TISSUE ENGINEERING
I. Overview

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TISSUE

• **Tissue** is a biological structure made up of cells of the same type.
  – Cells of the same phenotype (i.e., same genes expressed).
  – An aggregation of morphologically similar cells and associated extracellular matrix acting together to perform one or more specific functions in the body.
  – There are four basic types of tissue: muscle, nerve, epithelia, and connective.
  – An **organ** is a structure made up of 2 or more tissues.
TISSUE FORMATION PROCESSES

- Embryonic tissue formation
- Tissue growth and development (fetal and postnatal)
- Remodeling (degradation-formation)
- Healing (repair versus regeneration)
  - Repair: defect in the tissue fills with “scar” (generally fibrous tissue)
  - Regeneration: defect fills with tissue that is indistinguishable from the original tissue

Figures by MIT OCW.
TISSUE FORMATION PROCESSES

Response to **Permanent and Absorbable Implants**

- Tissue formation in the gap* between the implant and surrounding host tissue
- Tissue formation in pores of porous implant

* Gaps could be on the micrometer length scale

Questions Regarding the Response to **Permanent and Absorbable Implants**

- What tissue is desired in and around the implant: scar or the original host tissue?
- What strategy to employ for regeneration?*
  - recapitulate embryonic conditions: with embryonic cells and/or embryonic extracellular matrix molecules
  - provide conditions (cells and matrix) that favor tissue formation in the adult

* “Tissue engineering” and “regenerative medicine”
TISSUE ENGINEERING

What is tissue engineering?

- Production of tissue *in vitro* by growing cells in porous, absorbable scaffolds (matrices).

Why is tissue engineering necessary?

- Most tissues cannot regenerate when injured or diseased.
- Even tissues that can regenerate spontaneously may not completely do so in large defects (*e.g.*, bone).
- Replacement of tissue with permanent implants is greatly limited.

Problems with Tissue Engineering

- Most tissues cannot yet be produced by tissue engineering (*i.e.*, *in vitro*).
- Implantation of tissues produced *in vitro* may not remodel *in vivo* and may not become integrated with (bonded to) host tissue in the body.

Solution

- Use of implants to facilitate formation (regeneration) of tissue *in vivo*.
  - “Regenerative Medicine”
  - Scaffold-based regenerative medicine
### TISSUE ENGINEERING VS. REGENERATIVE MEDICINE

<table>
<thead>
<tr>
<th>TISSUE ENGINEERING</th>
<th>REGENERATIVE MED.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regeneration <em>In Vitro</em></td>
<td>Regeneration <em>In Vivo</em></td>
</tr>
<tr>
<td>Produce the fully formed tissue <em>in vitro</em> by seeding cells into a biomaterial matrix, and then implant the regenerated tissue into the body.</td>
<td>Implant the biomaterial matrix with, or without seeded cells, into the body to facilitate regeneration of the tissue <em>in vivo</em>.</td>
</tr>
</tbody>
</table>

### TISSUE ENGINEERING VS. REGENERATIVE MEDICINE

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<tr>
<th>TISSUE ENGINEERING</th>
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<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>• Evaluation of tissue prior to implantation</td>
<td>• Incorporation and formation under the influence of endogenous regulators (including mechanical strains)</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>• For incorporation, must be remodeling</td>
<td>• Dislodgment and degrad. by mech. stresses <em>in vivo</em></td>
</tr>
<tr>
<td>• Stress-induced architecture cannot yet be produced <em>in vitro</em></td>
<td></td>
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</tbody>
</table>
**TISSUE ENGINEERING/REGEN. MED.**

**Historical Perspective; Selected Milestones**

1980 **Yannas**: Collagen-GAG matrix for dermal regeneration (“artificial skin”); Integra

1984 **Wolter/Meyer**: 1st use of the term, TE; endothel-like layer on PMMA in the eye

1991 **Cima/Vacanti/Langer**: Chondrocytes in a PGA scaffold; the ear on the nude mouse

1993 **Langer/Vacanti**: Science paper on TE; cells in matrices for tissue formation *in vitro*; PGA

1994 **Brittberg/Peterson**: NEJM paper on human autologous chondrocyte implantation; Carticel

**TISSUE ENGINEERING**

**Current Status**

- No one has yet employed Tissue Engineering methods to fully regenerate any tissue that does not have the capability for spontaneous regeneration*.

- Experience has taught us that full regeneration may not be necessary to achieve a meaningful clinical result (*e.g.*, pain relief, recovery of function, esthetics)

- How close to regeneration is good enough?

*Many examples of bone regeneration*
### Which Tissues Can Regenerate?

