Extracellular Matrix I
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OBJECTIVES
After studying the material presented in this lecture, the student will be able to:

1. Describe the cellular and matrix components of connective tissue.
2. Discuss the structural and functional relationship of the matrix elements.
3. Outline the biosynthesis and degradation of collagen and the critical points of regulation for these processes.

RECOMMENDED READING
Molecular Cell Biology, 5th edition, Lodish et al., pages 216-219
Lehninger Principles of Biochemistry, 5th edition, pages 124-128
Marks Basic Medical Biochemistry, 3rd edition, Chapter 49

![Figure 1](image)

“Tissues are not made up solely of cells. A substantial part of their volume is extracellular space, which is largely filled by an intricate network of macromolecules constituting the extracellular matrix. This matrix is composed of a variety of versatile proteins and polysaccharides that are excreted locally and assembled into an organized meshwork in close association with the surface of the cell that produced them. In connective tissues, the extracellular matrix is frequently more plentiful than the cells that it surrounds, and it determines the tissue’s physical properties. Connective tissues form
the architectural framework of the vertebrate body, but the amounts found in different organs vary greatly: from skin & bone, in which they are the major components, to brain & spinal cord, in which they are only minor constituents”.

I. OVERVIEW

The extracellular matrix is composed mainly of collagen, elastin and proteoglycans. It is a very dynamic structure and plays an active role by influencing the development, proliferation, shape, and metabolism of cells that interact with it. This lecture will focus on COLLAGEN, the major protein of the extracellular matrix. Collagen is the most abundant protein in the animal kingdom and it accounts for 30% of the total protein in the human body. In normal tissue, collagen provides strength, integrity and structure. When tissues are disrupted following injury, collagen is needed to repair the defect and hopefully restore structure and thus function. If too much collagen is deposited in the wound site, then normal anatomical structure is lost, function is compromised and the problem of fibrosis results. Conversely, if insufficient amounts of collagen are deposited, the wound is weak and may dehisce. Therefore, in order to fully understand the process of normal tissue development and wound healing, it is first essential to understand the basic biochemical mechanisms involved in the process of collagen metabolism and how these pathways are regulated.

II. COLLAGENS

A family of fibrous proteins with a characteristic triple-stranded helical structure.

About one quarter of all of the protein in your body is collagen. Collagen is a major structural protein, forming molecular cables that strengthen the tendons and vast, resilient sheets that support the skin and internal organs. Bones and teeth are made by adding mineral crystals to collagen. Collagen provides structure to our bodies, protecting and supporting the softer tissues and connecting them with the skeleton. But, in spite of its critical function in the body, collagen is a relatively simple protein.

The Collagen Triple Helix
Collagen is composed of three chains, wound together in a tight triple helix. Each chain is over 1400 amino acids long and a repeated sequence of three amino acids (G-P-HP) forms this sturdy structure. Every third amino acid is glycine, a small amino acid that fits perfectly inside the helix. Many of the remaining positions in the chain are filled by two unexpected amino acids: proline and a modified version of proline, hydroxyproline.
A. Types

Where is collagen found in the body, which cells produce it, and how do the specific types relate to function?
B. How does the molecular structure of collagen dictate its biologic function?

We make many different kinds of collagen, which form long ropes and tough sheets that are used for structural support in mature animals and as pathways for cellular movement during development. All contain a long stretch of triple helix connected to different types of ends. The simplest is merely a long triple helix, with blunt ends. These "type I" collagen molecules associate side-by-side, like fibers in a rope, to form tough fibrils. These fibrils crisscross the space between nearly every one of our cells. There are at least 25 types of collagen but the major 5 are listed in Table 4 above.

The illustrations below depict a basement membrane, which forms a tough surface that supports the skin and many organs. A different collagen--"type IV"--forms the structural basis of this membrane. Type IV collagen has a globular head at one end and an extra tail at the other. The heads bind strongly together, head-to-head, and four collagen molecules associate together through their tails, forming an X-shaped complex. Using these two types of interactions, type IV collagen forms an extended network, shown here in light blue. Two other molecules--cross-shaped laminin (blue-green) and long, snaky proteoglycans (green)--fill in the spaces, forming a dense sheet.
Figure 5:

Figure 6

Molecular Cell Biology, 5th edition, Lodish et al. Figure 6-15 Structure & Assembly of Type IV Collagen
III. **BONE**

![Diagram of bone structure]

Figure 22-52. Molecular Biology of the Cell, 4th Edition.

**Figure 8**
"Bone is a very dense, specialized form of connective tissue. Like reinforced concrete, bone matrix is predominantly a mixture of tough fibers (type I collagen), which resist pulling forces, and solid particles (calcium phosphate as hydroxyapatite crystals), which resist compression. The volume occupied by the collagen is nearly equal to that occupied by the calcium phosphate. The collagen fibrils in adult bone are arranged in regular plywood-like layers, with the fibrils in each layer lying parallel to one another but at right angles to the fibrils in the layers on either side".

