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The structure and function of the periodontium

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Periodontium

The supporting apparatus of the tooth is known as the periodontium. The gingiva, periodontal ligament (PDL), cementum, and alveolar bone are the tissues of the periodontium (Figure 1.1). This unique collection of tissues has a functional role in the oral cavity beyond anchoring the tooth in the bone. Understanding the structural, functional, biochemical, immunological, and molecular aspects of the periodontium is necessary to understand the pathophysiology of periodontal disease, periodontal treatments, periodontal regeneration, and periodontal repair. The hard tissues (cementum and bone) and the soft tissues (PDL and gingiva) of the periodontium play active rolls in the local inflammatory and immune response by synthesizing and releasing cytokines, growth factors, and enzymes. This fascinating interrelation of tissues, along with the normal apoptosis of the cells of the periodontium, provides the backdrop for the continued battle between periodontal health and disease.

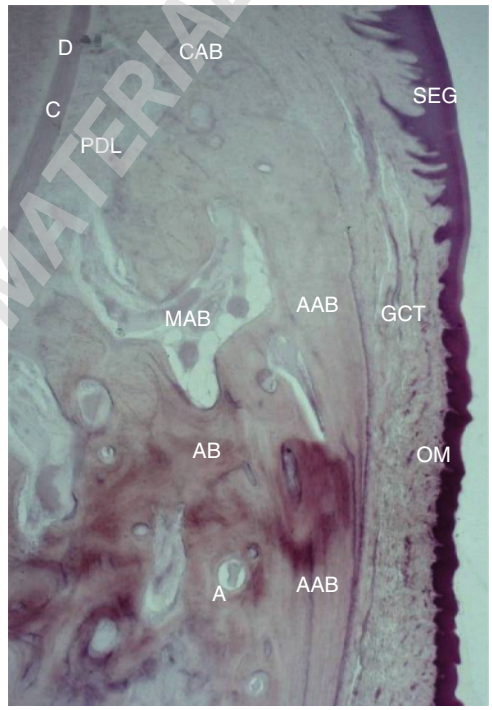


Figure 1.1 Histological image of the periodontium depicting dentin (D), cementum (C), periodontal ligament (PDL), alveolar bone (AB), apposition of buccal alveolar bone (AAB) associated with insertion of dental alveolar fiber, coronal crest of alveolar bone (CAB), medullary alveolar bone (MAB), arteriole (A), gingival connective tissue (GCT), stratified epithelium of gingiva (SEG), and oral mucosa (OM).

Odontogenesis and the periodontium

Odontogenesis is the embryological events in tooth development. Complete tooth development is described elsewhere.[1] However, the development of the tooth is not isolated from development of the periodontal tissues. During enamel development an outer enamel epithelium (OEE), inner enamel epithelium (IEE), and stellate reticulum are present. Adjacent to the enamel epithelium are the ectomesenchymal cells that form the dental follicle and papilla. The dental follicle gives rise to the cementum, PDL, and some alveolar bone.[2] This ectomesenchymal embryonic tissue forming the dental papilla and follicle is derived from neuroectoderm.[2]

The tooth root forms after the crown has developed, but before it is completely mineralized. The OEE and IEE, without the stellate reticulum, develop into *Hertwig's epithelial root sheath* (HERS). HERS proliferates into the underlying connective tissue to form the root. The dental papilla is stimulated to form odontoblasts, which

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produce dentin. At this stage, the root sheath breaks up and cementoblasts are formed from the adjacent ectomesenchymal tissue. This inductive, interactive ectodermal-ectomesenchymal pattern of tooth and periodontium development is conserved in most higher vertebrate species.[3] HERS cells can remain trapped in the periodontal ligament and are known as the epithelial rests of Malassez (ERMS) (Figures 1.2 and 1.3). These cells, if stimulated later in life, may become cysts or possibly odontogenic tumors.[4,5] However, it is debated whether the ERMS are sources of odontogenic tumors.

The enamel epithelium proliferates into a thick reduced enamel epithelium that fuses with the oral epithelium.[6] The gingiva forms as the crown of the tooth penetrates into the oral epithelium and erupts into the oral cavity. A dentogingival junction is created and the junctional epithelium is established.

