Aalto University
School of Chemical Technology

CHEM-E3225, Cell- and Tissue Engineering, 5 cr

TOPIC 3 :

Selected Cell-ECM Interactions, with implications for growing cells and tissues. This is a more detailed view that in the Book by Birla.

(ECM is covered both in Chapter 2 and Chapter 3, we already covered CHP 2 (Topic 2) , so also look at text on ECM in CHP 3 (3.12) , this is relevant to Biomaterials also)

We will look at:

1) ECM = Extracellular Matrix
2) ECM composition
3) Cell surface receptors for ECM molecules

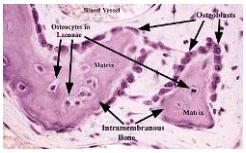
Why do we need to understand these ?

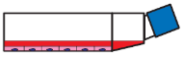
- Because it lays the foundation to understand what is needed for cell or tissue implants to function as "implants"
- Because, as "implants" they need to have normal functions (or at least mimic them)
- Because it shows that we may need materials and scaffolds to support the implant so that it can function

Extracellular environment

What is present outside the cell?

- Adjacent cells: cell-cell interaction
- ECM (substrate): cell-matrix interaction
- Soluble factors: e.g. growth factor-mediated signal transduction





Major types of macromolecules to be examined today (figure 10.1 next ppt)

1. Molecules important for their structural properties (collagens and elastin)
2. Multidomain molecules: both structural constituents as well as regulators of cell behavior (fibronectin, fibrillin, laminin, thrombospondin, tenascin, perlecan and other proteoglycans)
3. Matrix-bound signaling molecules (such as matrix-bound fibroblast growth factors (FGFs), transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs)).

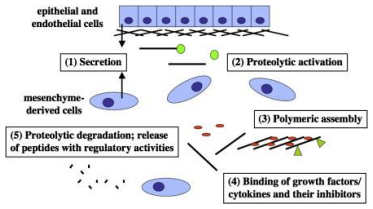


FIGURE 10.1. The life cycle of extracellular matrix molecules. Soluble matrix molecules are secreted by cells, modified by proteolysis, and assembled into polymeric complexes. Soluble matrix molecules are secreted by cells, modified by proteolysis, and assembled into polymeric complexes. These complexes serve as scaffolds for cells and as binding sites for small molecules such as growth and differentiation factors. Depending on the growth factor and cellular context, this may either inhibit or stimulate growth factor activity. Degradation of the scaffolds, during normal tissue turnover or during wound healing, may release bound growth factors and/or release peptide fragments from the larger scaffold proteins; such fragments may bind to cellular receptors and regulate cellular behavior.

Chapter 10 – Matrix Molecules and Their Ligands Bjorn Reino Olsen
Principles of Tissue Engineering (Fourth Edition), 2014, 189–208
http://dx.doi.org/10.1016/B978-0-12-398358-9.00010-0

3-Dimension cell culture (3D)

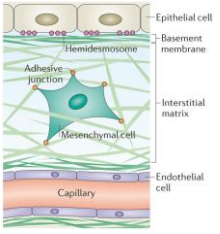
1. In the body cells experience a 3D environment, surrounded by other cells, membranes, proteins and matrices.
2. 3D environment provides another dimension for external mechanical inputs and for cell adhesion. Cells grown in 3D culture are capable of orientating and migrating into different directions and therefore form tissue-like architecture.
3. The 3D matrix affects solute diffusion and creates tissue-scale concentration gradients of ECM components and growth factors.
4. Signaling and other cellular functions therefore differ in 3D compared with 2D systems.
5. Cells grown in 3D culture are far more resistant to apoptosis.

1. Extracellular matrix (ECM)

A collection of extracellular molecules secreted by cells that provides structural and biochemical support to the surrounding cells.

Cells in tissues

- The ECM includes the interstitial matrix and the basement membrane (BM)
- Each tissue has a definite cellular composition and microarchitecture.
- Epithelial cells are attached to BM with hemidesmosomes
- Mesenchymal cells are surrounded by interstitial matrix
- Cells need nutrients and oxygen and removal of waste. This is supplied by the (blood) capillary system



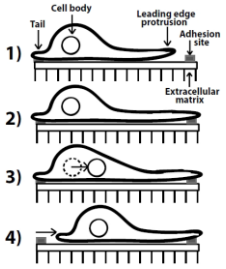
Nature Reviews Molecular Cell Biology 14, 467–473 (2013)

Cell behavior & how it is affected by the ECM dynamics

What does the cell do itself and in tissues ?

