Extracellular Matrix and Adhesion Molecules

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Contents

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What You will Learn in This Chapter and Associated Exercises

Students will gain a basic knowledge of extracellular matrix components and main classes of cell adhesion molecules. They will then learn how to treat coverslips with different cell adhesion proteins and the key steps of collagen isolation protocol.

3

3.1 The Content and Role of ECM

All tissues consist of cells and extracellular matrix (ECM) that surrounds them. ECM is a complex meshwork of *fibers* (collagen, elastin) and *ground substance*. Ground substance fills the space between cells and fibers and has high water content. During fixation and dying, water evaporates, making ground substance largely invisible on histology slides. The individual components of the ground substance vary depending on the tissue. They include *proteoglycans* (aggrecan, syndecan), *glycosaminoglycans* (dermatan sulfate, heparan sulfate, keratan sulfate, hyaluronan), and *multiadhesive glycoproteins* (fibronectin, laminin).

ECM content depends on the type of tissue. The role of ECM is to provide mechanical and structural support for the cells, give the tissue its appropriate tensile strength, and enable cell communication. ECM components anchor cells through cell-to-ECM attachment adhesion molecules discussed below. ECM also binds different growth factors, such as TGF-b, which are critical for cell growth.

3.2 Cell Adhesion Proteins

Adhesion is a property of cells to attach to surfaces, the latter being other cells, components of the extracellular matrix, natural and artificial scaffolds, or any other surroundings. The ability of the cell to adhere is critical for its differentiation, growth, migration, and even survival. By using different attachment pathways, cells are able to communicate with each other and perform their tissue-specific functions [1].

Points of cell attachment are called cell junctions (Fig. 3.1). They have various structures and are responsible for different functions. O*ccluding junctions,* also known as *tight* junctions or *zonulae occludentes,* seal off fluid passage between the adjacent cells. *Anchoring junctions* provide mechanical stability and are involved in cell-to-cell attachment, recognition, morphogenesis, and differentiation. *Communicating junctions* allow small molecules to diffuse in and out of the cells as well as between connected cells. They are critical to regulating cell homeostasis.

For the purposes of this course, we will mainly concentrate on anchoring junctions. These types of junctions play a key role in cell-to-cell and cell-to-extracellular matrix adhesion and are made of cell adhesion molecules or CAMs. CAMs are transmembrane proteins containing *intracellular*, *transmembrane,* and *extracellular* domains. In addition to their role in adhesion, CAMs affect cell migration and proliferation by binding to the intercellular components of the cytoskeleton, which then can trigger multiple downstream pathways.

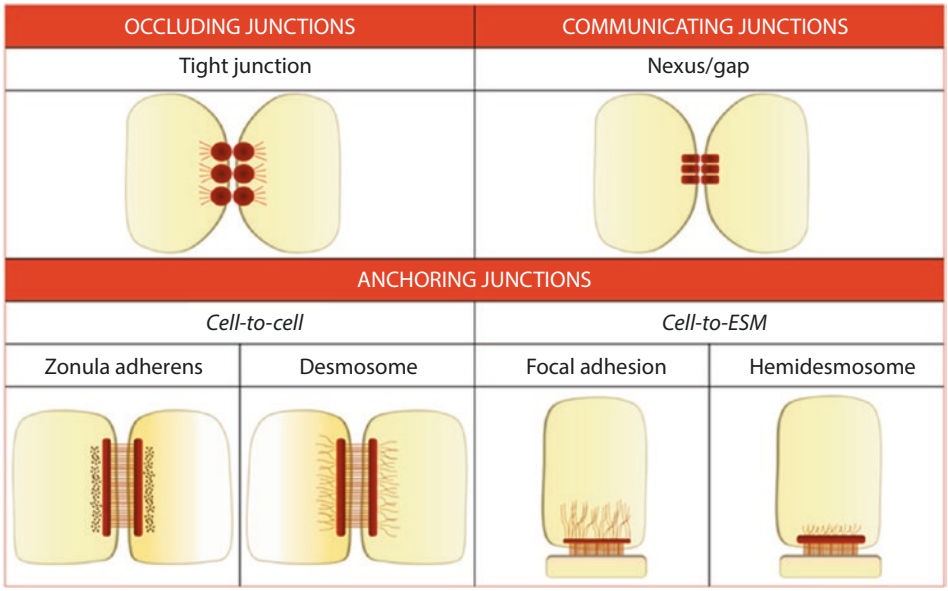

Fig. 3.1 Different types of cell junctions

3.3 Main Classes of CAMs

CAMs are usually classified into four big families: *cadherins*, *immunoglobulin superfamily* (IgSF), *selectins,* and *integrins* (Fig. 3.2). Most of them are Ca-dependent (cadherins, selectins, and integrins). When binding occurs between the same cell adhesion molecules, it is called *homophilic* (cadherins and IgSF); binding between non-identical proteins is called *heterophilic* (selectins, integrins).

