Massachusetts Institute of Technology
Harvard Medical School
Brigham and Women's Hospital
VA Boston Healthcare System


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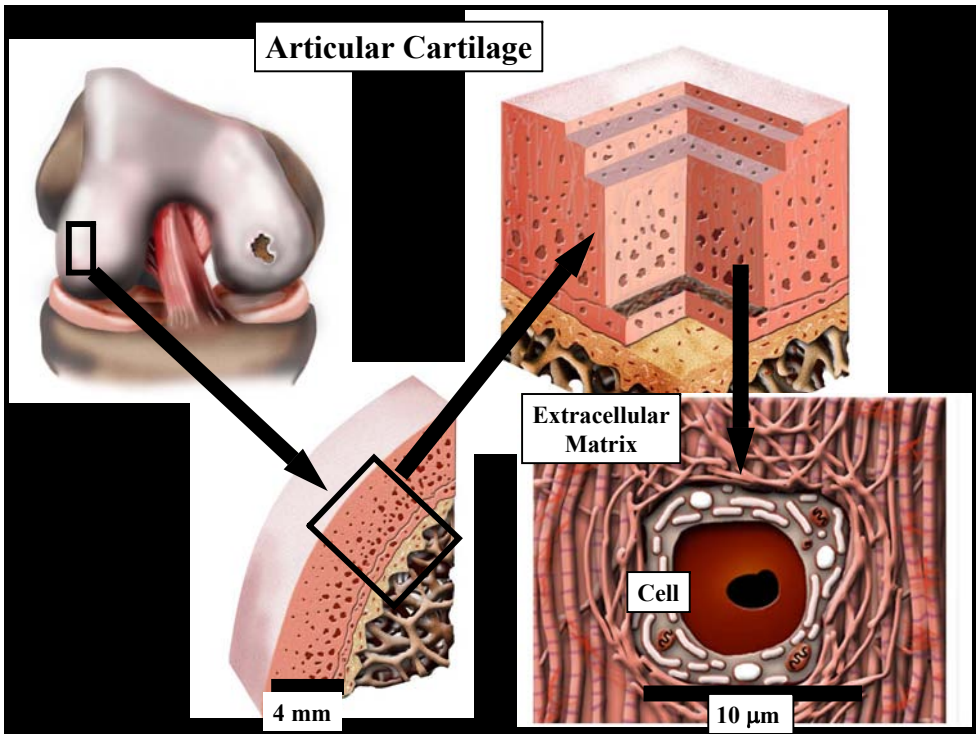
# TISSUE ENGINEERING
# I. Overview

M. Spector, Ph.D.

## 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.




Figures by MIT OCW.

## 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

## 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

## TISSUE FORMATION PROCESSES

**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.

## TISSUE ENGINEERING

**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

**TISSUE ENGINEERING**

Regeneration *In Vitro*

Produce the fully formed tissue *in vitro* by seeding cells into a biomaterial matrix, and then implant the regenerated tissue into the body.

**REGENERATIVE MED.**

Regeneration *In Vivo*

Implant the biomaterial matrix with, or without seeded cells, into the body to facilitate regeneration of the tissue *in vivo*.

## TISSUE ENGINEERING VS. REGENERATIVE MEDICINE

**TISSUE ENGINEERING**

Regeneration *In Vitro*

**Advantages**

- Evaluation of tissue prior to implantation

**Disadvantages**

- For incorporation, must be remodeling
- Stress-induced architecture cannot yet be produced *in vitro*

**REGENERATIVE MED.**

Regeneration *In Vivo*

**Advantages**

- Incorporation and formation under the influence of endogenous regulators (including mechanical strains)

**Disadvantages**

- Dislodgment and degrad. by mech. stresses *in vivo*

## 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?

| | Yes* | No |
|---|---|---|
| **Connective Tissues** | | |
| • Bone | √ | |
| • Articular Cartilage, Ligament, Intervertebral Disc, Others | | √ |
| **Epithelia (*e.g.*, epidermis)** | √ | |
| **Muscle** | | |
| • Cardiac, Skeletal | | √ |
| • Smooth | √ | |
| **Nerve** | | √ |

