Lecture #8:
ECM
Natural Scaffold Materials

Extracellular Matrix (ECM)

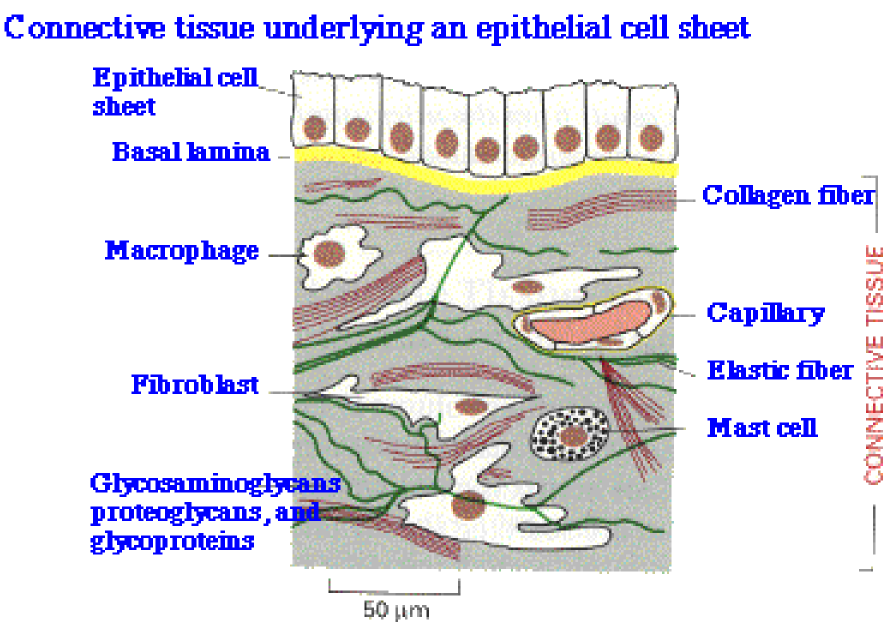
- ECM is a complex structural network surrounding and supporting cells
- Most natural polymers used as biomaterials are constituents of the ECM
- ECM vs connective tissue
  - ECM – structure network, *not including cells*
  - Connective tissue
    - Supportive tissues with large amounts of ECM
      - 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

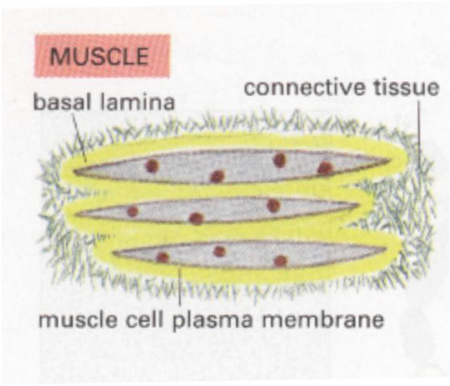
ECM Forms

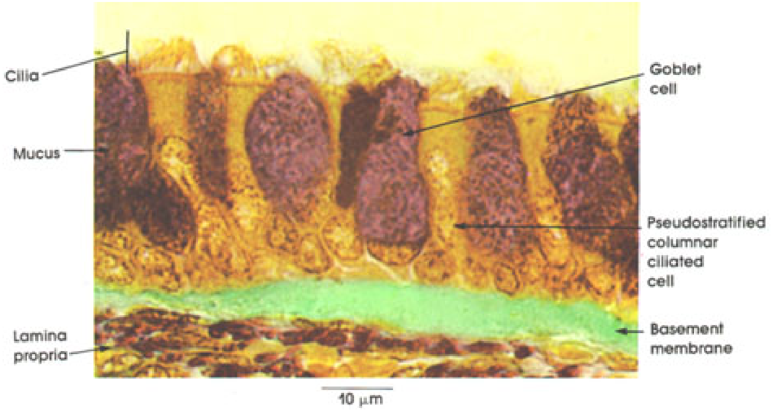
1. Interstitial matrix (in connective tissue):
   - Matrix can be calcified as in bone
   - Can be transparent as in the cornea
   - Ropelike organization as in tendons

