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COLLAGEN IN HEALTH

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ABSTRACT

Collagen is the largest and most abundant protein in the body, making up about 65% of our total protein. It is a fibrous protein, polypeptide molecule found mainly in connective tissues, exists throughout body tissue. It is found mainly in the skin, bones, muscles, cartilage, tendons, ligaments where it forms a scaffold to provide strength and structure. As collagen forms building block of body structures, any defect in collagen results in disorders. This review discusses the role of collagen in normal health.

KEYWORDS: Collagen, Extracellular matrix, Fibroblast, Health.

INTRODUCTION

Collagen is the unique, triple helical protein molecule which forms the major part of the extracellular matrix. It is the most abundant protein in the human body, representing 30% of its dry weight and is important to health because it characterizes the structure of skin, connective tissues, tendons, muscles, bones and cartilage.[1]

The word collagen is derived from a Greek word where "kola" means gum and "gen" means producing.[2] This is the major fibrous glycoprotein present in the extracellular matrix and in connective tissue and helps in maintaining the structural integrity of these tissues and provides rigidity, elasticity and strength.[1]

This super family includes 28 different types. These groups can also be classified into subtypes according to their structural and functional properties.[3] All kinds of connective tissue shows fibers of collagen. They are unbranched and in loose connective, they appear to be randomly oriented. When not under tension, they have an undulant course. In larger fibers, a faint longitudinal striation is evident, suggesting that these are bundles of smaller fibers.[4]

Collagens are produced by several cell types and are distinguishable by their molecular compositions, morphologic characteristics, distribution, functions and pathologies. Collagen is a triple helical structure. Various studies have proved that mutations that modify folding of the triple helix result in identifiable genetic disorders.[5] Therefore; this article highlights the role of collagen in normal health.

Types of collagen: (table 1)

Molecule type	Synthesizing cell	Function	Location in body
1. Fibril–forming; most common of all collagen	Fibroblasts, osteoblasts, odontoblasts, cementoblasts	Resists tension	Dermis, tendon, ligaments, capsules of organs, bone, detin, cementum
2. Fibril forming	Chondroblasts	Resist pressure	Hyaline & elastic cartilage
3.Fibril-forming:also known as reticular fibers. Highly glycosylated	Fibroblasts, reticular cells, smooth muscle cells	Resists pressure, forms structural frame work of spleen, liver, lymph nodes, smooth muscles, adipose tissue	Lymphatic system, spleen, liver, cardiovascular system, lung, skin
4. Network-forming: do not display 67nm periodicity and α-chains retain propeptides	Epithelial cells, muscle cells, Schwann cells	Forms meshwork of lamina densa of the basal lamina to provide support and filtration	Basal lamina
5. Fibril-forming	Fibroblasts,	Associated with type I	Dermis, tendon, ligaments,