* 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

| Required for regeneration | Vasc.[1] | # Cells[2] | Mitosis[2] | Migrate[2] | Synthesis[3] |
|---|---|---|---|---|---|
| Bone | + | + | + | + | + |
| Articular Cartilage | - | - | - | - | - |
| Ligament/Tendon | - | - | + | + | + |
| Intervertebral Disc | - | - | + | + | + |
| Meniscus | - | - | + | + | + |

**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*

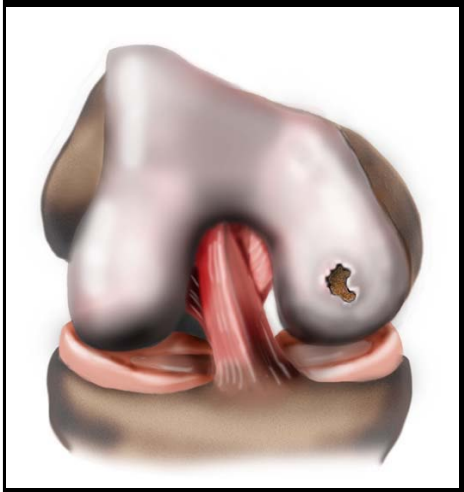
* Degree of maturation of the construct prior to implantation: relative to integration and stress-induce architecture?

## 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.



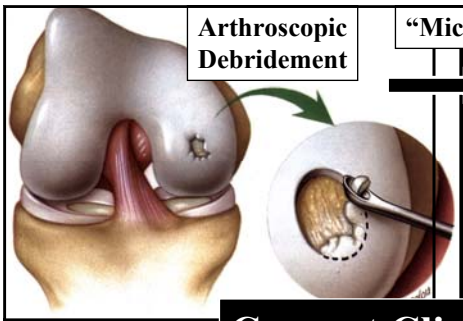
Figures by MIT OCW.

**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



Arthroscopic Debridement

"Microfracture" (closed)

