

# Collagen.

## Learning Objectives.

At the end of this course, you should be able to :

1. describe the basic structure of the collagen molecule
2. discuss the role of collagen within the body
3. describe the main steps in the synthesis of collagen
4. understand the balance between synthesis and degradation, and how this affects collagen function.

Collagen is the most abundant protein in the animal kingdom, and it accounts for around 30% of the total protein content of the human body, providing strength, integrity, and structure. Collagen is found in all connective tissues, such as dermis, bones, tendons, and ligaments, and also provides for the structural integrity of all of internal organs.

There are close to 20 different types of collagen in humans, each being encoded for by a specific gene. However, the various types of collagens have slightly different amino acid compositions and provide specific functions in the body. The major types of collagen are :

Type I - predominant, all tissues and organs

Type II - exclusive to cartilage

Type III - found in skin, blood vessels, organs, higher levels in newborns than adults

Type IV - found in basement membranes acting as a filtration system

Type V - in all tissues, functions as a cytoskeleton

Type I is the major collagen of tendon and bone, but it is also predominant in lung, skin, heart valves, fascia, scar tissue, cornea, and liver. It is essential for the tensile strength of bone - it is the final amount and distribution of these

collagen fibers that will determine the size, shape, and ultimate density of the bone.

Type II collagen is the form that is found exclusively in cartilaginous tissues. It is usually associated with proteoglycans, and functions as a shock absorber in joints.

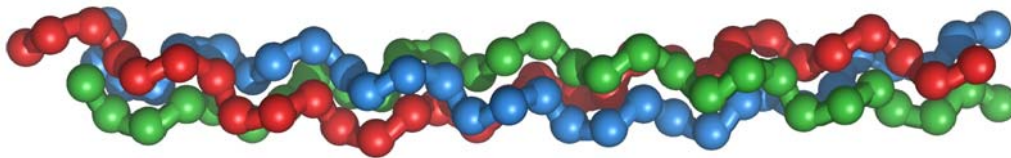
Type III collagen is also found in skin as well as blood vessels and internal organs. In the adult, the skin contains about 80-percent Type I and 20-percent Type III collagen. In newborns, the Type III content is greater than that found in the adult. It is thought that the supple nature of the newborn skin, as well as the flexibility of blood vessels, is due in part to the presence of Type III collagen. During the initial period of wound healing, there is an increased expression of Type III collagen.

Type IV collagen is found in basement membranes and basal lamina structures and functions as a filtration system. Because of the complex interactions between the Type IV collagen and the non-collagenous components of the basement membrane, a meshwork is formed that filters cells as well as molecules and light. For example, in the lens capsule of the eye, the basement membrane plays a role in light filtration. In the kidney, the glomerular basement membrane is responsible for filtration of the blood to remove waste products. The basement membrane in the walls of blood vessels controls the movement of oxygen and nutrients out of the circulation and into the tissues. Likewise, the basal lamina in the skin delineates the dermis from the epidermis and controls the movement of materials in and out of the dermis.

Type V collagen is found in essentially all tissues and is associated with Types I and III. In addition it is often found around the perimeter of many cells and functions as a cytoskeleton. It is of interest to note that there appears to be a particular abundance of Type V collagen in the intestine compared to other tissues.

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, 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, to fully understand wound healing, it is essential to understand the basic biochemistry of collagen metabolism.

## Structure



The collagen molecule is composed of three very long protein chains. Each protein chain is referred to as an "Alpha" chain. Two of the Alpha chains are identical and are called Alpha-1 chains, whereas the third chain is slightly different and is called Alpha-2. The three chains are wrapped around each other to form a triple helical structure called tropocollagen.

Collagen's triple helical structure is known as a Madras helix. The collagen molecule subunit (tropocollagen) is a rod made up of three left-handed helices (distinct from the right-handed alpha helix). These three helices are then twisted together into a right-handed coiled coil, forming tropocollagen. It is distinguished by the regular arrangement of amino acids in each of the three chains of these collagen subunits.

