Periodontal Ligament: Structural and Clinical Correlates

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Abstract: **This paper reviews certain structural aspects of the periodontal ligament. These include collagen (type, crimping and fibril diameters), ground substance (functions), cells (fibroblast morphology, cell heterogenicity and distinguishing of cell phenotypes, cell kinetics, 'foetal' characteristics), nerves (presence of neuropeptides) and blood vessels (presence of fenestrations). Attention is drawn to recent advances in our understanding of the development of the periodontal ligament, especially cementum formation, and in the presence of bioactive molecules such as growth factors and cytokines.**

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Clinical Relevance: **Basic knowledge concerning the structure and development of the periodontal ligament has relevance in understanding and achieving periodontal regeneration.**

The periodontal ligament (PL) is the dense fibrous connective tissue that occupies the periodontal space between the root of the tooth and the alveolus. Above the alveolar crest, the PL is continuous with the connective tissues of the gingiva, while at the apical foramen it is continuous with the dental pulp. The continuity with the gingiva is important when considering the progression of periodontitis from gingivitis. The continuity with the pulp explains why inflammation from this dental tissue (often related to dental caries) spreads to involve the PL and the other apical supporting tissues. The periodontal ligament impinges upon three major clinical dental disciplines, namely periodontics, orthodontics and endodontics.

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The PL has the following main functions:

- It is the tissue of attachment between the tooth and alveolar bone. It is thus responsible for resisting displacing forces (the tooth support mechanism), thereby protecting the dental tissues from damage caused by excessive occlusal loads (especially at the root apex).
- It is responsible for the mechanisms whereby a tooth attains, and then maintains, its functional position. This includes the mechanisms of tooth eruption and drift.
- Its cells form, maintain and repair alveolar bone and cementum.
- Its mechanoreceptors are involved in the neurological control of mastication.

In common with other dense fibrous connective tissues, the PL consists of an extracellular matrix of fibres (principally collagen) and ground substance that contains cells, blood vessels and nerves. However, the PL possesses features that, as a group, might justify consideration of the PL as a 'specialized' fibrous connective tissue when compared with other adult fibrous connective tissues. Much of the research on the PL has been undertaken using animal material and *in vitro* systems, so that care must be taken before extrapolating to the human *in vivo* situation.[1-3]

EXTRACELLULAR MATRIX

The extracellular matrix[3,4] is made up of the following:

- Collagen fibres;
- Oxytalan fibres;
- Ground substance.

Collagen Fibres

The majority of collagen is present as the fibrous collagens Types I and III, in the ratio of about 3:1. The functional significance of such comparatively high amounts of Type III collagen (normally present in young developing tissues and in granulation tissue) is unclear. These two types of collagen show no apparent spatial separation within the PL. However, Type III collagen may be found at the periphery of Sharpey fibres inserting into alveolar bone.

Small amounts of other types of collagen are present within the PL,

including Types V, VI, XII, as well as basement membrane collagen (Types IV and VII associated with the basement membranes of blood vessels and epithelial cell rests). Presumably, these largely non-fibrous collagens act to hold the fibrous collagens in a functional three-dimensional network. As evidence for this, transgenic mice with a mutation of collagen Type XII show disruption of the normal architecture of the periodontal ligament collagen fibre system.

Collagen fibril diameters are relatively small and unimodal, with a mean of about 50 nm, and show no significant change with age.

Features related to the spatial arrangement of the minor collagens of the PL include:

- Type VI collagen being absent from the middle of the ligament during the eruptive phase.
- Type XII collagen appearing relatively late in development when the tooth has more or less already erupted (This pattern is recapitulated on the pressure side of PL following orthodontic loading and remodelling of the PL.)

With periodontal disease, there may be a change in the nature of the collagen (with some evidence for the presence of more Type V collagen) and in collagen fibril diameters.

Perhaps one of the most specialized features of PL collagen is its high rate of turnover. Indeed, this rate is probably the fastest anywhere in the body, having a half-life in rodents of just a few days. As this high turnover rate does not appear to be related to functional considerations (such as occlusal loads or tooth movement), its significance awaits clarification.

Oxytalan Fibres

These pre-elastin-type fibres constitute no more than about 3% of the fibres of the PL. They are attached into the cementum of the tooth and course out into the PL in various directions, rarely being incorporated into bone. In the cervical region, they follow the course of gingival and trans-septal collagen fibres, but within the PL proper they tend to be more longitudinally orientated, crossing the oblique fibre bundles more or less perpendicularly. In the outer part of the ligament, the oxytalan fibres are said often to terminate around blood vessels and nerves.