Repair and/or regeneration of the periodontium share many of the same events that occur during development of these unique tissues. A complete understanding of these chemical messengers and cell origins may provide a basis for periodontal repair and regeneration. However, the complete biochemical and molecular changes and the origins of cells, particularly cementoblasts, are not fully understood. It may be argued that periodontal tissue cannot be regenerated (restored to normal architecture), rather it is repaired.[2] For example, there is little evidence to support that acellular (primary) cementum reforms. Instead, when repair occurs, cellular cementum is deposited; this is argued to not be a true odontogenic tissue.[2] In many regeneration studies, the newly formed cementum is cellular with low numbers of fibers resulting in a new attachment may be weaker than the normal acellular extrinsic fiber cementum.[7] Similarly, repair as opposed to complete regeneration of a PDL occurs following damage.[8]

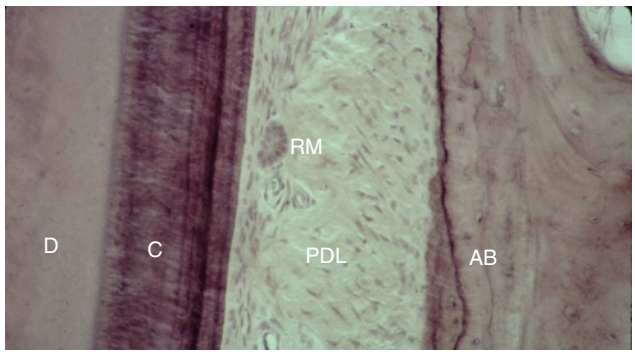


Figure 1.2 Epithelial rests of Malassez: dentin (D), cementum (C), periodontal ligament (PDL) with rests of Malassez (RM), and incremental apposition lines in alveolar bone (AB).

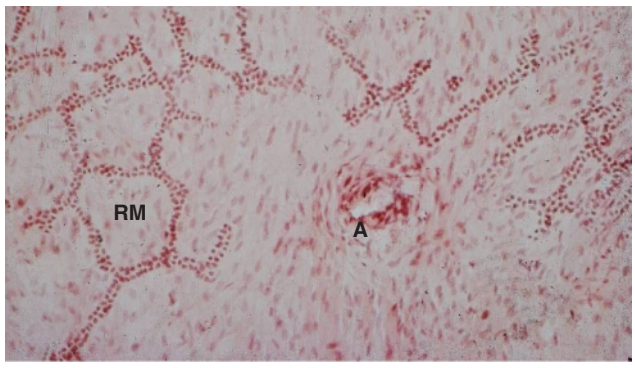


Figure 1.3 Connective tissue with an arteriole (A) with epithelial rests of Malassez (RM) displayed as a mosaic pattern.

Gingiva

The oral mucosa is classified into specialized mucosa (dorsum of tongue), the non-keratinized alveolar mucosa and the keratinized masticatory mucosa. The masticatory mucosa includes the hard palate mucosa and the gingiva. The gingiva is demarcated from the alveolar mucosa by the mucogingival junction (Figure 1.4).

General histology of the gingiva

A stratified squamous epithelium and deeper connective tissue (collagen fibers and ground substance) are the components of the gingiva. Gingival connective tissue consists of collagen fibers (type I and III collagen), fibroblasts, nerves, blood vessels, lymphatics, macrophages, eosinophils, neutrophils, T and B lymphocytes, and plasma cells.[1,9,10] This connective tissue is called *lamina propria* with a superficial papillary layer and deeper reticular layer. There are some variations in the lamina propria in relation to the type of gingiva. In the attached gingiva, the lamina propria has a papillary layer interdigitating with *rete pegs* of

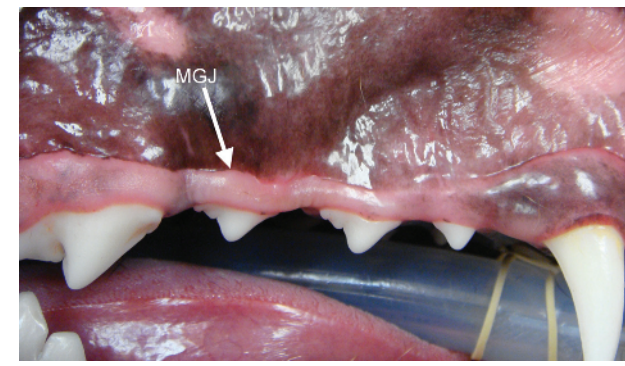


Figure 1.4 A photograph showing part of a dog's right maxillary arcade. The mucogingival junction (MGJ) demarcates between keratinized gingiva and non-keratinized alveolar mucosa.

the epithelium and a reticular layer adjacent to the periosteum of the alveolar bone.