- Attach: generate the contact site between the cell and substrate
- Spread: increase the contact area between the cell and substrate
- Proliferate: increase in cell number by division
- Differentiate: become specialized for particular functions.
- Apoptosis: programmed cell death, an active process requiring metabolic activity by the dying cell
- Migrate: move from one place to another
- Function: carry out certain tasks, e.g. molecule transport, molecule metabolism, and energy conversion

Cell migration-coordinated interaction with cytoskeletons, adhesion molecules (such as fibronectin, hyaluronan etc.) and ECM



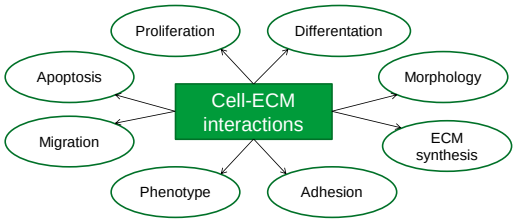
1. The protrusion of the leading edge driven by actin polymerization
2. The formation of new attachments in advance of its body
3. Contraction
4. Breaking the tail adhesions and moving forward

2006 Cytoskeletal Mechanics

Cytoskeleton

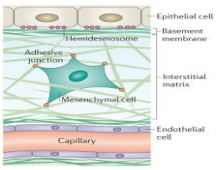
- The cytoskeletal filaments play roles in cell division, cell migration and cell shape and are fundamental to the spatial organization of cells.
- Three types of cytoskeletal filaments:
  - Microfilaments/actin filaments: 5-9 nm in diameter; universal constituent of eukaryotic cells; determining the shape of the cell's surface and necessary for whole-cell locomotion
  - Intermediate filaments: 10 nm in diameter, only found in animals, cell type specific, e.g. vimentin, cytokeratins; providing mechanical strength and resistance to shear stress
  - Microtubules: ~25 nm in diameter; universal constituent of eukaryotic cells; determining the positions of membrane-enclosed organelles and directing intracellular transport
- Cytoskeletal filaments are dynamic and self-assembling molecules.

Receptor-ligand binding and signal transduction



1. Basement membrane (BM)

Nature Reviews Molecular Cell Biology 14, 467–473 (2013)



- BMs are thin (50-150 nm in most tissues)
- extracellular matrices underlying cells
- Secreted by epithelial and endothelial cells.
- Most epithelial and endothelial cells
- The BM separates epithelial cells from the interstitial matrix/ mesenchymal connective tissue (compartmentalization)
- The BM provides an interactive surface for regulation of cell function (growth, differentiation and spatial orientation).
- The BM regulates permeability (a molecular barrier) and increases mechanical stiffness.

2. Interstitial Matrix

- Is the space between cells and is often depicted as being below the BM, however, it should be viewed as a 3D image, as it is a space that is part of the ECM
- Mesenchymal stem cells can move across the BM and move in and out of the ECM; they also participate in the formation of the mesenchymal connective tissue
- The form in which the mesenchymal connective tissue occurs with regards to the endothelial cells depends on the type of tissue and organ

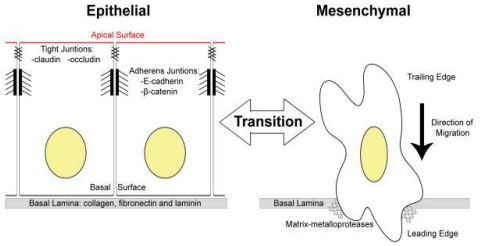


FIGURE 8.1. Epithelial vs. Mesenchymal.Epithelial cells adhere together by tight junctions and adherens junctions localized near the apical surface. Epithelial cells also have a basal surface that rests on a basal lamina (ECM). Mesenchymal cells in contrast do not have well-defined cell-cell adhesion complexes, have front-end/back-end polarity instead of apical/basal polarity, and mesenchymal cells are characterized by their ability to invade the basal lamina.

