Role Of Collagen In The Periodontal Ligament - A Review
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Citation

Abstract
As in most connective tissues the fibers of the periodontal ligament are mainly collagenous in nature. The fibers of the periodontal ligament appear similar to those of other supportive connective tissues in that they are composed of an integrated unit of fibrous components. Nevertheless, the fibers of the periodontium have a particular structural requirement to withstand intensive forces from mastication and to accommodate tooth eruption.

INTRODUCTION

LIGAMENT
The connective tissue fibers are produced by fibroblasts and can be divided into

a. Collagen fibers
b. Reticulin fibers
c. Oxytalan fibers
d. Elastic fibers

At least 18 species of collagen has been isolated from the extracellular matrix of connective tissues (Kielty et al., 1998). The Collagen of the periodontal ligament is largely type I, with lesser amount of type III, IV, V, VI & XII also present (Butler et al.; Walker et al.; Sloan et al.).

Collagen type I, III & V are ‘banded’ molecules which have a characteristic periodicity of 67 nm when stained and examined in the electron microscope, owing to the staggered arrangement of the molecules in the fibril.

Collagen type III is co-distributed with collagen type I but is found in higher proportion in fetal tissues (Berkovitz.). Collagen type V also co-distributes with type I but it can only be demonstrated immuno-histochemically by disrupting the fibril. Collagen type IV is found in the basement membrane of junctional epithelium and epithelial rests of the periodontal ligament (Sloan et al.).

The collagen fibril diameters of the human periodontal ligament are relatively small, with mean diameters of the order 45-55 mm (Berkovitz et al.) which is much less when compared to other connective tissues, e.g. Tendon fibril diameters may reach up to 250 mm. The small diameter of the fibrils in the periodontal ligament could be the result of either the high rate of collagen turnover or the absence of mature collagen fibrils (Berkovitz et al.). It has also been suggested that fibril diameter is regulated by the co-polymerization of different collagen molecules and hence the small diameter of the collagen fibrils in the periodontal ligament may be due to specific fibril composition, but there is no comparative evidence to prove it.

PLASTIC FIBERS OF THE PERIODONTAL LIGAMENT
The elastic fibers are associated with collagen fibers as an elastic meshwork and are composed of three fibrous components – Oxytalan, Elaunin, and elastin.

OXYTALAN AND ELAUNIN
Oxytalan and Elaunin fibers can be demonstrated with elastin stains in light microscope (Fullmer.). Oxytalan fibers form a three dimensional meshwork that extends from the cementum to the peripheral periodontal blood vessels. Depending on the site and species, oxytalan fibers measures between 0.2 and 1.5 μm in diameter in the electron microscope and is reported to occupy 3% of the periodontal ligament in humans (Shore, Moxham and Berkovitz.). In transmission electron microscope, oxytalan fibers appear similar to developing elastin fibers and are composed of groups of microfilaments embedded within amorphous material (Fullmer et al.; Shore, Moxham and Berkovitz.).

The similarities between the ultrastructure of Oxytalan microfibrils and fibrils of fibronectin have been commented
by Frank and Nalbandian, 1989, like fibronectin Oxytalan fibers are important for fibroblast adhesion and migration.

**ELASTIN**

Elastin fibers are present in only some periodontal ligament of some species and are composed of microfibrillar glycoprotein and amorphous elastin (Ross\(^\text{12}\)). These elementary units of elastin are rod like in shape and are aggregated into globular protein masses.

**ARRANGEMENT OF THE PERIODONTAL COLLAGEN FIBERS**

The fibers of periodontal ligament are arranged in 5 major groups.

The fiber bundles were considered to pass directly from bone to cementum, supporting the tooth in the manner of a sling. Grant et al.,\(^\text{8}\) demonstrated using light and transmission, electron microscopy that the principal bundles branch into small bundles to form a continuous plexiform arrangement.

Mashouf and Engel found highly organized birefringent collagen fibers in rat periodontal ligament, which reconciled the view that arrangement of the fibers is in a random manner a indifferent fibers plexus when examined by scanning electron microscopy.

