



Massachusetts Institute of Technology  
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# DENTAL TISSUE ENGINEERING

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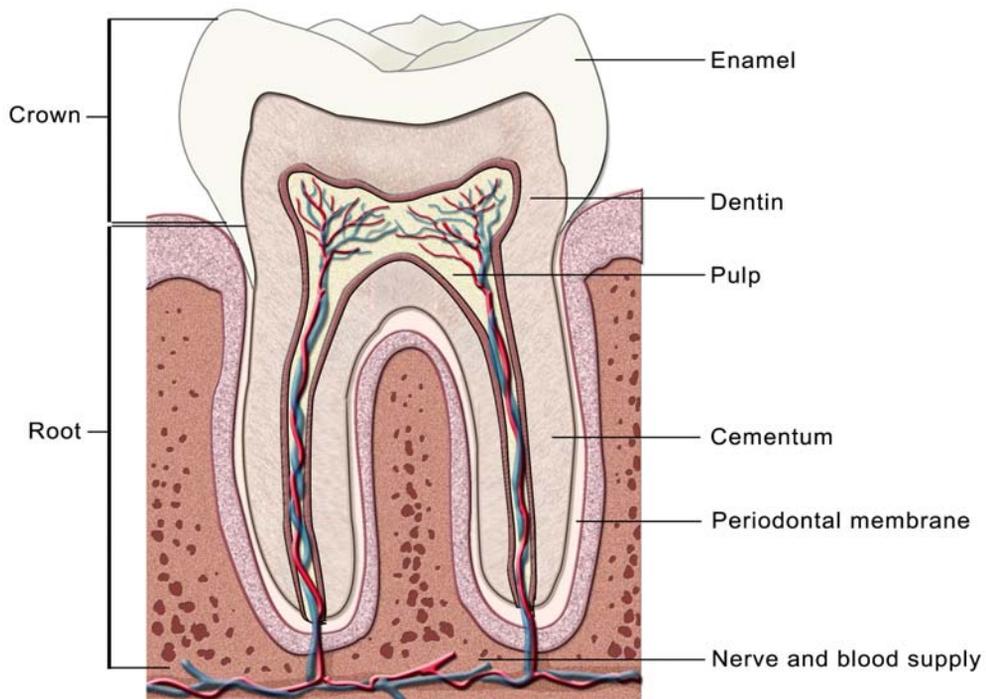


Figure by MIT OCW.

## REGENERATION STRATEGY

- “Tissue Engineering”
  - Formation of tissue *in vitro* for subsequent implantation
- “Regenerative Medicine”
  - Regeneration *in vivo*
- Coupled Approach
  - Cell-seeded matrix grown *in vitro* to meet certain design specifications, to facilitate regeneration *in vivo*