The functions of the oxytalan fibres remain unknown. They are said to be thicker and more numerous in teeth which carry abnormally high loads (including abutment teeth for bridges and teeth being moved for orthodontic reasons), although detailed quantitative support is lacking. Oxytalan is said, therefore, to have some role in tooth support (perhaps also indicated by its relationship with the periodontal vasculature), although some experimental evidence shows that the oxytalan fibres are little changed with age (and presumably occlusal loading) and in the periodontal ligaments of teeth with reduced masticatory loading.

Oxytalan microfibrils have a similar ultrastructure to the fibrils of fibronectin. In addition, oxytalan fibres are stained strongly by immuno-histochemical stains for fibronectin. As fibronectin is important in fibroblast adhesion and migration, this might support the suggestion of some authors that oxytalan fibres aid fibroblast migration in the periodontal ligament.

Ground Substance

Although we are used to thinking of the PL as a collagen-rich tissue, it is more a ground substance-rich tissue in the sense that the collagen fibre bundles seen on electron micrographs are composed of about 60% ground substance by volume. The two main proteoglycans isolated from the PL are proteodermatan sulphate and a proteoglycan containing chondroitin sulphate/dermatan sulphate hybrids. The turnover rate of the ground substance is even more rapid than that of the collagen.

The ground substance has many important functions. These include ion and water binding and exchange (including binding growth factors), control of collagen fibrillogenesis and fibre orientation. Tissue fluid pressure has been found to be high in the periodontal ligament (being about 10 mm Hg above atmospheric pressure) and has been implicated in the tooth support and eruptive mechanisms. The nature of the ground substance may also explain why the PL rarely mineralizes, as it may act as an inhibitor of this process. This is suggested by *in vitro* experiments whereby enzymes that degrade elements of the ground substance (hyaluronidase and chondroitinase) have been applied and, following the addition of mineralizing solutions, mineral crystals appear within the PL (but do not appear within the normal PL). Calcium-binding proteins such as S100A4 in the extracellular matrix have also been implicated in inhibiting mineralization in the PL. In cases of ankylosis, the homeostasis controlling PL width is disrupted. Experimentally, ankylosis can be produced by the administration of a bisphosphonate (that may act by reducing cell numbers and by inducing the expression of the osteogenic factors in the body of the PL).

The glycoprotein fibronectin is thought to promote attachment of cells to the substratum, especially to collagen fibrils. Furthermore, as cells preferentially adhere to fibronectin, it may be involved in cell migration and orientation. As loss of fibronectin has been observed during the terminal maturation of many connective tissue matrices, its presence within the PL may be indicative of the PL retaining 'immature', foetal-like characteristics. Another glycoprotein termed tenascin has also been identified in the PL. Like fibronectin, tenascin is more characteristic of a foetal-like connective tissue than a fully 'mature' connective tissue. Unlike fibronectin, tenascin is not uniformly localized throughout the ligament but is concentrated adjacent to the alveolar bone and the cementum. Fibronectin has been employed in the past to 'condition' roots in the hope of improving periodontal wound healing.

Analysis of the ground substance following the onset of periodontal disease indicates there may be a change in the content of the ground substance, with a decrease in the dermatan sulphate content and an increase in chondroitin sulphate. Occlusal hypofunction results in remodelling of the PL with a significant decrease of chondroitin sulphate, decorin and heparan sulphate. That there are differences in the ground substance (as well as in the nature of collagen) between the PL and adjacent alveolar bone may provide a clinical diagnostic tool when analysing gingival crevicular fluid to assess and predict patient susceptibility to further progress of the disease.[4]

CELLS

Although the predominant connective tissue cell within the PL is the fibroblast, the tissue presents a heterogenous cell population. Formative cells covering the surface of both cementum and alveolar bone are part of the ligament (i.e. cementoblasts and osteoblasts) and have a similar mesenchymal origin to fibroblasts. Resorbing cells on the surface of bone and cementum (osteoclasts and odontoclasts/cementoclasts), however, are derived from a monocyte/macrophage lineage from the blood. As well as the fully differentiated cells, the PL will also contain stem cells and precursors (e.g. preosteoblasts, precementoblasts). In addition, the periodontal ligament contains defence cells and epithelial cells (rests of Malassez).