The *keratinocyte* is the main cell type of the gingival epithelium.[11] The keratinocytes proliferate via mitosis in the basal layer of the epithelium. As cells migrate to the surface epithelium, they differentiate. The keratinization process includes flattening of the cuboidal cells, production of keratohyaline granules, and disappearance of the nucleus. The majority of gingival epithelium is parakeratinized and consists of the stratum basale, stratum spinosum (prickle cell layer), and stratum corneum. Pyknotic nuclei are present in the stratum corneum of *parakeratinized* epithelium. *Non-keratinized* epithelium lacks a stratum corneum and stratum granulosum with superficial keratinocytes containing nuclei. *Orthokeratinized* (complete keratinization with a *stratum granulosum*) gingiva was not observed in canine gingival samples.[12] The epithelial cells of the gingiva have cell-to-cell attachments consisting of desomosomes, adherens junctions, tight junctions, and gap junctions.[13]

Other cells found in the epithelium include *Langerhans cells*, *Merkel cells*, and *melanocytes*.[1,9–11] Melanocytes function to produce melanin granules, thereby providing a barrier to ultraviolet light damage. The melanin granules are phagocytosed and stored by melanophages in the epithelium and connective tissue.[10] Melanocytes also function as dendritic cells (antigen-presenting cells). The Langerhans cells are dendritic cells located in the suprabasal layers of the gingival epithelium. They originate from the bone marrow and function as antigen-presenting cells to the lymphocytes. Merkel cells are found in the deep layers of the gingival epithelium and are involved in tactile sensation.

The epithelium is attached to the lamina propria through the basement membrane consisting of the basal lamina (*lamina lucida* and *lamina densa*) and reticular lamina. Proteoglycans and laminin are present. Type IV and VII are the major collagens of the basement membrane.[14] Hemidesmosomes of the basal epithelial cells attach to the lamina lucida. Clinically, the distinction of the region is important. Mucous membrane pemphigoid cleavage occurs at the basement membrane, resulting in the entire epithelium, including the basal layer, cleaving off the lamina propria, whereas pemphigus vulgaris causes intraepithelial cleavage, leaving basal cells attached to the lamina propria.

Gingival fiber groups

The gingival fiber group is sometimes considered to be part of the periodontal ligament fiber group, which will be discussed later. The collagen fiber bundles of the gingiva are organized into groups (Figure 1.5):[6]

1. *Dentogingival group*: Fibers attach cervical cementum to free and attached gingiva. These are the most numerous fibers of the gingiva.
2. *Alveologingival group*: Fibers attach the alveolar bone to free or attached gingiva.
3. *Dentoperiosteal group*: Fibers attach the cervical cementum to the alveolar bone after traversing over the alveolar margin and toward the apex.
4. *Circular gingival group*: Fibers are interlaced with the other fiber groups within the marginal gingiva and encircle the tooth.

Vascular supply, nerves, and lymphatics of the gingiva

The blood supply to the gingival tissues arises from branches of the maxillary arteries and mandibular inferior alveolar arteries, terminating as supraperiosteal arterioles along the lingual and buccal surfaces of the alveolar bone, as well as from the periodontal ligament vasculature and arterioles from the marginal bone.[10] The gingival vascular system is a region of microcirculation.[15] The microvascular circulation of the human gingiva can be divided into the gingival region (where the capillaries run perpendicular to the surface) and the interdental papillae regions (where the capillaries run parallel to the surface).[16] The density of human gingival capillary loops increases with age and tends to be greater in females.[17] The vascular network in the dog gingiva was found to have a "glomerulus-like" form in the sulcular epithelium and a squamous mesh form in the junctional epithelium.[18] This network exudates fluid and allows leukocytes to pass into the gingival crevicular fluid.

These vascular networks are the primary defense against periodontal insults. The vascular beds are important in autoimmune and periodontal pathologies. The increased vascular densities in gingiva may be related to some of the first non-specific defenses against periodontitis. Capillaries of the marginal gingiva are the first vessels involved with inflammation.[15]

Innervation of the gingiva arises from the periodontal ligament and the regional trigeminal (cranial nerve V) branches. The mucosa of the oral cavity is richly innervated and primarily sensory.[1] The rich supply of neuronal axons is found in the lamina propria following the course of the vasculature in addition to nerve fibers in the vicinity of the epithelium.[19] Specialized sensory nerve endings are present (Meissner's or Ruffini's corpuscles, Krause's bulbs, and mucocutaneous endorgans) that detect heat, cold, touch, and pain.[1]