Cadherins are Ca-dependent homophilic molecules, which bind to the actin filaments through catenins. There are three subgroups of molecules: N-cadherin (neural), P-cadherin (placental), and E-cadherin (epithelial). The failure of cadherin-cadherin interaction can bring about the development of cancer. This occurs due to the disruption of a pathway called contact-inhibition, which limits the growth of cells connected to their neighbors [2].

Immunoglobulin superfamily (*IgSF)* plays an important role in inflammation and immune response. This family of adhesion proteins has various subgroups involved in many processes (ICAM – intercellular, C-CAM – cell, VCAM – vascular, DSCAM – Down syndrome, PECAM – platelet endothelial, and many other cell adhesion molecules).

Selectins are mostly found in white blood cells (L-selectin), endothelial cells (E-selectin), and platelets (P-selectin).

Integrins are transmembrane receptors that facilitate cell-extracellular matrix adhesion. They consist of two alpha- and beta-glycoprotein chains, which attach the cells to the components of the extracellular matrix (collagen, laminin, and fibronectin) and actin or intermediate filaments.

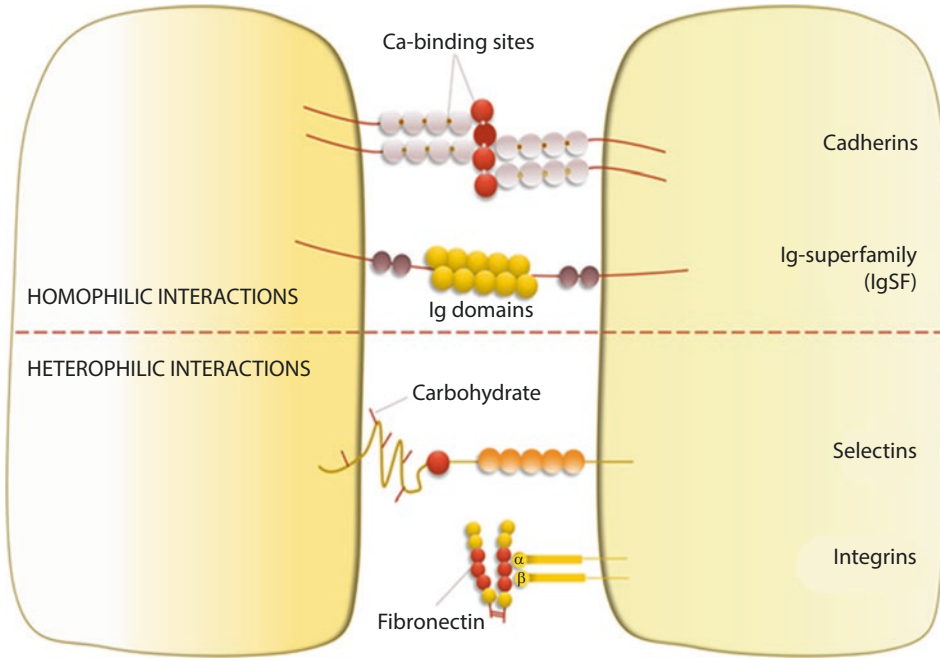

Fig. 3.2 A cartoon showing the main types of CAMs and their interactions

3.4 Attachment Glycoproteins

Multiadhesive glycoproteins stabilize the extracellular matrix, link it to cell surfaces, and connect collagen, proteoglycans, and glycosaminoglycans (Fig. 3.3). Here are some of the most important molecules of this class (Fig. 3.4).

Fibronectin has several domains; one of them binds to ECM molecules, another one to cell surface receptors. Fibronectin matrix assembly begins when soluble, compact fibronectin dimers are secreted from cells, most often fibroblasts. Fibronectin also participates in the formation of blood clots and is a key protein in wound healing.

