

# Evolution And History Of The Periodontal Ligament - A Review

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## Abstract

The periodontium is defined as those tissues supporting and investing the tooth, comprises of root cementum, periodontal ligament, bone lining the tooth socket and that part of the gingiva facing the tooth. The widespread occurrence of periodontal diseases and the realization that lost tissues can be repaired and perhaps regenerated has generated considerable interest in the factors and cells regulating their formation and maintenance. It is important to understand that each of the periodontal components has its very specialized structure and these structural characteristics directly define function. Indeed, proper functioning of the periodontium is only achieved through structural integrity and interaction between its components.

## EVOLUTION

Although the various methods by which teeth are fixed in their position upon the bones which carry them pass by gradational forms into one another, so that a simple and at the same time absolutely correct classification is, impossible, yet for the purpose of description for principal methods may be enumerated, namely, attachment by means of fibrous membrane, by a hinge, by ankylosis and by implantation in bony sockets.

There is a fundamental difference between the attachment of reptilian and mammalian teeth. In the ancestral reptiles the teeth are ankylosed to the bone. In mammals they are suspended in their sockets by ligaments. The evolutionary step from reptile to mammal included a series of co-ordinated changes in the jaws. The central point of these changes is the radical "Reconstruction" of the mandible. In reptiles the mandible consists of a series of bones united by sutures. Only the upper most bones, the dentary, carry the ankylosed tooth. The change from the many – boned reptiles to the single--boned mammalian mandible brought with it a radical change in the mode of growth. In the reptile, the mandibular and maxillary teeth "move" with the bones to which they are fused. In the mammal the teeth have to "move" as units independent of the bones, and this movement is made possible by the remodelling of the periodontium. The evolutionary change from the reptiles to the mammals replaces the ankylosis of tooth and bone to a ligamentous suspension of the tooth. This change permits

movement of mammalian teeth and the continued repositioning necessitated by jaw growth or tooth wear.

## CELLS OF THE PERIODONTAL LIGAMENT FIBROBLASTS

The fibroblasts lie between the collagen fibers and although various shapes have been described (Fullmer<sup>6</sup>; Roberts and Chamberlain<sup>14</sup>), it is likely that their appearance is governed by the surrounding matrix (Ross<sup>13</sup>).

The periodontal ligament fibroblast contains a prominent nucleus, which has single distinct nucleolus and clearly defined nuclear pores. When stained with colloidal silver (Crocker and Nar) it demonstrates either one or two regions of acidic proteins which are associated with nucleolar organizer regions (Shore et al., 1991). An internal dense lamina characteristic of connective tissue cells is also found.

The nucleus is of a flattened disc shape, and it has a diameter of approximately 10  $\mu\text{m}$  (Shore and Berkovitz<sup>2</sup>) and may occupy up to 30% of the cell volume. (Beertsen and Everts<sup>1</sup>; Yamasaki et al.<sup>28</sup>). Its outline is relatively smooth and no form of crenulation is present in the 'in vivo' state. However, in certain induced pathological conditions such as "Cyclosporin-induced hypertrophy"<sup>28</sup>, a crenulated nuclei may be present in a significant number of cells (Yamasaki et al.<sup>28</sup>).

As fibroblasts produce the extra-cellular matrix of the periodontal ligament, which demonstrates a very high rate of

turnover (Sodek<sup>23</sup>) the cells contain significant amounts of organelles involved in protein synthesis and degradation. The synthetic pathway in these cells is same as other protein matrix producing cells, such as odontoblasts, ameloblasts, etc.

Cho & Grant<sup>3</sup> have demonstrated using tritiated proline in a pulse-chase experiment that the synthetic pathway is from:

Rough Endoplasmic Reticulum → Golgi complex → Secretory vesicles → Cell membrane.

And a period of approximately 30 min is required for the completion of this process. The rough endoplasmic reticulum is dispersed throughout the cytoplasm, except for the finest cell processes, and occupies approximately 5% (In the human periodontal ligament (Yamasaki et al.<sup>28</sup>).

The periodontal ligament fibroblasts possess a Golgi complex found primarily in juxta nuclear position (Garant & Cho<sup>8</sup>) and are indistinct and not localized. This diffuse nature of the Golgi in periodontal ligament fibroblasts makes quantitation difficult. The Mitochondria appear to be distributed throughout the cell, except for the finest cell processes. Their shape varies from elongated to round. The mitochondria possess a characteristically dense intramitochondrial matrix. Quantitative analysis suggests that these organelles occupy approximately 3-3.5% of the cell by volume (Yamasaki et al.<sup>28</sup>).

Lysosomes are present in periodontal ligament fibroblasts in the form of large membrane bound vesicles containing a homogenous matrix that is more electron dense than the surrounding cytoplasm. Their numbers are considerably less than in actively phagocytic cells such as macrophages.

