Harvard-MIT Division of Health Sciences and Technology
HST.535: Principles and Practice of Tissue Engineering
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HST 535

PRINCIPLES AND PRACTICE OF TISSUE ENGNEERING:

Review of the Principles and Practice of Using Tissue Engineering Scaffolds

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SCAFFOLDS

Chemical Composition

- Collagen-GAG (Yannas)
- Polyglycolic/polylactic acid (Langer and Freed)
- Self-assemblying proteins (Zhang)
- Nano-Ap/Collagen Composite-self assembly (Cui)
- Chitin/chitosan (Xu and others)

Structure/Architecture

- Fiber mesh, like noodles (Langer and Freed)
- Free Form Fabrication-3-D printing (Yan)
- Sponge-like (Yannas and Cui)
- Fine filament mesh (Zhang)

COLLAGEN STRUCTURE

Primary;
amino acid structure

Secondary;
single chain

Diagram removed for copyright reasons

Collagen molecule; triple helix

Tertiary;
triple helix

Collagen fibril (fiber)

Quaternary;
fibril

"banding"

Yannas

Primary

Secondary

Diagram removed for copyright reasons

Tertiary

Platelets interact with the banded collagen resulting in clotting; disrupting the quaternary structure prevents this platelet activation

Quaternary

banding

Yannas

Normal banded (quaternary) structure

No enzyme

Figure removed for copyright reasons.
See Figure 4.7 in Yannas, I. V. *Tissue and Organ Regeneration in Adults*. New York: Springer, 2001. ISBN: 0387952144.

Degradation of collagen fibers by collagenase

Exposed to enzyme

Disruption of the quaternary structure

Yannas

Collagen
molecule;
triple helix

Diagram removed for copyright reasons. (Figure 4.2 in Yannas)

Spontaneous melting
to gelatin following degradation

Degradation of the collagen molecule (triple helix; tertiary structure) by collagenase releases the individual molecular chains that associate to form "gelatin." Gelatin itself degrades much faster than collagen.

Yannas

collagen

Diagram removed for copyright reasons. (Figure 4.2 in Yannas)

Cross-linking of gelatin.

Formation of covalent bonds among collagen chains (cross-linking) can increase strength and decrease degradation rate of collagen and gelatin.

Yannas

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Scaffold Structures

Langer and Freed

Fiber mesh, like noodles

Yan

3-D printed

Cui

Photos removed for copyright reasons.

Fine filament mesh

Yannas

Sponge-like

Zhang

100 μm

PRINCIPLES AND PRACTICE OF TISSUE ENGINEERING

Principles

- Scaffolds can regulate cell function by their chemical make-up
- Scaffolds can regulate cell function by their structure/architecture

Practice

- Methods for producing scaffolds with selected chemical composition and structure

PRINCIPLES

- Chemical Composition
- Pore Structure/ Architecture
- Degradation Rate
- Mechanical Properties

PRINCIPLES

Chemical Composition

- Scaffolds can regulate cell function by their chemical make-up
  - Affects cell attachment through integrin binding, or absence of attachment in the case of hydrogels
  - Affects cell behavior through interactions with integrins
- Degradation rate and mechanical properties are dependent on the chemical make-up

PRINCIPLES

Pore Structure/Architecture

- Percentage porosity
  - number of cells that can be contained
  - strength of the material
- Pore diameter
  - surface area and the number of adherent cells
  - ability of cells to infiltrate the pores
- Interconnecting pore diameter
- Orientation of pores
  - can direct cell growth
- Overall shape of the device needs to fit the defect

PRINCIPLES

Degradation Rate

- Too rapid does not allow for the proper regenerative processes.
- Too slow interferes with remodeling.
- For synthetic polymers regulated by blending polymers with different degradation rates (*e.g.*, PLA and PGA).
- For natural polymers (*viz.*, collagen) by cross-linking.

PRINCIPLES

Mechanical Properties

- Strength high enough to resist fragmentation before the cells synthesize their own extracellular matrix.
- Modulus of elasticity (stiffness) high enough to resist compressive forces that would collapse the pores.
- For synthetic polymers regulated by blending polymers with different mechanical properties and by absorbable reinforcing fibers and particles.
- For natural polymers (*viz*., collagen) by cross-linking and reinforcing with mineral (or by mineralization processes) or synthetic polymers (*e.g*., PLA).

