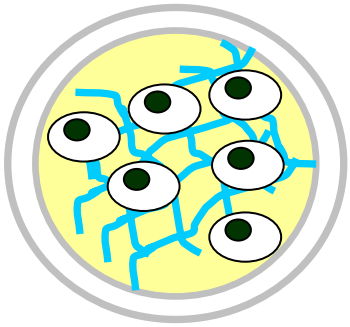
Objectives:

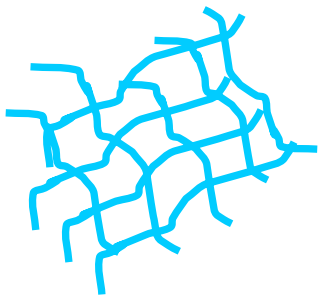
1. Fulfill a biomechanical role (bone, cartilage)
2. Replace physiological function (liver, nerve)
3. Deliver secretory products (insulin)
4. A combination of the above

3 Main Approaches:

1. Extracorpeal/cell encapsulation (3)
2. *In vitro* synthesis (1-4)
3. *In vivo* synthesis (1-4)



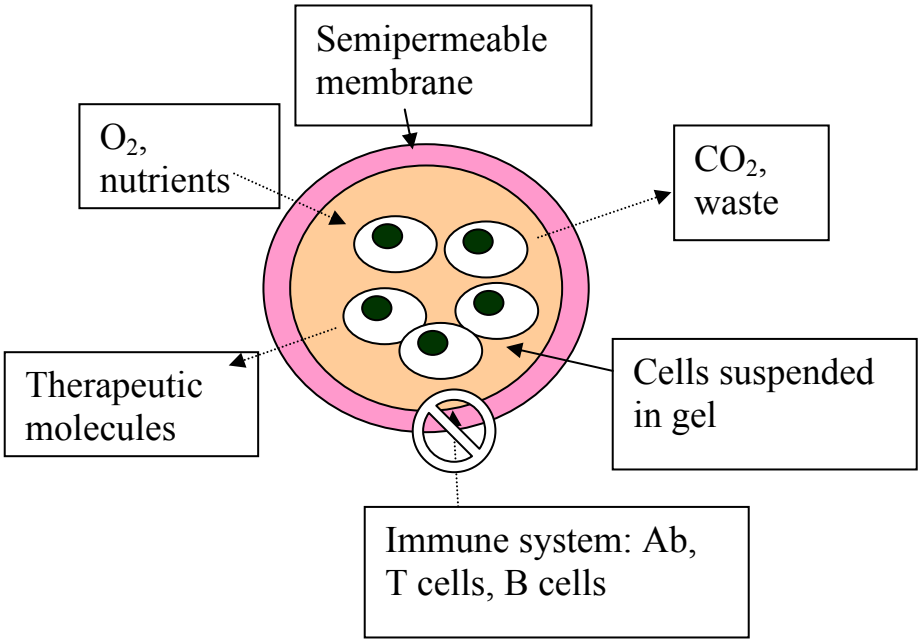




1. Extracorpeal/Cell Encapsulation

Method:

1. Encapsulate cells of interest in semipermeable membrane
2. Implant encapsulated device or connect *ex vivo*
3. Cells secrete product ⇒ therapy
4. Remove/disconnect device when therapy concluded



Advantages:

- Natural theraputic response from living cells
- Use of nonhost cells—immunoisolation

Issues:

- Potential for undesirable immune response from adsorption of complement proteins (similar to blood filtration membranes)
- Potential for thrombosis formation
  (Anti-coagulants used during *ex vivo* treatment)
- Potential for rupture of implanted devices

Applications Investigated:

- Diabetes treatment*
- Chronic pain*
- Neurodegenerative diseases: ALS (Lou Gehrig's disease, neuromuscular), Parkinson's, Alzheimer's, Huntington's disease (progressive brain death)
- Dwarfism
- Anemia/Hemophelia
- Macular degeneration (blindness)
- Cancer
- Liver Failure*

* = clinical trials

Device Examples

Encapsulated Islets (Islet Technology Inc., St Paul, MN)
CapCell: implantable membrane-encapsulated islets for glucose regulation

Cells: insulin-producing islets
Use: long-term treatment of diabetes
Device: alginate-based membrane confines islets
Treatment: islets transplanted into patient's pancreas; patients' blood flows thru membrane; islets detect glucose level variations & respond through insulin production
Status: preclinical trials (islet transplantation in clinical trials)