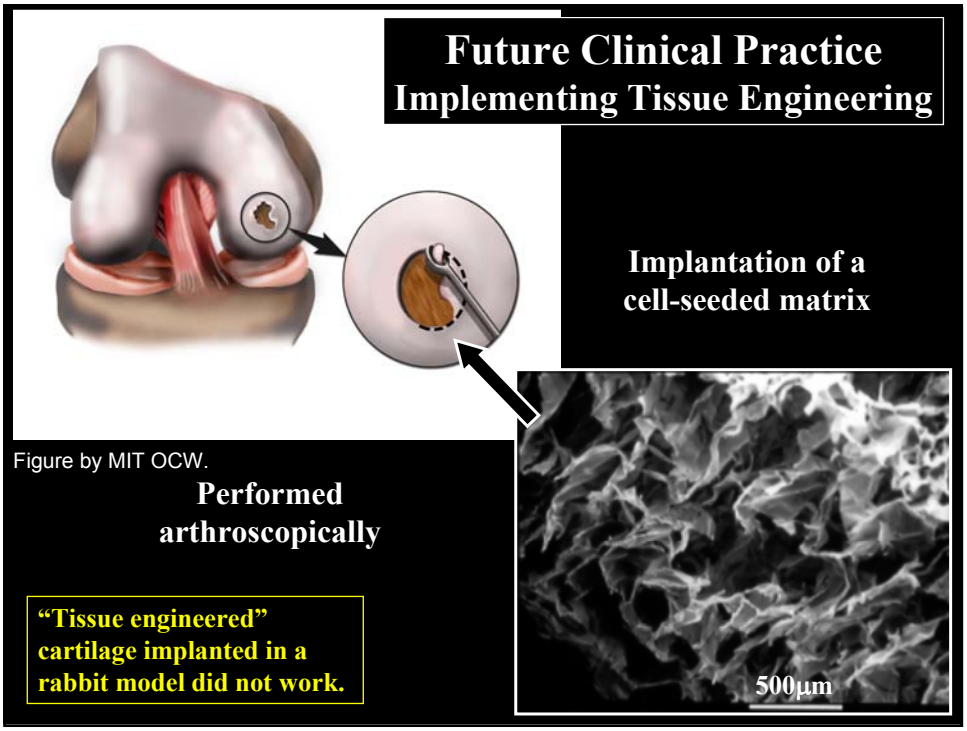
Osteochondral Autograft

**Current Clinical Practice**

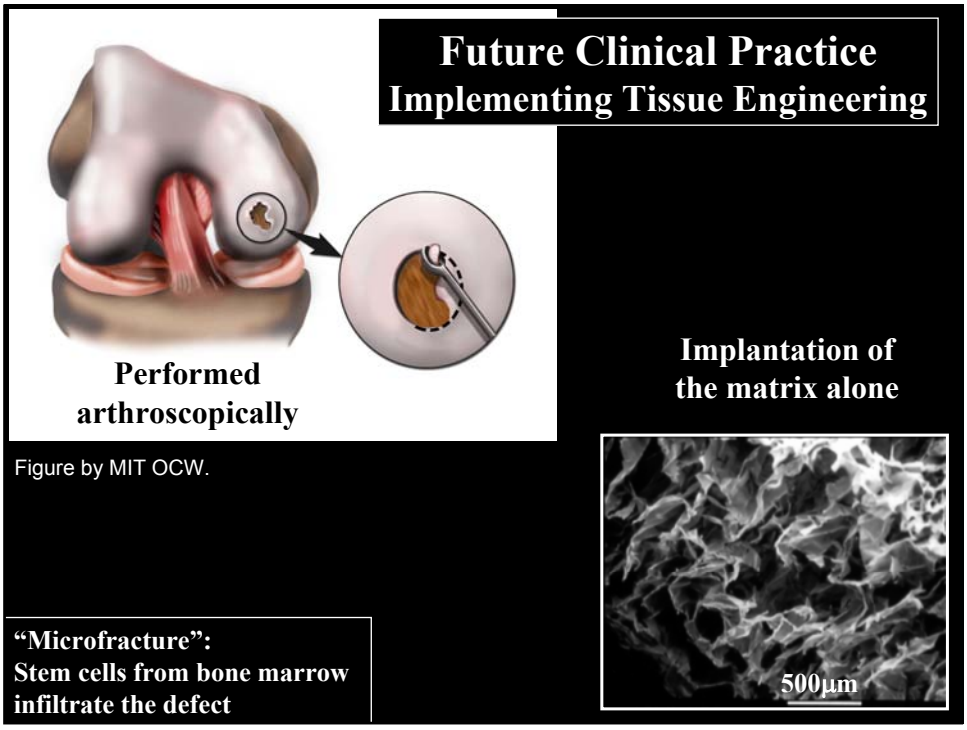
Figures removed due to copyright restrictions.

Total Knee Replacement

Autologous chondrocytes injected under a periosteal flap (open)




Yannas, et al. *PNAS* (1989).




Yannas, et al. *PNAS* (1989).

## 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.

## 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

# TISSUE ENGINEERING ADVANCES

| | |
|---|---|
| Scaffolds | • Novel polymers<br>– self-assemblying peptides<br>thermosensitive and photopolymerizing<br>• Controlled mechanical behavior<br>– undergo cell-mediated contraction<br>• Free-form fabrication |
| Cells | • Conditions for cell expansion; *ex vivo* gene transfer*<br>• Stem cells*; sources; expansion<br>• Scaffold-free cartilaginous constructs*<br>• Cell-seeded scaffolds*; bioreactor; mech. condition. |
| Regulators | • GFs (*e.g.*, BMP-2) incorporated into scaffolds<br>• Novel regulators<br>• 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

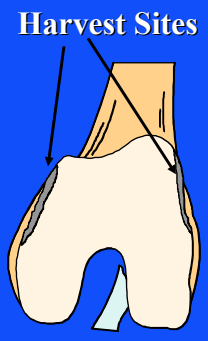
Exercise caution that the tissue engineering solution does not create larger problems that being solved.

- 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.