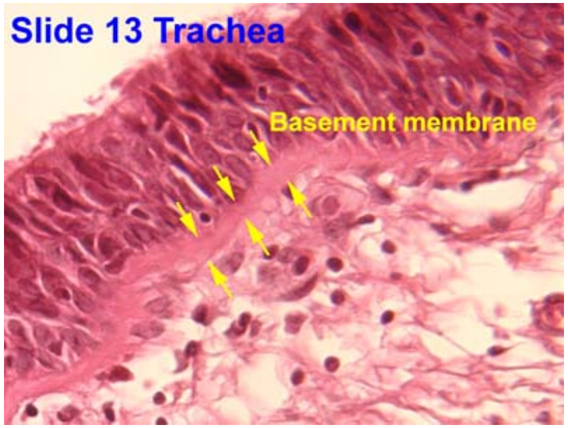


ECM Forms

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





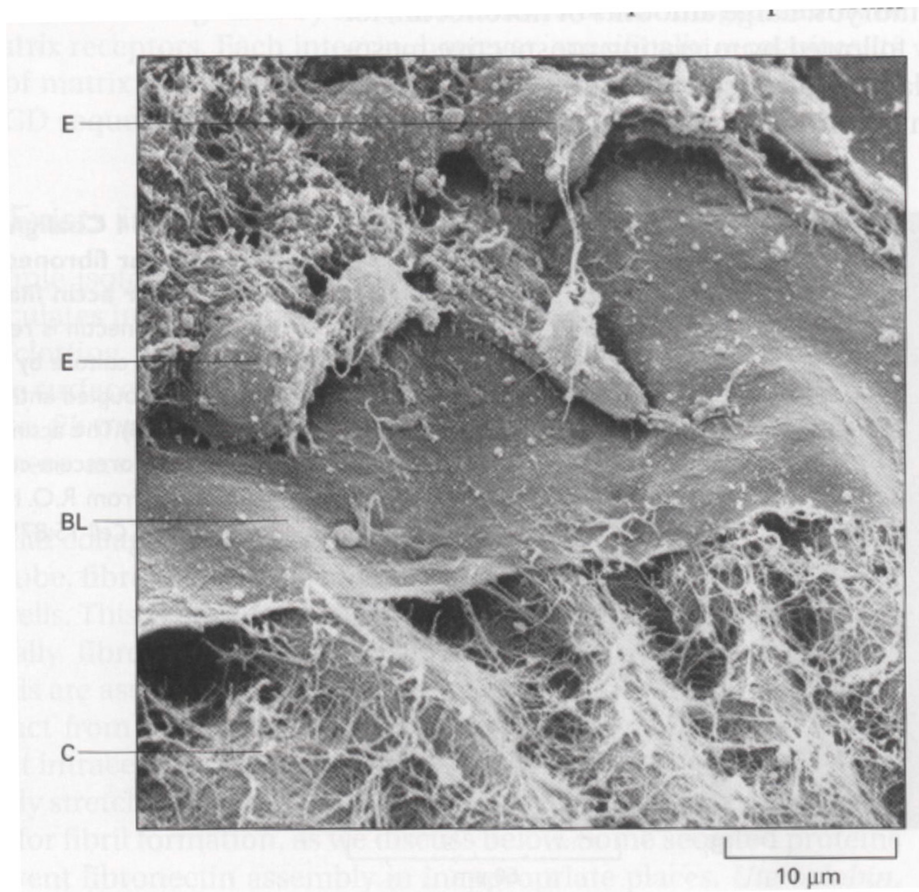


Basal Lamina in the Cornea of chick Embryo

Epithelial Cells

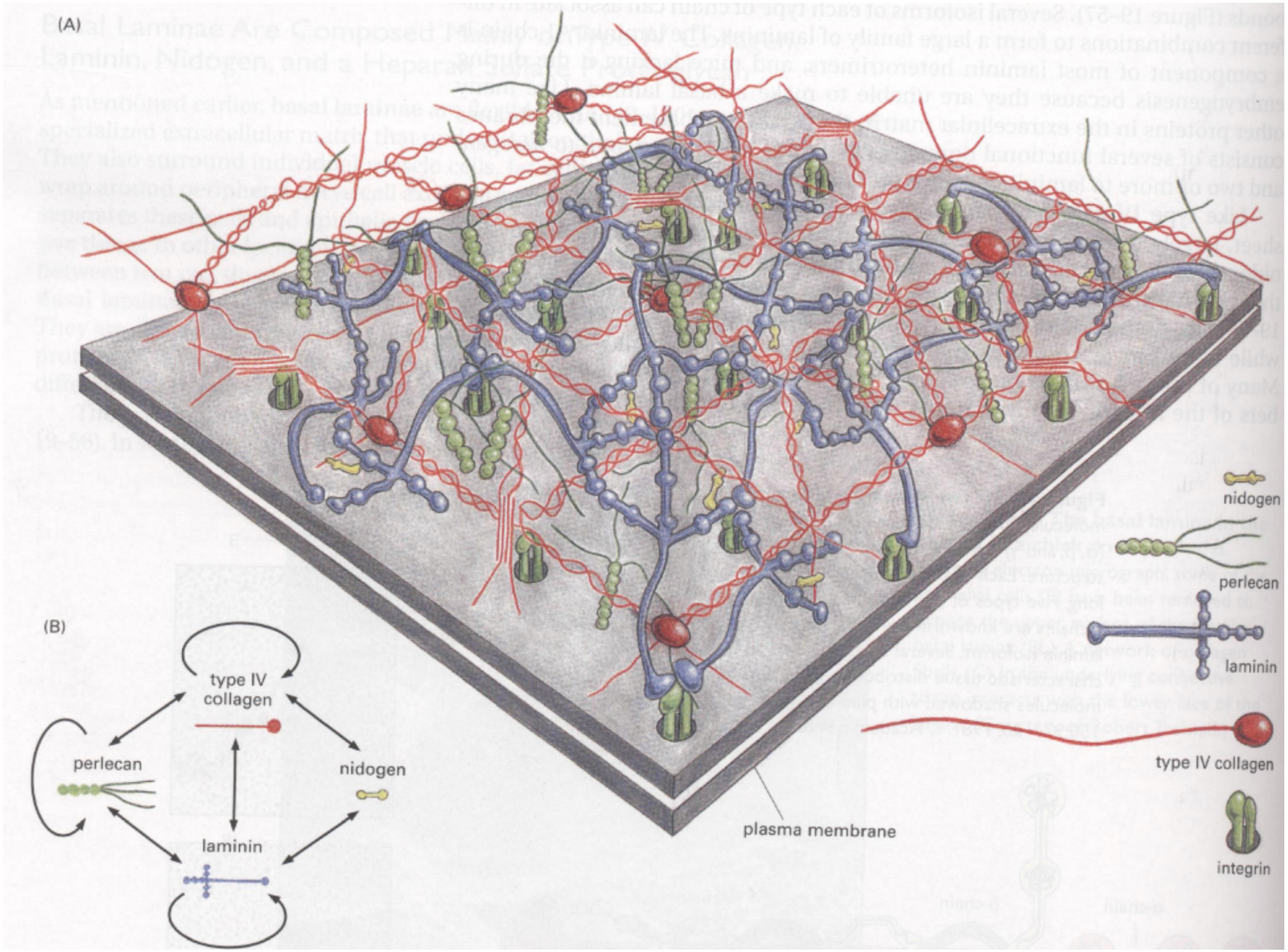
Basal Lamina

Collagen Fibers



Molecular Structure of a Basal Lamina

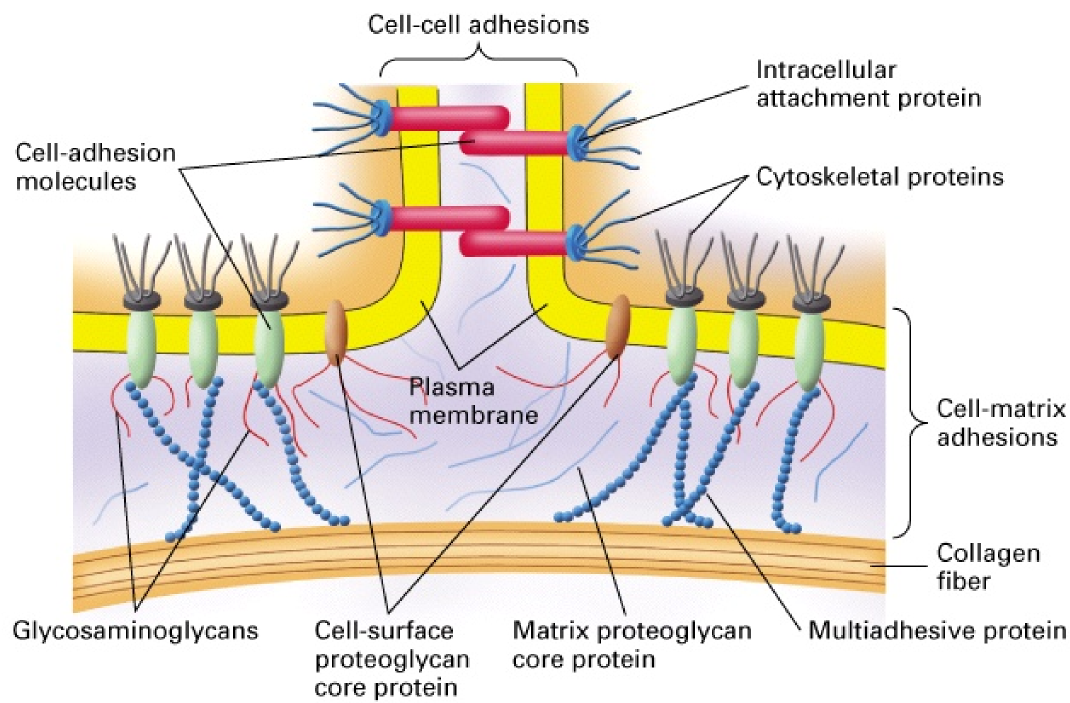
(A) Basal Lamina is formed by specific interactions



(B) Molecules that directly bind each other

Native ECM Functions

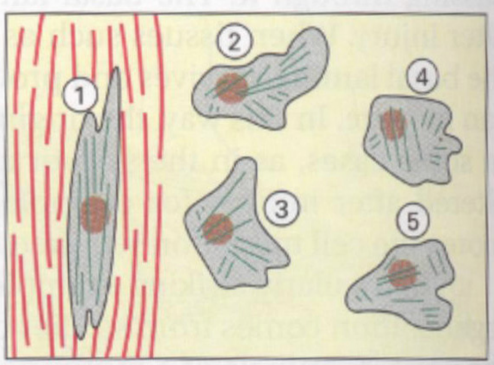
- Scaffold to support and organize cells
- Regulate cell function
- Provide mechanical support to the tissue



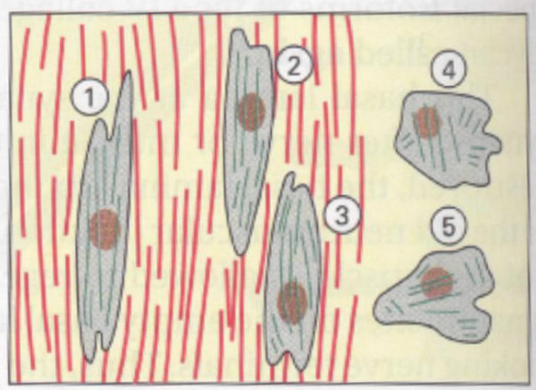
Schematic overview of the types of molecules that bind cells to each other and to the extracellular matrix

Extracellular Matrix

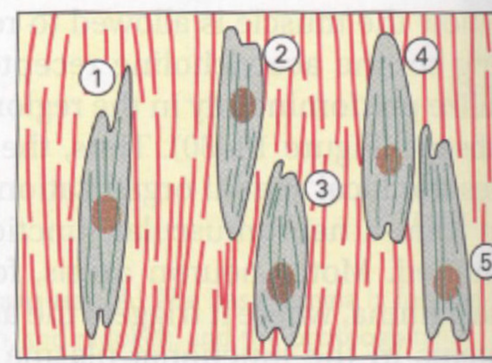
– ECM can influence
  - Cell shape
    – Cells align with the direction of fibers
    – Cell spreading
  - Cell survival
    – Anchorage dependent cells
    – Mediated by integrins and intracellular signals



orientation of cytoskeleton in cell 1 orients the assembly of secreted extra-cellular matrix molecules in the vicinity



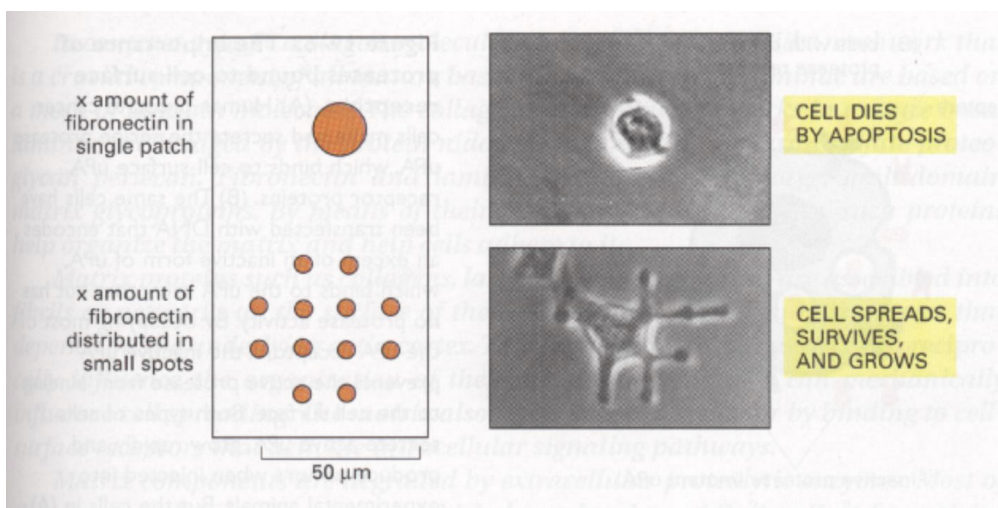
the oriented extracellular matrix reaches cells 2 and 3 and orients the cytoskeleton of those cells



cells 2 and 3 now secrete an oriented matrix in their vicinity; in this way the ordering of cytoskeletons is propagated to cells 4 and 5