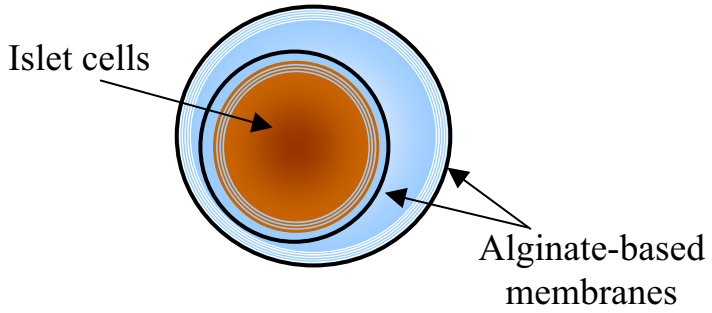


Figure by MIT OCW.

Arbios Systems, Inc. (recently acquired from Circe Biomedical)

HepatAssist System: an extracorporeal, bioartificial liver support system

Cells: primary porcine hepatocytes (pig liver cells)
Use: temporary liver function for transplant candidates
Device: hollow fiber bioreactor, oxygenator, pump
Treatment: plasma circulated through bioreactor and recombined with blood cells
Status: Phase I trials completed

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2. *In vitro* Synthesis

Method:

1. Cells seeded *in vitro* on scaffold device
2. Cells maintained in culture to expand population & develop tissue organization (in static culture or bioreactors)
3. Device implanted once cell colony is established
4. Device degrades, scaffold replaced by remodeled tissue