Laminin family of glycoproteins is an integral part of the structural scaffolding in almost every tissue of an organism, forming the basis of basal and external laminae. Laminins form independent networks and are associated with type IV collagen networks via entactin, fibronectin, and perlecan. They also bind to cell membranes through integrin receptors and other plasma membrane molecules.

Tenascin is mostly present during the embryogenesis. After this period, it switches off and reactivates only during the regenerative process such as wound healing.

Osteopontin also represents a family of multiadhesive glycoproteins and is mainly present in bones. It binds osteoclasts to the bone surface and plays an important role in calcification of ECM.

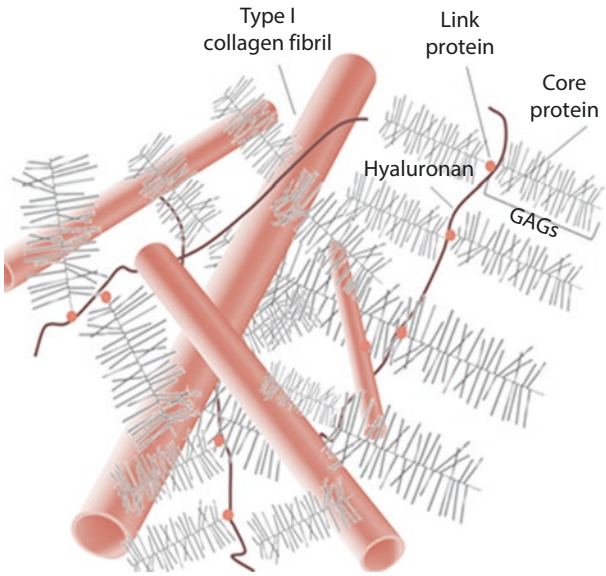

Fig. 3.3 A cartoon showing key multiadhesive glycoproteins

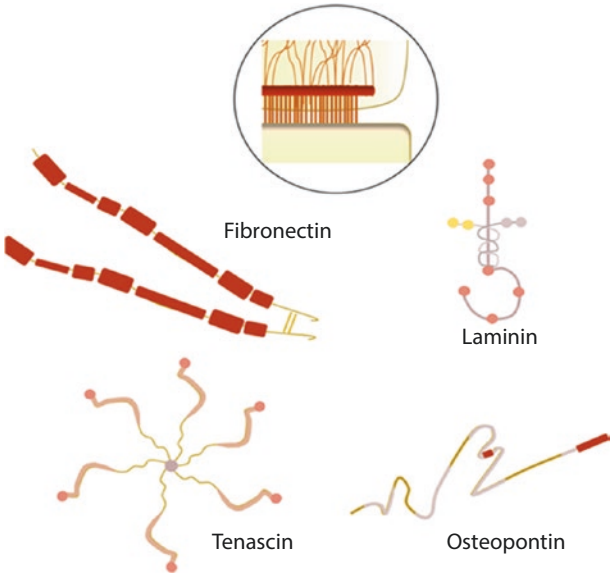

Fig. 3.4 A cartoon showing a simplified structure of an extracellular matrix

3.5 Macromolecular Protein Fibers

Collagen fibers are the most prevalent structure of the extracellular compartment (Fig. 3.3). They consist of collagen fibrils, which have different length and width. The structural unit is a collagen molecule, which is a triple helix of three alpha polypeptide chains. If these chains are the same, the molecule is called homotrimeric; if these chains are not the same, the molecule is called heterotrimeric. Currently, twenty-

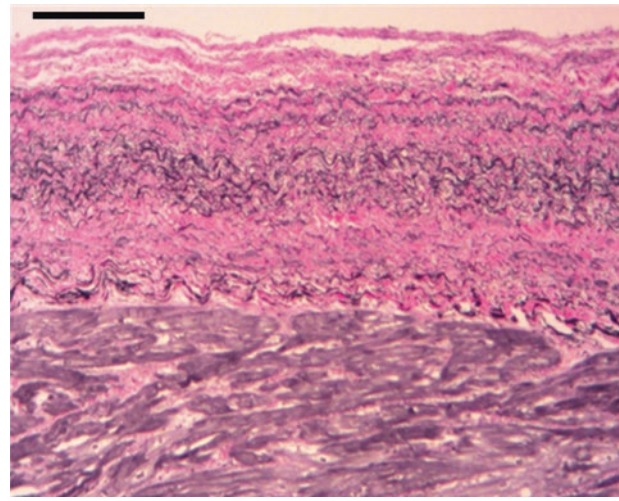

Fig. 3.5 Histology of epicardial surface of human left atria. Verhoeff-van Gieson staining: collagen fibers show in pink, elastin in black, and muscle in purple. Scale bar: 100 micron