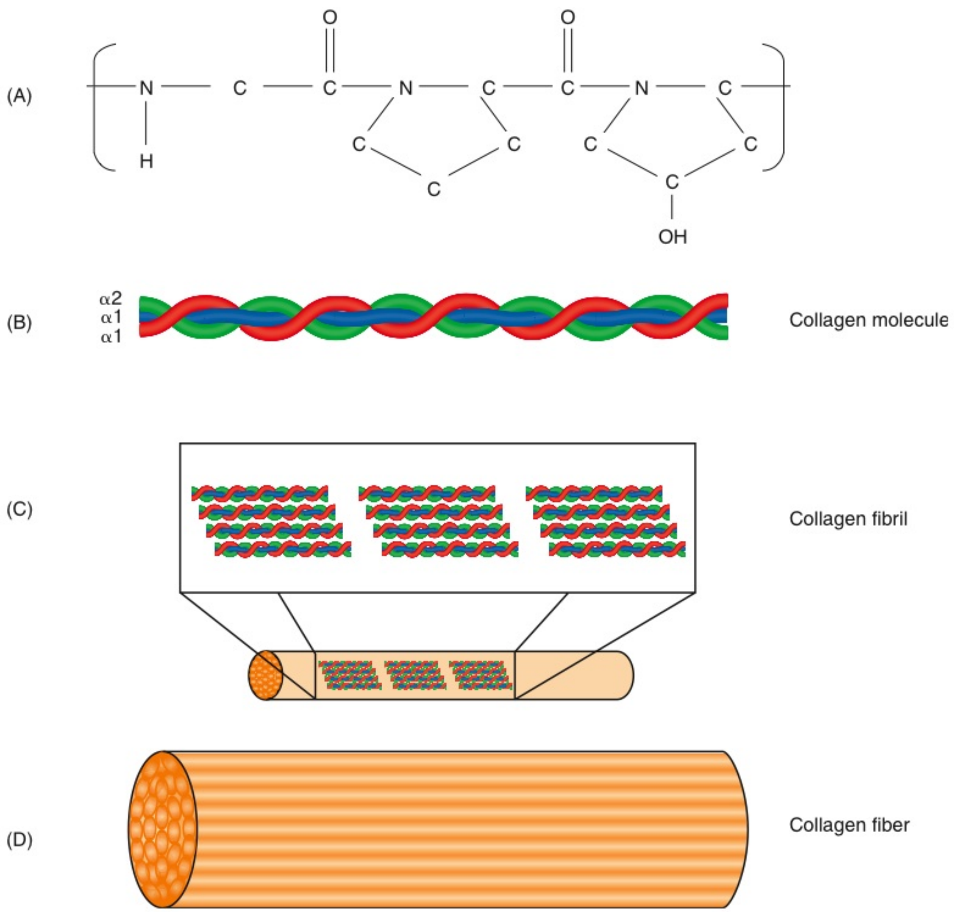
Extracellular Matrix Functions

- 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

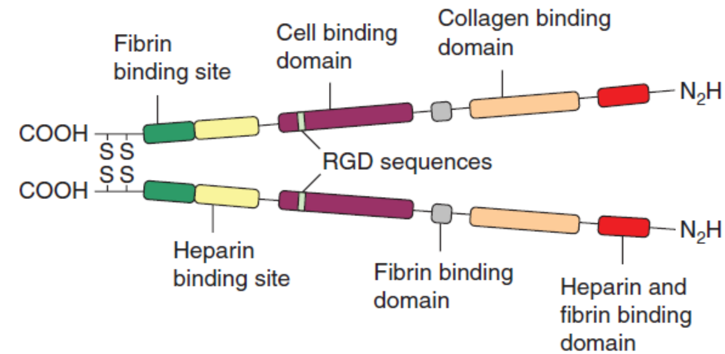
- 90% of ECM from most tissues
- Tensile strength
- More than 20 types
  - Bovine Type I collagen from Achilles tendon
    - most common therapeutic source
  - Type IV in vascular basement membrane



Fibronectin

– RGD (Arg-Gly-Asp) peptide is bound by $\alpha_5\beta_1$ integrin

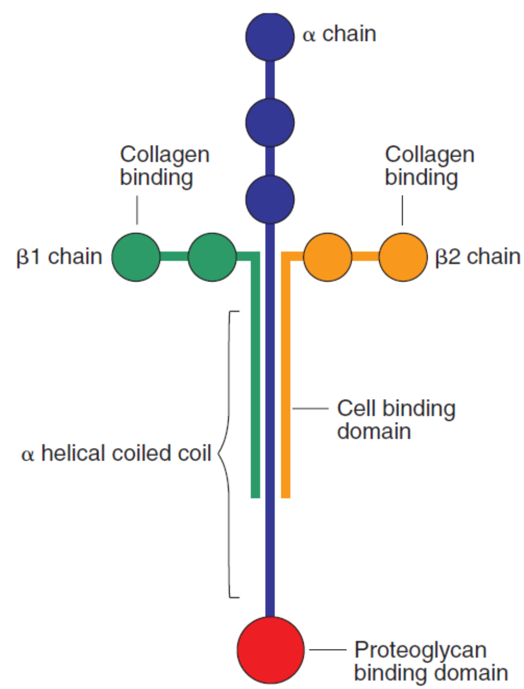
– Adhesive properties useful in cell culture and biocompatibility coatings



Laminin

– Complex adhesion protein

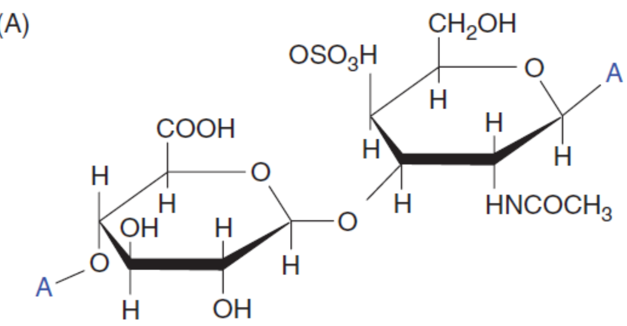
– Important in development and in cell/tissue differentiation



Glycosaminoglycans (GAGs)

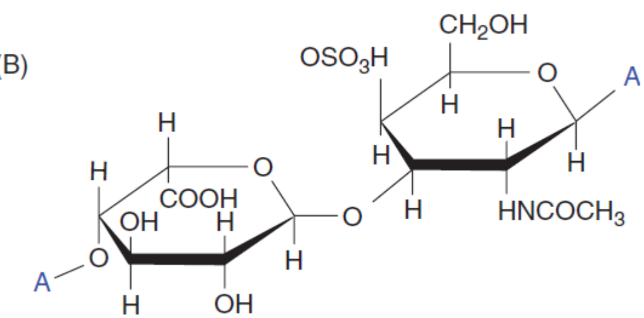
- Bind growth factors and cytokines
- Retain water to provide compressive strength (gel)
- Chondroitin sulfates A and B, heparin, heparan sulfate, and hyaluronic acid.

(A)



Chondroitin sulfate A

(B)



Dermatan sulfate (chondroitin sulfate B)