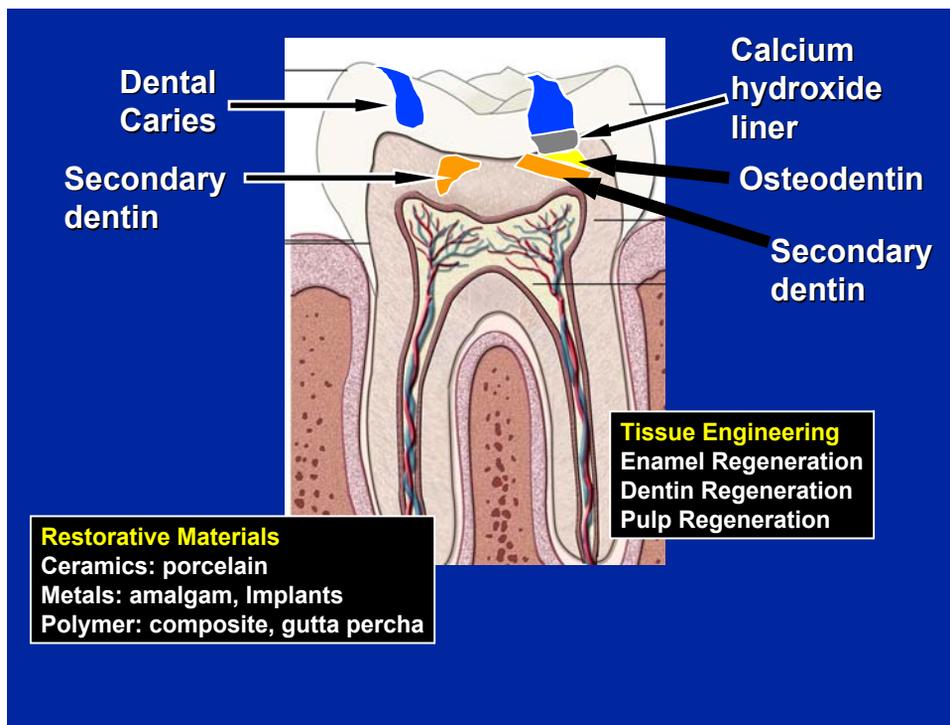


Figure by MIT OCW. After J-W. Hwang.

## TISSUE ENGINEERING

- **MATRIX (SCAFFOLD)**
  - Porous, absorbable synthetic and natural (collagen-GAG copolymers) biomaterials
- **CELLS (Autologous or Allogeneic)**
  - Differentiated cells of same type as tissue
  - Stem cells (bone marrow-derived)
  - Other cell types
- **SOLUBLE REGULATORS**
  - Cytokines (growth factors) or their genes
- **ENVIRONMENTAL FACTORS**
  - Mechanical loading
  - Static and “bioreactor”

## ELEMENTS FOR TISSUE REGENERATION/ENGINEERING

<b>CELLS</b>	Autologous, Adult (pulp and bone marrow stromal stem cell)
<b>MATRIX</b>	Collagen-GAG
<b>CYTOKINES</b>	Regulation of phenotype Matrix biosynthesis

## **Tissue Engineering of Complex Tooth Structures on Biodegradable Polymer Scaffolds**

- Cells dissociated from porcine third molar tooth buds.
- Cells seeded onto PLA fiber mesh and implanted in rats for 20 to 30 wks.
- Resulting tooth structures contained dentin (odontoblasts), a well-defined pulp chamber, putative cementoblasts, and a morphologically correct enamel.
- Results suggest the presence of epithelial and mesenchymal dental stem cells in porcine third molar tissues.

C.S. Young, et al. *J Dent Res* 81(10): 695-700, 2002

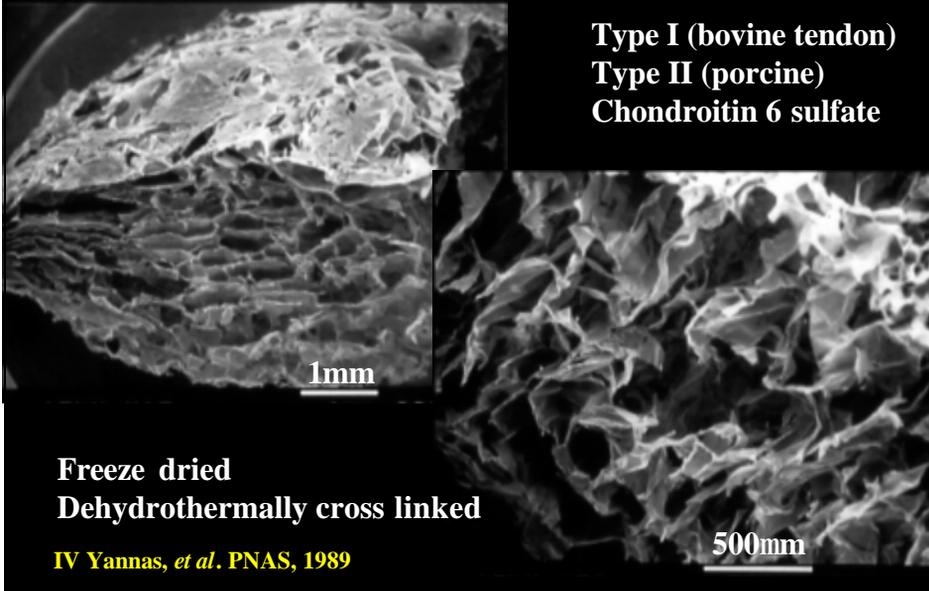
## **Growth of Porcine Enamel-, Dentin-, and Cementum-Derived Cells in Collagen-GAG Matrices *In Vitro***