Fibroblasts

Periodontal fibroblasts, being very active secretory cells, have low nuclear-cytoplasmic ratios. Reflecting the high rate of synthesis of collagen (and ground substance), the typical periodontal fibroblast shows a well-developed rough endoplasmic reticulum, Golgi complex and many mitochondria and secretory vesicles. As with other connective tissues, even the fibroblasts of the PL may represent a heterogeneous rather than a homogeneous population.[5] That PL fibroblasts *in vitro* can generate tension by their motility/contractility has been interpreted by some as indicating that the cells may play a role in generating the force of tooth eruption *in vivo*, although this is strenuously denied by others.

In addition to synthesizing and secreting collagen, fibroblasts also appear responsible for collagen degradation. The main evidence for this is the presence of intracellular collagen profiles within the cells.[6] These profiles contain collagen fibrils within an elongated membrane-bound vacuole with appearances varying from normal banded to unbanded fibrils. Although this collagen could represent newly synthesized collagen that has not yet been secreted, experimental evidence strongly suggests that these intracellular collagen vacuoles represent collagen that has been phagocytosed from the extracellular environment and that is undergoing enzymatic degradation in a phagolysosome. The time taken to degrade the collagen intracellularly is not known for certain, although some evidence suggests it to be about 30 minutes (a roughly similar time to that required for collagen synthesis).

There is little evidence for the presence of the enzyme collagenase within the normal PL, and other experimental data suggests that collagenase is not essential for collagen remodelling. If this is correct, then other enzymes (e.g. cysteine proteinase) and other metalloproteinases are involved in collagen remodelling in the normal physiological situation. As the amount of collagen present within the PL must represent a balance between the rate of synthesis and the rate of degradation, the loss of collagen (e.g. during periodontal disease) could result from either a more rapid rate of breakdown and/or a slower rate of synthesis, and/or the loss of fibroblasts. Furthermore, the process of collagen loss during periodontal disease probably represents a different process, as in the diseased inflammatory state there is evidence for the activity of collagenase. The presence of tissue inhibitors of metalloproteinases (TIMPs) in the PL, some produced by PL fibroblasts, provides the rationale for the use of drugs that have a similar activity to combat periodontal disease, such as the designer tetracycline, doxycycline.

The various cellular activities of PL fibroblasts can be modulated by numerous bioactive molecules. These molecules may be produced by the cells themselves, by local inflammatory cells, or be present within the extracellular matrix of the PL or of bone and cementum. They include growth factors and cytokines. The cell membrane also contains a multitude of receptors to bioactive molecules. At the cell/matrix interface are to be found a group of adhesion molecules, the integrins, that regulate many cellular activities, including those relating to cell spreading, cytoskeletal reorganization and apoptosis. Through the activities of bioactive molecules and receptors, the fibroblast is involved in maintaining homeostasis. There may be rapid up- or down-regulation of activities following, for example, the application of mechanical stress associated with orthodontic tooth movement[7] or the onset of periodontal inflammation.

Periodontal ligament fibroblasts produce numerous growth factors and cytokines, such as IGFI, BMPs, PDGF, IL-1, TGFβ. TGFβ, for example, stimulates the synthesis of collagen and inhibits the synthesis of metalloproteinases such as collagenase. It is not suprising, therefore, that increased production of cytokines is related to the onset of tissue damage. Fibroblasts may release factors that inhibit osteoclastic differentiation and function (e.g. osteoprotegerin), while supporting it by expressing RANKL (receptor activator nuclear factor kappa B ligand) on its surface. The cells also release prostaglandins, which may influence bone cell activity.

PL fibroblasts are rich in substances such as alkaline phosphatase (that might be related to the formation of acellular cementum), cellular retinoic acid-binding protein, and in receptors to epidermal growth factor (that may inhibit

	Cementoblasts	PL Fibroblasts	Osteoblasts
Bone Sialoprotein	+	-	+
Osteopontin	+	-	+
Osteocalcin	+	-	+
Osteonectin	?	+	+
EGF receptors	-	+	+/-
PTH receptors	+	-	+
Vitamin D responsive	+	?	+
Alkaline Phosphatase	+/-	+	+
CAP	+	-	-
Collagens:			
Type I	+	+	+
Type III	-	+	-
Type V	-	+	-

***Table 1**. Features helping to distinguish cementoblasts, fibroblasts and osteoblasts. CAP = Cementum attachment protein.*

the fibroblast from differentiating into a cementoblast/osteoblast that lacks such receptors). Presumably, PL fibroblasts have the genome to produce any of the proteins in the body, but the majority of these are inactivated. For example, whereas the cell does not normally produce elastin *in vivo* (or only in negligible amounts), *in vitro* tropoelastin mRNA is transcribed. This transcription is suppressed by basic fibroblast growth factor, suggesting one possible role for this growth factor.