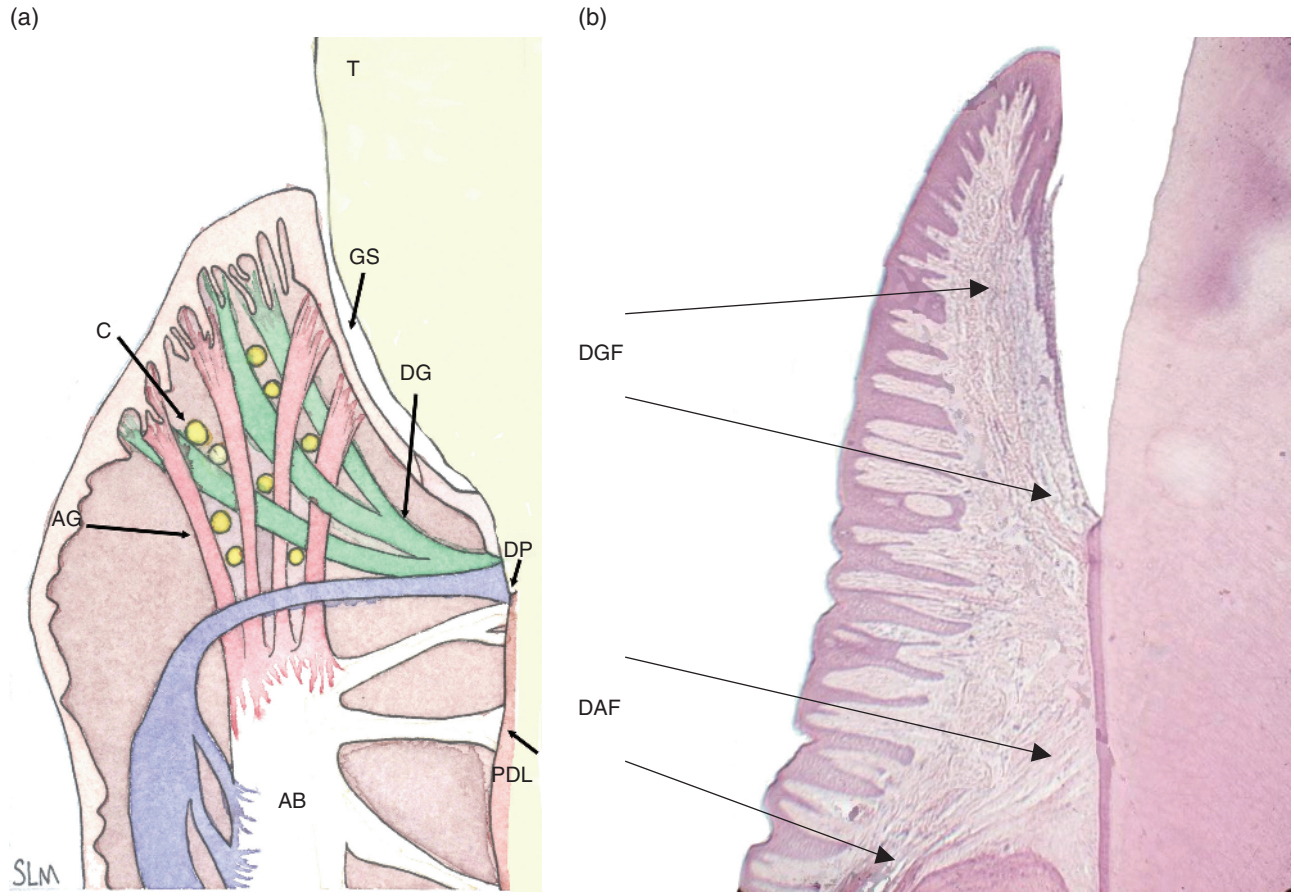


Figure 1.5 (a) Schematic drawing of the gingiva and gingival fiber groups. Dentogingival group (DG), alveologingival group (AG), dentoperiosteal group (DP), circular gingival group (C), alveolar bone (AB), periodontal ligament (PDL), gingival sulcus (GS). (Illustrated by Ms. Sarah L. Mann.) (b) Gingival fibers groups: Dentinal-gingival fibers (DGF) radiate from cementum into papilla, marginal gingival, and attached gingiva, while dentinal-alveolar fibers (DAF) radiate from cementum to insert into surface of alveolar bone.

Microscopic anatomy of periodontal lymphatics is challenging and difficult to study.[20,21] Within the oral mucosa the lymphatics play a primary role in the diffusion, control, and resolution of inflammatory processes. Lymphatics remove extracellular fluid, cellular debris, bacteria, and cells. The endothelial wall of gingival lymphatics is complex, with more intercellular junctions and intercellular channels with few open endothelial junctions.[22] The lymph of the gingival lymphatics drains into the PDL lymphatic system and follows the vasculature to a collective network, external to the periosteum of the alveolar bone, as it moves toward regional lymph nodes.[10,21]