Under scanning electron microscopy the periodontium exhibited a continuous band of fibrous tissue (100-250 µm in width) containing prominent neurovascular bundles (Sloan,\(^\text{14}\)) and the fiber bundles were 3-10 µm in diameter towards the cementum and close to the alveolar bone they were 10-20 µm in diameter, The remaining bundles in the periodontal ligament were 1-4 µm in diameter.

**COLLAGEN TURNOVER IN THE PERIODONTAL LIGAMENT**

Measurements of tritiated proline and glycein uptake in the periodontal ligament of erupting teeth have indicated that there is a high turnover of collagen with a half-life varying between 3 and 23 days The turnover rate of collagen in the periodontal ligament is estimated to be several times greater than in skin and oral mucosa (Sodek,\(^\text{15}\)).

**FUNCTIONAL ADAPTATIONS OF THE COLLAGEN FIBERS OF THE PERIODONTAL LIGAMENT**

Current theories of tooth support envisage a multiphasic system involving fibers, ground substances, blood vessels and fluid, all acting together to resist mechanical forces and it seems that there is both tension & compression of the tissues.

Minns et al. showed that the internal orientation of collagen fibers in the connective tissues influences the mechanical properties of the tissues and suggested that, in general the collagenous bundles could best resist axially directed forces. The arrangement of majority of the periodontal ligament collagen fibers are in horizontal & oblique direction and hence may be adapted to resist, axial forces. But the overlapping arrangement of the bundles seen in scanning electron microscopy & by polarizing microscopy demonstrated their ability to resist rotational forces. The overlapping arrangement of the bundles is also advantageous in resisting intrusive forces.

**COLLAGEN CRIMPING**

Diament demonstrated that collagenous tissues exhibit a quantifiable periodicity of structure of variable scale and this wave for that describes this periodicity has been referred to as a ‘crimp’. In polarizing microscope, crimping can be recognized by a regular banding of dark lines across a collagenous bundle observed when its axis lies perpendicular to the polarizer directions. Crimping may be due not only to a sharp zig-zag arrangement of collagen fibrils, but also to the micro anatomical organization of collagenous sheets and bundles.

**SHARPEY’S FIBERS**

The Collagen bundles of the periodontal ligament are embedded into cementum and alveolar bone in a manner similar to a tendon inserting into bone (ie.) in the form of Sharpey’s fibers. The orientation of the sharpey’s fibers in the alveolar bone is similar to that of the adjacent periodontal ligament bundles (Melcher and Eastoe,\(^\text{9}\)) and they tend to be concentrated in the crestal region. However, the orientation in the cementum is of various types as put forward by 65; Yamamoto 17. Light microscopic observations have suggested that some Sharpey’s fibers in mice, hamsters, monkey and human, pass right through alveolar bone, which implies that there may be continuity with the collagen fibers of the periodontal ligament of adjacent teeth. Although the existence of the trans alveolar fibers has been disputed (Atkinson, 1978) trans alveolar fibers appear to be a major structural feature of the interdental septum in the young mouse.

On the basis of ultrastructural and microradiographic
observations Selvig 1965 reported that Sharpey’s fibers that insert into the alveolar bone have unmineralized cores and are separated from each other by lamellar bone fibers which either run parallel to the mineral surface or are randomly arranged.

Wang et al., showed by immuno histochemical methods that Sharpey’s fibers are enclosed within a sheath of Collagen type III and this confer elasticity to the fibers even in alveolar bone and prevents their remineralization.

The examination of the surface of the alveolar bone and the cementum after removal of the soft connective tissue with scanning electron microscopy, shows that the peripheral bone surrounding the Sharpey’s fibers may be mineralized to a level slightly above or below the level of bone surface and exhibit a stippled appearance that indicate that the mineralization occurs approximately at right angles to the axis of the fibers and it offers mechanical advantage for transmitting axially directed forces and tensional forces.

AGING OF THE PERIODONTAL FIBERS
The main changes that occur with age is increased collagen fibrosis and decreased cellularity (Grant and Bernick). The fiber bundles were thicker and that the fiber groups were broader and more highly organized with areas of hyalinization and decreased argyrophilia increased fuschinophilia and a reduction in alcian blue-positive areas. Decrease in the number of periodontal fibers with increase in size of interstitial spaces.

