Lecture #8:
ECM
Natural Scaffold Materials
Extracellular Matrix (ECM)

- ECM is a complex structural network surrounding and supporting cells
- Most natural polymers used as biomaterials are constituents of the ECM
- ECM vs connective tissue
  - ECM – structure network, *not including cells*
  - Connective tissue
    - Supportive tissues with large amounts of ECM
      - Includes bone, cartilage, loose connective tissue
      - Excludes nerve, muscle, blood, epithelial tissues
ECM Composition

- The ECM is composed of 3 major classes of biomolecules
  1. Structural proteins: collagen and elastin
  2. Specialized proteins: e.g. fibrillin, fibronectin, and laminin
  3. Proteoglycans: protein core with attached polysaccharides, glycosaminoglycans (GAGs), forming a complex macromolecule
ECM Forms

1. Interstitial matrix (in connective tissue):
   - Matrix can be calcified as in bone
   - Can be transparent as in the cornea
   - Ropelike organization as in tendons
2. Basal laminae

- Flexible, thin (40-120 nm thick) mats of specialized ECM that
  - Underlie all epithelial and endothelial sheets and tubes.
  - Surround individual muscle cells, fat cells and Schwann cells
- Separate them from connective tissue or another layer of cells
- Basement membrane
  - Basal laminae plus a layer of anchoring collagen microfibrils that anchor the basal laminae to connective tissue
Basal Lamina in the Cornea of chick Embryo

Epithelial Cells

Basal Lamina

Collagen Fibers
Molecular Structure of a Basal Lamina

(A) Basal Lamina is formed by specific interactions

(B) Molecules that directly bind each other
Native ECM Functions

- Scaffold to support and organize cells
- Regulate cell function
- Provide mechanical support to the tissue

Schematic overview of the types of molecules that bind cells to each other and to the extracellular matrix.
Extracellular Matrix

- ECM can influence
  - Cell shape
    - Cells align with the direction of fibers
    - Cell spreading
  - Cell survival
    - Anchorage dependent cells
    - Mediated by integrins and intracellular signals
Extracellular Matrix Functions

• ECM can influence
  – Cell proliferation
    • Anchorage dependent cells
    • Physical spreading of a cell on the matrix also has a strong influence on intracellular events
    • Cells that are forced to spread over a large surface area survive better and proliferate faster than cells that are not so spread out
      – Even if the cells have the same area making direct contact with the matrix (equal number of matrix molecules in contact with the cells)
Collagen

- 90% of ECM from most tissues
- Tensile strength
- More than 20 types
  - Bovine Type I collagen from Achilles tendon
    - most common therapeutic source
  - Type IV in vascular basement membrane
Fibronectin
- RGD (Arg-Gly-Asp) peptide is bound by $\alpha_5\beta_1$ integrin
- Adhesive properties useful in cell culture and biocompatibility coatings

Laminin
- Complex adhesion protein
- Important in development and in cell/tissue differentiation
Glycosaminoglycans (GAGs)

- Bind growth factors and cytokines
- Retain water to provide compressive strength (gel)
- Chondroitin sulfates A and B, heparin, heparan sulfate, and hyaluronic acid.
Engineered Scaffold Functions

- Scaffold to support and organize cells
- Regulate cell function
- Provide mechanical support to the tissue
- Determine gross morphology of the engineered tissue
- Create space for potential tissue formation
- Provide specific cell adhesion receptors
- Act as a reservoir for immobilized growth factors
- Impart tensile and compressive strength
Degradation

- Natural ECM scaffolds degrade quickly after implantation (unless chemically crosslinked)
- Strength of scaffold can decrease in early weeks following implant
- Remodeling can result in final replacement tissue as strong or stronger than native
- Degraded ECM releases bound growth factors and peptides
Example: ECM + Cells

- Esophagus repair in dogs (5 cm length)
  1. ECM scaffold only (porcine urinary bladder)
  2. Autologous muscle tissue only
  3. ECM + 30% muscle
  4. ECM + 100% muscle

Healthy remodeled ECM scaffold (group 3)
Naturally Derived Scaffold Materials

- Collagen and Gelatin (single-stranded collagen)
- Hyaluronan (hyaluronic acid)
- Fibrin
- Alginate
- Agarose
- Chitosan
- Acellular tissue-derived matrices (Small intestinal submucosa)
Collagen