Chapter 8 – Molecular Organization of Cells Jon D. Ahlstrom

http://dx.doi.org/10.1016/B978-0-12-398358-9.00008-2
Principles of Tissue Engineering (Fourth Edition), 2014, 147–160

3. Extracellular matrix (ECM)

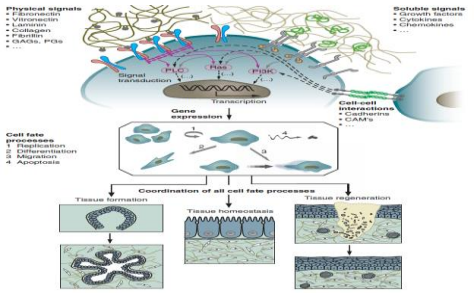
- ECM contains polysaccharides (such as glycosaminoglycans, GAGs) and proteins (such as collagen, elastin, fibronectin, and laminin) secreted by cells.
- The distribution and organization of ECM molecules varies from tissue to tissue →Developmental changes in matrix chemistry
- The common functions of ECM are cell adhesion, cell-to-cell communication and differentiation.

Major types of macromolecules to be examined today (figure 10.1. a reminder)

1. Molecules important for their structural properties (collagens and elastin)
2. Multidomain molecules: both structural constituents as well as regulators of cell behavior (fibronectin, fibrillin, laminin, thrombospondin, tenascin, perlecan and other proteoglycans)
3. Matrix-bound signaling molecules (such as matrix-bound fibroblast growth factors (FGFs), transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs)).

Cell signalling

2005 Nature Biotech 23:47



Cytoskeleton, adhesion molecules, and ECM function in a coordinated manner to transmit signals between inside and outside of the cell.

2. ECM composition

Proteins and glycosaminoglycans
- and their use in tissue engineering as supports and structures

ECM composition (a functional classification)

1. Structural Proteins: collagens and elastins (see 1.1. and 1.2. below)
2. Other functional proteins: Glycoproteins: proteins with one or more oligosaccharide chains covalently linked to amino-acid side chains, e.g. fibronectin, laminin, tenascin (see 2.1. and 2.2. below for fibronectin and laminin)
3. Glycosaminoglycans (GAGs): long, linear, negatively charged and highly hydrated polysaccharide composed of a repeating pair of sugars, one of which is always an amino sugar. For example, hyaluronan (see 3.1), heparan sulfate, chondroitin sulfate and dermatan sulfate, keratan sulfate. When covalently linked to a protein, they are called proteoglycans.
4. Proteoglycans (also present on cell surface): molecule consisting of one or more GAG chains covalently linked to a core protein. For example, aggrecan, syndecan (see 4.1.)

1. STRUCTURAL PROTEINS
1.1.Collagens– major constituents of ECM

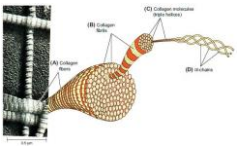
- Collagens: a family of fibrous insoluble proteins rich in glycine and proline and it is a major component of the ECM and connective tissues.
- Collagen fibril: extracellular structure formed by self-assembly of secreted fibrillar collagen subunits. An abundant constituent of the ECM in many animal tissues.
- Collagen fiber: a bundled group of collagen fibrils

Collagens

- Collagens are the most abundant proteins in the human body.
- A typical collagen molecule is long, stiff, and it has triple-stranded helical structure comprised of three polypeptide chains, called α-chains.
- 25 distinct collagen α-chains have been identified. About 28 types of collagens have been found.
- Collagens tend to form fibrils, filaments, or networks alone or with other ECM proteins.
- Fibril-forming collagens (rope-like structures) are associated with the structural functions.
- Nonfibrillar collagens are involved in the regulatory functions.

Collagen types

- Fibril-forming collagens, e.g. types I, II, and III, are the major products synthesized by connective tissue cells. (collagen fiber means a bundle of collagen fibrils)
- Sheet-forming collagens, e.g. IV and VII, are involved in forming basement membrane. They act as anchorage for cells, serve as molecular filters, or provide permeable barriers for developing embryos.



Immunologic properties of collagens

- Soluble collagen is a poor immunogen.
- Insoluble collagen is even less immunogenic.
- Xenogeneic collagenous tissue devices, such as porcine and bovine pericardial heart valves, are acceptable for long-term implantations in humans.
- Bovine and porcine type I collagen provide readily available sources of scaffold material for many clinical applications.

