IV. THE EXTRACELLULAR MATRIX

IV.1. INTRODUCTION

Most cells in multicellular organisms are in contact with an intricate meshwork of interacting, extracellular macromolecules that constitute the **extracellular matrix**.

The macromolecules that constitute the extracellular matrix are secreted by local cells, especially fibroblasts, wich are widely distributed in the matrix. In specialized matrix structures, such as cartilage and bone, these macromolecules are secreted locally by more specialized cells (chondroblasts and osteoblasts).

Two of the main classes of extracellular macromolecules that make up the matrix are:

- ***the collagens*** **-** collagen fibers strengthen and help to organize the matrix;
- the polysaccharide ***glycosaminoglycans***, which are usually covalently linked to protein to form ***proteoglycans***. The glycosaminoglycan and the proteoglycan molecules form a highly hydrated, gel-like "ground substance" in which collagen fibers are embedded. The aqueous phase of the polysaccharide gel permits the diffusion of nutrients, metabolites, and hormones between the blood and the tissue cells.

In many cases, fibers of the rubber like protein ***elastin*** are also present and impart resilience to the matrix. In addition, two high molecular weight glycoproteins are among the major components of extracellular matrices: ***fibronectin***, which is widely distributed in connective tissues (as well in blood), and ***laminin***, which has so far been found only in basal laminae.

The extracellular matrix consists of the fibrilar component, the amorphous ground substance, and the basement membrane or basal lamina.

The roles of extracellular matrix are:

-it is a scaffold that stabilized the phisical structure of tissue;

-regulates the behaviour of the cells that contact it;

-it influences the development, migration proliferation shape and metabolic functions.

IV.2. THE FIBRILAR COMPONENT

The fibrilar component is represented by the collagen fibers, the elastin fibers, the reticulin fibers, and the oxitalamic fibers.

IV.2.1. COLLAGEN. Is the most abundant type of protein present in higher vertebrates, accounting for a quarter or more of the total body protein. The collagen constitutes more than 70 % (dry weight) of tendons and the dermis of skin.

IV.2.1.1. Composition. Collagen is composed by three polypeptide chains bound into a superhelix:-several kinds of polypeptide chains are synthesized. Synthesis is directed by distinct genes. Some collagens have two kinds of polypeptide chains interwoven in the superhelix, while others contain only one kind of polypeptide chain. Collagen polypeptide chains are predominantly glycine, glutamic acid, aspartic acid, proline, hydroxyproline, and an assortment of other amino acids including arginine, lysine, hydroxylysine, and leucine. Polypeptide chain triple helices are assembled into collagen microfibrils that can be viewed microscopically.

IV.2.1.2. Types of collagen: at present, 11 different types of collagen have been identified (types I through XI). Types I-V are the most abundant varieties of collagen. Types VI-XI are sometimes called minor components, as they occur in relatively small quantities; however, they may have important functions Table IX summarized the major features of collagen types I-V.

Table IX -Types of Collagen and Their Properties

Type	Molecular Formula	Polymerized Form	Distinctive Features	Tissue Distribution
I	$[\alpha1(I)]_2\alpha2(I)$	fibril	low hydroxylysine low carbohydrate broad fibrils	skin, tendon, bone, ligaments, cornea, internal organs
II	$[\alpha1(II)]_3$	fibril	high hydroxylysine high carbohydrate usually thinner fibrils than type I	cartilage, intervertebral disc, notochord, vitreous body of eye
III	$[\alpha1(III)]_3$	fibril	high hydroxyproline low hydroxylysine low carbohydrate	skin, blood vessels, internal organs
IV	$[\alpha1(IV)]_3$ controversial	basal lamina	very high hydroxylysine high carbohydrate	basal laminae
V	$[\alpha1(V)]_2\alpha2(V)$	unknown	high hydroxylysine high carbohydrate	widespread (in small amounts)

Type I collagen is found in bone, tendon, skin, and the cornea; consists of two alpha 1 (I) chains and one alpha 2 (II) chain and is designated by the notation $[\alpha1(I)]_2\alpha2(I)$. It contains very little carbohydrate, and the lysines present are not highly hydroxylated and is synthesized by connective tissue fibroblasts, osteoblasts, smooth muscle cells, and some epithelial cells. Type I collagen forms microscopically visible fibrils that have a characteristic 67 nm periodicity under the electron microscope due to the asymmetrical arrangement of collagen molecules. Similar arrangements occur in types II and III collagen.