### **Unerupted Porcine Premolars and Molars**

- Lower mandibles from 6-month old pigs.
- In aseptic environment, mandibles were split in half, soft tissue removed, and overlying bone from lingual side chiseled away.
- Exposed teeth were excised and gingiva removed.

Marty-Roix R, et al., *Tiss. Engr.* (In press)

# COLLAGEN-GAG MATRICES

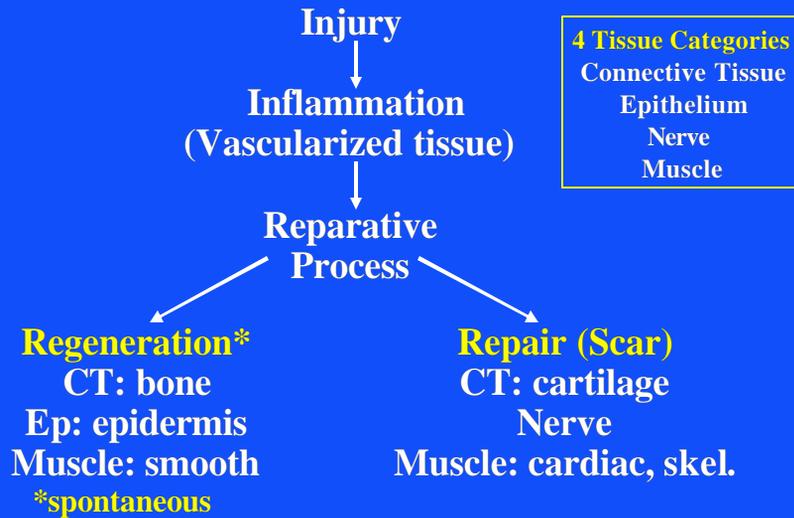


## DENTAL TISSUES

- Enamel
- Dentin
- Cementum
- Pulp
- Periodontal Ligament
- Bone

# WOUND HEALING

## Roots of Tissue Engineering



## TISSUE ENGINEERING TRIAD

<b>CELLS</b>	Autologous, Adult (pulp and bone marrow stromal stem cell)
<b>MATRIX</b>	Collagen-GAG
<b>CYTOKINES</b>	Regulation of phenotype Matrix biosynthesis Contractile actin expression

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## **MATERIALS AND METHODS**

### **Specimen Procurement and Tissue Dissection**

#### **Unerupted Porcine Premolars and Molars**

- Lower mandibles from 6-month old pigs
- In aseptic environment, mandibles were split in half, soft tissue removed, and overlying bone from lingual side chiseled away.
- Exposed teeth were excised and gingiva removed.

## **MATERIALS AND METHODS**

### **Cell Isolation**

**Dentin** - from developing cusp tips. Mineralized enamel removed and pulp cut away.

**Enamel** - Mineralized enamel removed from cusp tips and chiseled into small pieces.

**Cementum** - from erupted 2nd molars and unerupted premolars. Chiseled away from tooth and into smaller pieces. Pulp removed with sterile gauze.

**Pulp** - from base of teeth and cut into small pieces with scalpel.

## **MATERIALS AND METHODS**

### **Methods of Cell Culture**

- **Cell Isolation from Digested Tissue**
  - Tissue digested for 12 hours in collagenase.
  - Suspension filtered, and cells plated into tissue culture dishes.
- **Explants/Cell Outgrowth**
  - Small pieces of tissue plated onto tissue culture dishes.

