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Collagen Metabolism

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Abstract and Introduction

Abstract

The process of wound healing consists of an orderly sequence of events characterized by the specific infiltration of specialized cells into the wound site. The platelets and inflammatory cells are the first cells to arrive, and they provide key functions and signals needed for the influx of connective tissue cells and a new blood supply. These chemical signals are known as growth factors or cytokines. The fibroblast is the connective tissue cell responsible for collagen deposition needed to repair the tissue injury. Collagen is the most abundant protein in the animal kingdom, as it accounts for 30 percent of the total protein in the human body. In normal tissues, 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, 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.

Introduction

Collagen is found in all of our connective tissues, such as dermis, bones, tendons, and ligaments, and also provides for the structural integrity of all of our internal organs.[1,2] Therefore, because of its wide distribution throughout our bodies, it represents one of the most abundant naturally occurring proteins on earth.[3] In addition to its natural abundance, there are well over 1,000 commercial products on the market today that contain collagen and collagen enhancers. These products are represented by body and hand lotions, nail treatments, firming gels, wrinkle injections, eye pads, and even anti-cancer treatments to name but a few. In recent years, new high-tech wound dressing materials and skin substitutes have become available for the treatment of partial-thickness injuries as well as full-thickness and chronic dermal ulcers.

There are close to 20 different types of collagen found in our bodies.[4,5] Each one of these collagens is encoded by a specific gene. The five major types are summarized in Table 1 . The predominant form is Type I collagen. This fibrillar form of collagen represents over 90 percent of our total collagen and 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 a collagen monomer (Figure 1). This configuration imparts tremendous strength to the protein. To understand the overall structure of the collagen molecule, think of it as the reinforcement rods called re-bar that are used in concrete construction. Indeed if one converts the molecular dimensions of the collagen molecule to measurements that we can relate to, the molecule when scaled up would measure one inch in diameter to approximately 17 feet long. Therefore, collagen is indeed nature's re-bar, because it is responsible for the strength and integrity of all of our connective tissues and organ structures.

(Enlarge Image)

Figure 1.

The basic structural unit of collagen is a triple-stranded helical molecule. From Molecular Cell Biology by Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. © 1986, 1990, 1995, 2000 by W. H. Freeman and Company. Used with permission.

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 our bodies. Type II collagen is the form that is found exclusively in cartilaginous tissues. It is usually associated with proteoglycans or "ground substance" and therefore functions as a shock absorber in our joints and vertebrae. Type III collagen is also found in our skin as well as in 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.[6]

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 noncollagenous 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 glomerulus 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.[7]

Collagen Synthesis

The biosynthetic pathway responsible for collagen production is a very complex one.[4,8] 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 messenger RNA (mRNA) 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 pro-alpha chain mRNA 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 also synthesized on the rER (Figure 2, step 1).

(Enlarge Image)

Figure 2.

The intracellular and extracellular events involved in the formation of a collagen fibril. Copyright 1994 from Molecular Biology of the Cell, Third Edition , by Alberts, Bray, Lewis, Raff, Roberts, Watson (eds). Reproduced by permission of Routledge, Inc., part of The Taylor & Francis Group.

A precursor form of collagen called procollagen is produced initially.[9] Procollagen contains extension proteins on each end called amino and carboxy procollagen extension propeptides. These nonhelical 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.[4,8] These modifications take place in the Golgi compartment of the ER.

Collagen, like most proteins that are destined for transport to the extracellular spaces for their function or activity, is produced initially as a larger precursor molecule called procollagen.[9] Procollagen contains 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 insure 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.

One of the first modifications to take place is the very critical step of hydroxylation of selected proline and lysine amino acids in the newly synthesized procollagen protein (Figure 2, step 2). Specific enzymes called hydroxylases are responsible for these important reactions needed to form hydroxyproline and hydroxylysine. The hydroxylase enzymes require Vitamin C and Iron as cofactors.[10] If a patient is Vitamin C deficient, then this reaction will not occur. In the absence of hydroxyproline, the collagen chains cannot form a proper helical structure, and the resultant molecule is weak and quickly destroyed.[11] The end result is poor wound healing, and the clinical condition is called scurvy.[12]The current recommended daily allowance for Vitamin C is 60mg; however, 200mg may be optimal.[13,14]

Some of the newly formed hydroxylysine amino acids are glycosylated by the addition of sugars, such as galactose and glucose.[15] The enzymes that catalyze the glycosylation step, galactosyl and glucosyl transferases, require the trace metal manganese (Mn+2). The glycosylation step imparts unique chemical and structural characteristics to the newly formed collagen molecule and may influence fibril size.[16] 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.[17]

While inside the cell and when the procollagen peptides are intact, the molecule is about 1,000 times more soluble than it is at a latter stage when the extension peptides are removed.[18] This high degree of solubility allows the procollagen molecule to be transported easily within the cell where it is moved by means of specialized structures called microtubules to the cell surface where it is secreted into the extracellular spaces.[19]

As the procollagen is secreted from the cell, it is acted upon by specialized enzymes called procollagen proteinases that remove both of the extension peptides from the ends of the molecule.[20] 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.[21,22] The processed molecule is referred to as collagen and now 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 (Figure 1) 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 (Figure 3).[23]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. On a per weight basis, the strength of collagen approaches the tensile strength of steel!

(Enlarge Image)

Figure 3.

The intramolecular and intermolecular cross-links formed within a collagen fibril. Copyright 1994 from Molecular Biology of the Cell, Third Edition , by Alberts, Bray, Lewis, Raff, Roberts, Watson (eds). Reproduced by permission of Routledge, Inc., part of The Taylor & Francis Group.

One can visualize 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. The structure is just like the steel rope cables on the Golden Gate bridge. This highly organized structure is what is responsible for the strength of tendons, ligaments, bones, and dermis.

When the normal collagen in our tissues is injured and replaced by scar collagen, the connective tissue does not regain this highly organized structure. That is why scar collagen is always weaker than the original collagen. The maximum regain in tensile strength of scar collagen is about 70 to 80 percent of the original.[24] Collagen synthesis and remolding (see below) continue at the wound site long after the injury. 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.[25] If only we understood more about the biology and mechanisms responsible for the rapid and optimal wound healing response seen in the fetus, we would have greater insight into the management of adult wounds.[26]

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 nonhealing 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 (Figure 1), the molecule is resistant to attack by most proteases. Under these normal healthy conditions, only

specialized enzymes called collagenases can attack the collagen molecule.[27] The group of collagenases belong to a family of enzymes called matrix metalloproteinases or MMPs.

Many cells in our bodies can synthesize and release collagenase including fibroblasts, macrophages, neutrophils, osteoclasts, and tumor cells. One of the reasons that some neoplastic cells can be so invasive is because they release potent collagenases and can break down the collagen around them. Then they can break down the basement membranes of blood vessels and spread throughout the body. In chronic pressure ulcers, there is a massive invasion of neutrophils, and they release a very potent collagenase called MMP-8 that is responsible for connective tissue breakdown.[28] Some exciting new research suggests that members of the tetracycline family of antibiotics, such as doxycycline, when given systemically, may be useful to treat pressure ulcers, because at low doses, they inhibit MMP-8.[29]

Conclusion

Collagen metabolism is indeed one of the most complex and highly regulated processes in our bodies. As we move forward in the future to design new strategies and technologies to treat the many challenging clinical problems associated with wound healing, we need to keep in mind how our connective tissues are assembled and how they are remodeled.