CR Lee, *et al.*, JOR, 2000;18:790-799

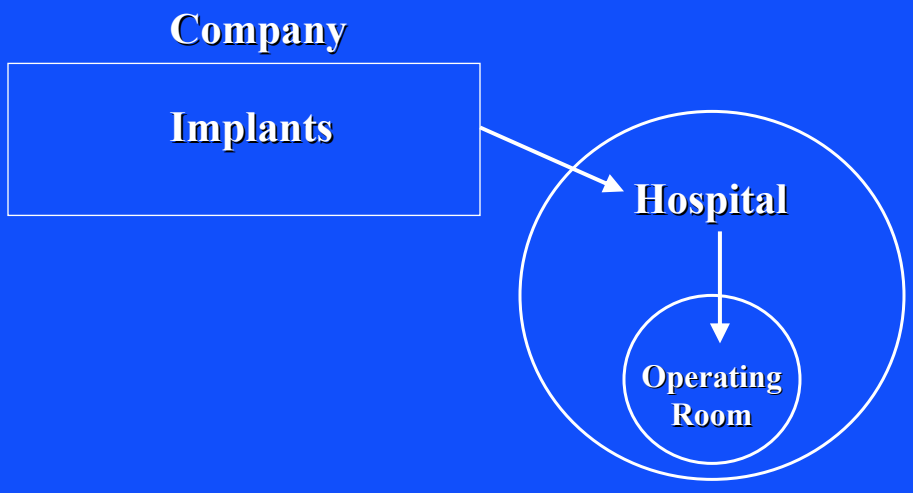
## TISSUE ENGINEERING Product Considerations

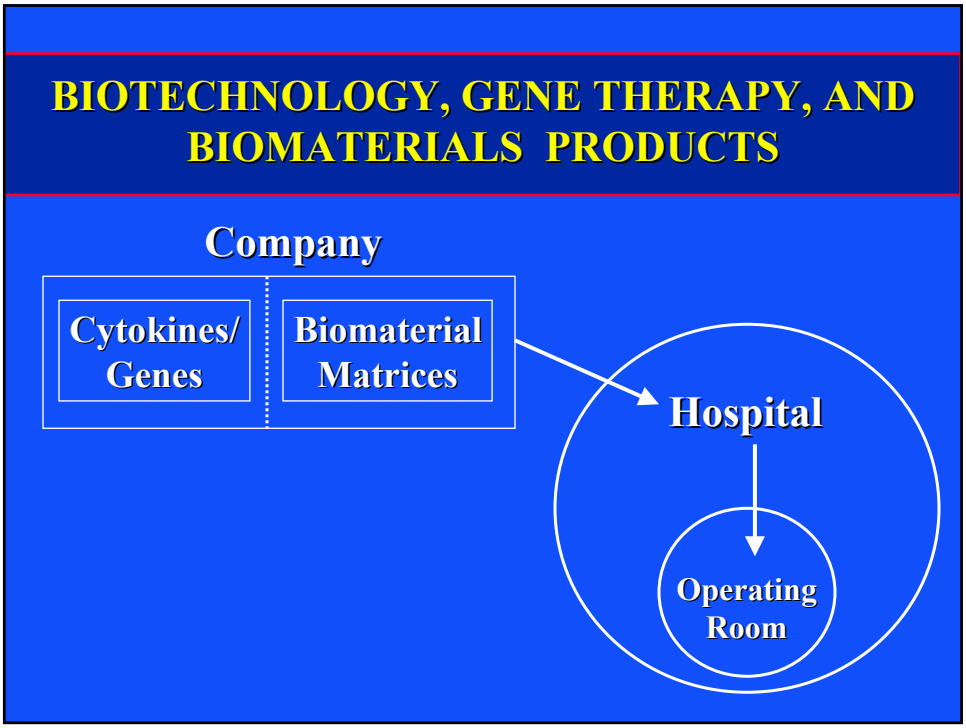
- MATRIX
  - Produced by companies
- CELLS
  - From the patient
- SOLUBLE REGULATORS
  - Growth factors produced by biotechnology companies or their genes cloned in the laboratory