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Yes*</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective Tissues</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Bone</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>• Articular Cartilage, Ligament, Intervertebral Disc, Others</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>Epithelia (e.g., epidermis)</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Cardiac, Skeletal</td>
<td></td>
<td>√</td>
</tr>
<tr>
<td>• Smooth</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>Nerve</td>
<td></td>
<td>√</td>
</tr>
</tbody>
</table>

* If defects are large, regeneration may not be complete.

### FACTORS THAT CAN PREVENT REGENERATION

- **Size of defect**
  - *e.g.*, bone does not regenerate in large defects
- **Collapse of surrounding tissue into the defect**
  - *e.g.*, periodontal defects
- **Excessive strains in the reparative tissue**
  - *e.g.*, unstable fractures
**Elements of Tissue Engineering/Regenerative Medicine**

- **Matrix (Scaffold)**
  - Porous, absorbable synthetic (e.g., polyglycolic acid) and natural (e.g., collagen) biomaterials
- **Cells** (Autologous or Allogeneic)
  - Differentiated cells of same type as tissue
  - Stem cells (e.g., bone marrow-derived)
  - Other cell types (e.g., dermal cells for cartilage)
- **Soluble Regulators**
  - Growth factors or their genes
- **Environmental Factors**
  - Mechanical loading
  - Static versus dynamic (“bioreactor”)

**Tissue Features Relating to Regeneration of Musculoskeletal Connective Tissues**

<table>
<thead>
<tr>
<th>Tissue Feature</th>
<th>Required for regeneration</th>
<th>Vasc.¹</th>
<th># Cells ²</th>
<th>Mitosis ²</th>
<th>Migrate ²</th>
<th>Synthesis³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Articular Cartilage</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ligament/Tendon</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Intervertebral Disc</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Meniscus</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Guide to tissue engineering*

1. Lack of a fibrin clot requires use of a *matrix*
2. Lack of cells requires a procedure to bring *cells* to the defect
3. Low biosynthesis may require use of a *growth factor*
THERAPEUTIC APPROACHES
IMPLEMENTING TISSUE ENGINEERING

• Injection of cells alone
  – contained in defect or uncontained
• Injection of growth factor alone
• Implantation of scaffold alone (with microfracture)
• Implantation of scaffold incorporating GFs or genes
• Implantation of scaffold-free tissue construct*
• Implantation of cell-seeded scaffold*

* Degree of maturation of the construct prior to implantation: relative to integration and stress-induce architecture?

CELL THERAPY FOR LOCAL REPAIR

Injection of Exogenous Cells;
Cells Expanded in Number in Monolayer Culture

• Chondrocytes for cartilage repair (ACI)
• Intervertebral disc cells for herniated disc
• Stem cells into spinal cord lesions
• Stem cells into brain lesions*
• Myoblasts and stem cells for myocardial infarction*
• Stem and other cells into the retina
• Stem cell injection into the joint

* Evidence of stem cell migration to the site of injury.
Articular Cartilage Defects

**Important Clinical Problem**
- Incidence is high and increasing due to increasing activity levels
- Causes pain and disability
- Profoundly impacts the quality of life

**Articular Cartilage Defects Do Not Heal**
- Avascular
- Aneural
- Low cell density
- Cells of low mitotic activity
- Cells cannot freely migrate through the extracellular matrix

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Current Clinical Practice

*Figures by MIT OCW.*

- Arthroscopic Debridement
- **“Microfracture” (closed)**
- Osteochondral Autograft
- Total Knee Replacement
- Autologous chondrocytes injected under a periosteal flap (open)

*Figures removed due to copyright restrictions.*
Implantation of a cell-seeded matrix

Performed arthroscopically

“Tissue engineered” cartilage implanted in a rabbit model did not work.


Future Clinical Practice
Implementing Tissue Engineering

“Microfracture”: Stem cells from bone marrow infiltrate the defect

Implantation of the matrix alone


Future Clinical Practice
Implementing Tissue Engineering

Perform arthroscopically

Figure by MIT OCW.

Figure by MIT OCW.
TISSUE ENGINEERING / REGENERATIVE MEDICINE

- **Science (acquisition of new knowledge)**
  - Response of cells to matrices and environmental factors.

- **Engineering (making a product - tissue)**
  - How cells, matrices and regulators can be combined to facilitate tissue formation.

- **Technology (means of production)**
  - Methods for producing porous matrices.

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TISSUE ENGINEERING
Why tissue engineering now?