The structures so far described are typical of those seen in fibroblasts in connective tissues in general. However periodontal ligament fibroblasts also contain significant numbers of other organelles only infrequently seen in adult connective tissues.

These are small fragments of Collagen fibrils within membrane bound vesicles (Shore and Berkovitz<sup>2</sup>). These are termed "intracellular Collagen profiles".

Three broad types can be observed within the cell:

### DEGRADATION OF COLLAGEN FIBRILS BY LYSOSOME

According to Tencate et al., the cell initially phagocytoses a fragment of the collagen fibril and the resultant phagosome

then fuses with one or more lysosomes to form a phagolysosome. The lysosomal enzymes then degrade the fibril, which consequently loses its characteristic structures.

So, the degradation of periodontal ligament collagen is indeed an intracellular process, and the question arises as to why this is so as normally the degradation of collagen has historically been regarded as an "extra-cellular process", in which a specific enzyme, collagenase (Matrix metalloproteinase – M – MMP – I) is thought to be responsible for cleaving the triple helical portion of the molecules within the fibrils into 1/4-3/4 fragments and together with MMP-IV, it leads to spontaneous denaturation under physiologic conditions. The rest of the molecule is then responsive to further proteolysis by gelatinase (MMP-II) and MMP-V. However, before any of this collagenase activity can occur, the glycoproteins such as fibronectin & proteoglycans residing on the fibril surface masking the collagenase binding site must first be removed by stromelysin (MMP-III).

However even though collagen degradation is an extra-cellular process, degradation of periodontal ligament collagen is an intra-cellular process due to the following reasons.

But Tencate and Deporter<sup>7</sup> have suggested that this form of degradation is not unique to periodontal ligament but is found in all healthy tissues where there is controlled turnover and remodeling. Only in the tissues where the changes are "pathological" or when degradation is rapid and involves a whole tissue simultaneously does the extracellular pathway have a role.

The microfilaments and microtubules are present in the cytoplasm of the cell either as a network that fills the cell processes (Beertsen et al.,<sup>1</sup>) or as bundles beneath the cell membrane that resemble stress fibres seen in fibroblasts in vitro (Beertsen et al.<sup>1</sup>; Shore & Berkovitz,<sup>2</sup>). They are 5-7 nm in diameter and are found both in motile and non-motile cell types. They are composed predominantly of polymerized actin (F-actin) although other proteins are also present. They are important in membrane ruffling, amoeboid movement, chromosome movement, endocytosis, exocytosis and cell surface receptor mobility.

Their presence within the periodontal ligament, together with the apparent migration of cells in the occlusal direction has led to the proposal that the fibroblasts generate the eruptive force (Melcher & Beertsen<sup>12</sup>). Moxham et al.<sup>15</sup>

suggested that these filament bundles are not polarized and hence do not have a highly polarized migratory activity in eruption but this local motility is of considerable significance in maintaining the periodontal ligament integrity.

Microtubules have also been linked to fibroblast motility, and disruption of microtubules leads to internal accumulation of pro collagens (Cho & Garant<sup>5</sup>). The microtubules are characterized as non-branching cylinders of approximately 22 nm diameter. They are often seen to radiate from centrioles.

Centrioles are structures, which consists of a hollow tube of microtubules. A structure frequently associated with centriole of periodontal ligament fibroblasts is a solitary cilium.

The final element of the cytoskeleton that must be considered are the “intermediate filament” (IF) so called because of their size (approximately 11 nm) which is intermediate between that of the thin (actin) and thick filaments of muscle. There are five classes of Ifs, depending on the tissue of origin.

Mesenchymal tissues typically express Vimentin, a subtype of class III Ifs (Sterniert et al.,<sup>19</sup> ; Stewart,<sup>20</sup> ). Human & sheep periodontal ligament may possess significant accumulation of Ifs, particularly within cell processes (Yamasahi et al.<sup>28</sup> ; Berkovitz,<sup>2</sup>)

Webb et al.,<sup>21</sup> suggested that periodontal fibroblasts (but not gingival fibroblasts) and cementoblasts co-express Vimentin and cytokeratin immediately before and during the active phase of eruption. Once the eruption has ceased, the expression of cytokeratin ceases also.

One further feature of Periodontal ligament fibroblasts is the presence of numerous intercellular contacts not normally found in adult connective tissues ( Moxham et al.<sup>15</sup>). Such structures are normally observed in fetal connective tissue (Ross & Greenler<sup>17</sup>) between myofibroblasts of healing wounds (Gabbiani<sup>17</sup>), and between fibroblasts in culture.

In the Periodontal ligament, two major types of contacts are seen:

( Beertsen & Everts<sup>1</sup>; Shore et al<sup>16</sup>.)