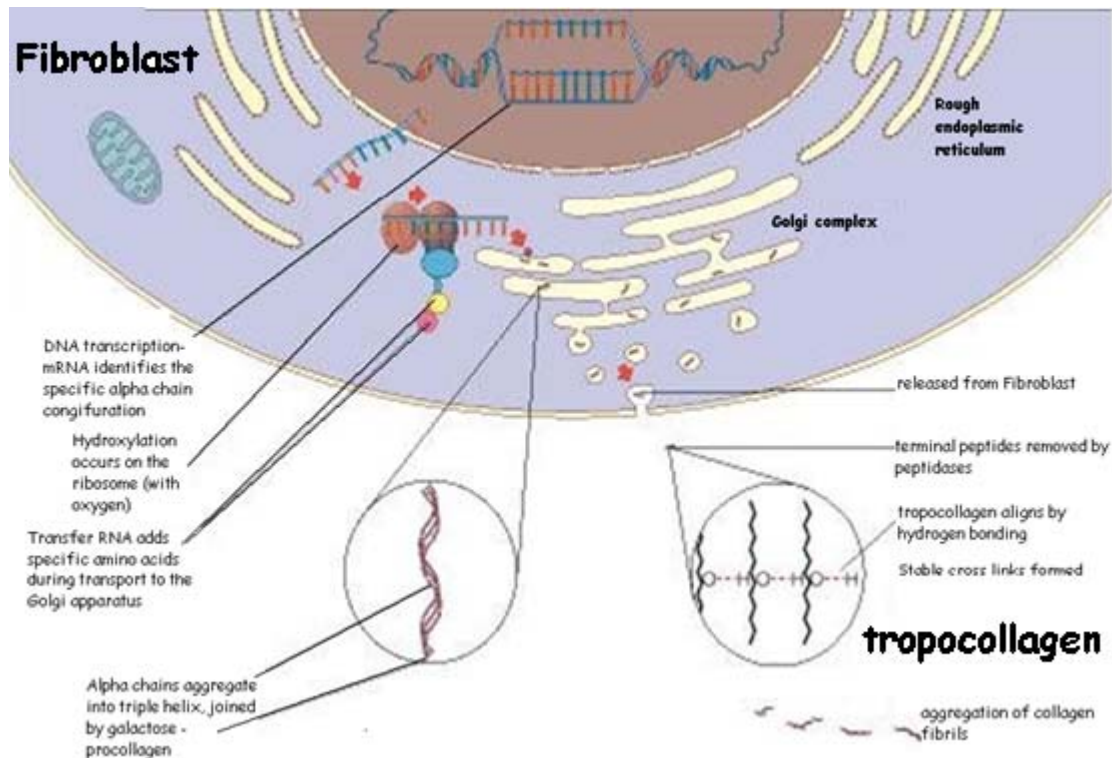
This configuration imparts tremendous strength to the protein. To understand the overall structure of the collagen molecule, think of it as the reinforcement rods used in concrete construction.

Basically all of the collagens share this triple-helical molecular structure as described above. However, the various other types of collagens have slightly different amino acid compositions and provide other specific functions in the body.

Collagen has an unusual amino acid composition. It contains large amounts of glycine and proline, as well as two amino acids that are not inserted directly by ribosomes - hydroxyproline and hydroxylysine - the former composing a rather large percentage of the total amino acids. They are derived from proline and lysine in enzymatic processes, for which vitamin C is required. This is related to why vitamin C deficiencies can cause scurvy, a disease that leads to loss of teeth and easy bruising caused by a reduction in strength of connective tissue due to a lack of collagen or defective collagen.

Another rare feature of collagen is its regular arrangement of amino acids in each of the alpha chains of the collagen sub-units. The sequence generally follows the pattern Gly-X-Y, where Gly for glycine, and X and Y for any amino acid residues. Most of the times, X is for proline and Y is for hydroxyproline. There are very few other proteins with such regularity. The inordinate number of Gly residues allows the tight coiling of each of the alpha chain subunits of tropocollagen. Hydroxylysine and hydroxyproline play important roles in the stabilisation of the tropocollagen globular structure as well as the final fibre shaped structure by forming covalent bonds. The resulting structure is the collagen helix.

## Collagen synthesis



Collagen is produced by fibroblasts in a complex biosynthetic pathway. Each specific collagen type is encoded by a specific gene; the genes for all of the collagen types are found on a variety of chromosomes. As the mRNA (messenger ribonucleic acid) for each collagen type is transcribed from the gene, or DNA "blueprint," it undergoes many processing steps to produce a final code for that specific collagen type. This step is called mRNA processing.

Once the final pro-alpha chain mRNA is produced, it attaches to the site of actual protein synthesis. This step of the synthesis is called translation. This site of translation is found on the membrane-bound ribosomes, also called the rough endoplasmic reticulum, or rER. Like most other proteins that are destined for function in the extracellular environment, collagen is synthesised on the rER.