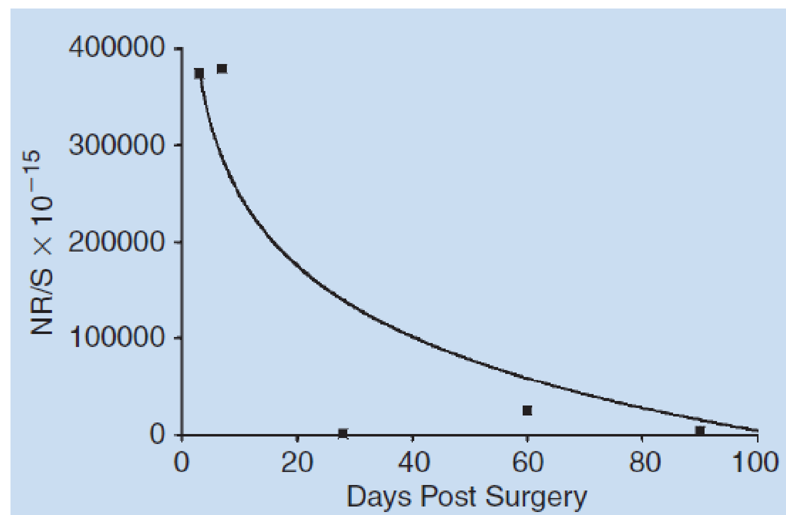
Engineered Scaffold Functions

- 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

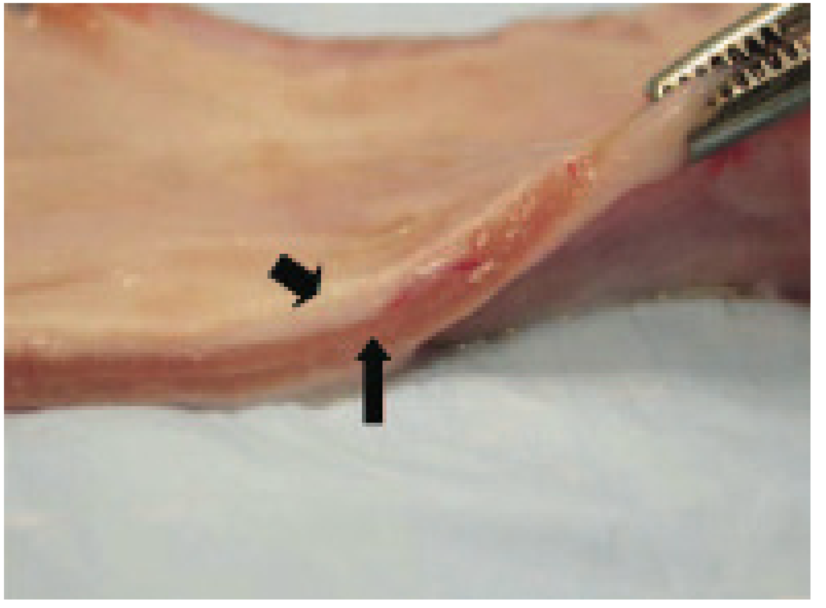


Degradation of radiolabeled ECM scaffold implanted into bladder

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)

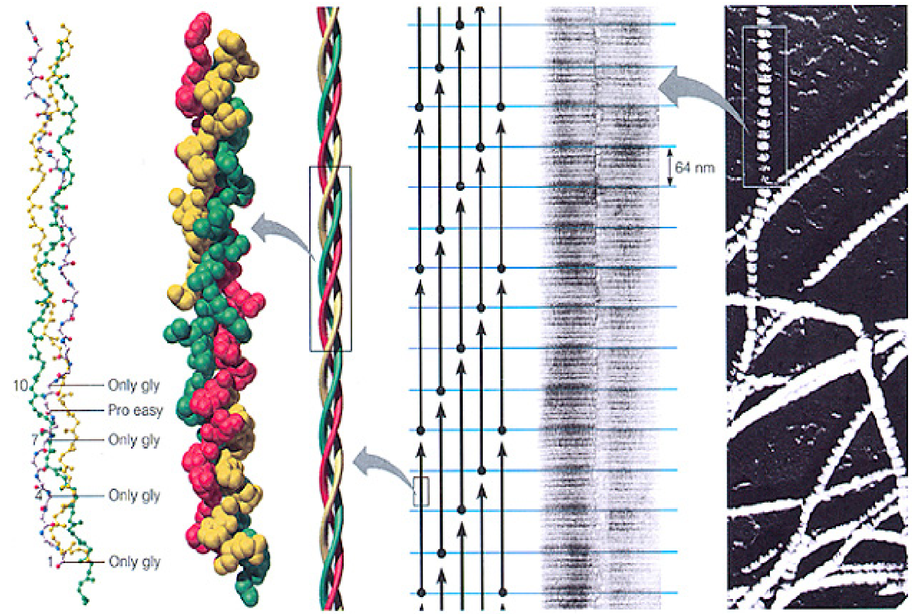
Naturally Derived Scaffold Materials

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

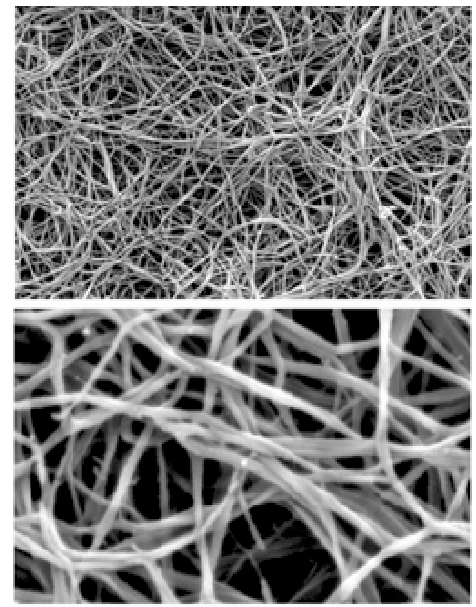
Collagen

- 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

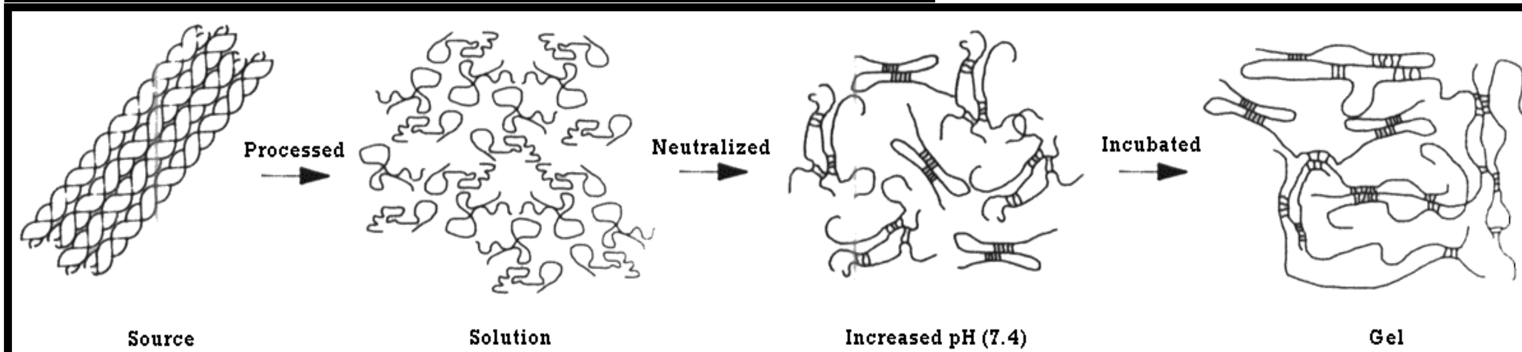
Type-I Collagen Gels



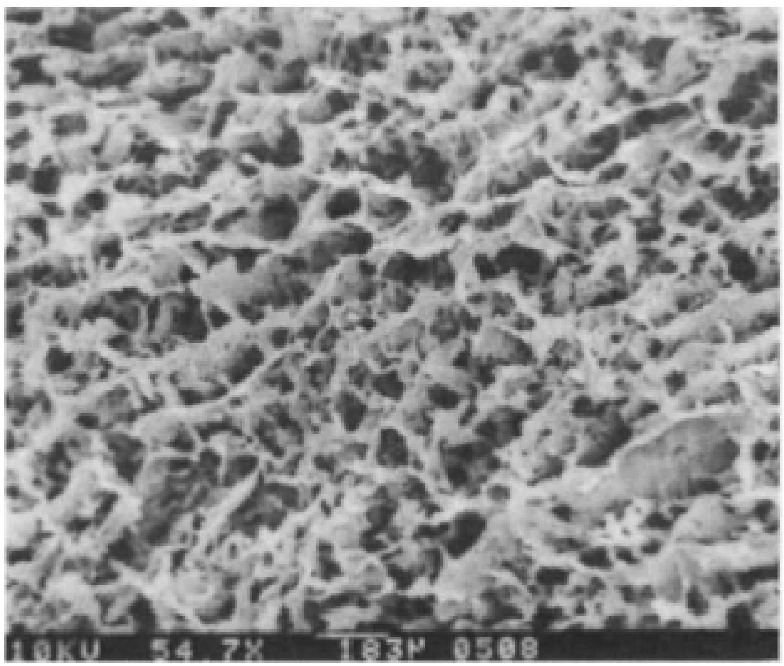
Benjamin/Cummings, an imprint of Addison Wesley Longman, Inc.



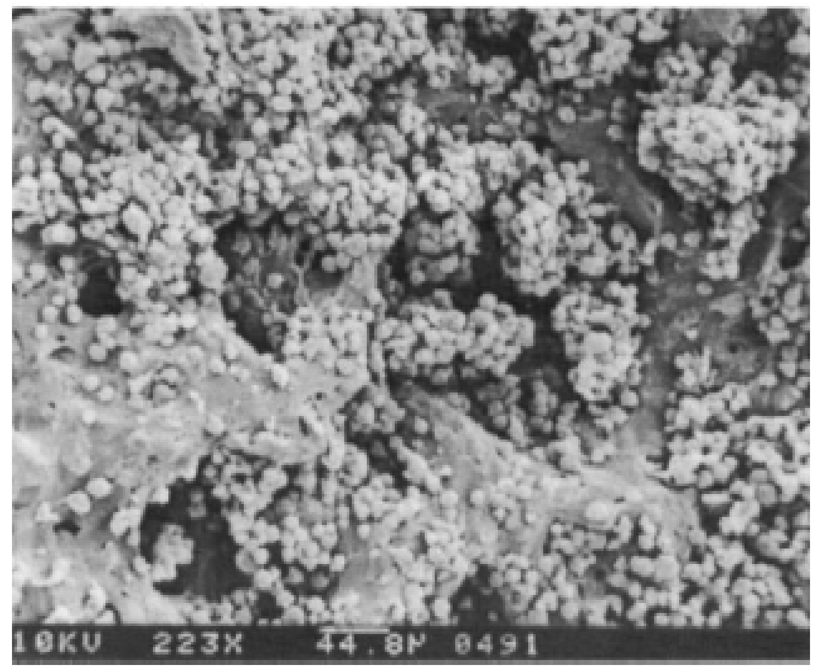
Raub CB, et al. - Biophys J. 2007 Mar 15;92(6):2212-22.