Classification of gingiva and types of gingival epithelium

The gingiva is divided into the *free gingiva* (synonyms include marginal and unattached gingiva), *attached gingiva*, and *interdental gingiva*. The gingiva protects the underlying alveolar bone and tooth roots from mechanical trauma, and provides an epithelial barrier to help prevent bacteria from reaching the underlying tissues. The gingiva plays an active role in cellular communication, responds to infection, and integrates the innate and acquired immune responses when challenged by bacteria.[10] Epithelial cells excrete interleukin-8 and other cytokines and produce antimicrobial peptides as part of an innate defense mechanism.[13]

Free gingiva and the sulcular epithelium

The surface of the free gingiva adjacent to the tooth forms the wall of the gingival sulcus. The gingiva on the opposite side of this layer is exposed directly to the oral cavity (Figure 1.6). The epithelial layer exposed directly to the oral cavity is a parakeratinized stratified squamous epithelium. The free gingiva of young dogs is delicate

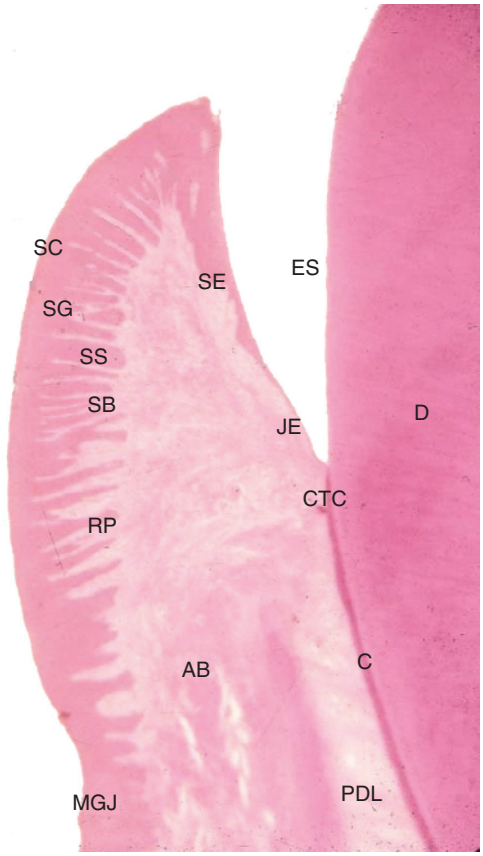


Figure 1.6 Healthy periodontium depicted with stratified squamous oral epithelium exhibiting Rete pegs (RP), stratum corneum (SC—keratinzed), stratum granulosum (SG—granular), stratum spinosum (SS—prickle cell), and stratum basalar (SB—germinativum). The sulcular epithelium (SE) consists of SB and SS with a limited amount of granulosum in coronal aspect, while junctional epithelium (JE) contains SB and SS and no granulosum. The mucogingival junction is identified as MGJ. ES represents the enamel space. CTC is the connective tissue attachment to cementum, AB the crest of the alveolar bone, PDL the periodontal ligament, C the cementum, and D the dentin, respectively.

and flat with a knife-edge marginal termination, whereas older dogs have a more curved appearance.[23] The free gingival height was measured at 1.80–1.92 mm and the width (most apically) at 1.31–1.34 mm.[23]

The gingival sulcus is lined with a non-keratinized stratified squamous epithelium that is referred to as *sulcular epithelium*. The gingival sulcus is bound by the junctional epithelium apically, the tooth, and the sulcular epithelium. Sulcular epithelium lacks rete pegs.[10] It is suggested that the non-keratinized nature of the sulcular epithelium is the result of the local irritation and inflammation within the gingival sulcus.[10]

The normal gingival sulcus depths are $< 3\,mm$ in dogs and $< 0.5\,mm$ in cats.[24] In plaque-free dogs and/or when plaque is well controlled, the gingival sulcus is essentially

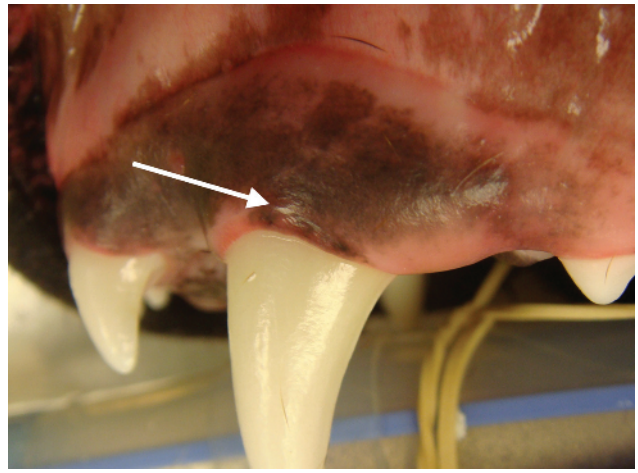


Figure 1.7 Photograph of a left maxillary canine tooth of a dog, gingiva, and alveolar mucosa. The free gingival groove within the keratinized gingiva is identified with an arrow.