A precursor form of collagen, procollagen, is produced initially. This has additional peptides at both ends that are unlike collagen. On one end of the

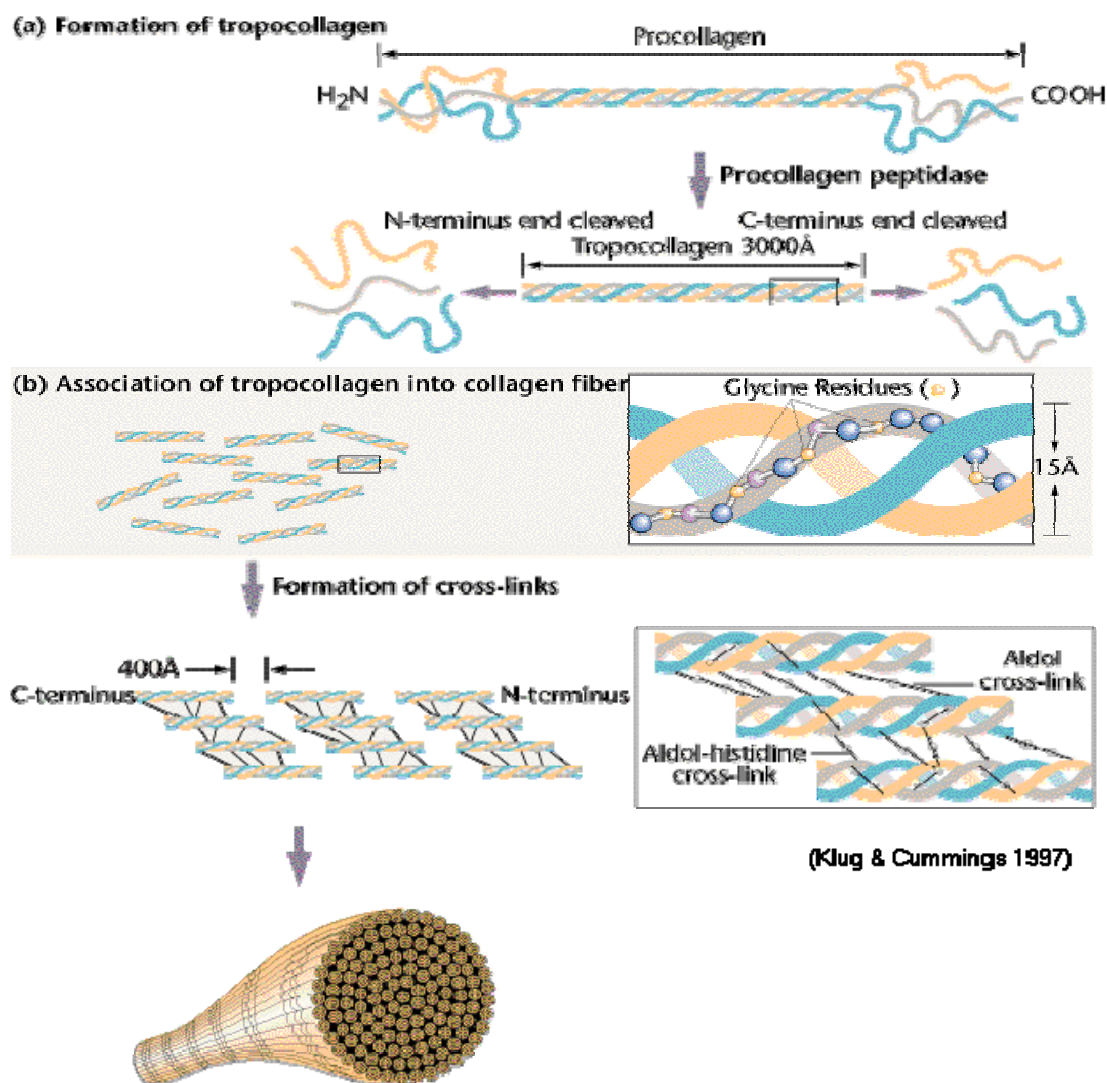
molecule, called the amino terminal end, special bonds called disulfide bonds are formed among three procollagen chains and ensure that the chains line up in the proper alignment. This step is called registration. Once registration occurs, the three chains wrap around each other forming a string-like structure. These portions of the procollagen molecule make it very soluble and therefore easy to move within the cell as it undergoes further modifications. As the collagen molecule is produced, it undergoes many changes, termed post-translational modifications.