PRACTICE

Methods for Producing Scaffolds*

- Fibers (non-woven and woven)
- Freeze-drying
- Self-assembly
- Free-form manufacturing

* Need to consider the advantages and disadvantages with respect to the production of scaffolds with selected chemical composition and structure

PRACTICE

Methods for Producing Scaffolds*

- Fibers (non-woven and woven)
- Freeze-drying (collagen-GAG) -Yannas
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Collagen-GAG Scaffolds

Synthesis of active ECM analogs:

--- Ionic complexation of collagen/GAG <u>at acidic pH.</u>

--- Freeze-drying for the formation of pore structure.

--- Cross-linking.

COLLAGEN-SCAFFOLDS FREEZE DRYING

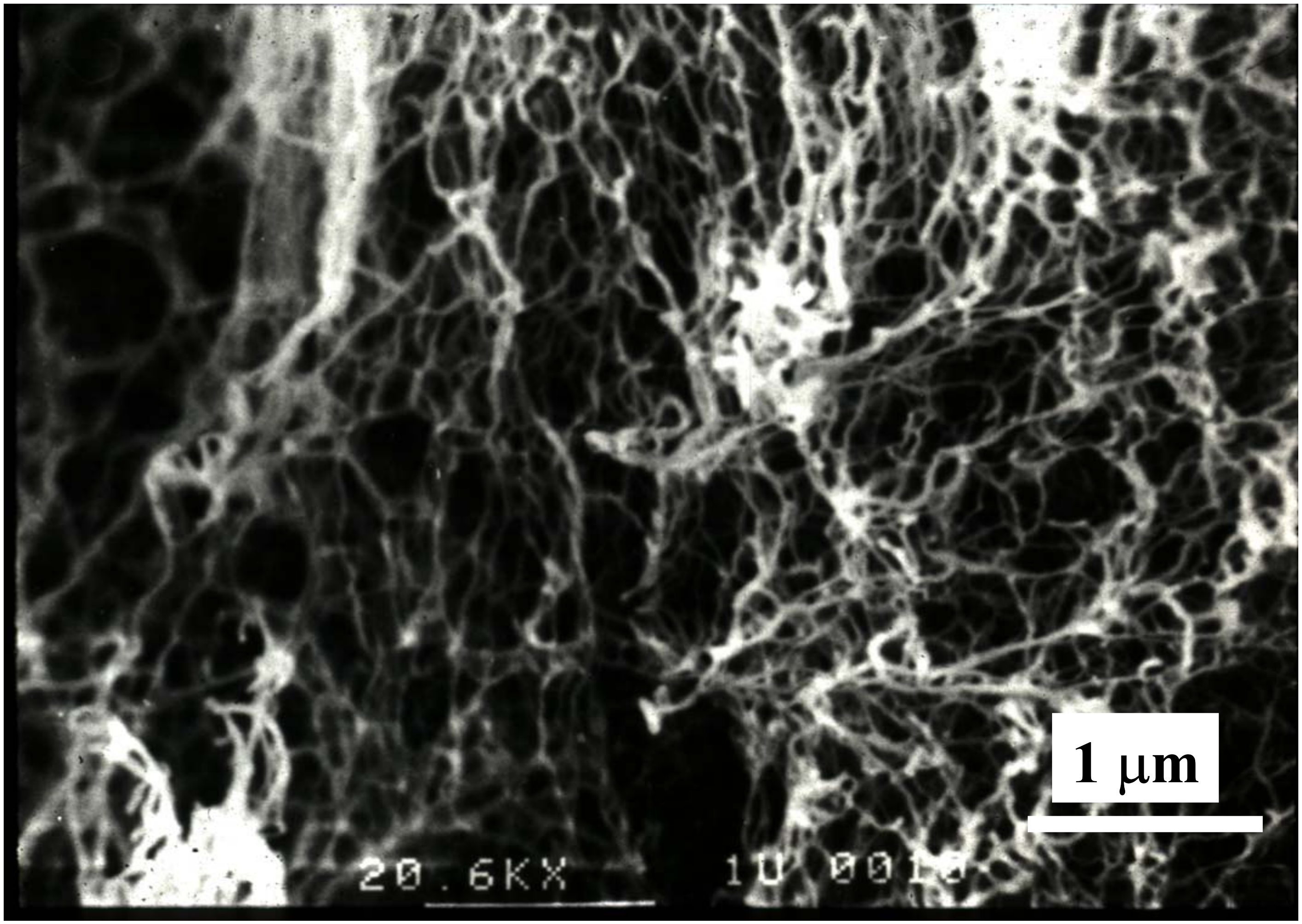
- Pore Diameter
  - Lower the temperature of freezing the smaller the pore diameter
- Cross-Linking; many methods of cross-linking
  - Dehydrothermal treatment
  - Ultraviolet light
  - Chemical agents (glutaraldehyde and carbodiimide)