	mesenchymal cells	collagen, also with placental ground substance	capsules of organs, bone, cementum, placenta
6.Microfiber-forming collagen	-	Bridging between cells and matrix(has binding properties for cells, proteoglycans, a type I collagen	Ligaments, skin, cartilage
7. Network-forming : from dimmers that assemble into anchoring fibrils	Epidermal cells	Forms anchoring fibrils that fasten lamina densa to underlying lamina reticularis	Junction of epidermis and dermis
8. Meshwork	-	Tissue supports, porous meshwork, provide compressive strength	Basal laminae of endothelial cells and smooth muscle cells Descemet's membrane of cornea
9. Fibril-associated: decorate the surface of type II collagen fibers	Epithelial cells	Associated with type II collagen fibers	Cartilage
10. Meshwork	-	Calcium binding	Hypertrophic zone of cartilage growth plate
11. Fibril collagen fibers	-	Forms core of type II fibers, provides tensile strength	Cartilage and vitreous humor
12. Fibril-associated: decorate the surface of type I collagen fibers	Fibroblasts	Associated with type I collagen fibers	Tendons, ligaments and aponeuroses
13. Transmembrane protein	-	Cell matrix and cell adhesion	Cell surfaces, focal adhesion and intercalated disks
14. FACIT	-	Modules fibril interaction	
15. Endostatin forming collagen	Endothelial cells	Proteolytic release of antiangiogenic factor	Endothelial basement membrane
16. Cartilage and placenta	-j	Unknown	Endothelial, perineural muscle and some epithelial basement membrane, cartilage and placenta
17. Collagen-like protein: a transmembrane protein, formerly known as bullous pemphigoid antigen	Epithelial cells	Cell to matrix attachment	Hemidesmosomes
18. Collagen-like protein: cleavage of its C-terminal forms endostatin and angiogenesis inhibitor	Endothelial cells	Proteolytic release of antiangiogenic factor	Endothelial basement membrane
19. FACIT	-	Unknown	Endothelial, perineural muscle and some epithelial basement membrane, cartilage and placenta
20. FACIT	-	-	Cornea
21. FACIT	-	-	Stomach, kidney
22. FACIT	-	-	Tissue junctions
23. Membrane-associated collagen with interrupted triple helix	-	-	Heart, retina
24. Fibrillar	-	-	Bones, cornea
25. Membrane-associated collagen with interrupted triple helix	-	-	Brain, heart, testis
26. FACIT	-	-	Testis, ovary
27. Fibrillar	-	-	Cartilage
28. Microfiber forming collagen	-	-	Dermis, sciatic nerve

Structure of collagen

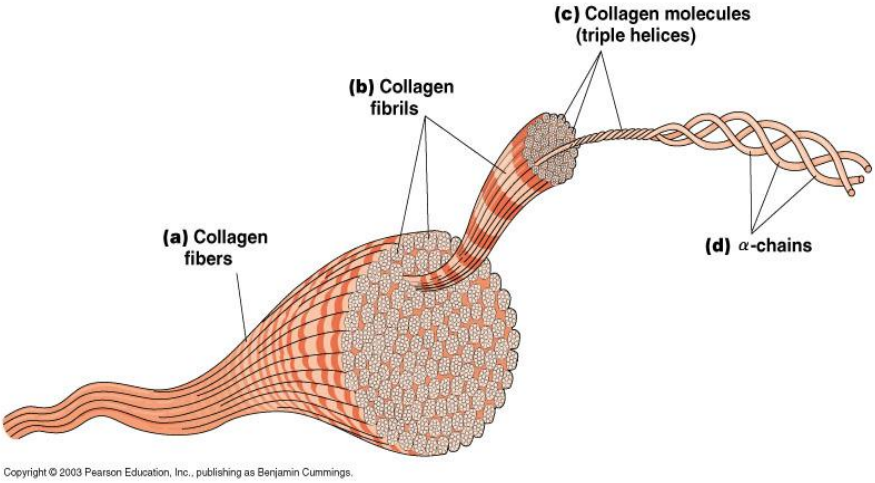

Figure 1: Structure Of Collagen

One-third of the total protein in humans encompasses collagen, accounts for three-quarters of the dry weight of skin and is the prevalent constituent of the extracellular matrix. There are Twenty-eight different types of collagens composed of at least 46 distinct polypeptide chains have been recognized in vertebrates.[1]

The unit fibrils are polymers of collagen molecules, each 300nm in length and 1.4nm in diameter. They are made up of three polypeptide chains, called α-chains(figure 1). The chains have a left-handed helical configuration, and the three are entwined to form a right-handed triple helix, in which each turn spans a distance of 8nm.The α-chains are held together within the triple helix by hydrogen bonds. A distinctive feature is regular arrangement of amino acids in each of the three chains of collagen subunits. Often followed pattern Gly-X-Pro or Gly-X-Hyp, where X may be having various other amino acid residues.