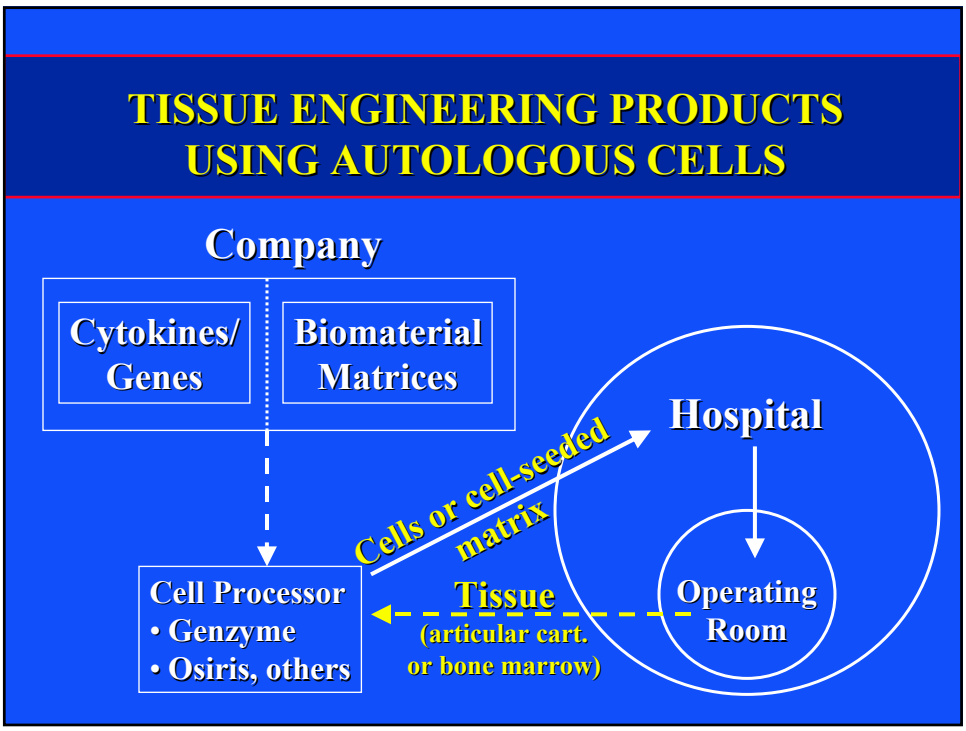
## How will tissue engineering products be commercialized?

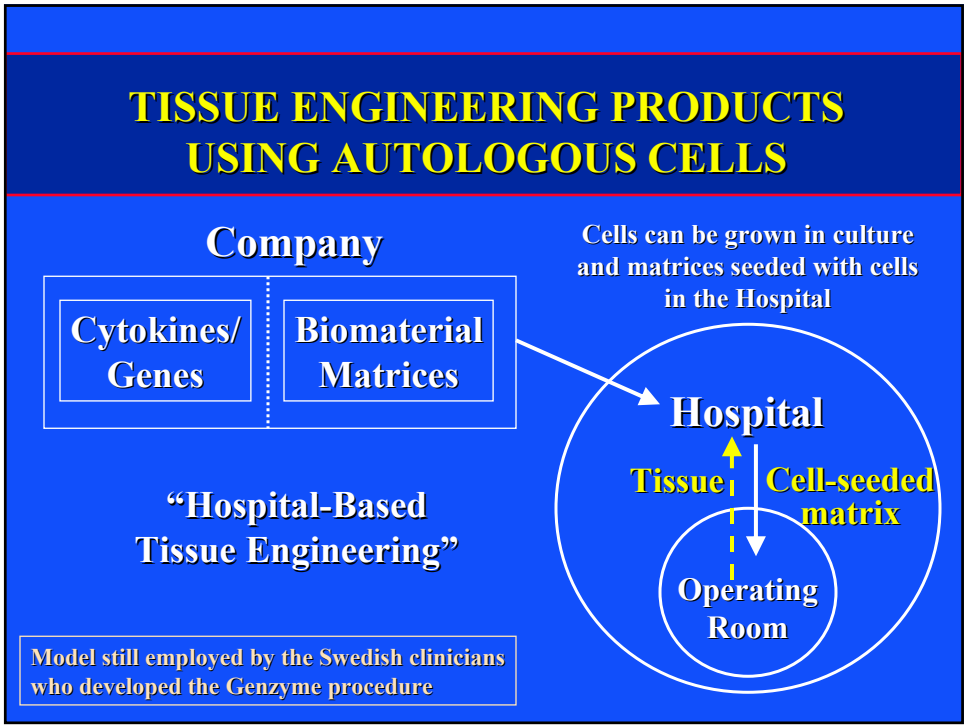
- What are the models that can be used for the sales/purchase of tissue engineering products?