absent with a probing depth close to 0 mm.[25] Clearly, there are breed variations in dogs, with giant breed dogs having deeper "normal" sulcular measurements as compared to toy breed dogs, which have shallower "normal" sulcular measurements. Likewise, in dogs without periodontal inflammation, the gingival sulcus is often minimal and there is minimal gingival crevicular fluid and leukocytes.[25] The *cementoenamel junction* (CEJ) is normally positioned just apical to the free gingival margin. In young dogs, the junctional epithelium extends from the CEJ to the oral epithelium with minimal gingival sulcus observed.[23,25]

Attached gingiva

The free gingiva is continuous to the attached gingiva and may be demarcated by a *free gingival groove* (Figure 1.7).[23] The thickness of attached gingiva in large breed dogs (as determined by transgingival probing) was measured as 1.10–2.20 mm, with the thinnest areas in the region of the incisors and the thickest regions around the canine, maxillary fourth premolar, and mandibular first molar teeth.[26] These measurements may not correlate with small and toy breed dogs. Individual variation of masticatory mucosa thickness has been observed in humans.[27] Likewise, the gingival width (as measured from the free gingival margin to the mucogingival junction) varies in the canine oral cavity with less gingival width in the region of the maxillary premolars compared to the canine tooth.[28] It has been suggested that 2 mm of attached gingiva (apical-coronal height) must be maintained for periodontal health.[29] The clinical significance of gingival width and thickness in various regions in the oral cavity is not known. It could be speculated that these thinner regions are more likely



Figure 1.8 Photograph of the attached gingiva of a left maxillary fourth premolar in a young dog. Stippling illustrated within keratinized gingival tissue.

predisposed to periodontal disease, and the thicker and wider regions would be more ideal sites to use for periodontal surgical procedures (periodontal flap procedures and harvesting free gingival grafts).

The attached gingiva is a parakeratinized stratified squamous epithelium and has strong connections to the underlying periosteum of the alveolar bone. The attached gingiva is demarcated from the alveolar mucosa by the *mucogingival junction* (MGJ), which remains stationary throughout life.[10] Significant clinical loss of gingiva toward the MGJ (gingival recession) and/or probing depths of the gingival sulcus beyond the MGJ require periodontal surgery to help re-establish periodontium that can be maintained for periodontal health.

The attached gingival connective tissue and epithelium interdigitate. The connective tissue and gingival epithelial extensions are termed the *dermal papilla* and rete pegs, respectively. Well-developed rete pegs provide strong attachment to the underlying connective tissue. This well-developed interdigitation of the epithelial rete pegs and papillary layer can result in a clinical appearance of *gingival stippling* on the labial surface of attached gingiva (Figure 1.8).[12] In humans, the stippling is most prominent in the maxillary subpapillary region, whereas dogs do not have a proper interdental papillae and stippling is absent in these interdental spaces.[12] In one study, gingival stippling was found most commonly in older dogs (8–9 years of age), with younger dogs having absent stippling suggestive of less developed tissues.[23] In a more recent study, the stippling was found most commonly in middle-aged dogs and less in young and older patients.[12]

Interestingly, the most prominent signs of stippling were found at the canine teeth, upper fourth premolar, and first molar teeth with absence in the premolar teeth.[12] These are the same regions that were found to have the thickest width of attached gingiva. Therefore, the attachments will be stronger in these regions. These regions are considered to be the strategic teeth in carnivores and are challenged with the greatest mechanical forces during mastication.

In humans, stippling of the attached gingiva has been associated with the interdigitation of the rete pegs and dermal papillae.[10] This appears not to be the case in canine patients. The microscopically detectable pits in the canine gingiva do not correspond to the structures of the rete pegs and dermal papillae.[12] Loss of stippling has been suggested as a sign of gingival disease in humans.[10,12] In the dog, however, stippling can still be present in inflamed gingiva.[12] Regardless, the parakeratinized layer of the gingiva in dogs was found to be 5–10 cell layers thick whether it was in a region of stippled or unstippled gingiva.[12]

Interdental gingiva

The interdental gingiva between the teeth can form a pyramidal or col shape.[10] The col is a non-visible concavity between buccal and lingual gingival papillae. The col is covered with non-keratinized epithelium and is most commonly found between the maxillary fourth premolars and first molars, the mandibular first and second molars, and the incisors in dogs.[29] This region of non-keratinized epithelium is more susceptible to irritation and trauma, resulting in inflammation and possible early loss of periodontium.