Common features of collagen

1) Family of fibrous insoluble structural proteins making 1/3rd of body's protein
2) Most abundant protein in mammals
3) Collagen family consists of 30 genes producing 28 types of collagen
4) Presence of **glycine at every 3rd position** higher proportion of Proline residues, Hydroxyproline, Hydroxylysine

Variations among the collagens are due to

1. Differences in the assembly of the basic polypeptide chains.
2. Different lengths of triple helix.
3. Interruptions in the helix.
4. Differences in the terminations of helical domains

Microscopic structure & stains

Light microscopic structure

After staining with hematoxylin and eosin, they appear as long, wavy, pink fiber bundles. Unstained collagen fibers of connective tissue are usually less than 10µm in diameter and are colorless.

Electron microscopic structure

Appear as bundle or bundles of fine, thread like subunits collagen fibrils which are of uniform diameter. Fibrils differ in size in different location & different stages of development.

- In immature tissues : small as 15-20 nm in diameter
- In dense regular connective tissue: 200nm in diameter
- 68 nm banding pattern of collagen
- Appearance of dark & light bands
- Penetration of contrast medium into the gaps results in uniformly spaced dark bands across the fibril.
- Light bands are the regions in which molecular overlap prevents penetration of the stain.
- Collagen fibrils when stained with Osmium tetra oxide or other heavy metals exhibits sequence of closely spaced transverse bands that repeat every 68 nm along the length of the fibril.[6,7]

Stains

Different types of stains used for collagen are Hematoxylin & Eosin, Masson's trichrome, Mallory's trichome, Van gieson's stain, Picro-sirius red stain.

Synthesis of collagen

The chief producers of collagen are mesenchymal cells and their derivatives (fibroblasts, osteoblast, odontoblast, chondroblasts and cementoblasts). Other cell types synthesizing collagen are epithelial, endothelial, muscle and Schwan cells.[1]

Fibroblast

The most common cell of connective tissue is fibroblast that produces and maintains the extracellular matrix. Fibroblast maintains the structural integrity of connective tissues by continuously secreting precursors of the extracellular matrix, primarily the ground substance and a variety of fibers. They are recognized by their

association with collagen fibers bundles and are usually spindle shaped.[8] they also exhibit contractility and motility which are important during connective tissue remodeling, formation and during wound repair. Fibroblasts in certain tissues have significant contractile properties and are called as myofibroblasts.[6,7]

Biosynthesis of collagen fibers

Collagen fiber formation involves events that occur both within and outside the fibroblast. The production of fibrillar collagen I, II, III, V and XI involves a series of events within the fibroblast that leads to production of procollagen**,** the precursor of the collagen molecule. These events take place in membrane – bounded organelles within the cell. Production of the actual fibril occurs outside the cell and involves enzymatic activity at the plasma membrane to produce the collagen molecule, followed by assembly of the molecules into fibrils in the ECM under guidance by the cell.

Collagen fibrils often consist of more than one type of collagen. Usually different types of fibrillar collagens assemble into fibrils composed of more than one type of collagen molecule. For example, type I collagen fibrils often contain small amounts types II, III, IV and XI. Current studies indicate that assembly of type I collagen fibrils is preceded by formation of a fibrillar core containing type V and type XI molecules. Subsequently, type I collagen molecules are deposited and polymerized on the surface of the fibrillar core. In addition, small amounts of type II and III collagen molecules are incorporated into type I collagen fibrils. Collagen types V and XI are important regulators of fibrillogenesis. They control the thickness of type 1 fibrils by limiting the deposition of collagen molecules after the fibril has reached the desired diameter.

Degradation of collagen

Collagen fibres are degraded either by proteolytic or phagocytic pathways. All proteins in the body are being continually degraded and resynthesized. These processes allow tissues to grow and to undergo remodelling. Initial fragmentation of insoluble collagen molecules occurs through mechanical wear, the action of free radicals, or proteinase cleavage. Further degradation is continued by specific enzymes called **proteinases.** The resulting collagen fragments are then phagocytosed by cells and degraded by their lysosomal enzymes. Excessive collagen degradation is observed in several diseases for example, degradation of cartilage collagen in rheumatoid arthritis or bone collagen in osteoporosis.