The gap junctions vary in size about 0.1 μm & 0.5 μm in diameter and macula adherence are generally smaller ranging from 0.1 – 0.4 μm. (Beertsen & Everts<sup>1</sup>; Shore et

al.). One of the important features of these contacts is that they lack the intercellular specialization and insertion tonofilaments that are characteristic of the epithelial macula.

The third type of contact reported in rat periodontal ligament is the ‘close contact’ (Shore et al., ) where the cell membranes of opposing cells come to lie in close association without any visible membrane specialization.

Periodontal ligament fibroblasts also have cell surface receptors for –

**Figure 1**

EGF	-	Epidermal growth factor ( <i>Theslaff et al.<sup>25</sup>; Cho et al<sup>6</sup></i> )
IL - 0	-	Interleukin I - .
I-LGF	-	Insulin like growth factor.
PDGF	-	Platelet derived growth factor
		growth hormone ( <i>Blom et al, Matsuda et al<sup>1</sup></i> )
		Parathyroid hormone ( <i>Ngan et al.</i> )

The cells of periodontal ligament also shows positive staining reaction to cellular retinoic acid binding protein (CRABP-1) (Berkovitz and Maden<sup>2</sup>) and is important in modulating the action of retinoic acid by binding to excess within the cell . Within the fully differentiated periodontal ligament, a lack of retinoids (Vit A deficiency) may lead to necrosis and decreased vascularity .

**CEMENTOBLASTS**

They are the cells responsible for secreting the organic (mainly collagenous) matrix of cementum. They appear as a distinct layer of cells on the root surface, similar to osteoblastic layer but not regular in arrangement. They are often indistinguishable from periodontal ligament fibroblasts but they appear near the cementum and have less rough endoplasmic reticulum but more mitochondria than periodontal ligament fibroblasts (Yamasaki et al<sup>28</sup>; Berkovitz,<sup>1</sup>).

They have accumulation of numerous glycogen granules and contain significant quantities of both intermediate and actin filaments. The cell membrane may demonstrate numerous intercellular contacts of both the gap junction and simplified desmosome type (Yamasaki et al.,<sup>28</sup>) and also possess receptors for growth hormone and EGF (Cho et al.<sup>4</sup>).

**OSTEOBLASTS**

These cells within the periodontal ligament are found on the surface of the alveolar bone. Their gross appearance and

ultrastructure is same as that seen in osteoblasts anywhere in the body. In active state they form a layer of cuboidal cells, which exhibit strong basophilic cytoplasm. A prominent nucleus lies towards the basal end of the cell and a pale juxta nuclear area indicates the site of Golgi complex.

Like fibroblasts they are seen to contain a prominent rough endoplasmic reticulum and numerous mitochondria and vesicles. The Golgi complex however appears more localized and, extensive than the fibroblast. Microtubules and microfilaments are present. The cells do not appear to possess receptors for EG. Cell to cell contact is via gap junctions and also via simplified desmosomes. They also contact via gap junctions with osteocytes lying within lacunae in the adjacent bone, thus forming a co-ordinated system through the bone tissue.

Osteoblast precursor cells are often seen beneath the osteoblast layer in the vicinity of adjacent blood capillaries. These cells have a reduced cytoplasm and few organelles. During stages of differentiation from

Precursor – committed osteoprogenitor – pre osteoblast

They first migrate away from the bone surface into the body of periodontal ligament before eventually taking up their functional position (Roberts et al.<sup>18</sup>). In functional state the cells remain active for a period of up to 20 days and when osteogenesis is not occurring, a distinct layer of osteoblasts is absent and the bone surface is covered upto 85-90% by flattened cells with scanty cytoplasm, the so called bone-lining cells and remaining 10-15% only by osteoblasts.

### OSTEOCLASTS

Although it has been claimed that bone resorption is mediated via osteocytes (Belanger), resorption of bone surface is accomplished via a distinct cell type, the “osteoclast”.

The features are same as cells elsewhere in the skeleton, in that:

They are found within the resorption lacunae

They are large multinucleated.

They have a ruffled border adjacent to resorbing surface, enclosed by a smooth (annular or ‘clear’ zone).

The cytoplasm adjacent to ruffled border has numerous mitochondria suggesting extreme metabolic activity.

Tightly packed infolding of the cell membrane, coated with fine’ bristle like structure (Kallio)

cytoplasm stains intensely than adjacent active osteoblast, suggesting the presence of only small amounts of rough endoplasmic reticulum.