Bioactive molecules, such as TGFβ, IGFI, PDGF, BMP-2, BMP-7, FGF-2, that can regulate the proliferation and differentiation of fibroblasts, osteoblasts and cementoblasts, as well as promoting angiogenesis, have been applied in animal models, alone and in combination, in the hope of inducing/improving periodontal regeneration, with varying degrees of success being reported. However, whether any of these growth factors will be found suitable for promoting periodontal regeneration in humans remains to be seen.

Cell Kinetics and Cell Phenotype

Fibroblasts within the PL need replenishing. As osteoblasts and cementoblasts of the periodontal ligament become incorporated into alveolar bone and cellular cementum, replacement cells must also be provided within the PL to permit osteogenesis and cementogenesis to continue. During the early development of the periodontal ligament from the dental follicle, there is evidence that cells (ectomesenchyme?) populating the follicle migrate out from the dental papilla. In the mature PL, progenitor cell populations are located adjacent to the blood vessels near the surface of the alveolar bone and may also be found in the contiguous endosteal spaces of the alveolar bone. In normally functioning periodontal tissues, the mitotic index is between 0.5 and 3% (the greatest number being found in the central part of the periodontal ligament where there is the least cell density) and, as with other tissues, there is a reduction of the labelling index with age. Cell formation and cell differentiation increases markedly with wounding or after the application of orthodontic loads, while different stimuli may recruit progenitors giving rise to different cell types (e.g. an osteoblastic rather than fibroblastic response following orthodontic loading).

Knowledge of the basic cell biology of the PL is important to clinical practice in enabling us to have a detailed understanding of the principles involved in obtaining periodontal regeneration (as opposed to repair) and to develop successful clinical strategies towards this end. During regeneration, cells such as fibroblasts, cementoblasts and osteoblasts must be induced in appropriate numbers, at the right time and in the right place, and then synthesize their appropriate extracellular matrices. New Sharpey fibres must gain a functional attachment into new bone and cementum. In addition, epithelial downgrowths must be excluded from the cementum surface.

Among current research, *in vitro* studies are being undertaken to determine factors affecting the induction and differentiation of each of the three main mesenchymal cell types. Although it may be possible to distinguish between fibroblasts and osteoblasts or cementoblasts in the fully differentiated state, it is obviously less easy to distinguish between cementoblasts and osteoblasts.[8] The distinction between the three cell types becomes more difficult at earlier stages in the differentiation process when considering their various precursors. Thus, when papers describe the results of studies obtained by culturing cells scraped off from the roots of extracted teeth (and often calling them PL fibroblasts), it is difficult to know precisely what cell types are involved and how homogeneous the population is. Also, as different culture techniques (with different passage numbers and different culture media) are used, it is not surprising that different authors may claim different results.

It is not clear whether periodontal fibroblasts, cementoblasts and osteoblasts all arise from a common precursor, or whether each cell type has its own specific precursor cell. As far as being able to separate the fully differentiated cells, Table 1 lists some of the markers used in this identification process. Although phenotypically cementoblasts and osteoblasts are quite similar, different responses for osteocalcin, collagen Type I and bone sialoprotein genes to mechanical loading have been observed in the two cell phenotypes.

A clinical procedure said to be important in the attainment of periodontal regeneration is that of tissue guided regeneration. This is

	Gingiva	Periodontal Ligament
Collagen Type III	9%	20%
Collagen turnover	5 weeks	1 week
Cell volume	8%	40%
Ground substance	More	Less
Alkaline phosphatase	Less	More
Contractile proteins	Less	More
Prostaglandin release (in response to histamine) – *in vitro*	More	Less
Collagen production – *in vitro*	Less	More