Proteolytic degradation

Occurs outside the cells through the activity of enzymes called **matrix metalloproteinase's (MMPs)**. These enzymes are synthesized and secreted into the ECM by a variety of connective tissue cells (fibroblasts, chondrocytes, monocytes, neutrophils and macrophages), some epithelial cells (keratinocytes in the epidermis), and cancer cells. The matrix metalloproteinases (MMPs) include – collagenases, gelatinases, stromelysins, matrilysins.

Collagenases degrade type I, II, III and X collagens.
Gelatinases – degrade most types of denatured collagens, laminin, fibronectin elastin
Stromelysins – degrade proteoglycans, fibronectin, and denatured collagens
Matrilysins – degrade type IV collagen and proteoglycans
Membrane – type MMPs which are produced by cancer cells and have a potent pericellular Fibrinolytic activity
Macrophage metalloelastases – degrade elastin, type IV collagen and laminin

Phagocytotic degradation

Occurs intracellularly and involves macrophages to remove components of the ECM. Fibroblasts are also capable of phagocytosing and degrading collagen fibrils within the lysosomes of the cell.[9]

COLLAGEN IN HEALTH

Collagen is the one that holds everything in place and sometimes referred to as the body's cement. Collagen is important to health because it dictates the structure of skin, connective tissues, tendons, bones and cartilage.[1]

1. Skin

Collagen plays an important role in skin health. In normal young skin tissue Type I and III collagen are formed in a higher proportion relative to other types and are maintained in a fixed proportion relative to one another. Type I Collagen constitutes approximately 70% of collagen in the skin, with type III being 10% and trace amounts of collagen type IV, V, VI, VII. It helps to maintain firmness and elasticity of skin. As age advances lack of collagen becomes obvious as skin begins to sag and lines and wrinkles begin to form. Skin aging is a complex biological process, in which Type III collagen synthesis decreases resulting in changes in skin tension, elasticity and healing. Collagen in the form of collagen hydrolysate keep skin hydrated.[10] Autoimmune diseases, ageing and stress can change the quantity and integrity of collagen in the skin as they impair collagen quality and consequently affect the overall skin function.[11] During formation of scar tissue as a result of age or injury, there is change in the abundance of types I and III collagen as well as their proportion to one another.[10]

2. Wound healing

Collagen plays an imperative role in wound healing by repair and formation of scar.[1] The principal function of collagen is to act as a scaffold in connective tissue, mostly in its type I, II and III forms. In early healing wounds, type III is laid down first, with the proportion of type I increasing as scar formation progresses and is remodelled. Tensile strength of the wound is increased by collagen deposition and remodelling, which is approximately 20% of normal by three weeks after

injury, gradually reaching a maximum of 70% of that of normal skin. Although epithelial structures can heal by regeneration, connective tissues cannot and depend on the process of repair mostly by the formation of collagenous scar tissue predominantly of type I, which serves to restore tissue continuity, strength and function. For unwounded tissue, collagen is a brittle substitute and scar tissue rarely exceeds 70% of unwounded tissue strength.[12]

Collagen is the major protein in the extracellular matrix (ECM) and is known to be deficient in chronic wounds.[13] In chronic wounds involving skin, defined as those which do not heal in optimal conditions within six weeks, the complex interactive processes described above are deranged. Conversely, there is not a lack of normal healing but often hyperactivity which is out of phase, non-progressive and with a persistent, uncoordinated, mixed acute and chronic inflammatory response.[12]

Overproduction of collagen can form abnormal scars, which impede wound healing. A chronic wound burden among the elderly has been documented and much of this age- related, delayed wound healing is caused by impaired collagen synthesis and increased degradation. Increase in fibroblasts and collagen during healing suggested that a correlation might exist between number of fibroblasts, quantity of collagen and tensile strength of a scar.[1]

3. Bone

Bone is a complex and dynamic tissue, both light and strong and that provides structural support for the body, protection of internal organs and acts as levers to which muscles are attached, allowing movement.