Type II collagen - is found in cartilage, the cornea and the vitreous body of the eye; consists of three alpha 1 (II) chains and is designated by the notation $[\alpha1(II)]_3$. It contains a higher degree of lysine hydroxylation than type I collagen. It is synthesized by chondroblasts and chondrocytes, neural retinal cells.

Type III collagen - is found in fetal dermis, around blood vessels, and in many organs. It consists of three alpha 1 (III) chains and is designated by the notation $[\alpha1(III)]_3$. Type III collagen contains little lysine hydroxylation and some cysteine; it is synthesized by fibroblasts and myoblasts.

Type IV collagen - is an important structural component of the basement membrane (the fibrous meshwork of extracellular matrix on the basal surface of almost all epithelial layers).Two varieties of type IV collagen exist; they are designated by the notations $[\alpha1(IV)]_4$ and $[\alpha2(IV)]_3$.

It has highly hydroxylated lysine residues and is highly glycosylated. Type IV collagen is synthesized by a variety of epithelial cells.

Type V collagen - is abundant around blood vessels and smooth muscle cells; consists of two alpha 1 (V) chains and one alpha 2 (V) chain and is designated by the notation $[\alpha1(V)]_2\alpha2(V)$. It is synthesized by smooth muscle cells and chondrocytes. Types IV and V collagen are amorphous and probably exist as diffuse networks of polypeptide chains rather than as assembled, microscopically recognizable fibrils.

IV.2.1.3. Collagen synthesis

a) Early stages of collagen synthesis - All collagen molecules have structural similarities:

- Each is composed of three alpha chains interwind in a triple helix about 300 nm long and 1.5 nm thick.
- Multiple triple helices are bundled into collagen fibrils many micrometers long, with diameters of 10-300 nm. Collagen fibrils assemble into much larger collagen fibers that are several micrometers in diameter. Collagen fibers are a prominent component of many connective tissues and are visible under the light microscope.

Collagen polypeptide chains are **synthesized on ribosomes** bound to endoplasmic reticular membranes. The chains elongate and pass into the lumen of the rough endoplasmic reticulum as large polypeptide chains called **pro-alpha chains**:

- Pro-alpha chains have signal peptides, which help the polypeptide chain pass through the ribosome into the rough endoplasmic reticulum.
- Pro-alpha chains also have **extension peptides** at either end, which help form the triple helix during the assembly of **procollagen**. Extension peptides on the carboxyl terminus of procollagen molecules are cross-linked by interchain disulfide bonds.

Interchain hydrogen bonding also aids in procollagen molecule assembly. The proline and lysine residues are hydroxylated after translation but before the pro-alpha chains are assembled into procollagen. This procollagen modification is important for collagen cross-linking and requires several cofactors, including vitamin C. Scurvy**,** the deficiency of dietary vitamin C, results in abnormal pro-alpha chain formation, indirectly causing skin and blood vessel fragility.

Lysine hydroxylation is important for glycosylation, a second post-translational modification of procollagen. Procollagens have unusual dissacharide residues that usually contain glucose and galactose but lack the sialic acid characteristics of many other glycoprotein secretion products. Glycosylation in collagen varies. Type I has very little carbohydrate and type V has abundant carbohydrate.

b) Late stages of collagen synthesis consist of:

- After procollagen is synthesized and before it is released from the cell, a **procollagen peptidase** cleaves the extension peptides, leaving a collagen molecule (tropocollagen).
- Collagen molecules have a tendency to assemble spontaneously into collagen fibrils, but they will do so only after the extension peptides are cleaved by procollagen peptidase. This prevents intracellular fibrillogenesis.
- Types I, II and III procollagen are converted to collagen; types IV and V are not. This may explain why the former assemble into fibrils while the latter do not.

- Collagen molecules are 300 nm long and have a head and a tail end. Numerous collagen molecules are packed end-to-end and side-to-side, with regular gaps between heads and tails and an approximate one-third stagger to the molecules. This configuration results in a regular 67 nm periodicity that is clearly visible under the electron microscope.