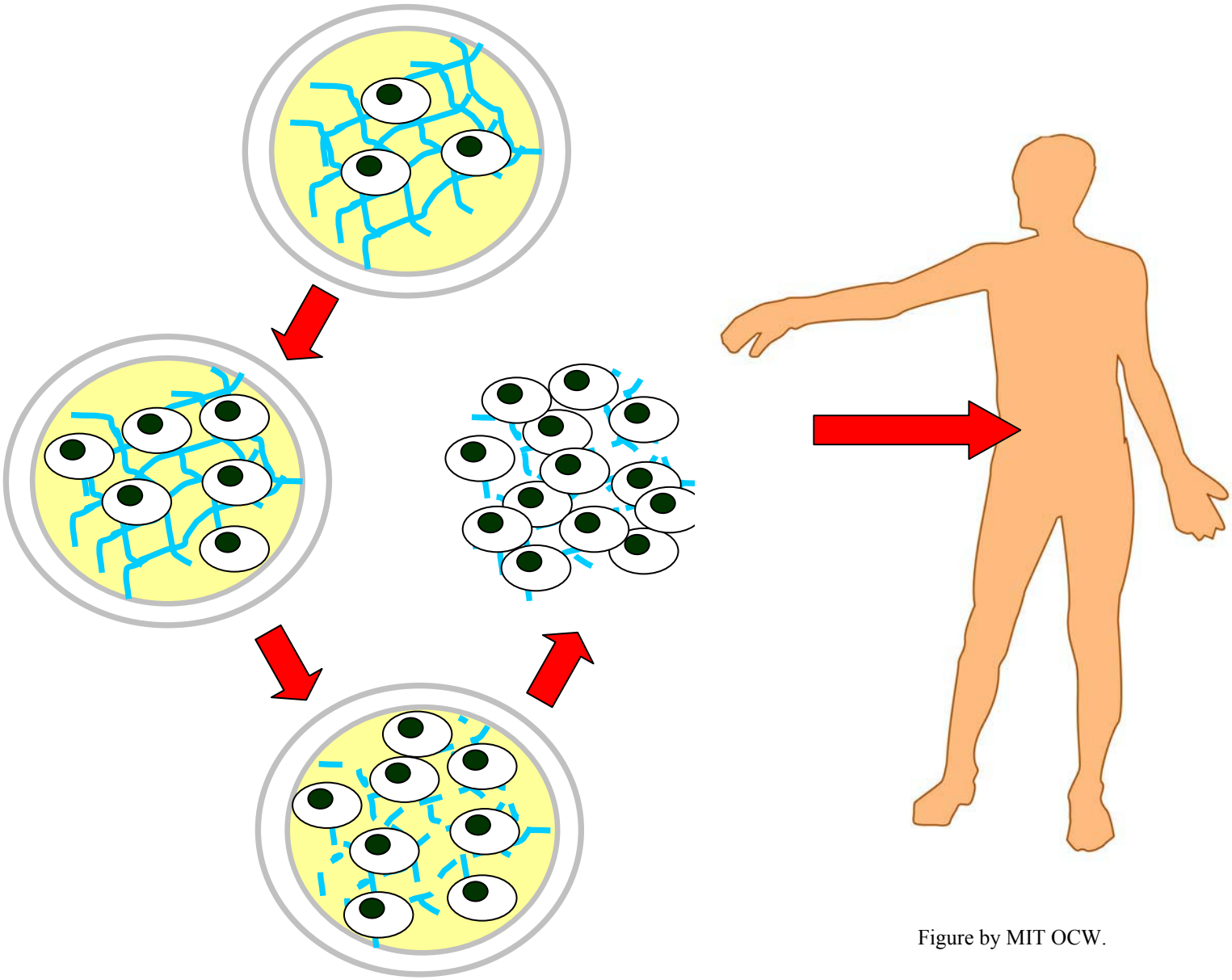


Figure by MIT OCW.

Advantages:

- Natural theraputic response from living tissues
- Permanent therapy
- Allows control and quantification not easily obtained *in vivo*

Issues:

- Cell sources
  - possibility of rejection
  - tumorogenicity—cell lines
- Full organ restoration challenges (e.g., skin)

Applications Investigated:

- Vasculature (resorbable & nonresorbable)
- Liver tissue
- Nerve tissue
- Cartilage*
- Cornea*
- Bladder*
- Skin*
- Bone
- Ligament
- Tendon
- Muscle
- Heart valve
- Heart

Link to list of websites of tissue engineering companies:

http://www.cs.cmu.edu/~webwatch/text_only_industry.html

3. *In vivo* synthesis

Method:

1. Implant porous scaffold device
2. Cellular ingrowth *in vivo*
3. Scaffold replaced by remodeled tissue

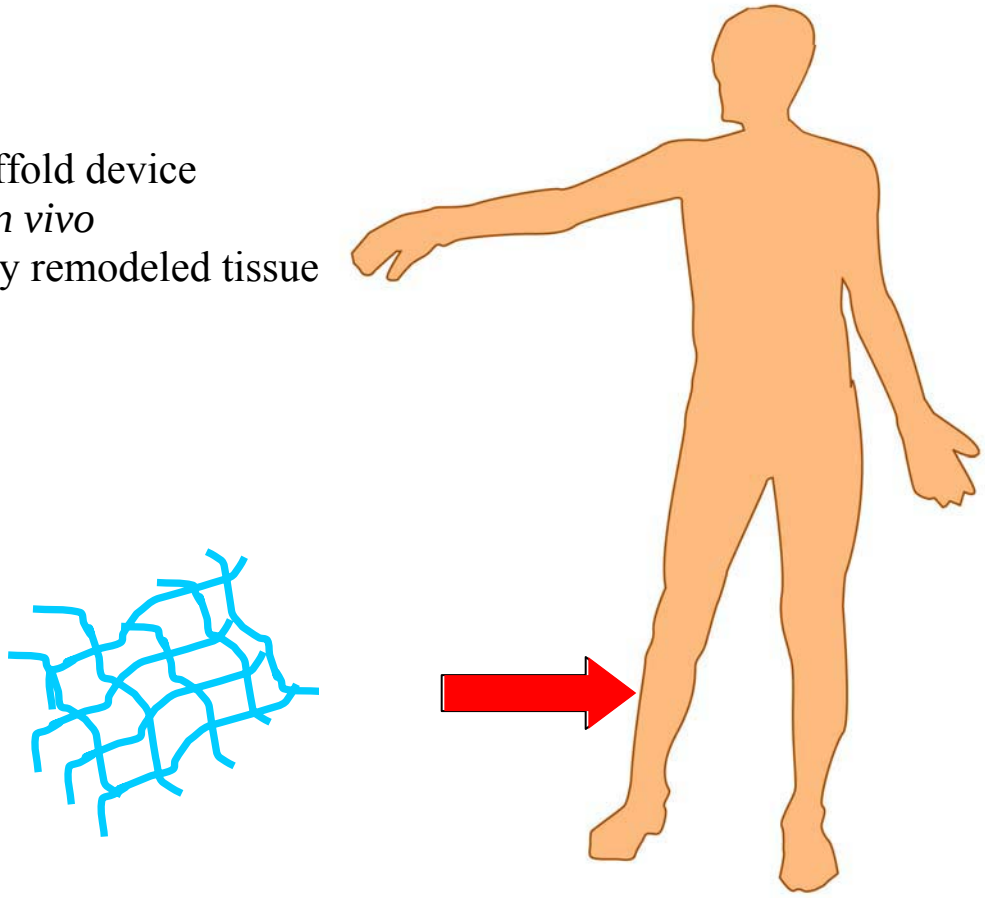


Figure by MIT OCW.