Collagen also acts as mineral reservoir and participates in acid-base balance. Bone is composed of 70% inorganic component (of which 95% is hydroxyapatite and 5% impurities impregnated in hydroxyapatite), 22% to 25% of organic component (of which 94-98% is mainly collagen type I and other non-collagen proteins and 2%-5% are cells) and 5 to 8% is water.[14] The combination of hard mineral and flexible collagen makes bone harder than cartilage without being brittle. Combination of collagen mesh and water forms a strong and slippery pad in the joint that cushions the ends of the bones in the joint during muscle movement.

Properties of bone

Bone is a linear and brittle, yet anisotropic and viscoelastic material. Mechanical properties are determined by its porosity, degree of mineralisation, collagen fibre orientation and other structural details.[15] Long bones are connected to skeletal muscles via tendons and Bones connect at joints by ligaments.

Woven bone (also called immature bone) is the weakest and cortical (also called compact) bone is the strongest bone.

Cancellous bone (also called trabecular or spongy bone) is intermediate in properties, compares compact bone's density and elastic modulus to those of a variety of other materials and ranks them according the ratio of modulus to density.[16]

4. Cartilage, tendon, ligaments

In tendon and ligament, collagen is found in the form of elongated fibrils. It is flexible and stretchy protein that is used by the body to support tissues and thus it plays a vital role in the maintenance of the cartilage, tendons and ligaments. Normal tendon consists of soft and fibrous connective tissue that is composed of densely packed collagen fibers bundles aligned parallel to the longitudinal tendon axis and surrounded by a tendon sheath also consisting of extracellular matrix components. 75% of the dry tendon weight constitutes collagen and functions chiefly to withstand and transmit large forces between muscle and bone.[17] Collagen also forms a major constituent of cartilages. Cartilages consist of collagen II, and quantitatively minor collagens IX and XI.[18,19]

Dense irregular connective tissue forms the capsule around joints and this is often reinforced by ligaments, which are strong bands of parallel collagen bundles that serve to attach bone to bone and to limit the degree of movement at the joint.[4]

5. Muscles

In muscle tissue, 1 to 2% constitutes collagen and it also serves as a major component of the endomysium. It accounts for 6% of the weight of strong, tendinous muscles.[20]

6. Dental tissues

A. Dentin

Dentin is first deposited as a layer of unmineralized matrix called predentin that varies in thickness (10 to 50µm) and lines its innermost (pulpal) portion. Predentin consists principally of collagen and is similar to osteoid in bone. Predentin gradually mineralizes into dentin as various noncollagenous matrix proteins are incorporated at the mineralization front.

Mature dentin is made up of approximately 70% inorganic material, 20% organic material and 10% water by weight and 45%, 33% and 22%, respectively, by volume. The inorganic component of dentin consists of substituted hydroxyapatite in the form of small plates. The organic phase is about 90% collagen (mainly type I with small amounts of types III and V) with fractional inclusions of various noncollagenous matrix proteins and lipids.

The noncollagenous matrix proteins pack the space between collagen fibrils and accumulate along the periphery of dentinal tubules. These proteins make up the following: dentin phosphoprotein/phosphophoryn (DPP), dentin sialoprotein (DSP), dentin glycoprotein (DGP), dentin matrix protein-1 (DMP1), osteonectin, osteocalcin, bone sialoprotein (BSP), osteopontin, matrix extracellular phosphoglycoprotein, proteoglycans, and some serum proteins. DPP and DSP represent the major noncollagenous matrix proteins in dentin. The noncollagenous matrix proteins regulate mineral deposition and can act as inhibitors, promoters and /or stabilizers. Large proportion (estimated at 56%) of the mineral in the holes and pores of fibrils accommodates collagen type I that acts as a scaffold.[21]

B. pulp

The extracellular compartment of the pulp or matrix consists of collagen fibers and ground substance. The fibers are principally types I and III collagen. The overall collagen content of the pulp increases with age, the ratio between types I and III remains stable and the increased amount of extracellular collagen organizes into fiber bundles. The greatest concentration of collagen generally occurs in the most apical portion of the pulp. This fact is of practical significance when a pulpectomy is performed during the course of endodontic treatment. Engaging the pulp with a barbed broach in the region of the apex affords a better opportunity to remove the tissue intact than does engaging the broach more coronally, where the pulp is more gelatinous and liable to tear.[22]

C. cementum

In cementum type I collagen is predominantly present (forms 90% of the organic matrix). Other collagens associated with cementum includes s type III, a less crosslinked collagen found in high concentrations during development and repair and regeneration of mineralized tissues and type XII that binds to type I collagen and also to noncollagenous matrix proteins. Collagens found in trace amount in cementum are types V, VI and XIV. During early stages of cementogenesis and during development and repair, type III collagen is present in high amounts but is reduced with maturation of this tissue.