Osteoblasts secrete bone while osteoclasts erode it. Once the osteoblasts become imprisoned in the hard bone matrix they are called osteocytes and they no longer divide but continue to secrete further matrix in small quantities around itself. Osteocytes occupy small cavities called lacuna and small channels called canaliculi radiate from each lacuna. The canaliculi contain cell processes connecting the osteocytes with neighboring cells via gap junctions. (see figure on previous page). Osteoclasts are large, multinucleated cells that originate from hemopoietic stem cells in the bone marrow. The precursor cells are released into the blood stream.
Osteoclasts can tunnel deep into compact bone and form cavities that are then invaded by other cells. A blood capillary can then grow down the center of such a tunnel and the walls of the tunnel become lined with a layer of osteoblasts.

**Cartilage** is also a highly specialized form of Collagen (Type II)
**Biosynthesis**: how is collagen synthesized and why is this information important for the clinical care of patients?

1. The collagen gene is complex. Contains a large number of introns and the mRNA undergoes extensive processing prior to translation. In addition there are many specific **post translation modifications** needed to produce collagen.

2. Membrane-bound polysomes are the site of collagen synthesis.

![Figure 12](image12.png)

**Figure 12**

3. Formation of hydroxyproline & hydroxylysine and essential cofactors: These are **critical steps** in the production of collagen and require **Vitamin C**, iron and oxygen.

![Figure 13](image13.png)

**Figure 13**
**Hydroxylysine formation.** A very similar reaction takes place whereby lysine is converted to hydroxylysine by the action of the enzyme **Lysyl Hydroxylase** using the same co-factors and molecular oxygen. Hydroxylysine participates in some of the initial cross link bonds and it is also the site for glycosylation (discussed below).

**Vitamin C**
Hydroxyproline, which is critical for collagen stability by enhancing Hydrogen bond formation, is created by modifying normal proline amino acids after the collagen chain is synthesized. This is one of several **Post Translational Modifications** that are characteristic of the collagen biosynthetic pathway. The reaction requires vitamin C to assist in the addition of oxygen. Unfortunately, we cannot make vitamin C within our bodies, and if we don’t get enough in our diet, the results can be disastrous. Vitamin C deficiency slows the production of hydroxyproline and stops the biosynthesis of new collagen, ultimately causing **scurvy**. The symptoms of scurvy—loss of teeth and easy bleeding & bruising-- are caused by the lack of collagen to repair the wear-and-tear caused by everyday activities.

![Figure 14](image)

4. Addition of sugars to collagen – this is another **Post Translational Modification** where selected hydroxylysine residues are “glycosylated” by the addition of galactose and glucose. Diabetics who are “out of control” have too much sugar added to their collagen and the filtration function of the basal lamina is lost. The process responsible for this abnormality is called “**non-enzymatic glycosylation.**”
5. Movement of collagen within the cell and secretion to the extracellular spaces – structures within the cell called microtubules are important for this process - drugs like colchicines and vinblastine can inhibit this process.

Figure 15

6. Procollagen processing and its relationship to the control of collagen synthesis: The C terminal domain contains both inter- and intra- molecular disulfide bonds whereas the N terminal peptides contain only intra- disulfide bonds. The procollagen extension peptides have several important functions. Disulfide bonds form in the C-terminal extension peptides and ensure proper REGISTRATION or alignment of the 3 alpha chains. When both the C and N terminal extension peptides are present, the molecule is 1,000 times more soluble than it is after they are cleaved off by the specific procollagen proteinases. After they are removed, the peptides are further digested and are taken up by the cell to regulate collagen synthesis in a “feedback” type of mechanism. The removal of the N and C terminal domains is another Post Translational Modification.
7. Cross-link and fiber formation provide the essential steps needed for the remarkable tensile strength that is characteristic of collagen. This Post Translational Modification is a critical step needed for proper wound healing. Both inter- and intramolecular cross-links are formed by the action of lysyl oxidase on selected lysine and hydroxylysine residues using copper as a co-factor.

The collagen molecule is like the steel bars called “rebar” that are used in building construction. If you were to scale up the molecular dimensions of the collagen molecule using units of measure more familiar to you, the molecule would be 1 inch in diameter and 17 feet long. When it is highly
organized in our tissues it has the tensile strength approaching that of steel.

Figure 18

Collagen degradation and remodeling (a final Post Translational Modification)

1. Collagenases; specific enzymes belonging to the family of enzymes termed Matrix Metallo Proteinases (MMPs) essential for collagen remodeling.

2. How is collagenase activity controlled in tissues? First, the genes controlling the synthesis of the Procollagenase (Zymogen) need to be expressed. The inactive “Pro” enzyme then needs to be activated. There are several mechanisms whereby the Procollagenase can be activated including Plasmin cleavage, Low pH and Reactive Oxygen Species. Once in an active state, the collagenase can be inhibited by forming a complex with alpha-2-macroglobulin or with TIMP (Tissue Inhibitor of Metallo Proteinases). Mammalian collagenases are very specific whereas gelatinases are less specific and bacterial collagens have a very broad specificity for collagen. All of the various collagenases can come into play in a host of human diseases and pathologies.
Collagenases also play a critical role in tumor metastasis.
Genetic disorders of collagen metabolism and their relationship to abnormal wound healing.