Enabling Technologies

- **Cells**
  - Cell proliferation *in vitro* with recovery of phenotype

- **Matrices**
  - Synthesis of porous, absorbable scaffolds

- **Regulators**
  - Genetically engineered growth factors
TISSUE ENGINEERING

Emerging Enabling Technologies

- **Cells**
  - Stem cell sources and cues for differentiation
  - Genetically modified cells

- **Matrices**
  - Chemistries that regulate selected cell functions

- **Regulators**
  - Incorporation of GF genes into matrices
  - Control of selected cell behavior (contraction)
  - Mechanical loading to regulate cell function

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TISSUE ENGINEERING
ADVANCES

| Scaffolds | • Novel polymers
  | - self-assembling peptides
  | - thermosensitive and photopolymerizing
  | • Controlled mechanical behavior
  | - undergo cell-mediated contraction
  | • Free-form fabrication

| Cells | • Conditions for cell expansion; *ex vivo* gene transfer*
  | • Stem cells*; sources; expansion
  | • Scaffold-free cartilaginous constructs*
  | • Cell-seeded scaffolds*; bioreactor; mech. condition.

| Regulators | • GFs (e.g., BMP-2) incorporated into scaffolds
  | • Novel regulators
  | • Genes for GFs incorporated into scaffolds

* Large animal model
TISSUE ENGINEERING ENDPOINTS

• Morphological/Histological/Biochemical
  – Match the composition and architecture of the tissue.
  – Problem: A complete analysis is difficult and no clear relationships yet with functional and clinical endpoints.

• Functional
  – Achieve certain functions; display certain properties (e.g., mechanical properties).
  – Problem: Difficult to measure all properties; Which properties are the most important?

• Clinical
  – Pain relief.
  – Problems: Can only be evaluated in human subjects and the mechanisms (including the placebo effect) and kinetics of pain relief (e.g., how long it will last) are unknown.

TISSUE ENGINEERING RISKS

Exercise caution that the tissue engineering solution does not create larger problems that being solved.

• Tissue harvest for the isolation of cells places the donor site and surrounding tissue at risk of degeneration.
• Implants that accelerate the breakdown of surrounding tissues.
EFFECTS OF THE CARTILAGE REPAIR PROCEDURES ON UNINVOLVED CARTILAGE?

Effects of Harvest (Canine Model)

- Changes in the mechanical properties of AC at sites away from the harvest, 4-mo post-op (up to 3-fold).
- Changes were consistent with hypertrophy, predisposing to osteoarthritis.

Harvest Sites


TISSUE ENGINEERING
Product Considerations

- MATRIX
  - Produced by companies
- CELLS
  - From the patient
- SOLUBLE REGULATORS
  - Growth factors produced by biotechnology companies or their genes cloned in the laboratory
How will tissue engineering products be commercialized?

• What are the models that can be used for the sales/purchase of tissue engineering products?
TISSUE ENGINEERING PRODUCTS
USING AUTOLOGOUS CELLS

Company

Cytokines/Genes  Biomaterial Matrices

“Hospital-Based Tissue Engineering”

Hospital

Tissue

Cell-seeded matrix

Operating Room

Cells can be grown in culture and matrices seeded with cells in the Hospital

Model still employed by the Swedish clinicians who developed the Genzyme procedure

Operating Room-Based Tissue Engineering
“Intraoperative Tissue Engineering”

TISSUE ENGINEERING PRODUCTS
NEW MODEL

Company

Cytokines/Genes  Biomaterial Matrices

Cells can be “processed” and matrices seeded with cells in the OR during surgery

Hospital

Operating Room

Cells

Operating Room
TISSUE ENGINEERING/REGEN. MED.
BACK TO THE FUTURE

1800s-1970s
“Wound Healing”
Repair (scar)
vs. Regeneration

1980s-1990s
“Tissue Engineering”
Formation of “tissue”
in vitro

2000s
“Regenerative Medicine”
Regeneration of tissue
in vivo

CONSIDERATIONS IN TISSUE ENGINEERING

• Clearly define the specific clinical problem to be solved.
• Implement the simplest procedure for treating the problem to achieve a meaningful clinical benefit.
  – Benefit-Risk Ratio (e.g., risks of using cells: cell transformation, morbidity of a 2nd surgical procedure)
  – Cost
• Need to evaluate tissue engineering products in animal models that come closest to human problems to be treated.
TISSUE ENGINEERING

Issues to be Addressed

- Should the tissue be produced *in vitro*, for subsequent implantation, or *in vivo*?
- What scaffold should be used?
  - Material of fabrication, pore characteristics, absorbability, mechanical properties?
  - How to be manufactured?
- What cells are to be used?
  - Source of cells?
  - Under what conditions can cells be expanded in number *in vitro* while retaining their phenotype?
- What regulators are required to stimulate cell proliferation and matrix synthesis or to facilitate differentiation of stem cells?