Advantages:

- Natural theraputic response from living tissues
- Permanent therapy
- No cell source problems

Issues:

- Uncontrolled biological response to implanted scaffold

Applications Investigated:

- Vasculature
- Skin*
- Bone*
- Nerve
- Ligament
- Cartilage (Knee Meniscus)

Scaffolds for Tissue Generation

Purpose: replace functions of extracellular matrix (ECM)

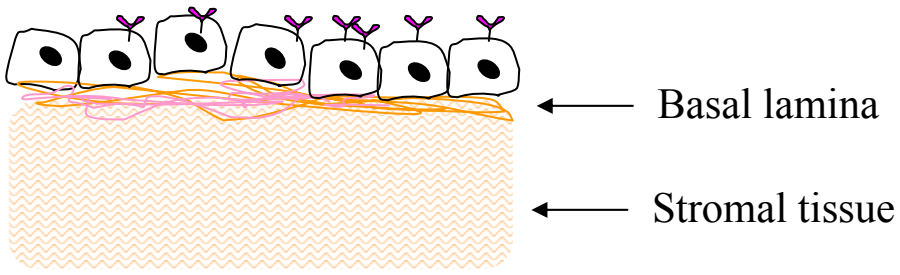
ECM functions:

1. cell anchorage
2. cell orientation
3. cell growth
4. mechanical integrity to neo-tissue
5. tissue microenvironment
6. cell differentiation
7. sequester, store & present soluble regulatory proteins
8. blueprint for tissue organization (e.g., biomineralized tissue)

ECM types:

Basal Lamina (basement membrane): directly underlying epithelial cells; contains laminin, collagen, fibronectin, vitronectin

Stromal tissue (interstitial matrix): provides structural integrity; contains matrix-secreting cells (fibroblasts, osteoblasts), collagen, elastin, fibrillin, fibronectin, vitronectin, GAGs, glycoproteins, regulatory proteins



Resorbable Tissue Engineering Scaffolds:

1. Collagen-matrix
   e.g., artificial skin
   drawback: immunogenic

2. Biodegradable polymers: PLA, PGA, PLGA
   e.g., cartilage
   drawback: no adhesion sites (can build in RGD)

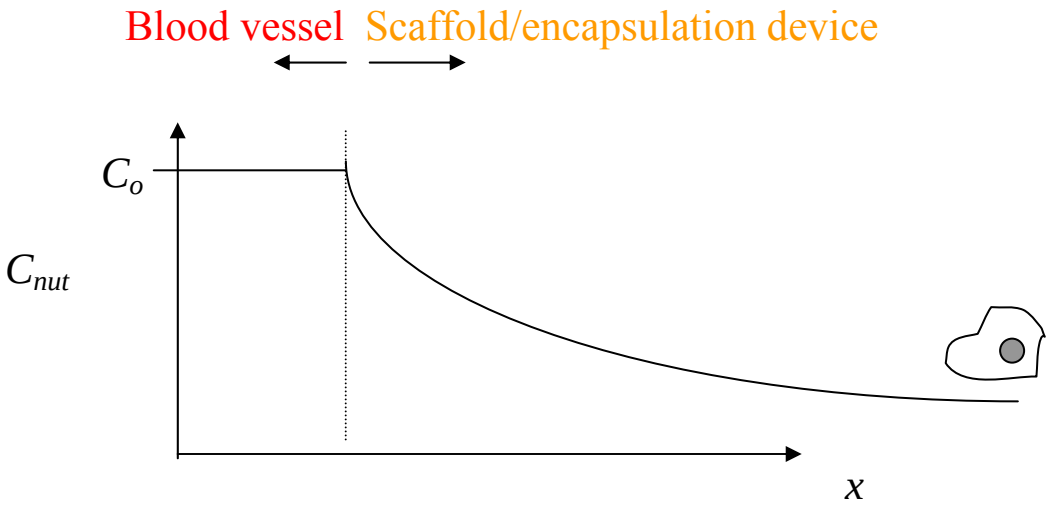
3. Hydroxyapatite, Bioglass
   e.g., bone regeneration
   drawback: brittle, low strength