Numerous free ribosomes are present suggesting considerable protein synthesis for internal use.

bone matrix is primarily fibrillar collagen but there is no evidence of presence of intercellular collagen profiles. This may be related to the controlled extracellular environment available to the osteoclast or the removal of collagen by subjacent periodontal ligament fibroblasts (Deporter,<sup>11</sup>) or by perivascular macrophages. The multinucleated cells associated with the resorption of cementum and dentin has sometimes been referred to as cementoblasts & odontoclasts.

### EPITHELIAL CELLS

Epithelial cell aggregates are a normal feature of the periodontal ligament. They represent the remains of the developmental Hertwig’s epithelial root sheath which is involved in differentiation of root odontoblasts (Thomas & Kollar,<sup>24</sup>) and also secrete enamel-like proteins on to the root surface (Siavkin et al.,<sup>22</sup>; Leo et al.).

The Epithelial cell rest (ECR) can be distinguished from the fibroblasts of the periodontal ligament in routine histological sections by the close packing of the cuboidal cells and tendency to stain more deeply. They are unique in being completely surrounded by connective tissue cells.

Initially Epithelial Cell Rests is found as one or two cells with partial basal lamina and subsequently the epithelial rests become more cellular and are contained within an almost complete basal lamina with narrow intercellular spaces. As laminin is chemotactic to epithelial cells the basal lamina may therefore play a role in formation differentiation and maintenance of the epithelial cell rest (Hamamoto et al.<sup>27</sup>).

Epithelial Cell Rests have a high nuclear – cytoplasmic ratio & exhibit basal cell-like undifferentiated and hyper proliferative characteristics as indicated by expression of cytokeratins 5, 6,14,16 & 19 (Salonen et al).

Epithelial Cell Rest are located closer to cementum than to alveolar bone surface, the average distance being 27 µm in the apical region, gradually increasing cervically to 41 µm

(Valderhaug & Zander,). As age increases the epithelial cell rest move cervically and are located mostly in the cervical region in the gingiva and according to there is continuity between epithelial cell rest and reduced enamel epithelium before eruption and the junctional epithelium after eruption, which may be of significance in chronic inflammatory periodontal disease.

**EPITHELIAL, CELL REST-ROLE IN ROOT RESORPTION**

Loe & Waerhaug, after re-implantation studies observed that ankylosis and subsequent root resorption never occurred when a periodontal ligament that contained epithelial cell rest was retained. These authors suggest that epithelial cell rest may be the factor in limiting the resorption and may therefore play a role in the maintenance of periodontal space a view later supported by Lindskog.

However, Wesselink & Beertsen,<sup>1</sup> after administration of drug 1-hydroxyethylidene -1, 1-bisphosphonate found that it resulted in marked reduction of the width of the periodontal ligament and produced ankylosis at several sites in the presence of the normal' distribution of the epithelial cell rest.

**ULTRASTRUCTURE OF EPITHELIAL CELL REST**

Ultrastructurally, epithelial cell rest is separated from connective tissue by a basal lamina, which may be fragmented (Listgarten,<sup>10</sup>). The nucleus of each cell is prominent, contains condensed heterochromatin and often shows invaginations.

The Scanty cytoplasm is characterized by the presence of filaments, which insert into the desmosomes frequently found between adjacent cells and into the hemidesmosomes between the cells and the basal lamina. Tight junctions are also found between cells. Mitochondria are abundant and distributed throughout the cell while scarcity of rough endoplasmic reticulum and golgi complex indicated lack of significant protein secretion (Valderhaug & Nylen<sup>26</sup>).

**CELLULAR CHANGES WITH AGE**

A decrease in cellularity of the periodontal ligament with age has been reported in

**Figure 2**

Premolars of Dogs	(Berglundh et al., 1991)
Molars of rats	(Jenser & Toto, <sup>19</sup> )
Hamsters	(Klingsberg & Butcher)
Mice	(Toto & Berg, <sup>9</sup> )
Monkeys	

Grant & Bernick, reported a decreased cellularity in the Periodontal ligament with age. Severson described the presence of fat cells while reported evidence of degenerative vascular changes. There is a decrease in mitotic index (Jenson & Toto,<sup>9</sup>) and the fibroblasts in aging tissues have longer 'life' than those in younger tissues. As age increases large multinucleated fibroblast cells appear (Cho & Garant,<sup>5</sup>) but differ from osteoblasts at electron microscopic level in that they have considerable amount of rough Endoplasmic reticulum, a conspicuous golgi complex and intracellular collagen profiles.

**DEFENCE CELLS**

The periodontal ligament contains defence cells, including macrophages, mast cells and eosinophils. These cells achieve more importance during inflammatory periodontal disease.

Mc Culloch et al.<sup>14</sup> described the detailed distribution of the macrophage and found that it has numerous microvilli, lysosomes and other membrane bound vesicles of varying density. Mc Culloch in 1989 raised the possibility of lymphokines released from macrophages, which may be involved in cell kinetics.

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