Severson et al found that the periodontal alveolar bone surface was smooth and regular in young adults but in older adults the corresponding surfaces were jagged and uneven and an irregular insertion of the fibers was seen. Cementum was thicker in aged tissues and the cementum surface also became irregular with time.

This irregularity of the fiber insertion together with replacement of some of the periodontal ligament space by interstitial areas and fat cells suggests that the structural organization of the periodontal ligament degenerates with age.

BIOCHEMISTRY OF THE FIBERS OF THE PERIODONTAL LIGAMENT
The extracellular matrix of the periodontal ligament comprises of two major compartments

These components are seen in electron microscope as an insoluble fibrillar network surrounded by an apparently less ordered, thixotropic gel. The fibrous elements are able to provide the tissue with tensile strength, while the ground substance is capable of dissipating compressional forces.

Collagen and the elastin family together make up the fibrous elements of the periodontal ligament. Collagen is the most abundant of these and it is often presumed to be the most important in terms of tooth support

COLLAGEN FIBER
Collagen which is the most abundant protein in the animal kingdom, is now understood to comprise a large family of related but genetically distinct proteins ( Miller).

Collagens are secreted primarily but not exclusively by fibroblasts, though many other cell types have also been shown to produce collagen (eg. Epithelial cells - Slovkin ).

Although some 18 different collagen types have now been described, all share to greater or lesser degree common features by basic molecular structure and homology of condensed amino acid sequence. This is the collagen triple helix or ‘super helix’, which arises because of the primary sequence of the component polypeptide chains (known as ‘a-chains’).

GENETIC ORGANIZATION
Review by Chu & Prockop demonstrates that the primary sequence of aminoacids in the fibrous collagen is directly related to their genetic organization. The a1 (1) chains of type I collagen are encoded by 41 small exons, most of which comprise of 54 bp and code for 6 (GLY-X-Y) repeats. The remainder either code for two or three (GLY-X-Y) repeats or contain a 9 bp deletion, corresponding to one (GLY-X-Y).

This evidence suggest that fibrillar collagens are evolved from an ancestral molecule based upon the repetition of a (GLY-X-Y) six folds repeat and those modern collagens are the result of gene duplication.

CLASSIFICATION OF COLLAGEN TYPES
At least 25 different gene sequences has been described which give rise to 13 distinct collagen type designated as I to XIII.

All the collagen types consist of three polypeptides (a-chains) and although homologous in part, they vary widely in the amount of their structure, which is in the form of the classic triple helix.
The collagens can be divided into three groups:

The first and most abundant group is the fibrous collagens. These collagens are all in the form of uninterrupted helices that are highly conserved. These collagens are types I, II, III, V and XI.

The second group is the high molecular weight collagens, which contain numerous intervening non-helical sequences, resulting in many stitches of interrupted helices. These collagens are those found in association with basement membranes (e.g. Types IV & VII). They are cross linked by disulphide bridges.

The third group is the short-chain collagens and consists of type VI, VIII, IX, X, XII and XIII. All contain numerous intervening non-helical domains. Type IX collagen may be classified as a proteoglycan, with chondroitin sulfate or dermatan sulphate side chains.

### Collagen Types Within the Periodontal Ligament

The periodontal ligament contains mostly fibrils of type I collagen as well as significantly high proportion of type III fibrils (Butler et al., 3; Sodek 15). This amount of type III collagen is unusually high for a mature connective tissue (approximately 20% of total collagen) and is more characteristic of a fetal, connective tissue. Becker et al. found that minor collagens, type V and VI are also present in periodontal ligament.

### General Structure of Fibrous Collagens

All collagens are made up of three polypeptide chains (ie. They are trimeric). These chains (α-chains) may be identical (homotrimers) are different (heterotrimers). These three polypeptide chains are organized into a three-stranded helical structure known as the collagen triple helix (Ramachandran ).