five various types of collagen, which differ in their location and function, have been described. The most abundant type is collagen type I. It is found in skin, bone, tendon, ligaments, dentin, sclera, fascia, and organ capsules and accounts for nearly 90% of body collagen. Collagen fibers can be histologically visible and appear blue when using Masson's trichrome stain or pink when H&E or Verhoeff-van Gieson staining is used.

Reticular fibers also consist of collagen fibrils, but only type III. They can be found in reticular organs, such as the liver, bone marrow, and lymphatic system. The more reticular fibers are found in the tissue, the more mature the tissue is. Reticular fibers can be stained with PAS (periodic acid-Schiff).

Elastin fibers provide tissues with the ability to stretch and return to their original shape when pressure is removed. It is the main component of ECM in the vertebral ligaments, epiglottis, external ear, and elastic arteries. Elastin fibers can be visualized on histology slices using dyes like orcein or resorcin-fuchsin. Staining with Verhoeff-van Gieson shows elastin in black color being coiled into multiple spiral strands (Fig. 3.5).

3.6 Use of Adhesion Molecules in Tissue Engineering and Cell Culturing

After cells are isolated from tissue, in order to survive and proliferate, they need to be attached to flat surfaces of culture plates or to scaffolds in cases of 3D cultures [3]. Since cells do not attach well to plastic or glass surfaces, they have to be coated with adhesion molecules or other substances, which help cells to stick to the surface material. The well-chosen coating agent can greatly affect in vitro cell viability and behavior.

Today, the main sources of coating reagents are different components of native ECM. The most prevalent one is collagen type I. It can be easily isolated from a rat tail or bovine tendons. Hydrolysis of collagen results in the breakup of protein fibrils into a mixture of smaller peptides, called gelatin. Gelatin is probably the most cost-efficient, yet very effective coating agent. Other purified adhesion molecules include laminin and fibronectin. A relatively new approach is to coat cell culture dishes with

a crude mixture of ECM proteins derived from decellularized scaffolds [4]. More information about decellularized scaffolds can be found in ► Chap. 9.

Negatively charged synthetic peptides, such as polylysine, can also serve as efficient coating agents. They have an additional benefit of reduced contamination risk due to the absence of biological components. Owing to the rapid development of the tissue engineering field, a large variety of commercially coated cell culture plates is now available.

Session I

Demonstration

Students are shown key steps on how to isolate rat tail collagen and to cover plates with solutions of different adhesion molecules such as collagen, laminin, polylysine, or gelatin. The latter are dissolved in PBS at 10–100 μg/mL concentrations, distributed evenly at 50–100 μl/well or glass coverslips, placed in an incubator for 30 minutes, followed by removal of excess fluid and drying under the hood. UV sterilization for 10 minutes can be then used to sterilize coated surfaces. Labeled, sterile coverslips are put into Petri dishes, sealed with Parafilm, and stored in the fridge to be used during subsequent weeks.

Homework

Teams are tasked with reading any recent review article on cell adhesion molecules and their role in creating engineered tissues.

Session II

Team Exercises

Students execute the first part of the collagen isolation protocol. In addition, each team covers cell culture and multiple glass coverslips with solutions of gelatin, collagen, fibronectin, or laminin using steps shown during DEMO session and labels and stores them for the next week's experiment.

Homework

Team members complete collagen isolation protocol and document all the steps involved in treating and storing coverslips. The latter will be used by the teams in the following sessions.

Sample Protocols

Collagen preparation from rat tail. The skin of the rat tail is removed with a clamp, and collagen fibers that look like silky white filaments are exposed. Fibers are immersed in ethanol for 3 minutes, wiped dry, and put in a UV-box for 10 minutes. Fibers are then chopped into small pieces and weighed. 100 mL of a 0.1% acetic acid solution is added to per gram of fibers followed by stirring at low speed for 2–3 days at 4 °C. More sophisticated protocols can be found in Reference [5].