The three-dimensional arrangement of collagen molecules and the factors that regulate fibrillogenesis are poorly understood, although fibrillogenesis appears to be regulated by the interaction of molecules such as glycosaminoglycans and fibronectin with collagen. In tissue, collagen fibers may be arranged randomly (in skin), in parallel arrays (in tendons), or in nearly orthogonally overlapping arrays (in the cornea). The cells that secrete collagen may be involved in the order or disorder observed in collagen fibers in situ.

c) Collagen cross-linked:

- After fibrils form outside of the cells, extensive cross-linking occurs within and between collagen molecules.
- Lysine and hydroxylysine residues are oxidatively deaminated by **lysyl oxidase**, an unusual extracellular enzyme, to form reactive aldehyde groups.
- The aldehyde groups react with each other and other amino acids to form both intramolecular and intermolecular covalent cross-links. The mechanism greatly increases the tensile strength of individual collagen fibrils so that structures composed of many collagen fibers and fibroblasts (tendons connecting muscles to bones) are especially durable and strong.

Abnormalities in collagen synthesis underlie several human diseases. For example, deficiencies in vitamin C intake results in decreased proline hydroxylation and aberrant collagen production. Patients with a vitamin C deficiency often exhibit scurvy, a disease in which blood vessels become fragile, teeth fall out, and wounds fail to heal properly. In addition, several human genetic diseases, such as Ehlers-Danlos syndrome and osteogenesis imperfecta, are due to abnormal collagen production.

IV.2.2. ELASTIN AND ELASTIC FIBERS

Elastin is an extracellular matrix polypeptide with peculiar elastic properties; it is the main component of **elastic fibers**, which are found in skin, blood vessels, the nose, the external ear, gastrointestinal organs, and the lungs. Elastic fibers allow these organs to resume their original shape after a distorting force has temporarily changed their shape.

Elastin is a glycoprotein with a molecular weight of about 70 kD. Like collagen, it is rich in glycine and proline; however, it lacks the hydroxyproline and hydroxylysine found in collagen. It contains many hydrophobic amino acids and desmosine and isodesmosine, two unique amino acids involved in creating intramolecular and intermolecular cross-links.

Synthesis of elastic fibers and elastin:

- Elastic fibers contain a microfibrillar protein in addition to the amorphous elastin molecules. Elastic fibers are synthesized by fibroblasts and smooth muscle cells.
- Elastin is synthesized on the rough endoplasmic reticulum and, like collagen, is packaged in the Golgi apparatus. Cross-linking of elastin molecules occurs in the extracellular space.
- Lysyl oxidase creates aldehydes groups on three lysines, resulting in the formation on three allysyl residues. Then, three allysyl residues condense with one lysyl residue to form the heterocyclic ring characteristic of desmosine. The resulting desmosyl residues form intermolecular cross-links between elastin polypeptide chains.

➢ Elastin molecules exist as cross-linked random coils. When cross-linked, they form a rubber-like network of molecules that returns to its original shape after distortion.
➢ The microfibrillar protein assembles into anastomosing networks of fibrous proteins surrounded by amorphous elastin. Apparently, microfibrillar protein is synthesized and secreted before elastin and may help organize elastin into deformable networks.

IV.2.3. RETICULIN FIBERS

Are founded in young tissue, liver, kidney, lungs, haematopoetics organs; contains glucides and lipids and are joined together in a network.

IV.2.4. OXITALAMIC FIBERS

Oxitalamic fibers have an intermediary molecular structure between collagen and elastin; are founded in non tensile tissue.

IV.3. THE AMORPHOUS GROUND SUBSTANCE

It contains mucopolysaccharides, glycoproteins, noncollagenous proteins.

IV.3.1. MUCOPOLYSACCHARIDES (GLYCOSAMINOGLYCANS)

Glycosaminoglycans (GAGs) are long unbranched polyssacharide chains composed of repeating disaccharide units. They are called GAGs, because one of the two sugar residues in the repeating disaccharide is always an amino sugar (N-acetylglucosamine or N-acetylgalactosamine). GAGs are highly negative charged due to the presence of sulfate or carboxyl groups or both on many of the sugar residues. Seven groups of GAGs have been distinguished by their sugar residues, the type of linkage between these residues, and the number and location of sulfate groups (Table X).