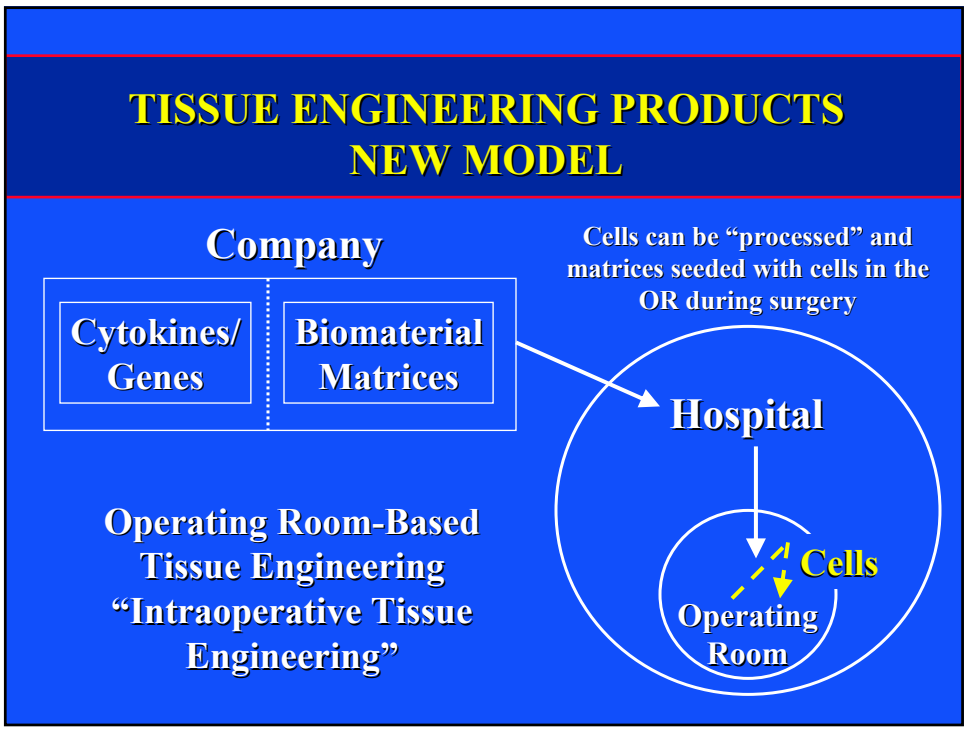
## CONVENTIONAL PROSTHESES CURRENT COMMERCIAL MODEL



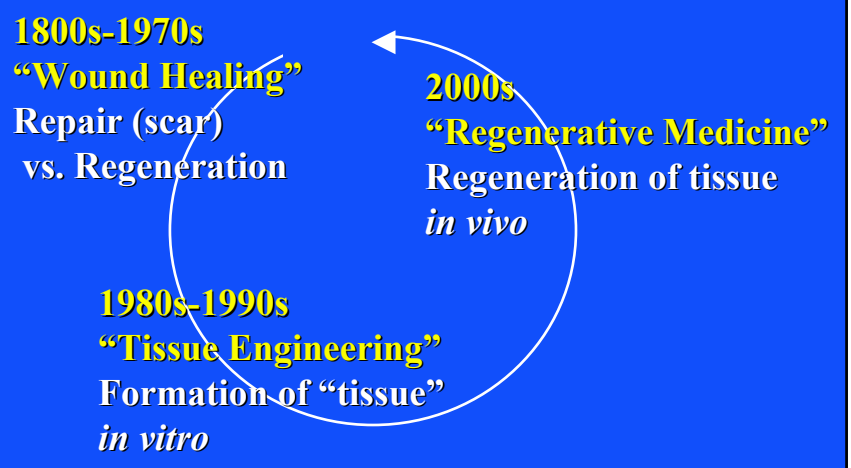

BIOTECHNOLOGY, GENE THERAPY, AND BIOMATERIALS PRODUCTS
Company
Cytokines/ Genes
Biomaterial Matrices
Hospital
Operating Room


TISSUE ENGINEERING PRODUCTS USING AUTOLOGOUS CELLS
Company
Cytokines/ Genes
Biomaterial Matrices
Hospital
Cells or cell-seeded matrix
Cell Processor
• Genzyme
• Osiris, others
Tissue
(articular cart. or bone marrow)
Operating Room


TISSUE ENGINEERING PRODUCTS
USING AUTOLOGOUS CELLS
Company
Cytokines/ Genes
Biomaterial Matrices
Cells can be grown in culture and matrices seeded with cells in the Hospital
Hospital
Tissue
Cell-seeded matrix
Operating Room
"Hospital-Based Tissue Engineering"
Model still employed by the Swedish clinicians who developed the Genzyme procedure


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
Cells
Operating Room
Operating Room-Based Tissue Engineering "Intraoperative Tissue Engineering"

## TISSUE ENGINEERING/REGEN. MED. BACK TO THE FUTURE




## 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?