A Type I Collagen Sponge Used as an ECM Analog



Porous Sponge without Cells



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

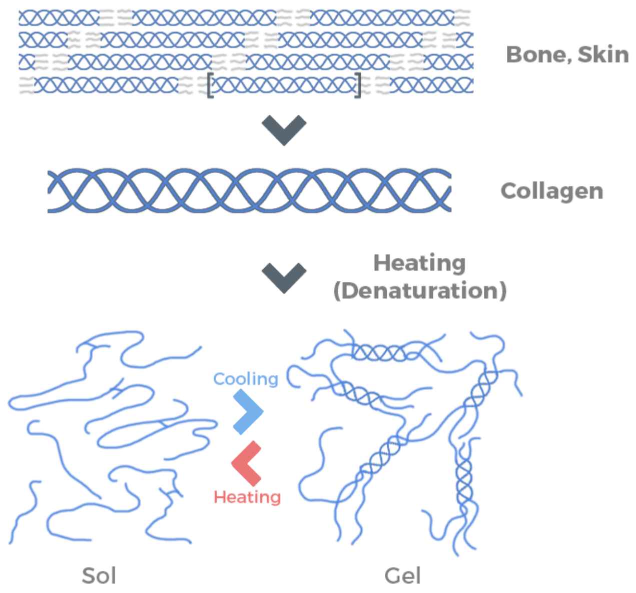
Reference: Kim BS, Nikolovski J, Bonadio J, Smiley E, Mooney DJ. Engineered smooth muscle tissues: regulating cell phenotype with the scaffold. *Exp Cell Res.* 1999 Sep 15;251(2):318-28.

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

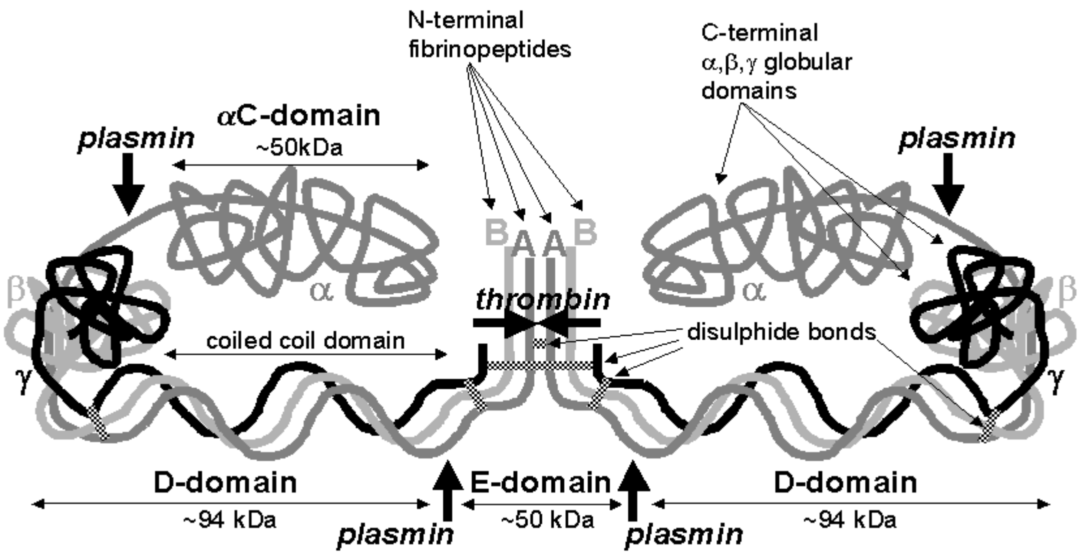
Gelatin

- 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)



www.well.ox.ac.uk/~fionag/fibrinogen.shtml

Factor XIII

Fibrinogen (Factor I)

Thrombin (Factor IIa)

Fibrin (Factor Ia)