Processing of Tissue Engineering Devices

A. Design Issues

1. Cell density
   must be sufficiently high to enable tissue formation, deliver therapy

2. Transport of nutrients/oxygen/waste
   nutrients must reach cells within the scaffold/encapsulation device



Limiting distance from nutrients can be gauged from the *Thiele modulus, S* (dimensionless ratio of consumption to supply)

$$S = \frac{k\rho x^2}{DC_o}$$

D = nutrient diffusivity in device (cm^2/sec)
C_o = nutrient concentration at source (mol/cm^3)
k = cell nutrient uptake rate constant ($mol/sec/cell$)
x = distance from nutrient source (bloodstream) (cm)
ρ = cell density in device ($cells/cm^3$)

$S>>1 \Rightarrow$ cells consume more than can be delivered
$S<<1 \Rightarrow$ supply greater than demand
$S = 1 \Rightarrow$ supply balances demand; use as limit estimate for device design

*A **rule of thumb** in designing tissue engineering devices:* $\mathbf{x_{max}}$**= 500 μm.**

3. Mechanical support

- Critical problem for hard tissue scaffolds

- Influenced by
  - materials choices
  - processing (orientation of polymers & composites)

4. Tissue organization blueprint

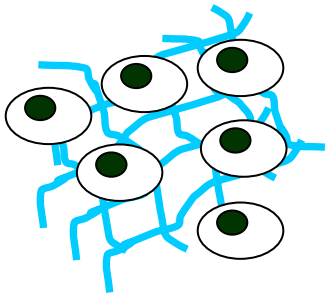
Cell migration guidance
chemical & morphology effects (chemo/hapto/durotaxis)

Cell Patterning
microcontact printing, microlithography

Spatial Organization of Muliple Cell Types
- most organs of more than one cell type
- pattern based on different ligands, ligand densities, ligand affinities

B. Scaffold Fabrication

Objective: Continuous, high-surface area scaffolds



1. Fabrics

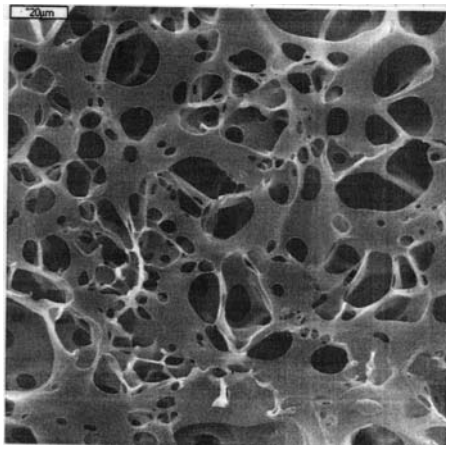
- Woven/nonwoven fibers
- Mechanical interlocking ⇒ pliable, 3D matrix
- Porosity and pore size roughly controlled

2. Bonded fibers

- PGA fibers dipped in PLLA/CH_2Cl_2 solution
- Heat treat fibers at $T_{g,PGA} < T < T_{m,PLLA}$ to bond PGA to PGA
- Dissolve away PLLA
- Improved mechanical properties over fabrics; similar porosity

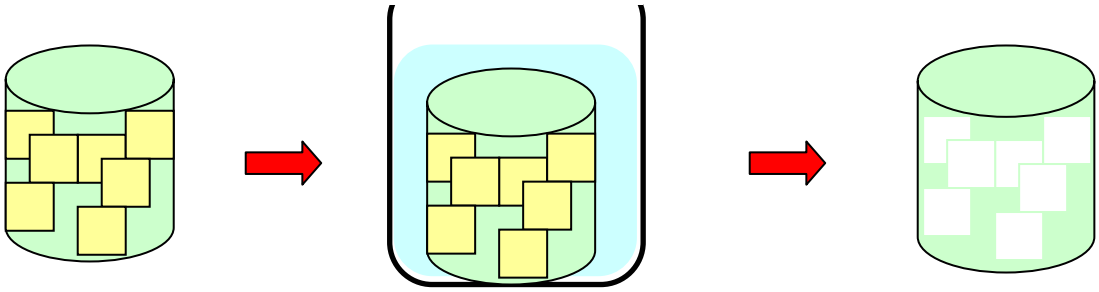
3. Freeze-dried Foams

- Polymer solution immersed in liquid $N_2 \Rightarrow$ phase separation
- Frozen solvent sublimates leaving porous scaffold
- Pore size ~ λ of spinodal decomposition $\Rightarrow$ controlled pore structure