D. periodontal ligament

The periodontal ligament is that soft, specialized connective tissue situated between the cementum covering the root of the tooth and the bone forming the socket wall (alveolo-dental ligament). It ranges in width from 0.15 to 0.38 mm, with its thinnest portion around the middle third of the root, showing a progressive decrease in thickness with age.

The predominant collagens of the periodontal ligament are type I, III and XII, with individual fibrils having a relatively smaller average diameter than tendon collagen fibrils, a difference believed to reflect the relatively short half-life of ligament collagen and hence less time for fibrillar assembly. The vast majority of collagen fibrils in the periodontal ligament are arranged in definite and distinct fiber bundles and these are termed principal fibers. Each bundle resembles a spliced rope; individual strands can be continually remodeled while the overall fiber maintains its architecture and function. In this way the fiber bundles are able to adapt to the continual stresses placed on them. The extremities of collagen fiber bundles are embedded in cementum or bone. The embedded portion is referred to as Sharpey's fibers. Sharpey's fibers in primary acellular cementum are fully mineralized; those in cellular cementum and bone are generally only partially mineralized at their periphery.

The main function of type I collagen is to structure the fiber bundles that anchor the tooth to the bone and distribute masticatory forces. The periodontal ligament has also the capacity to adapt to functional changes. When the functional demand increases, the width of the periodontal ligament can increase by as much as 50% and the fiber bundles also increase markedly in thickness.[23]

7. basement membrane

The epithelial basement membrane and adjacent area is termed the epithelial basement membrane zone (BMZ). Conventionally the BMZ can be divided into four Components from the epithelium inward toward the connective tissue:(figure 2).

1. The keratinocyte plasma membrane with the hemidesmosomes and integrin.

2. The lamina lucida

Consisting of laminin, an adhesive glycoprotein that mediates not only attachment between type IV collagen and the lamina densa, but also keratinocyte differentiation, migration, and morphogenesis.

3. The lamina densa

Consisting of type IV collagen that is coated by heparan sulfate, a glycosaminoglycan and anchoring fibrils, that are composed of type VII collagen and extend from the lamina densa to the connective tissue.

4. The sublamina densa

Containing collagen fibers, anchoring fibrils, and microfibrillar bundles that extend more deeply into the mesenchyme. Keratinocyte-epithelial basement membrane contact is largely via hemidesmosomes, which link the keratinocyte cytoskeletons to the lamina lucida – the superficial part of the epithelial basement membrane. The deeper aspect of the epithelial basement membrane – the lamina densa – is anchored to the underlying papillary dermis by cross-banded anchoring fibrils.[24]

CONCLUSION

Collagens are the major structural element of all connective tissues where they contribute to the stability

of tissues and organs and maintain their structural integrity. A balanced synthesis, regulation and degradation of collagen ensure a good health.

It is unavoidable that the undesirable effects of molecular changes in the structure of this fibrous protein may affect many systems of the human body, from the central nervous system to the musculoskeletal and cardiovascular systems leading to collagen disorders.

In recent years, despite the increasing information obtained about the structure and synthesis of collagen, the genetic and molecular bases of the collagen disorders, which are still accepted as untreatable or incurable clinical syndromes, remain undetermined. In addition to having the opportunity to understand numerous molecular disorders, progressive biochemical tests, molecular findings and also the technological resources will facilitate an explanation of the systematic and physiopathologic processes. Hence, future research and molecular studies are required in this field in order to provide the best treatment modalities to the patients with collagen disorders.

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