Factor XIIIa

X-linked Fibrin

Plasmin

MMPs

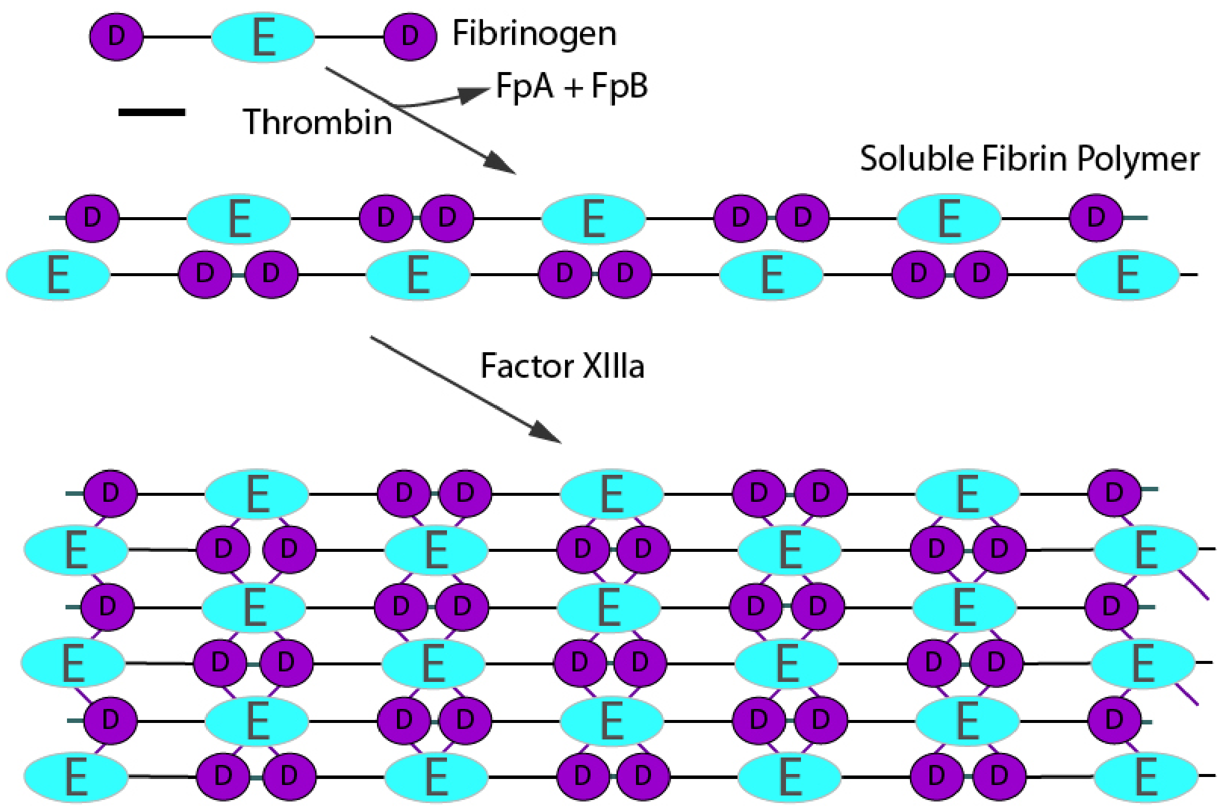
Fibrinolysis

Aprotinin

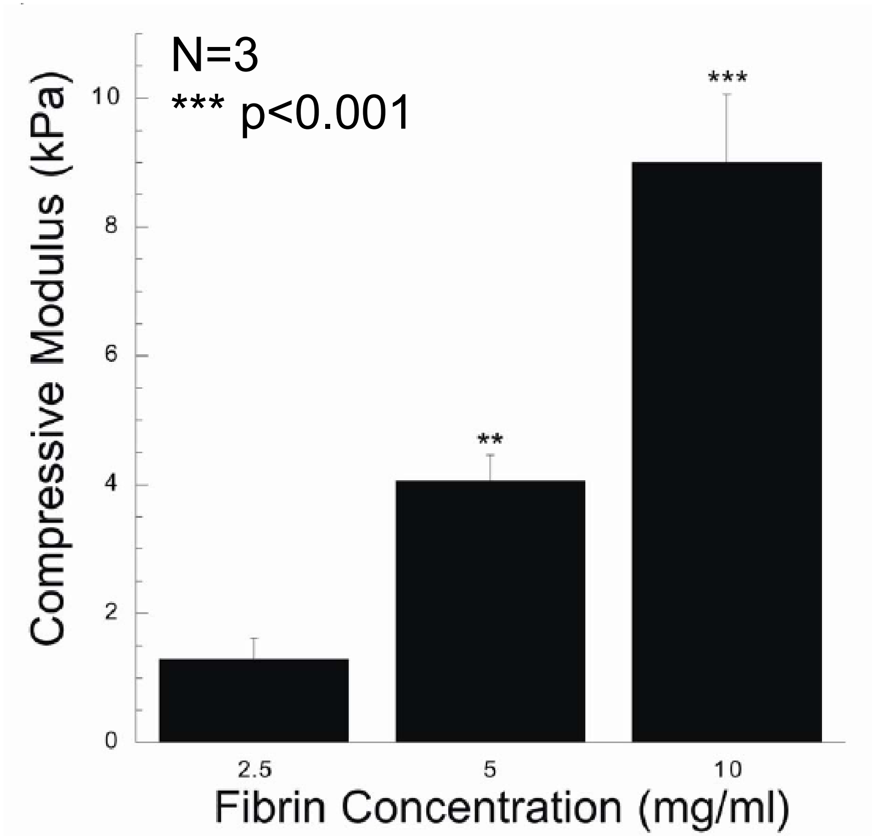
TIMPs

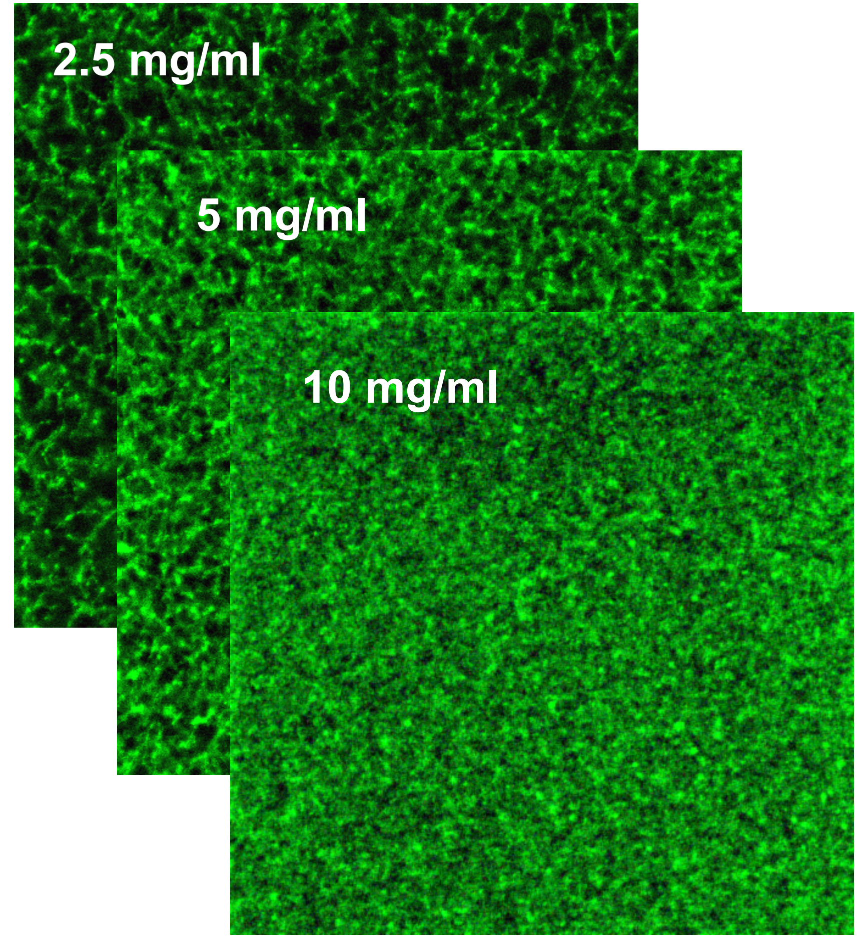
Factor XIIIa: transglutaminase
Covalent bond between free amine (e.g. lysine) and acyl group (e.g. glutamine)

Fibrin and the Coagulation Cascade



Increasing Fibrin Concentration Influences the Mechanical Properties and Porosity



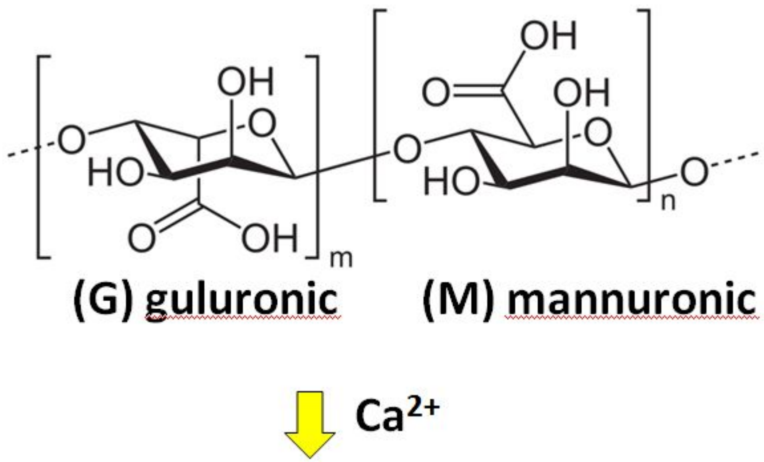


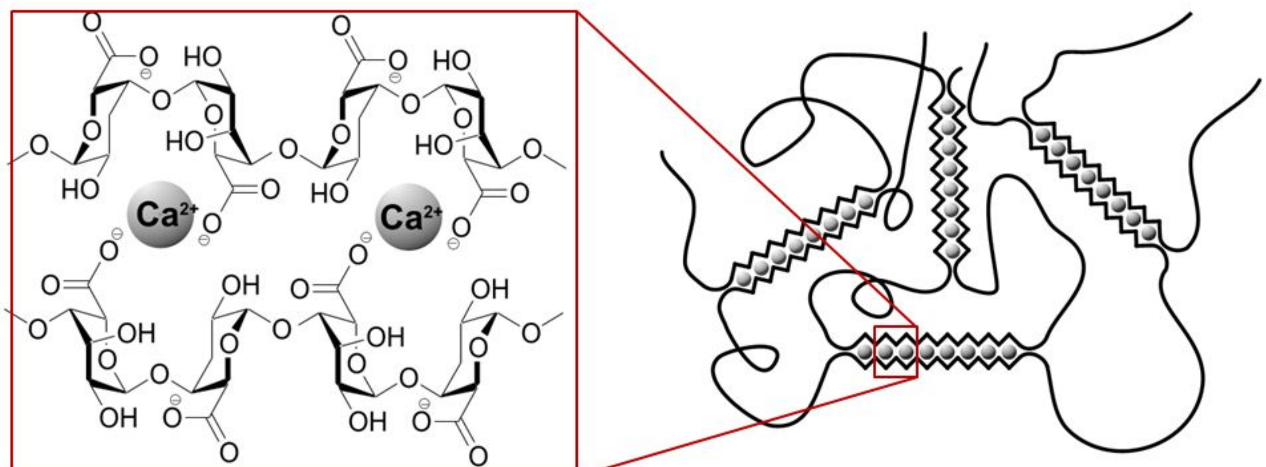
C.M. Ghajar, K.S. Blevins, C.C. Hughes, S.C. George, and A.J. Putnam. *Tissue Engineering* (2006)

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

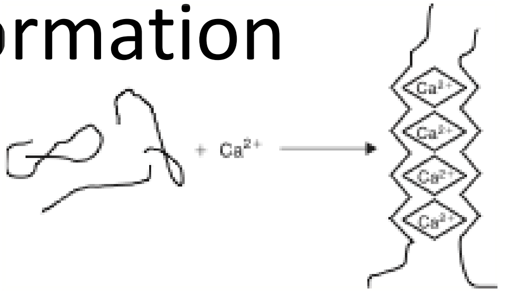
Alginate: A Naturally Derived GAG Analog



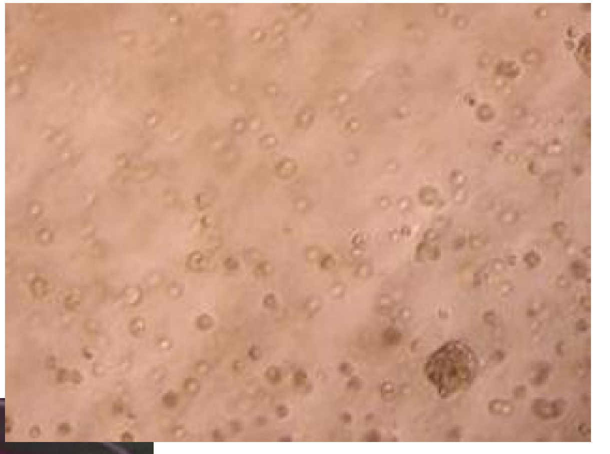


Amelman
Clarkson Univ.