Collagen preparation from bovine or porcine Achilles or other major tendons. Tendons are cleaned from any other connective tissues, rinsed with PBS, and

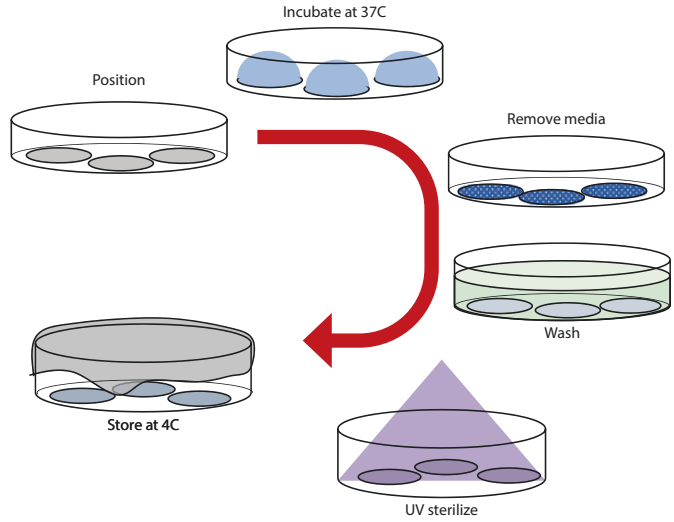

Fig. 3.6 Main steps to cover glass coverslips with adhesion proteins

manually chopped or homogenized using a blender with beaker placed in the ice basket to prevent overheating. The pieces are then resuspended in 0.01 M HCl and stirred overnight for 2–3 days at 4 °C.

Samples are centrifuged for 10 minutes at 250 g to remove undigested fibrous material and filtered using a 100–200 μm filter. Sterilized collagen stock solutions can be stored in the fridge for several weeks. More details can be found in Reference [6].

Plate coating (Fig. 3.6)

Reagents and supplies:

- 6-well or 12-well cell culture plates
- #1 Coverslips and forceps
- Incubator or UV-box
- 1% Gelatin solution. Weigh 1 g gelatin and put it into sterile glassware. Add 100 mL distilled water and warm up inside the glassware to get a homogeneous solution.
- If commercial solutions of laminin or fibronectin are available, dilute them in distilled water or as per product manual.

Procedure

- Take coverslips and put them into the dry Petri dish using forceps. Be careful and avoid overlapping of coverslips during the whole procedure. Coverslips can also be put into wells individually.
- Add 10 μg/mL solutions of gelatin, laminin, collagen, or fibronectin on top of each coverslip. Alternatively, fill individual wells.
- Incubate samples for 10–30 minutes at 37 °C.
- Aspirate the solutions making sure not to scratch the coverslips and avoid their overlapping.
- Use PBS or saline to rinse several times.
- Remove the excess of fluid and let dry under the hood.
- Sterilize the covered surfaces under a UV lamp for 10 minutes.

Take-Home Message/Lessons Learned

After reading this chapter and performing the requested assignments and exercises, students should be able to:

- Name the key components of extracellular matrix
- Distinguish between occluding, anchoring, and communicating junctions
- Identify the main classes of cell adhesion molecules
- List several adhesive glycoproteins and macromolecular protein fibers
- Cover glass coverslips with solutions of adhesive molecules
- Isolate crude collagen fraction

Self-Check Questions

Q.3.1. The main components of the ground substance include the following, EXCEPT
A. Proteoglycans
B. Glycosaminoglycans
C. Cadherins
D. Glycoproteins

Q.3.2. Choose the correct statement.
A. Occluding junctions are involved in cell-to-cell recognition, morphogenesis, and differentiation.
B. Communicating junctions allow small molecules to diffuse in and out of the cells.
C. Anchoring junctions seal off fluid passage between the adjacent cells.
D. Selectins are found in all types of cells and tissues.

Q.3.3. The role of ECM is to
A. Mechanically anchor the cells
B. Enable communication between different cells
C. Bind growth factors
D. All of the above

Q.3.4. These three molecules do not belong to the same class/family:
A. Cadherin, selectin, integrin
B. Fibronectin, laminin, osteopontin
C. Collagen, elastin, immunoglobulin
D. Dermatan sulfate, heparan sulfate, hyaluronan

Q.3.5. Which protein should not be used for cell attachment to plastic or glass surfaces?
A. Collagen
B. Albumin
C. Polylysine
D. Laminin

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