4. Salt-leached Foams

- polymer solution mixed with uniform salt crystals
- Solvent evaporates leaving solid polymer/salt composite
- Immerse in H_2O to leach out salt
- Controlled porosities up to 93% (< 2 mm thick)



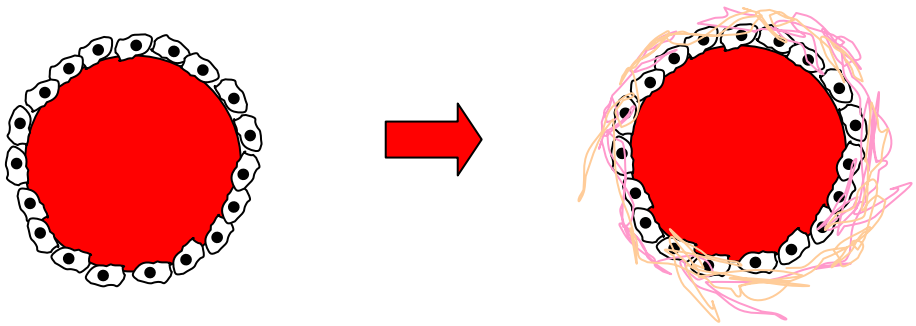
5. 3D Printing

- Cast a bed of polymer powder (e.g., PLGA)
- "Print" micron-sized droplets of solvent at desired points (chloroform)
- Congealed powder solidifies as solvent evaporates
- Repeat process, building up 3D structure
- Shake out uncongealed powder
- Precisely structured micron-porous polymer or ceramic scaffolds

C. Encapsulation Methods

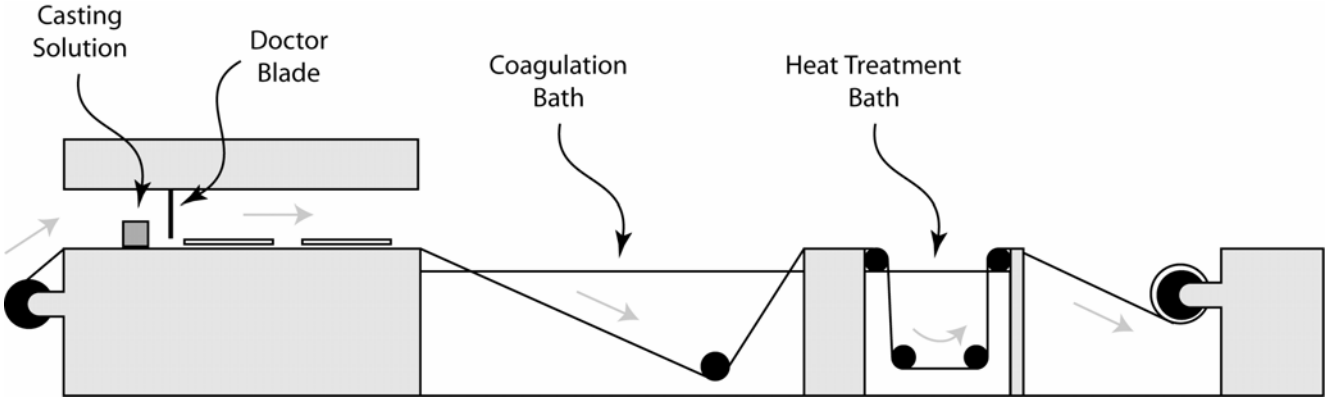
1. Encapsulation Microspheres

- Cells attach to surface of polymer microspheres
- Cell-coated spheres suspended in weak polycation (polylysine $-NH_3^+$)
- Add polyanion (e.g. sodium alginate, $-COO^-$)
- Polyelectrolytes form precipitated, porous complex around cells (Complex coacervation)
- Single microbeads contain a few hundred cells (thousands needed for therapy)