1.2. Elastic fibres and microfibrils

a) Elastin

- Highly elastic protein in connective tissue
- Allows tissues and organs to resume shape after streching
- On the surfaces of elastic fibbers one finds a cover of microfibrils

b) Microfibrils occur on the surface of elastic fibres, beaded filaments with fibrillin as their major components

- Fibrillin microfibres are found in eg. Growth plate cartilage → affect chondrocyte proliferation and /or maturation
- In lung tissue, blood vessel walls is a regulator of TGF-β activity
- Also proteins associated with elastin and microfibrils

2. OTHER MULTIFUNCTIONAL PROTEINS IN ECM

2.1. Fibronectin is a multidomain, multifunctional adhesive glycoprotein*

- Fibronectin can bind to ligands such as collagen, fibrin and heparan sulfate proteoglycans
  - Can therefore assemble into a fibrous network in the ECM
  - The ability to bind collagen and other ligands → association between the fibronectin network and the scaffold of collagen fibrils
  - Binding sites for heparin and chondroitin sulfate make fibronectin an important binding molecule between collagens and other matrix molecules

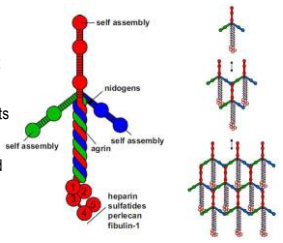
* (glycoprotein = protein with one or more oligosaccharide chains linked to amino-acid chains) (oligosaccharide = a saccharide polymer of (3 to10) monosaccharides)

Fibronectin - summary

- It is a multidomain, multifunctional adhesive, fibrilforming glycoprotein
- It is a globular protein present in nearly all tissues.
- There are 20 isoforms of fibronectin in human.
- It serves as a substrate for cell adhesion, spreading, and migration.

2.2. Laminin

- A non-filamentous glycoprotein
- The major components of basement membrane
- Laminins are able to selfassemble and to interact with other components of the ECM.
- Laminin consists of three different polypeptide chains, termed α, β, and γ, which form an asymmetrical four-armed molecule.
- The laminin network is formed through polymerization.
- There are many different laminin receptors. Several integrins are laminin receptors.



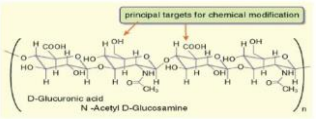
2010 Cell Tissue Res 339:259

3. GLYCOSAMINOGLYCANS – multifunctional molecules in the ECM and on cell surfaces

3.1. Hyaluronan or hyaluronic acid (HA)

- An important and simplest glycosaminoglycan (GAG) of most extracellular matrices
- Flexible, bending, and twisting into many conformations
- Plays roles in cell motility and morphogenesis
- Present in all vertebrates and readily available
- Strong affinity to water

Hyaluronan or hyaluronic acid (HA)



- Biocompatible, biodegradable, bioactive
- Non-immunogenic
- Easily manipulated and chemically modified: carboxylic acids and alcohols for modification and crosslinking
- HA-based hydrogels, sponges, meshes
- Applications: wound healing, bone and cartilage repair, nerve and brain repair, drug delivery

4. PROTEOGLYCANS

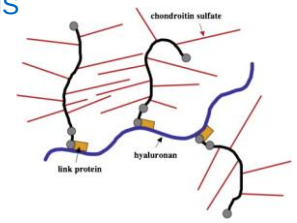


FIGURE 10.10. Diagram of a portion of a large proteoglycan complex from cartilage. Monomers of aggrecan, composed of core proteins with glycosaminoglycan side chains (mostly chondroitin sulfate) are bound to hyaluronan. The binding is stabilized by link proteins. For clarity, only some of the glycosaminoglycan side chains are shown in the monomers.

Bjorn Reino Olsen Principles of Tissue Engineering (Fourth Edition), 2014, 189–208
Chapter 10 – Matrix Molecules and Their Ligands
http://dx.doi.org/10.1016/B978-0-12-398358-9.00010-0

3. Cell surface receptors for ECM molecules

Integrins, proteoglycans, cell adhesion molecules

Effects of extracellular matrixes and biomaterials on cells

1. Direct biochemical effect via receptorbinding epitopes
2. Direct mechanical effect via receptorbinding epitopes
3. Indirect biochemical effect by regulating matrix dynamics and remodeling
4. Indirect biochemical effect by regulating the activity of soluble factors (growth factors and cytokines)

Summary: Interaction between cells and native extracellular matrix (ECM)

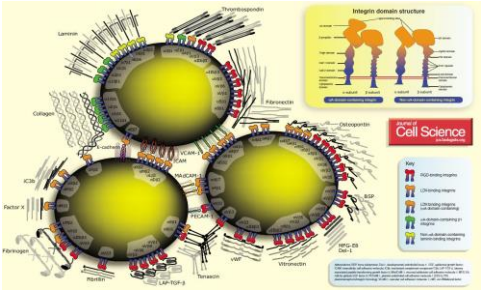
Binding of cell surface receptor to ECM proteins

Receptors on the cell surface:
- Integrins
- Selectins
- Cadherins
- Immunoglobulin cellular adhesion molecules
- Transmembrane proteoglycans

Ligands present in the ECM:
- Fibronectin
- Laminin
- Collagen
- Vitronectin
- Fibrinogen
- Proteoglycans
- Elastin

Receptor-ligand binding and signal transduction

2006 J Cell Sci 119:3901



Integrins bind to many ECM molecules, including collagen, laminin, and fibronectin.