### Primary Structure

Each polypeptide chain in type I collagen contains 1056 aminoacid residues, over 90% of which are in the form of the repeating tri-residue Motif (Gly-x-y), where x is often proline and y is often hydroxyproline. Together, these aminoacids account for over 20% of all residues. It is this high proportion of aminoacids, coupled with the presence of glycine at every third residue, which confers the characteristic conformation to collagen molecules (Ramachandran & Ramakrishnan, ).

### General Biosynthesis of Fibrous Collagens

Now the question arises as to how these tropocollagen molecules are transported to the extracellular matrix in an unaggregate form?

The presence of a soluble precursor form of collagen was predicted many years before its ultimate isolation and characterization.

Studies by Fessler & Smith have found the precursor to be “procollagen” synthesized from fibroblasts of tissue explants in vitro.

Pro collagens have a molecular weight which is up to 50% greater than that of the final tropocollagen molecule. These are due to the peptide extensions on the C & N termini and are called propeptides which are non-helical.

The propeptides are not identical with C-terminal propeptide being much larger (approximately 246 amino acids) than the N-terminal propeptide (139 amino acids for the pro α1 (1) chains and 57 amino acids for the shorter pro α2 (1) chain).

Both propeptides are susceptible to proteolytic cleavage. Indeed, cleavage of the propeptide is a necessary prerequisite for fibrillogenesis in the extracellular matrix. Failure to remove the N-terminal propeptide results in the inherited condition of dennatosparaxis in cattle and sheep in which an accumulation of partially processed procollagen molecules arise with concomitant failure to form fibers (Schofield & Prockop).

The ultimate fate of the cleared propeptides remains a matter of some conjecture, however, and there is some evidence to suggest that they may have a role in the control of collagen biosynthesis at the pre-translational level.

### Synthesis of Pro Collagen

Procollagen biosynthesis begins at the ribosomes on the rough endoplasmic reticulum (RER) and involves extensive co-translation & post-translational modifications which are controlled by 9 to 10 different enzymes (Kivirikko & Myllyla).

### Hydroxylation of Proline and Lysine

Hydroxylation of PRO & LYS residues is apparently initiated as a co-transitional event that occurs on the nascent α-chains during chain elongation at the ribosome (Kivirikko & Myllyla.). Hydroxylation is catalysed by three hydroxylase enzymes with specific target sequences.
All the hydroxylases require Fe^{2+}, a-ketoglutarate, O_2 and ascorbic acid. The reaction produces Co^{2+} and succinate (Kipirikko & Myllyla). The role of ascorbic acid is indirect; it probably plays a part in the regeneration of Fe^{2+} following uncoupling of the a-ketoglutarate decarboxylation reaction. The role of hydroxyproline and hydroxylysine in helix stability and intermolecular cross-linkage is an important one and failure of this mechanism (eg. in vit-c. deficiency) results in a range of pathologies (Bailey, et al.), including scurvy, hydroxylsine-deficiency disease, lathyrism & Menkes’ Kinky hair syndrome.

**GLYCOSYLATION OF HYDROXYLYSINE AND ASPARAGINE**

Collagen is a glycoprotein and the addition of α-linked carbohydrate to some hydroxylysine residues in the triple helical domain also occurs at the co-translational and post-translational levels (Kirvirikko and Myllyla). The amount of glycosylation varies with tissue and age, but essentially two carbohydrate residues are involved. These are:

The reactions are catalysed by hydroxylysyl galactosyl transferase and galactosyl hydorxylysyl glucosyl transferase respectively, utilizing the activated (UDP) sugars in the presence of bivalent cations (preferably M^{2+}). The significance of these sugars is not yet fully understood although their role in fibril organization is suggested.

**HELIX FORMATION**

Triple helix formation is initiated via the association of the three c- terminal propeptides. A role for the signal peptide in the assembly of two a1 chains and one a2 chain at a common site at the rough endoplasmic reticulum membrane has been suggested, (Kirk et al), chain alignment begins by non-covalent (hydrophobic) interaction at the c-terminal propeptide (Fessler). The c-terminal end of the triple helical domain consists of a highly conserved stretch of 3-10 repeating GLY-PRO-HYP motifs that stabilize the propagating helix. This alignment is further stabilized by interchain disulphide bond formation in the propeptide domain (Foster & Freedman).