- The prototypical scaffold material
- Facilitates cell adhesion
- Can be proteolytically remodeled by cells
- Can be solubilized into a liquid form conducive to injection in a minimally-invasive fashion
- Minimal inflammatory and antigenic responses
Type-I Collagen Gels

[Images and diagrams related to Type-I Collagen Gels]
A Type I Collagen Sponge Used as an ECM Analog

Porous Sponge without Cells

Porous Sponge with Smooth Muscle Cells (1 Day Post-Seeding)

Limitations to Collagen

- Collagen gels are thermally reversible and possess limited range of mechanical properties.
  - Collagen cross-linked with glutaraldehyde has improved mechanical properties but can have cytotoxic effects
- Source issues (xenogeneic, allogeneic, autologous?)
  - If from a non-autologous source, cells embedded within it have to be removed to reduce the chance of an adverse immune response
Gelatin

- Single-stranded collagen, produced by partial hydrolysis of collagen
- (Boil down bones and cartilage)
- Forms gel at lower temperatures, liquid at higher temperatures
- Can be crosslinked to stabilize
Fibrin(-ogen)

Fibrinogen (Factor I)

Factor XIII

Fibrinogen (Factor I)

Thrombin (Factor IIa)

Fibrin (Factor Ia)

Factor XIIIa

Fibrinolysis

Plasmin

MMPs

Aprotinin

TIMPs

X-linked Fibrin

Factor XIIIa: transglutaminase
Covalent bond between free amine (e.g. lysine) and acyl group (e.g. glutamine)

www.well.ox.ac.uk/~fionag/fibrinogen.shtml
Fibrin and the Coagulation Cascade

Fibrinogen → Thrombin → FpA + FpB → Soluble Fibrin Polymer

Factor XIIIa
Increasing Fibrin Concentration Influences the Mechanical Properties and Porosity

Alginate: A Naturally Derived GAG Analog

• A linear polysaccharide material isolated from brown seaweed
  – Cheap and plentiful
  – Can be cross-linked with calcium to form a hydrogel
  – Offers compression resistance like native GAGs
  – Cells do not have receptors for “alginate”
  – Can be modified with RGD
Alginate: A Naturally Derived GAG Analog

\[
\begin{align*}
(G) \text{ guluronic} & & (M) \text{ mannuronic} \\
\end{align*}
\]

\[\text{Ca}^{2+}\]
Calcium-Dependent Alginate Gel Formation

- Mix cells with alginate and drip into Ca$^{2+}$ ion solution
- Can recover gels with Calcium chelator (EDTA)
- Also can inject and form gel in situ

Chondrocytes encapsulated in alginate beads

http://articular.com/default_files/Page1604.htm
RGD Modification of Alginate

![Images of alginate modifications with and without RGD](image.png)

![Graph showing mass comparison](image.png)

Alsberg, Mooney+, PNAS 2002
Hyaluronan (HA)

- Sometimes called hyaluronic acid
- Not to be confused with hydroxyapatite (the other HA)
- Is a linear polysaccharide composed of a repeating disaccharide
- Can be formed into hydrogels by covalent cross-linking with hydrazide derivatives
- Can be combined with other natural biopolymers to form novel composite hydrogels
Chitosan: Another naturally derived GAG analog

- From shells of crustaceans and cell walls of fungi
- Bioadhesive, wound dressing, drug delivery, and TE scaffold
- Bone and Cartilage; also skin, neural, others
- Can be cross-linked to form gel (glutaraldehyde)
Scaffolds from Decellularized Organs: Heart

Ott et al, Nat Medicine, 2008
Scaffolds from Decellularized Organs:
Heart

Seeded with neonatal cardiac cells and cultured in perfusion bioreactor for up to one month.

Ott et al, Nat Medicine, 2008
Scaffolds from Decellularized Organs: Lung

Petersen et al, Science, 2010
Scaffolds from Decellularized Organs: Lung

http://www.jove.com/video/2651/procedure-for-lung-engineering
Injectable ECM from Decellularized Organs


http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3159653/
Limitations of Naturally-Derived Materials

- Isolation and processing of native materials contribute to:
  - Variable product
  - Large expense
  - Limited range of properties
  - Possibility of inflammatory response (foreign proteins even though cells have been removed)
Chapter 5
The extracellular matrix as a biologic scaffold for tissue engineering

Stephen Badylak, Thomas Gilbert and Julie Myers-Irvin

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