Junctional epithelium and the gingival crevicular fluid

The gingival epithelium lining the sulcus is continuous to the *junctional epithelium* (JE) at the apical extent of the sulcus. The JE is a non-keratinized stratified squamous epithelium that attaches the gingiva to the tooth (Figure 1.9). JE contains fewer desmosomal junctions compared to other oral epithelial tissues.

JE provides a physical epithelial barrier to the apical periodontal structures. It allows passage of *gingival crevicular fluid* (GCF) and inflammatory cells (via diapedesis) into the sulcus. The JE has a rapid cell turnover rate that helps maintain a favorable host-parasite equilibrium and rapid repair of damaged tissue.[30] The JE replaces every 4–6 days, whereas the sulcular epithelium takes several more days to do so. The gingival epithelium takes 9–12 days to replace.[6,30] It has been suggested that JE cells have endocytic capacity similar to neutrophils and macrophages.[6]

Apical to the JE, the densely packed collagen bundles of the connective tissue anchor to the acellular extrinsic fiber cementum, which plays a key role in limiting the migration of the JE (Figure 1.10).[6] The apical aspect of the JE is 1–2 cell layers thick, whereas the thickness



Figure 1.9 The junctional epithelium is composed of the stratum basalar (SB) and stratum spinosum (SS) and constitutes the interface between the cementum (C) and underlying connective tissue (CT).

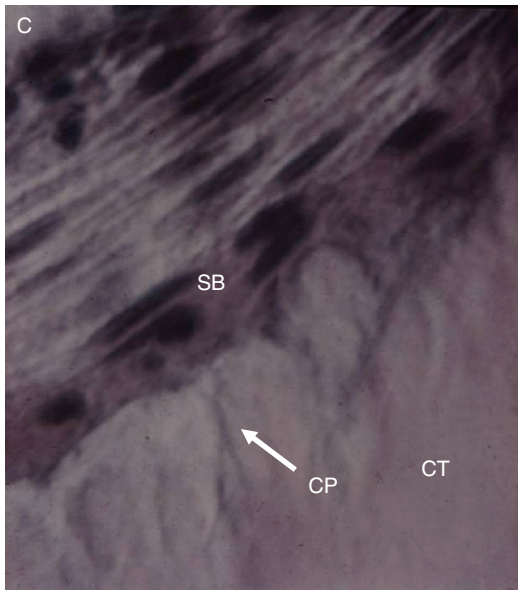


Figure 1.10 Cytoplasmic projections (CP) from the stratum basalar (SB) cells of the junctional epithelium extend into intercellular spaces of connective tissue (CT) cells and along cementum surface (C).

increases to 10–20 cells coronally.[6] It measures approximately 2 mm in apical-coronal direction. As junctional epithelial cells migrate coronally to be shed into the sulcus, a continuous attachment to the tooth surface is

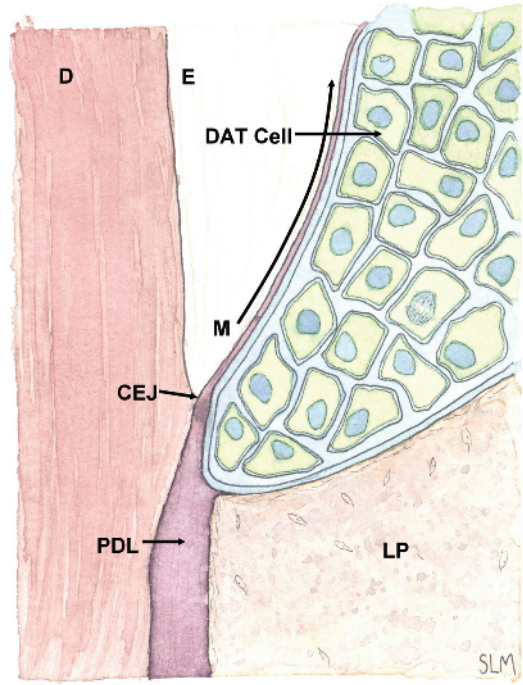


Figure 1.11 Schematic drawing of junctional epithelium. Lamina propria (LP), periodontal ligament (PDL), cementoenamel junction (CEJ), dentin (D), enamel (E), directly attached to tooth cell (DAT cell), movement of junctional epithelial cells coronally (M). (Illustrated by Ms. Sarah L. Mann.)

maintained, and these JE cells remain undifferentiated (Figure 1.11). The JE produces an internal basal lamina and anchors to this lamina via hemidesmosomes (Figure 1.12). The external basal lamina separates the JE from the gingival connective tissue.