The enzyme protein, disulphide isomerase, which has wide substrate specificity (Freedman & Hillson), is required for correct cis/trans conformational alignment and re-arrangement of (incorrect) disulphide bridges. Subsequent further folding of the collagen triple helix proceeds rapidly after the initial stabilization of the c-terminal propeptide, which is the rate-limiting step for helix formation. The fully associated trimeric procollagen molecule is then exported via the golgi apparatus in the classical secretory pathway. Once outside the cell, in the extracellular matrix, further processing occurs by endopeptidase activity which cleaves the N- Emd C- propeptides leaving the tropocollagen molecules which can then form aggregate.

Removal of the propeptidas is achieved via at least two specific endopeptidas, procollagen-c-proteinase and procollagen-N-proteinase, which cleave at the C- & N- termini respectively. These are both enzymes of matrix metalloproteinases class.

There is also some evidence to suggest that at least in case of type I procollagen, degradation may proceed via an intracellular route. Suggested that fibroblasts may produce collagen in excess of normal requirements, a fraction of which is broken down intracellularly before becoming incorporated into fibrils. A role for this route in rapidly meeting increased demand for collagen has been suggested (Berkovitz).

**METABOLISM OF THE FIBROUS COLLAGENS**

**General Features of Collagen Degradation**

Breakdown of collagenous matrix is a normal event in tissues undergoing morphogenesis, morphostasis and growth but failure to maintain an appropriate balance between degradation and synthesis can lead to net destruction or net gain, resulting, for example, in pathological conditions such as chronic inflammatory periodontal disease or hypertrophic scar formation.

The fibrillar collagens are subjected to fragmentation, owing to physical wear and tear of the tissues and to the action of highly reactive free radicals (Murphy and Reynolds). Collagen breakdown is also under stringent cellular control mediated via a group of proteolytic enzymes the matrix metalloproteinases (reviewed by Murphy & Reynolds). This is a group of endopeptidas which share some degree of sequence homology with ability to bind to metals and maintain neutral pH optima.

Matrix metalloproteinases (MMPs) all contain Zn^{2+} at their active site and require Ca^{2+} as stabilizer. Are all secreted as inactive precursors, which are often self-activated and are inhibited by tissue inhibitors of matrix metalloproteinases (TIMP). MMPs are secreted by connective tissue cells predominantly fibroblasts, but are also produced by some leucocytes (PMNs & Macrophages).
Fibrillar collagen is very resistant to proteolytic degradation in its triple-helical domain (Kuhn, 1986). Two matrix metalloproteinases (collagenases) are capable of cleaving the helix at a single locus, resulting in two fragments of 3/4 and 1/4 molecular lengths from the N-terminus. The triple helical conformation is rapidly lost after cleavage, and the resulting denatured molecule is then exposed to the action of less specific proteases which degrade the collagen further.

The six major matrix metalloproteases effective in collagen degradation are:

The control of these enzymes is very important in maintaining tissue morphostasis. Primary control is exerted by the production of the inhibitor molecule, TIMP which forms irreversible complexes with the MMPs via non-covalent interactions.

TIMP is a highly conserved glycoprotein (Mr is approximately 30 K). It is secreted by fibroblasts and macrophages co-ordinated production of both Matrix metalloproteinases and TIMP is presumably under both temporal and spatial control.

Profiles of collagen fibrils are also seen in fibroblasts, probably as a result of phagocytosis (Ten cate & Deporter, 1975; Garant, 1997). These can be further degraded by lysosomal enzymes (cysteine proteases). In contrast to the extracellular degradation of collagen, this intracellular pathway of collagen phagocytosis and subsequent breakdown does not appear to involve matrix metalloproteinases (Everts & Beertsen, 1997).

Some of the growth factors and hormones also induce production of MMPs such as IL-1, TNF, PDGF, TGF-B, FGF; TGF-B also increases TIMP production. Glucocorticoids and retinoids have been shown to inhibit MMP production and they also increase the production of TIMP.

References

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