COLLAGEN-GAG SCAFFOLDS

- Regeneration is dependent on pore diameter and degradation rate
  - Mechanisms to be discussed later in the term

Self-assembling Peptide Nanofibers

Scanning EM Image, EKA16-II



See Zhang, et al., "Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane." *PNAS* 90 no. 8 (1993 Apr 15): 3334-8.

FFF Technologies

1. SL – Sterolithography
2. LOM---Lamilated Object Manufacturing
3. FDM Fused Deposition Modeling
4. SLS Selected Laser Sintering
5. 3DP Three-Dimensional Printer

2 most important for tissue engineering scaffolds

Y Yan

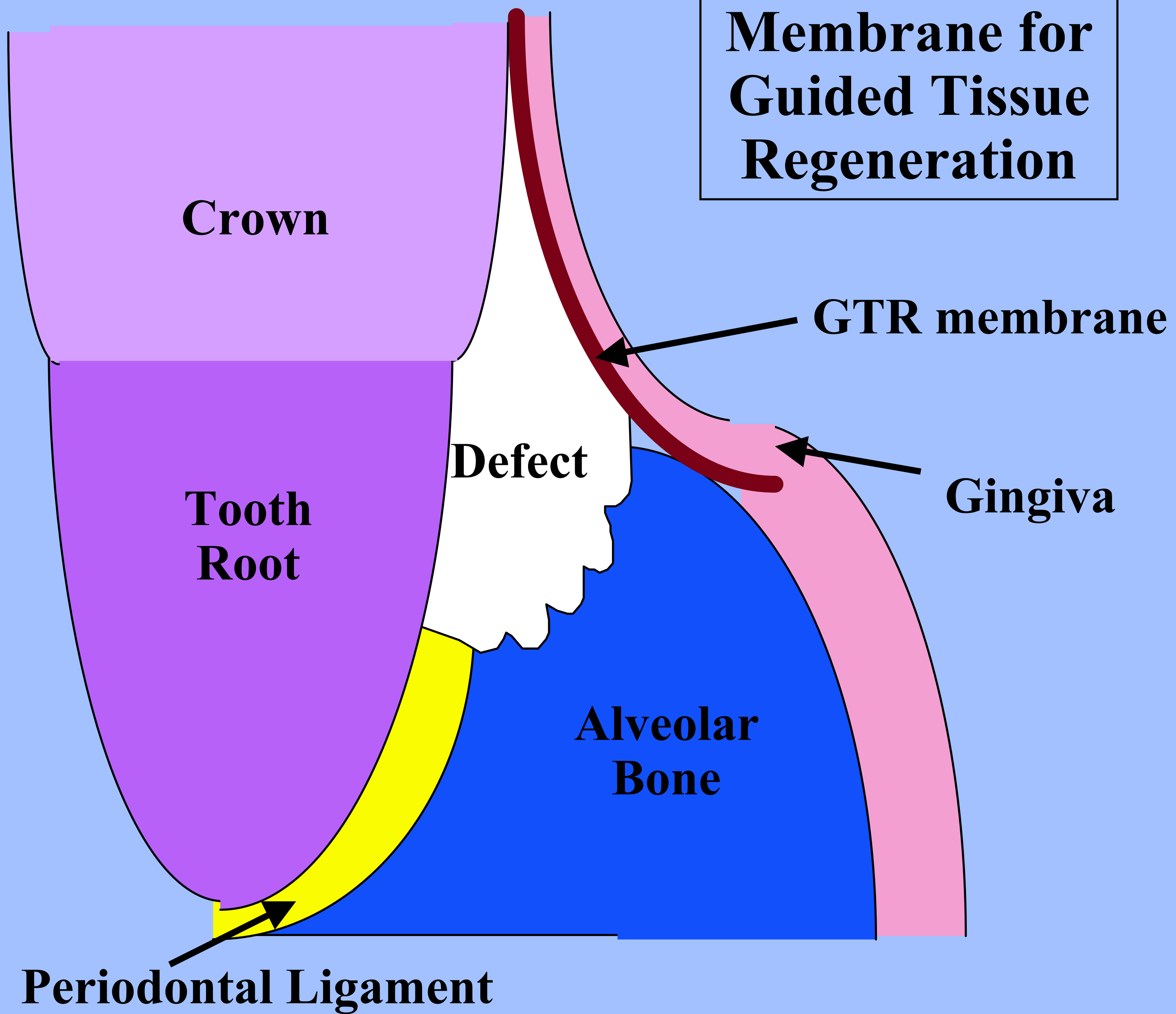
ROLES OF THE BIOMATERIALS/ SCAFFOLDS

1) before they are absorbed they serve as a matrix for cell adhesion to facilitate/“regulate” certain unit cell processes (*e.g.*, mitosis, synthesis, migration) of cells *in vivo* or for cells seeded *in vitro*.
2) structurally reinforce the defect to maintain the shape of the defect and prevent distortion of surrounding tissue.
3) serve as a barrier to the ingress of surrounding tissue that may impede the process of regeneration.
4) serve as a delivery vehicle for cells, growth factors, and genes.

ROLES OF A SCAFFOLD (MATRIX)
Cell Interactions

- **Facilitate cell-matrix interactions that are involved with tissue regeneration, by providing or binding the appropriate ligands.**
  - **Cell adherence**
  - **Mitosis (proliferation)**
  - **Migration**
  - **Synthesis**
  - **Contraction (favor or resist cell contraction)**
  - **Endocytosis (*e.g.*, of genes)**

Membrane for Guided Tissue Regeneration



PROPERTIES OF MATRICES
Change of Properties with Degradation