***Table 2**. Qualitative features distinguishing fibroblasts from gingiva or periodontal ligament.*

based on the proposition that if the wound is populated by connective cells derived principally from the periodontal ligament, with those from the gingiva (as well as the junctional epithelium) being excluded by a barrier (that may be resorbable or non-resorbable), PL regeneration is likely to be more successful. This would imply that important differences exist between PL fibroblasts and gingival fibroblasts that ultimately affect the clinical outcome, even though the tissues are in continuity. Studies comparing the two cell populations, often using *in vitro* methods, have indeed reported differences, some of which are listed in Table 2. However, which are the important differences responsible for the presumed basis of guided tissue regeneration awaits clarification. Bone associated macromolecules such as osteopontin, osteocalcin and bone sialoprotein are absent from gingival connective tissue but are present in the PL, although it is not always clear whether the positive cells in the PL represent fibroblasts rather than cells of the cementoblast/osteoblast lineage. Unlike PL fibroblasts, cultured gingival fibroblasts are unable to regenerate a new periodontal ligament when added to the surface of a root that is then implanted back into bone. Recently, it has been shown that a large number of genes are differentially expressed between PL and gingival fibroblasts: genes encoding transmembrane proteins tend to be up-regulated in PL fibroblasts, while genes encoding cell-cycle regulation proteins tended to be up-regulated in gingival fibroblasts.[9] As peridontal regeneration does not occur presently on a clinically predictable basis following guided tissue regeneration, there is still debate concerning the value of this procedure.

CEMENTOGENESIS AND PERIODONTAL REGENERATION

Although cementogenesis is a key component of periodontal regeneration, there are still gaps in our knowledge concerning this process in the normal state, especially during the initial stages. For example, the origin and characterization of the cells associated with the development of acellular (primary) cementum are not clear and these cells appear to differ from those associated with the later development of cellular (secondary) cementum (Table 3). Recent advances in our understanding of normal root development may have clinical implications in helping to achieve periodontal regeneration. During the development of the initial layer of cementum at the cement-dentine interface, the epithelial root sheath secretes enamel-related proteins into the interface (hyaline layer), perhaps helping to bond the two tissues together and playing some role in the induction of cementum-forming cells. This finding has led to studies in which enamel matrix-derived proteins (EMP) have been applied to the cleaned root surfaces of periodontally-affected teeth with beneficial effects on periodontal regeneration.[10] Although the underlying mechanisms are not known, it has been postulated that the EMP may interact with PL fibroblasts via integrins. In addition to osteocalcin, osteopontin and bone sialoprotein, the non-collagenous proteins of the extracellular matrix of cementum appear to include a unique cementum attachment protein (CAP) that *in vitro* enhances the recruitment of cementoblastic populations to the root surface (probably via an integrin-mediated mode of attachment). Although it may be assumed to be inactive when bound up in cementum, CAP may play a role during cementogenesis and during periodontal regeneration.

Major advances in tissue engineering have resulted in the prospect of tissues and organs being constructed *in vitro* to replace injured or diseased parts. The application of such methods to achieve periodontal regeneration is now under active consideration.[11]

NERVES AND VESSELS

Nerves

For a fibrous connective tissue, the PL has an unusually rich nerve and blood

CEMENTOBLASTS (ACELLULAR CEMENTUM)	CEMENTOBLASTS (CELLULAR CEMENTUM)
Recognizable for only a short time	Present for longer period
Fibroblast-like	More rounded
Expressed cytokeratin (8–18) and vimentin	Express vimentin
Derived from cells of epithelial root sheath?	Derived from mesenchyme in periodontal ligament
Parathyroid hormone receptors absent	Parathyroid hormone receptors present
Do not express TGFβ1	Express TGFβ1

***Table 3**. Features helping to distinguish cells associated with the formation of acellular and cellular cementum.*

supply. In addition to the usual autonomic nerves asociated with the vasculature, the sensory nerves show endings of the Ruffini type that play an important role in the reflex control of mastication. In addition, sensory nerve endings in the periodontal ligament release neuropeptides such as substance P, vaso-active intestinal peptide and calcitonin gene-related peptide. These substances can have widespread effects on both blood vessels and cells and presumably have an important, but as yet undetermined, role in the biology of the PL, with many of them being up-regulated during orthodontic tooth movement and inflammation.

Blood Vessels

The major blood vessels of the PL lie between the principal fibre bundles, close to the wall of the alveolus. The majority of vessels appear to be post-capillary venules. An unusual feature for adult connective tissue is the presence of large numbers of fenestrations in the capillaries. Fenestrated capillary beds differ from continuous capillary beds in that the diffusion and filtration capacities are greatly increased and this might be related in some way to the presence of a high tissue fluid pressure within the PL. It is possible that the fenestrations are related to the high metabolic requirements of the PL. The number of fenestrations is not fixed and varies according to the stage of eruption.[3]

PERIODONTAL LIGAMENT: A FOETAL-LIKE CONNECTIVE TISSUE?

When comparisons are made between the PL and other adult connective tissues, many differences appear to exist, giving the impression that the PL is a specialized connective tissue. However, when compared to foetal-like connective tissues, many of these differences disappear, particularly those related to its high turnover rate. An appreciation of this characteristic may aid our understanding of inflammatory periodontal disease and periodontal regeneration.

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