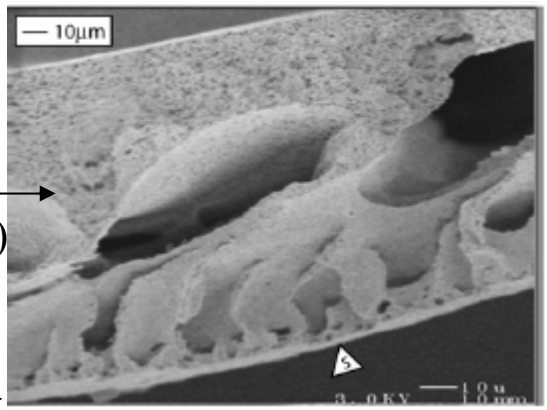
2. Encapsulation Membranes

- Cast concentrated solution onto substrate (flat or tubular)
- Substrate immersed into a nonsolvent bath
- Coagulation of asymmetric membrane results

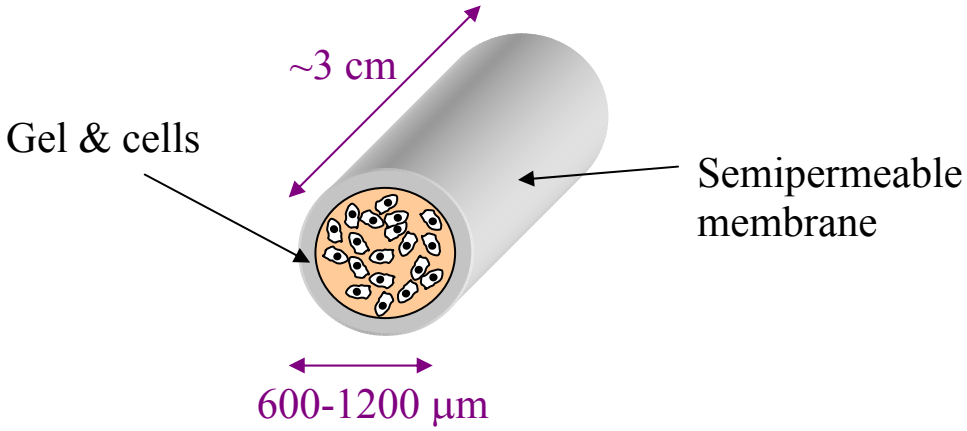


Microporous substructure
(mechanical rigidity, high flux)

Dense surface layer (bath side)
(semi-permeable)



- Cells suspended in gel within sealed membrane tube (length ~ 3 cm) or disk (dia ~ 2-3 cm)



- Membrane characteristics

  - Molecular weight cutoff: typically ~30-70 kg/mol (<100 nm dia. pores)

Note: Ab ~150 kg/mol

Molecule/Moiety	**Size**
O_2, H_2O, salts	2-3 Å
Lipids, glucose	10 Å
Serum proteins, endotoxins	100 Å
Viruses	1000 Å
Bacteria	10^4 Å
White blood cells, platelets	10^5 Å

  - Matrix examples: polysaccharides, alginate/chitosan coacervate, collagen

  - Body: PAN-PVC, PP, polycarbonate, cellulose nitrate, acrylic

  - Shape & Size: disks vs. tubes

	Disks	Tubes
Mass transport	Favored	diameter restrictions
Susceptibility to clotting	high surface area increases clot propensity	favored
Cell #	50-100M	5M

	Cell # required
Diabetes	10^9
Clotting factor	10^7-10^8
CNS therapies	10^6-10^7

D. Current Challenges

1. Micromechanical effects
   Cell differentiation and growth (especially in load-bearing tissues) can be affected by micromechanical stresses transmitted by the scaffold

2. Cell function deterioration

3. Cross-application to other areas (gene therapy, drug delivery)

4. Multicellular tissues and organs
   - Complex, multicomponent structures (vascularized tissues)
   - Regeneration-inducing factors (proteins) only known for blood & bone

Basic Tissue Cell Types and Functions

Cell type	Tissue Function	Example
epithelial	covers external (ex, skin) & internal (ex, intestine, blood vessel) organ surfaces	endothelial cells
connective	supports other body tissues; houses nerves & blood vessels	fibroblasts (ECM generation), cartilage, bone
muscle	specialized for contraction;	smooth, skeletal, cardiac
nerve	generate electrical signals & secrete neurotransmitters	brain cells, peripheral nerve