- Physical
  - Overall size and shape
  - Pore characteristics: % porosity, pore size distribution, interconnectivity, pore orientation
- Chemical
  - Biodegradability and substances released; degradation rate synchronized to the formation rate
  - Provide or bind ligands that affect cell function
- Mechanical
  - Strength (and related prop., *e.g.*, wear resistance)
  - Modulus of elasticity; stiffness
- Electrical and Optical ?

SCAFFOLDS (MATRICES) FOR TISSUE ENGINEERING

Categories

- **Synthetic Polymers**
  - *e.g.*, polylactic and polyglycolic acid
  - others
- **Natural Polymers**
  - fibrin
  - collagen
  - collagen-glycosaminoglycan copolymer
  - others

SCAFFOLD (MATRIX) MATERIALS
Synthetic

- Polylactic acid and polyglycolic acid
- Polycarbonates
- Polydioxanones
- Polyphosphazenes
- Poly(anhydrides)
- Poly(ortho esters)
- Poly(propylene fumarate)
- Pluronic (polaxomers)
  - Poly(ethylene oxide) and poly(propylene oxide)

SCAFFOLD (MATRIX) MATERIALS
Natural

- Collagen
  - Gelatin and fibrillar sponge
  - Non-cross-linked and cross-linked
- Collagen-GAG copolymer
- Albumin
- Fibrin
- Hyaluronic acid
- Cellulose
  - Most abundant natural polymer
  - Mechanism of absorbability *in vivo*?

SCAFFOLD (MATRIX) MATERIALS
Natural (Continued)

- Chitosan
  - Derived from chitin, 2nd most abundant natural polymer
  - Mechanism of absorbability *in vivo*?
- Polyhydroxalkanoates
  - Naturally occurring polyesters produced by fermentation
- Alginate (polysaccharide extracted from seaweed)
- Agarose
- Polyamino acids

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