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 extracellular 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. Extracorporeal/cell encapsulation (3)
2. *In vitro* synthesis (1-4)
3. *In vivo* synthesis (1-4)
1. Extracorporeal/Cell Encapsulation

Method:
1. Encapsulate cells of interest in semipermeable membrane
2. Implant encapsulated device or connect \textit{ex vivo}
3. Cells secrete product $\Rightarrow$ therapy
4. Remove/disconnect device when therapy concluded

\begin{tikzpicture}
  \node[draw] (seal) {Semipermeable membrane};
  \node[draw, below=1cm of seal] (o2) {$O_2$, nutrients};
  \node[draw, right=1cm of o2] (co2) {CO$_2$, waste};
  \node[draw, below=1cm of co2] (gel) {Cells suspended in gel};
  \node[draw, above=1cm of o2] (ther) {Therapeutic molecules};
  \node[draw, below=1cm of ther] (imm) {Immune system: Ab, T cells, B cells};
  \draw[->] (o2) -- (seal);
  \draw[->] (seal) -- (co2);
  \draw[->] (seal) -- (gel);
  \draw[->] (seal) -- (ther);
  \draw[->] (seal) -- (imm);
\end{tikzpicture}

Advantages:
- Natural therapeutic 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 \textit{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

Figure removed for copyright reasons.
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
Advantages:
- Natural therapeutic response from living tissues
- Permanent therapy
- Allows control and quantification not easily obtained in vivo

Issues:
- Cell sources
  - possibility of rejection
  - tumorigenicity—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

*Advantages:*
- Natural therapeutic 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)

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

- $D$ = 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)
- $\rho$ = cell density in device (cells/cm$^3$)

$S \gg 1 \Rightarrow$ cells consume more than can be delivered
$S \ll 1 \Rightarrow$ 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*: $x_{max} = 500 \ \mu$m.
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 Multiple 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/CH₂Cl₂ 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 N₂ ⇒ phase separation
- Frozen solvent sublimates leaving porous scaffold
- Pore size ~ λ of spinodal decomposition ⇒ controlled pore structure

4. Salt-leached Foams

- Polymer solution mixed with uniform salt crystals
- Solvent evaporates leaving solid polymer/salt composite
- Immerse in H₂O 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

![3D Printing Diagram]

C. Encapsulation Methods

1. Encapsulation Microspheres

- Cells attach to surface of polymer microspheres
- Cell-coated spheres suspended in weak polycation (polylysine –NH₃⁺)
- 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)

![Encapsulation Microspheres Diagram]
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

<table>
<thead>
<tr>
<th>Molecule/Moiety</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$, H$_2$O, salts</td>
<td>2-3 Å</td>
</tr>
<tr>
<td>Lipids, glucose</td>
<td>10 Å</td>
</tr>
<tr>
<td>Serum proteins, endotoxins</td>
<td>100 Å</td>
</tr>
<tr>
<td>Viruses</td>
<td>1000 Å</td>
</tr>
<tr>
<td>Bacteria</td>
<td>$10^4$ Å</td>
</tr>
<tr>
<td>White blood cells, platelets</td>
<td>$10^5$ Å</td>
</tr>
</tbody>
</table>

- Matrix examples: polysaccharides, alginate/chitosan coacervate, collagen
- Body: PAN-PVC, PP, polycarbonate, cellulose nitrate, acrylic
- Shape & Size: disks vs. tubes
<table>
<thead>
<tr>
<th>Mass transport</th>
<th>Disks</th>
<th>Tubes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Favored</td>
<td>diameter restrictions</td>
</tr>
<tr>
<td>Susceptibility to clotting</td>
<td>high surface area increases clot propensity</td>
<td>favored</td>
</tr>
<tr>
<td>Cell #</td>
<td>50-100M</td>
<td>5M</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cell # required</th>
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<tbody>
<tr>
<td>Diabetes</td>
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<tr>
<td>Clotting factor</td>
</tr>
<tr>
<td>CNS therapies</td>
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</tbody>
</table>

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

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

Figure by MIT OCW.
### Cell Regeneration Capability

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