One of the first modifications to take place is hydroxylation of selected proline and lysine amino acids in the procollagen. Specific enzymes, hydroxylases, are responsible for these reactions, needed to form hydroxyproline and hydroxylysine. The hydroxylase enzymes require Vitamin C and Iron as cofactors. In the absence of hydroxyproline, the collagen chains cannot form a proper helical structure, and the resultant molecule is weak and quickly destroyed. This ultimately will affect the strength of any collagen subsequently produced.

Some of the newly formed hydroxylysine amino acids are glycosylated by the addition of sugars, such as galactose and glucose. This imparts unique chemical and structural characteristics to the newly formed collagen molecule and may influence fibril size. It is of interest to note that the glycosylation enzymes are found with the highest activities in the very young and decrease as we age.

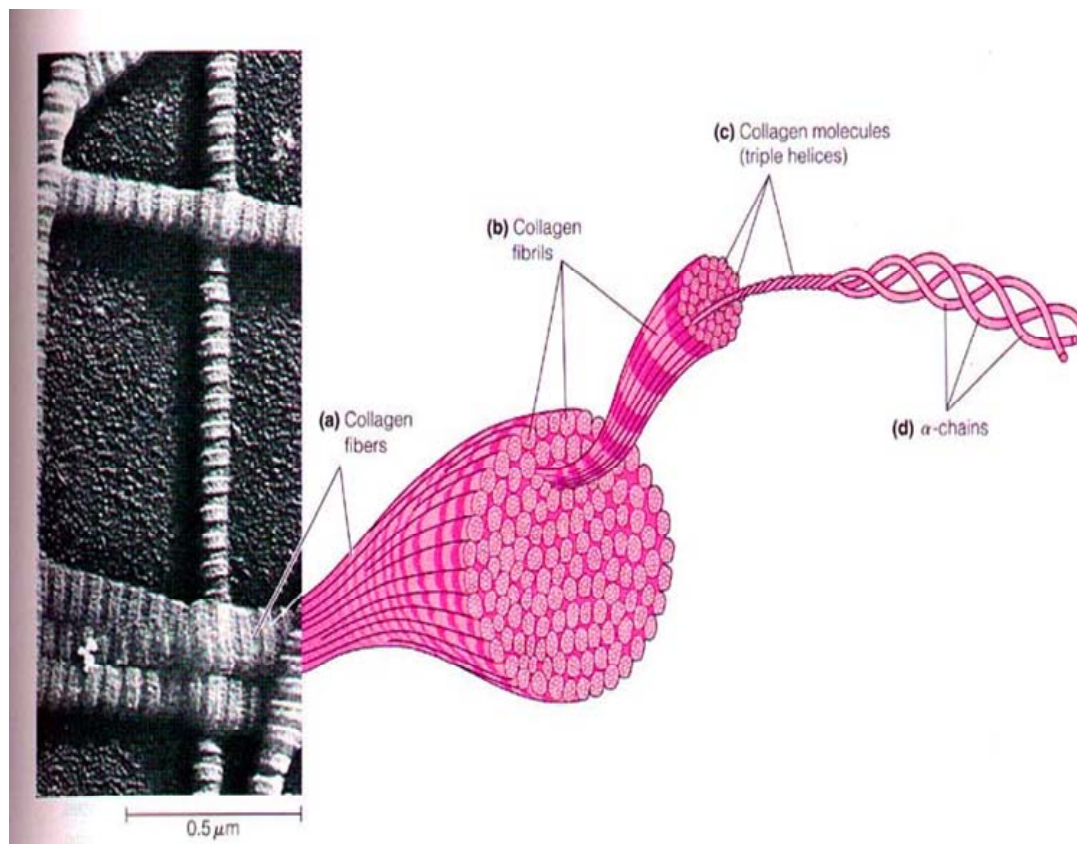
As the procollagen is secreted from the cell, it is acted upon by specialised enzymes called procollagen proteinases that remove both of the extension peptides from the ends of the molecule. Portions of these digested end pieces are thought to re-enter the cell and regulate the amount of collagen synthesis by a feed-back type of mechanism. The processed molecule is now referred to as collagen and begins to be involved in the important process of fiber formation

In the extracellular spaces, another post-translational modification takes place as the triple helical collagen molecules line up and begin to form fibrils and then fibers. This step is called crosslink formation and is promoted by another specialized enzyme called lysyl oxidase. This reaction places stable crosslinks within (intramolecular crosslinks) and between the molecules (intermolecular crosslinks). This is the critical step that gives the collagen fibers such tremendous strength.