## **DENTAL TISSUES**

### **Tissue-Specific Matrix Molecules**

- **Enamel**            Amelogenin
- **Dentin**            Dentin Matrix Protein-1
- **Cementum**        ?
- **Pulp Cells**        ?

### **Amelogenin Immunohistochemistry**

**Amelogenin**

**Pre-ameloblasts**

**Negative Control**

**Dentin**

**Odontoblasts**

Photos removed due to copyright restrictions.

## **DMP-1 Immunohistochemistry of Odontoblast-Seeded Collagen-GAG Matrices**

Photos removed due to copyright restrictions.

### **SUMMARY**

- Cells can be isolated from digested tissue and grown from explants: enamel, dentin, cementum, and pulp.
- Cells display distinctive characteristics.
- Ameloblasts expanded in monolayer and grown in collagen-GAG matrices express amelogenin.
- Odontoblasts in collagen-GAG matrices express DMP-1

## MUSCULOSKELETAL CELLS THAT CAN EXPRESS $\alpha$ -SMOOTH MUSCLE ACTIN AND CAN CONTRACT

- Articular chondrocyte
- Osteoblast
- Meniscus fibroblast and fibrochondrocyte
- Intervertebral disc fibroblast and fibrochondrocyte
- Ligament fibroblast
- Tendon fibroblast
- Synovial cell
- Mesenchymal stem cell

M. Spector,  
Wound Repair Regen.  
9:11-18 (2001)

### $\alpha$ -Smooth Muscle Actin Content Western Blot Analysis

Figure removed due to copyright restrictions.  
Matrix comparing smooth muscle cell controls with pulp cells.

Brock, *et al.*, J Dent Res  
2002;81:203-208

**Porcine Pulp Cells in Monolayer  
 $\alpha$ -Smooth Muscle Actin (red)  
Immunohistochemistry**

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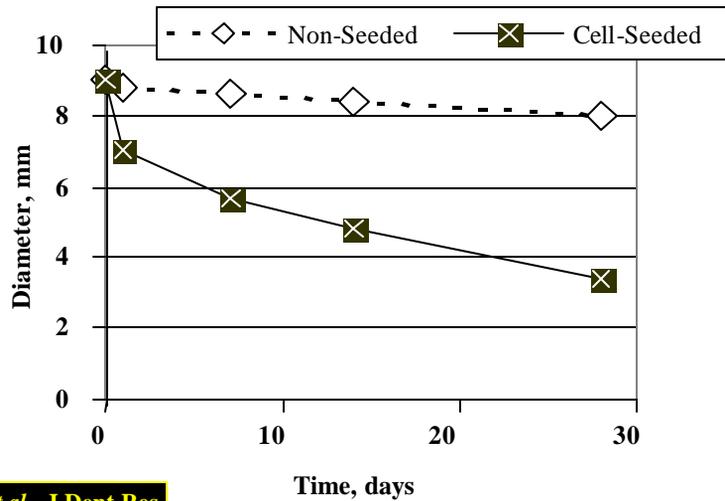
**Brock, *et al.*, J Dent Res  
2002;81:203-208**

**Brock, *et al.*, J Dent Res 2002;81:203-208**

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**Porcine Pulp Cell-Seeded Collagen-GAG Matrices**

## Contraction of Pulp Cell-Seeded Collagen-GAG Matrices



Brock, *et al.*, J Dent Res  
2002;81:203-208

## Pulp Cell-Seeded Collagen-GAG Matrices (28 days)

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Brock, *et al.*, J Dent Res  
2002;81:203-208

## SMA Immunohistochemistry

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*Marty-Roix, et al.*  
Tiss. Engr. 2003;9:175-186

## Ameloblasts in Monolayer Culture

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*Marty-Roix, et al.*  
Tiss. Engr. 2003;9:175-186

**Odontoblasts in  
Monolayer  
Culture**

Photos removed due to copyright restrictions.