Adhesion peptide sequences within ECM proteins and their receptors

- Various ECM molecules contain specific amino acid motifs that allow them to bind directly to cellsurface receptors.
- The first characterized motif is the tripeptide RGD (Arginine-Glycine-Aspartic acid), which was first found in fibronectin in 1984, and later in several other ECM molecules.
- These adhesion peptides have been grafted to synthetic biomaterials to promote cell adhesion and regulate cell behavior (functional biomaterials).

Influence of the ECM on growth factors and cytokines

- Many growth factors and cytokines are secreted in matrix-binding forms.
- The matrix protects growth factors and cytokines from being degraded
- It allows more efficient presentation of growth factors to their receptors
- It leads to their localization to particular regions within tissues
- Various ECM molecules have specific domains that bind to growth factor receptors.
- All of these actions eventually regulate cell behavior.

Growth factors – what are they ?

- Naturally occurring substance which stimulates cell growth, healing, proliferation and cell differentiation
- Usually they are proteins or a steroid hormones
- Act as signaling molecules between cells, and bind with receptors on the surfaces of their target cells
- Promote cell differentiation and maturation
- Examples: Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor , Bone morphogenetic proteins (BMPs), Brain-derived neurotrophic factor (BDNF), Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Foetal Bovine Somatotrophin (FBS), Glial cell line-derived neurotrophic factor (GDNF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Growth differentiation factor-9 (GDF9), Hepatocyte growth factor (HGF), Hepatoma-derived growth factor (HDGF), Insulin-like growth factor (IGF), Keratinocyte growth factor (KGF), Migration-stimulating factor (MSF), Myostatin (GDF-8), Nerve growth factor (NGF) and other neurotrophins . Platelet-derived growth factor (PDGF) ("Healing Factor), Thrombopoietin (TPO), T-cell growth factor (TCGF), Transforming growth factor alpha (TGF-α), Transforming growth factor beta (TGF-β), Tumor necrosis factor-alpha (TNF-α), Vascular endothelial growth factor (VEGF), Wnt Signaling Pathway , Placental growth factor (PGF), IL-1 - Cofactor for IL-3 and IL-6. Activates T cells, IL-2 - T-cell growth factor. Stimulates IL-1 synthesis. Activates B-cells and NK cells., IL-3 - Stimulates production of all non-lymphoid cells., IL-4 - Growth factor for activated B cells, resting T cells, and mast cells, IL-5 - Induces differentiation of activated B cells and eosinophils, IL-6 - Stimulates Ig synthesis. Growth factor for plasma cells., IL-7 - Growth factor for pre-B cells, Regalase- RNLS -Anti-apoptotic survival factor ..... And so on – which is the reason, why we will not cover any of these in detail !

Cytokines – what are they ?

- A broad and loose category of small proteins (~5–20 kDa)
- Important in cell signaling
- Released by cells and affect the behavior of other cells.
- Cytokines can also be involved in autocrine signaling
- Include chemokines, interferons, interleukins (note some overlap with growth factors) , lymphokines, tumour necrosis factor but generally not hormones or growth factors (despite some overlap in the terminology).
- Produced by a broad range of cells, including immune cells like macrophages, B lymphocytes, T lymphocytes and mast cells, as well as endothelial cells, fibroblasts etc.
- Act through receptors to regulate the maturation, growth, and responsiveness of particular cell populations
- They are different from hormones, which are also important cell signaling molecules, in that hormones circulate in less variable concentrations and hormones tend to be made by specific kinds of cells.

Summary

- Cells in the tissue are surrounded by a 3D ECM.
- The ECM molecules regulate cell behavior via cell-surface receptor-mediated signal transduction. The most important receptors are integrins.
- Adhesion peptides in ECM proteins are responsible for the interaction between cells and ECM.
- The ECM regulates activity of growth factors and cytokines.
- The ECM is a dynamic structure.