One can visualise the ultrastructure of collagen by thinking of the individual molecules as a piece of sewing thread. Many of these threads are wrapped around one another to form a string (fibrils). These strings then form cords; the cords associate to form a rope, and the ropes interact to form cables. This

highly organised structure is what is responsible for the strength of tendons, ligaments, bones, and dermis.



When the normal collagen in tissues is injured and replaced by scar collagen, the connective tissue does not regain this highly organised structure. This is why scar collagen is always weaker than the original collagen. The maximum regain in tensile strength of scar collagen is about 70- 80% of the original. Collagen synthesis and remodeling continue at the wound site long after the injury, and can continue for up to 2 years. The body is constantly trying to remodel the scar collagen to achieve the original collagen ultrastructure that was present before the injury. This remodeling involves ongoing collagen synthesis and collagen degradation. Anything that interferes with protein synthesis will cause the equilibrium to shift, and collagen degradation will be greater than collagen synthesis. For example, patients who are malnourished or patients receiving chemotherapy may experience wound dehiscence, because the wound site will become weak due to a shift in the balance toward collagen

degradation. It is of interest to note that when wounds in the fetus heal, they do so in such a manner that the original collagen ultrastructure is achieved.

## Collagen degradation.

Of equal importance in the total picture of collagen metabolism is the complex process of collagen degradation. Normally, the collagen in our connective tissues turns over at a very slow and controlled rate. However, during rapid growth and in disease states, such as arthritis, cancer, and chronic non-healing ulcers, the extent of collagen degradation can be quite extensive. In normal healthy tissues where the collagen is fully hydroxylated and in a triple helical structure, the molecule is resistant to attack by most proteases. Under these normal healthy conditions, only specialized enzymes called collagenases can attack the collagen molecule. The group of collagenases belong to a family of enzymes called matrix metalloproteinases or MMPs. Many cells in our bodies can synthesise and release collagenases including fibroblasts, macrophages, neutrophils, and osteoclasts.

Collagen remodeling by fibroblasts is regulated by various factors such as fibroblast-myofibroblast transformation factor, transforming growth factor- $\beta$ , platelet-derived growth factor (PDGF), lysophosphatidic acid, mechanical stimulus by the surrounding matrix as well as by cell number in collagen. There is plenty of evidence indicating that collagen remodeling is also dependent on the function of MMPs.

The transition from granulation tissue to scar involves shifts in the composition of the extra-cellular matrix (ECM); even after its synthesis and deposition, scar ECM continues to be modified and remodeled. The outcome of the repair process is a balance between ECM synthesis and degradation. The degradation of collagens and other ECM components is accomplished by a family of MMPs, which are dependent on zinc ions for their activity. MMPs include interstitial collagenases, which cleave fibrillar collagen (MMP-1, -2 and -3); gelatinases

(MMP-2 and 9), which degrade amorphous collagen and fibronectin; and stromelysins (MMP-3, -10, and -11), which degrade a variety of ECM constituents, including proteoglycans, laminin, fibronectin, and amorphous collagen.

MMPs are produced by a variety of cell types (fibroblasts, macrophages, neutrophils, synovial cells), and their synthesis and secretion are regulated by growth factors, cytokines, and other agents. Their synthesis is inhibited by TGF- $\beta$  and may be suppressed pharmacologically with steroids. Given the potential to wreak havoc in tissues, the activity of the MMPs is tightly controlled. They are produced as inactive precursors that must be first activated; this is accomplished by certain chemicals or proteases (e.g., plasmin) likely to be present only at sites of injury. In addition, activated collagenases can be rapidly inhibited by specific tissue inhibitors of metalloproteinases (TIMPs), produced by most mesenchymal cells. MMPs and their inhibitors are spatially and temporally regulated in healing wounds. They are essential in the debridement of injured sites and in the remodeling of the ECM.

## Key Learning Points :



1. Collagen is the most abundant protein found in the body.
2. It is responsible for providing strength and support for a wide range of tissues.
3. It has a helical structure, consisting of three alpha chains.
4. The combination of left-handed and right-handed helices within the molecule is responsible for its ability to withstand stress.
5. Collagen is synthesised within fibroblasts in a complex series of steps.
6. Procollagen is produced initially, which is modified after it has been produced and released by the fibroblast, and then becomes collagen.
7. Once in the extra-cellular environment, collagen molecules line up and form cross-links with each other.
8. When damaged, collagen is replaced with scar tissue, which contains a weaker version of collagen.
9. Collagen breakdown is usually a controlled process involving collagenases.
10. In disease states, collagen can be broken down more easily, leading to an inability to undergo repair.