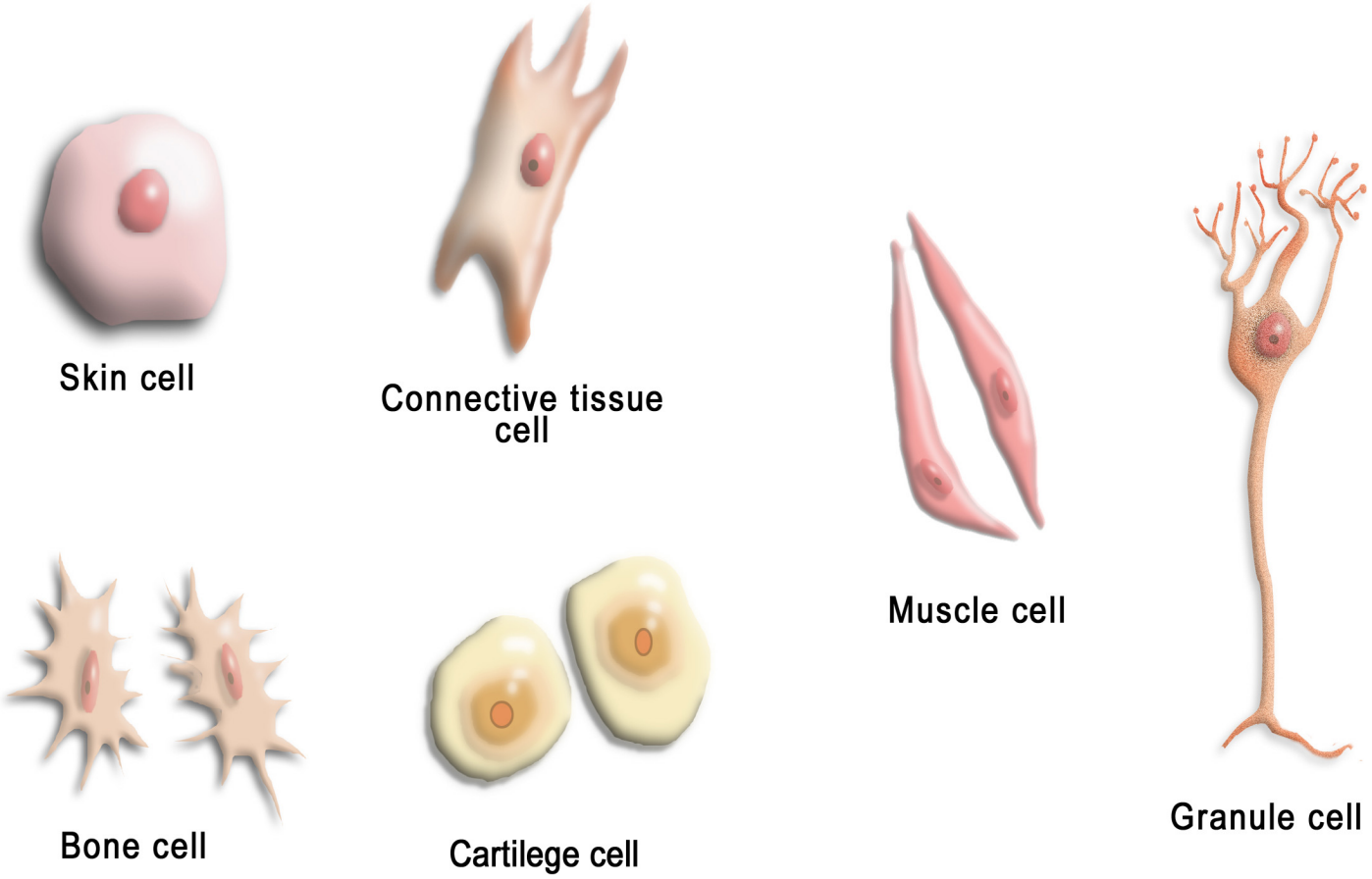


Figure by MIT OCW.

Cell Regeneration Capability

Category	Normal replic. rate	Response to injury	Examples
renewing/ labile	High; via stem cell differentiation	modest ↑	skin, intenstinal mucosa, bone marrow
Expanding/ stable	Low	large ↑	endothelium, fibroblasts, hepatocytes, osteoblasts
Static/ permanent	None	No replication; replaced by scar tissue	heart muscle cells, nerve cells