**Marty-Roix, *et al.*  
Tiss. Engr. 2003;9:175-186**

**Cementoblasts in  
Monolayer  
Culture**

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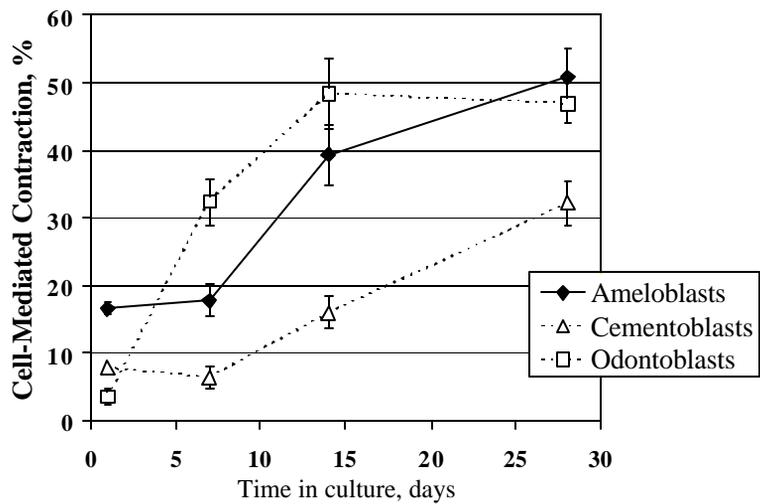
**Marty-Roix, *et al.*  
Tiss. Engr. 2003;9:175-186**

## $\alpha$ -Smooth Muscle Actin: Western Blot Analysis

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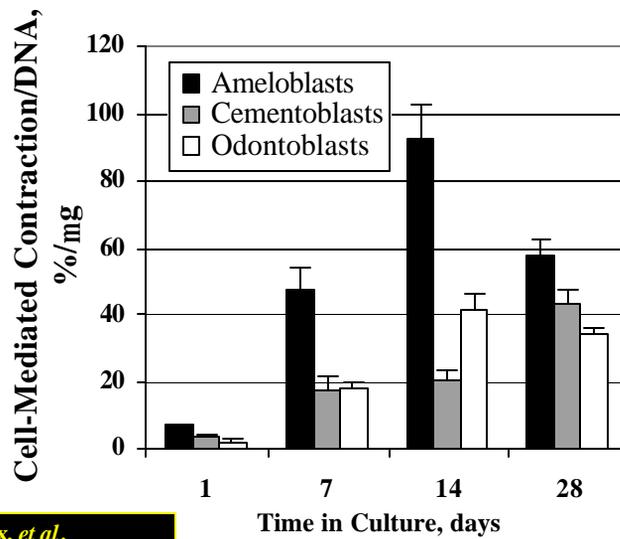
Marty-Roix, *et al.*  
Tiss. Engr.  
2003;9:175-186

## Contraction of Collagen-GAG Matrices by Dental Tissue Cells



Marty-Roix, *et al.*  
Tiss. Engr. 2003;9:175-186

## Contraction of Collagen-GAG Matrices by Dental Tissue Cells



Marty-Roix, *et al.*  
Tiss. Engr. 2003;9:175-186

## SMA Immunohistochemistry of Cell-Seeded Collagen-GAG Matrices

Ameloblasts

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Marty-Roix, *et al.*  
Tiss. Engr. 2003;9:175-186

## DENTAL TISSUE CELLS WITH MUSCLE

- |                      |   |
|----------------------|---|
| • Tissue formation   | <b>impart ECM architecture</b><br><b>tooth eruption</b> |
| • Remodeling         | <b>alter ECM architecture</b>                           |
| • Disease processes  | <b>contracture</b>                                      |
| • Healing            | <b>wound closure</b>                                    |
| • Tissue engineering | <b>contraction of scaffolds</b>                         |

## FINDINGS TO DATE

- Enamel-, dentin-, cementum-, and pulp-derived cells can be grown in monolayer culture and seeded into collagen-GAG matrices.
- Enamel- and dentin-derived cells retain certain phenotypic traits.
- Discovery that ameloblasts, odontoblasts, cementoblasts, and pulp cells can express SMA and can contract a collagen-GAG analog of extracellular matrix.