Table X - The Glycosaminoglycans

Glycosamino-glycan	Repeating Disaccharide $(A\text{-}B)_n$		Sulfates per $(A\text{-}B)_n$	Tissue Disttributtion
	Mono-saccharide A	Mono-saccharide B		
Hyaluronic acid	Glucuronic acid	N-acetyl-D glucosamine	0	Various connective tissues, skin, vitreous body, cartilage, synovial fluid
Chondroitn 4-sulfate	Glucuronic acid	N-acetyl-D galactosamine	0.2 - 1.0	Cartilage, cornea, bone, skin, arteries
Chondroitin 6-sulfate	Glucuronic acid	N-acetyl- D galactosamine	0.2 - 2.3	Cornea, bone, skin, arteries
Dermatan sulfate	Glucuronic acid	N-acetyl-D galactosamine	1.0 – 2.0	Skin, blood vessels, heart, heart valves
Heparan sulfate	Glucuronic acid	N-acetyl-D glucosamine	0.2 – 3.0	Lung, arteries, cell surfaces
Heparin	Glucuronic acid	N-acetyl-D glucosamine	2.0 -3.0	Lung, liver, skin, mast cells
Keratan sulfate	D-galactose	N-acetyl-D glucosamine	0.9 – 1.8	Cartilage, cornea, intervertebral disc

Glycosaminoglycans, with the exception of hyaluronic acid, are covalently bound to protein to form **proteoglycans**. These macromolecules consist of a core protein to which numerous unbranched GAG side chains are covalently attached.

IV.3.2. GLYCOPROTEINS. The exracellular matrix contains a number of adhesive glycoproteins that bind to both cells and other matrix macromolecules and thereby help cells attach to the extracellular matrix. The best characterized of these is **fibronectin**, which is found around collagen fibers and the pericellular environment of many connective tissues, blood, and other body fluids. Fibronectin is a dimer consisting of two 60-70 nm polypeptide chains that have a molecular weight of 230-250 kD each. These chains are joined at one end by several disulfide bonds.

Fibrinonectin receptors-integrin-on the cell surface are anchored in the plasma membrane. Integrin recognizes and binds to extracellular fibronectin-containing fibrils, thus anchoring the cells to the extracellular matrix. A careful study of fibrinonectin peptide fragments reveals separate domains in the molecule: one devoted to cell binding, a second for collagen binding, a third for actin binding, a fourth for heparin binding, and a fifth for fibrin binding. The discovery that the fibrinonectin content of tumor cells is often reduced compared to that of a normal cells has led the speculation that the loss of fibrinonectin is responsible for some of the abnormal properties of cancer cells.

IV.4. BASEMENT MEMBRANE - BASAL LAMINA

IV.4.1. STRUCTURE. The basal surface of most epithelia has a thin layer of material lying between it and the subjacent connective tissue domains. This layer is visible in the light microscope. In the EM a substructure may be apparent. In some locations, the basement membrane is an exceedingly thin, gossamer network around an epithelium (around capillaries). In location where epithelial basal surfaces are opposite to one another (in the renal glomerulus), the basement membrane can be several micrometers thick. Basal lamina also surrounds muscle and Schwann cells.

IV.4.2. FUNCTIONS. The basement membranes perform four important functions:
1. the most important function is to provide a surface that epithelial cells (above) and connective tissue cells (below) can attach to, thus holding these two distinct tissues together;
2. the basement membrane is a barrier that prevents microorganisms from entering the organism's inner domain;
3. prevents the loss of cells and fluids from the body;
4. performs selective filtration (example: the glomerular basement membrane filters wastes from blood passing through the kidneys).

IV.4.3. FOUR IMPORTANT CONSTITUENTS OF THE BASEMENT MEMBRANE

Peripheral parts of the basal lamina appear to be rich in laminin and proteoglycans, while the central portion is relatively rich in type IV collagen.

Laminin is a glycoprotein with a molecular weight of 1000 kD. It is shaped like a cross and is composed of two different subunits that have molecular weight of 220 kD (A chain) and 440 kD (B chain); the chains are joined together by disulfide bonds. Molecules contain distinct domains including a 50 kD heparin-binding domain at the end of the B chain, and cell-binding and collagen-binding domain in the A chain (Figure 26). Like fibronectin, laminin is an

extracellular matrix glycoprotein that binds to cells and components of the extracellular matrix. The molecules have no obvious fibrous substructure under the electron microscope.

Type IV collagen - the pro-alpha chains of type IV collagen have unusually long extension peptides that are not cleaved from the molecule. Therefore, type IV collagen does not assemble into microscopic microfibrils in vivo.

Heparan sulfate proteoglycans are abundant in basement membranes.

Fibronectin is also present in some basement membranes.