1. Ehlers-Danlos syndrome: There are at least 12 types and most all of these patients with connective tissue abnormalities also have wound healing problems.

2. Marfan’s syndrome – another group of individuals with connective tissue defects and they frequently have aneurysm. Defect appears to be due to abnormal fibrillin in the elastin of their blood vessels.

3. Osteogenesis imperfecta – this group of connective tissue defects have frequent bone fractures.

4. Epidermolysis bullosa – characterized by blistering and ulcerations of the dermis due to excessive collagenase and/or abnormal attachment of the epidermis to the dermis.

Clinical correlation (Do Not Memorize)

IV. EHLERS-DANLOS SYNDROME

Ehlers-Danlos Syndrome (EDS) is a heterogeneous group of heritable disorders of connective tissue, characterized by skin extensibility, joint hyper mobility and tissue fragility. There are different types of EDS and these were reclassified in 1997 into six major types, they are classified according to their symptoms and signs with each type running true in a family thus an individual with one type will not have a child with a different type.

EDS is caused by a defect in the collagen (connective tissue), which is the main building block in the body. Collagen provides strength for the different parts of the body. Some types are firm to give support, others are elastic to allow movement and strength, and still others resemble glue binding protein together. Consequently, if it is defective, it can produce many problems.

A. Prevalence

EDS is known to affect both males and females of all races and ethnic backgrounds.

B. Diagnosis
Diagnosis is based on the presenting symptoms and family history. Diagnosis can be delayed or overlooked in some cases as they do not fit conveniently into a specific type. A skin biopsy may be taken to study the connective tissue. Specific tests are available for certain types of EDS.

C. Prognosis

The prognosis depends on the specific type of EDS. Life expectancy can be shortened in the Vascular Type (type IV) due to the rupture of vessels and organs. Pregnancy can be life-threatening in the Classical and Vascular Types (types I, II, IV).

D. Symptoms

1. Skin

Cutaneous hyper extensibility characterizes all EDS except for the Vascular Type (type IV), which has noticeably translucent skin with visible veins. When skin is over-stretched it still retains normal elastic recoil and snaps back once released. This is best tested at the neck, elbows or knees.

**Cutaneous fragility.**

Easy splitting of the skin is particularly common in Classical Type (Types I and II). Gaping, 'fish-mouth' or 'cigarette paper' scars follow minimal trauma over sites of bony prominence and areas prone to trauma such as the forehead, chin, elbows, knees and shins.

**Epicanthic folds.**

These are additional symmetrical folds of skin at the inner aspects of the eyes producing apparent broadening of the nose.

**Molluscoid pseudotumours.**

These are firm, fibrous lumps measuring up to 2 - 3 cm which develop over pressure points such as the elbows and knees.

**Spheroids.**

Approximately one third of affected individuals describe small, firm nodules like 'ball bearings' just beneath the skin (subcutis). These consist of fibrotic and calcified fat which overlay bony areas such as the shins.
Piezogenic papules.

These small, soft, skin-colored lumps appear on the side of the heel when standing and disappear when the foot is elevated. Although usually symptom-less they can occasionally be painful.

Varicose veins.

These are more common in many types of EDS

2. Joints

Hyper mobility should be assessed using the Beighton scale. A score of 5/9 or defines hyper mobility.

Dislocation and subluxation.

This is common due to unstable joints.

Chronic joint and limb pain.

Pain is common even when skeletal X-Rays are normal.

3. Bruising and Haematomas

Easy bruising, at sites of trauma, accompanies most forms of EDS. This implies increased fragility of dermal blood capillaries and poor structural integrity of the skin. When bruising presents in a child it may be incorrectly attributed to non-accidental injury.

4. Mitral Valve Prolapse

This is quite common and should be diagnosed by echocardiography, CT or MRI.

5. Less Common Features

Rupture. Arterial/uterine/ intestinal due to tissue fragility. Hernia are also relatively common. Scoliosis. Which may be present at birth or can develop in later life. Gum disease. Gastrointestinal diverticulae.

E. Treatment and Management

This depends on the presenting symptoms but simple precautionary
measures will greatly lessen the chances of accidental trauma, scarring and bruising. It is important to carefully balance the advantages of less frequent injuries and the disadvantages of overprotection in a child. Simple measures like padding of the lower legs and elbows in children may reduce the number of injuries. Surgery and skin suture should be undertaken with great care as fragile tissues may tear. Sutures need to be left in longer than normal. Bracing may be used to support unstable joints. Orthopaedic surgery may be necessary but is not always successful. Physiotherapy and Occupational Therapy advice may be sort to strengthen muscles and teach aids to daily living.

F. Psychological

The main problem with having Ehlers-Danlos Syndrome is that the person can look very fit and may often not be believed that they have the joint pain etc. Diagnosis is often delayed and misdiagnosis is relatively common. Some forms of EDS may be misdiagnosed as child abuse/self-inflicted injury. Where there is severe skin involvement scarring can be severe and the person needs to learn to cope with disfigurement.

Valerie Burrows, Founder of the Ehlers-Danlos Support Group