Calcium-Dependent Alginate Gel Formation



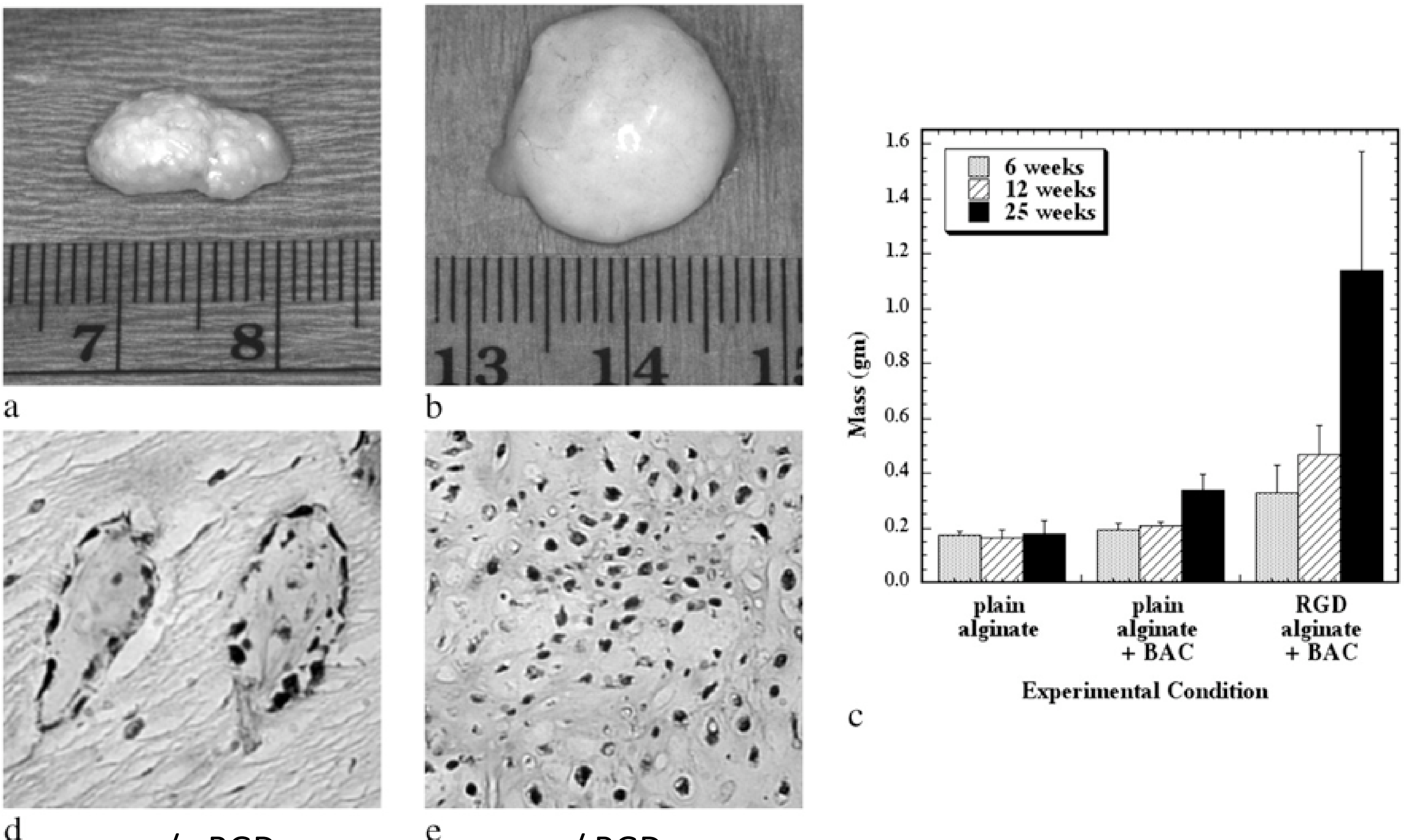
- Mix cells with alginate and drip into Ca^{2+} ion solution
- Can recover gels with Calcium chelator (EDTA)
- Also can inject and form gel *in situ*



Chondrocytes encapsulated in alginate beads

http://articular.com/default_files/Page1604.htm

RGD Modification of Alginate

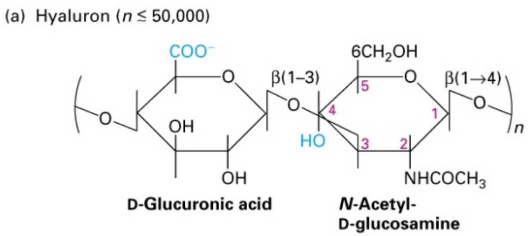


w/o RGD

w/ RGD

Alsberg, Mooney+, PNAS 2002

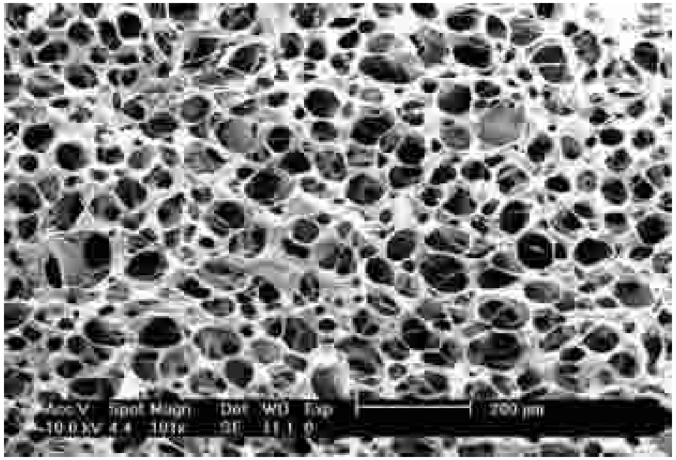
Hyaluronan (HA)

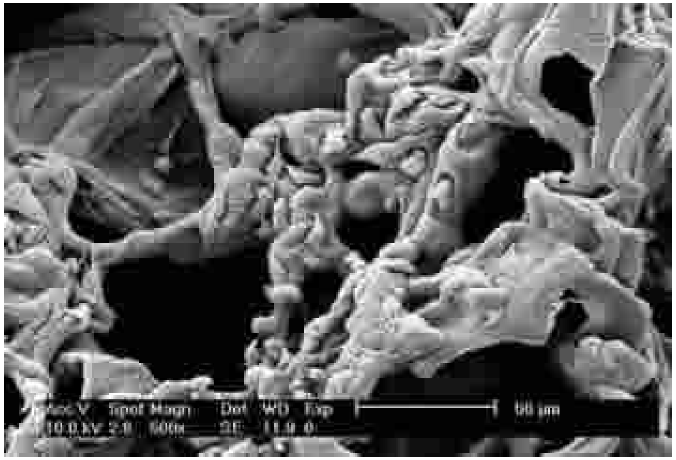


- Sometimes called hyaluronic acid
- Not to be confused with hydroxyapatite (the other HA)
- Is a linear polysaccharide composed of a repeating disaccharide
- Can be formed into hydrogels by covalent cross-linking with hydrazide derivatives
- Can be combined with other natural biopolymers to form novel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

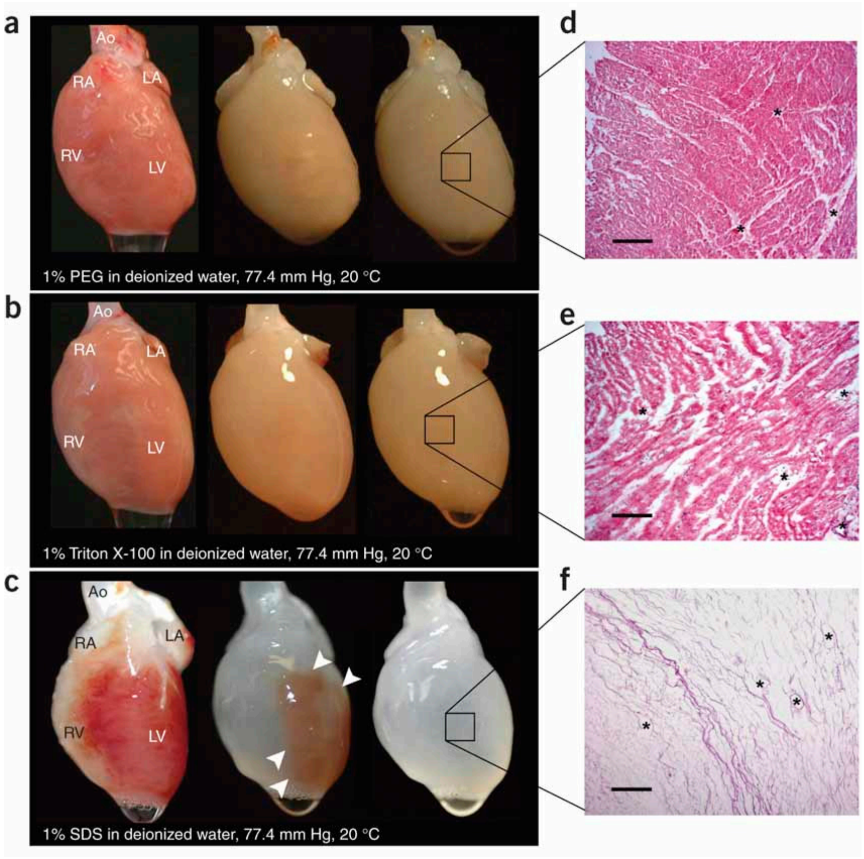
Chitosan: Another naturally derived GAG analog

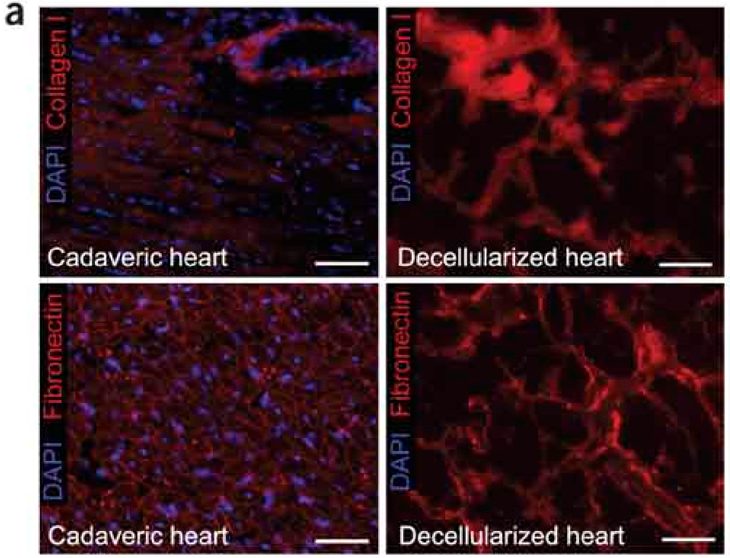
- From shells of crustaceans and cell walls of fungi
- Bioadhesive, wound dressing, drug delivery, and TE scaffold
- Bone and Cartilage; also skin, neural, others
- Can be cross-linked to form gel (glutaraldehyde)





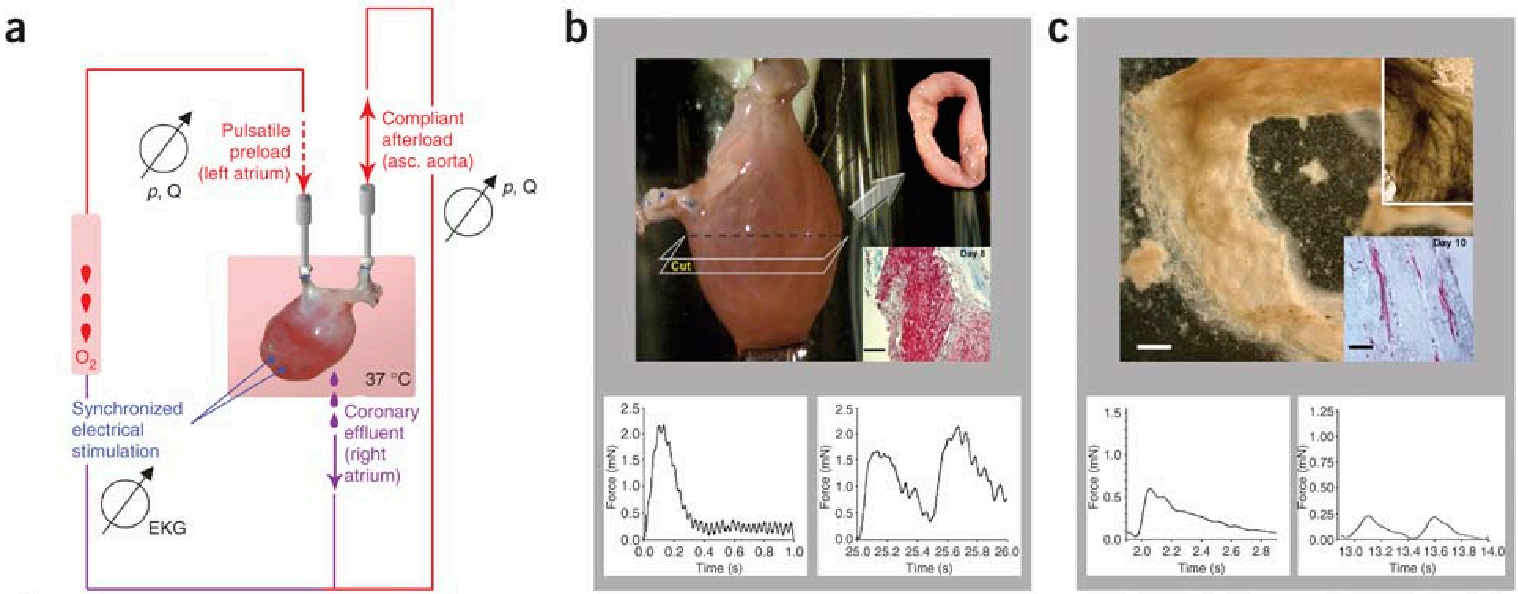
Scaffolds from Decellularized Organs: Heart





Ott et al, Nat Medicine, 2008

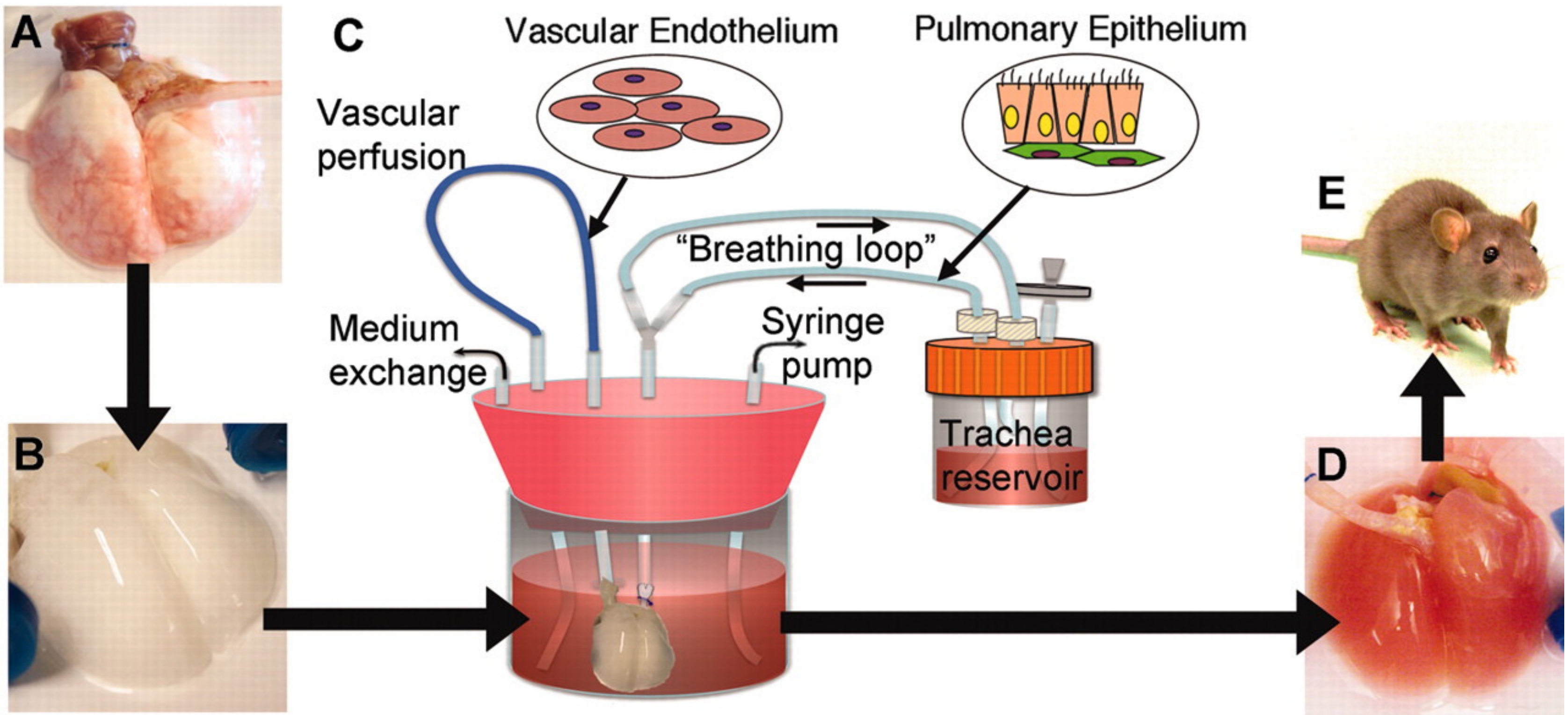
Scaffolds from Decellularized Organs: Heart



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.jove.com/video/2109/fabrication-biologically-derived-injectable-materials-for-myocardial

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)

Additional Reading

Chapter 5

The extracellular matrix as a biologic scaffold for tissue engineering

Stephen Badylak, Thomas Gilbert and Julie Myers-Irvin

Chapter contents