Hemidesmosomes of the JE cells plasma membrane directly attach to the tooth (DAT cells). An internal basal lamina on the tooth surface interacts with the epithelial attachment.[30] The DAT cells synthesize the internal basal lamina, since there is no connective tissue present. The JE is a stratified epithelium with a basal layer facing connective tissue and a suprabasal layer extending to the tooth surface.[30] Interestingly, evidence suggests these DAT cells have mitotic activity, which is unique, since they are several cellular layers away from the basal layer of the epithelium.[30]

The JE vessels are fenestrated, which functions to allow exchange of substances through the openings.[18] The GCF moves through these fenestrations into the cellular spaces and then into the sulcus. The movement of GCF coronally helps clean the sulcus. Additionally, leucocytes move between the cellular gaps of the stratified squamous epithelium and into the sulcus in an organized manner.[18]

The GCF may be a transudate or exudate.[10,30] The composition of the GCF depends on the presence of a

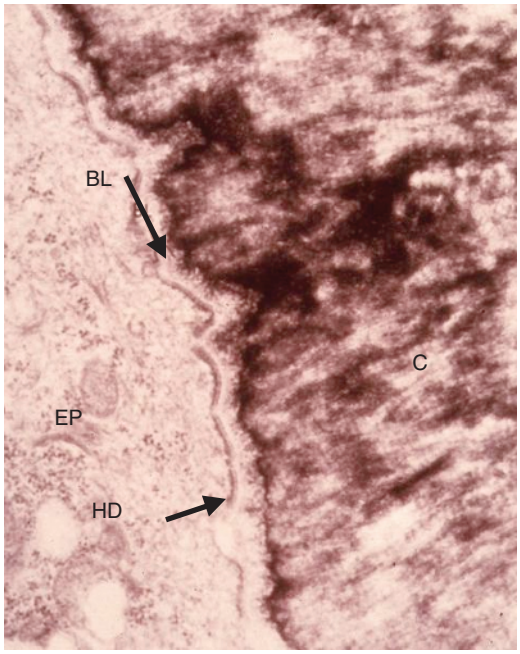


Figure 1.12 An electron microscopic image depicting hemidesmosomes on the periphery of an epithelial cell with the basal lamina (BL) consisting of the lamina densa [adjacent to cementum (C)] and lamina lucida [adjacent to periphery of epithelial cell (EP)] interspersed between the hemidesmosome and cementum (HD).

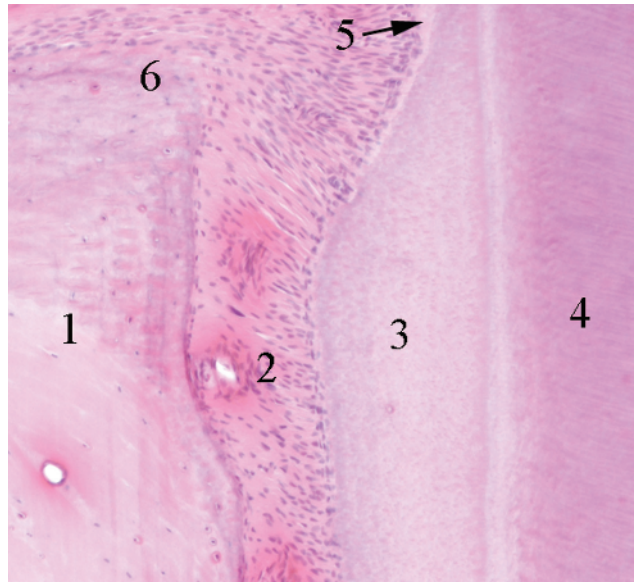


Figure 1.13 Histomicrograph of a feline periodontal ligament space. Alveolar bone (1), periodontal ligament (2), cementum (3), dentin (4), acellular afibrillar cementum (5), marginal alveolar bone (6). Reprinted with permission, Roux P, et al. Observations of the periodontal ligament and cementum in cats with dental resorptive lesions. J Vet Dent. 22(2):74–85, 2005.

plaque biofilm. Normally, there will be minimal fluid production. With inflammation, however, the fluid production increases and the GCF will contain breakdown products of connective tissue, epithelial cells, inflammatory cells, bacteria, and serum. The GCF mechanically flushes the sulcus, as well as contains antimicrobial products, immunoglobulins, and plasma proteins; the latter also assists in epithelial adhesion.[10] It is important to note that the polymorphonuclear leukocytes at the gingival margin do not remove plaque. These leukocytes provide a protective wall against bacteria.[30] The primary granules of these leukocytes contain myeloperoxidase, lysozyme, elastase, cathepsin G, urokinase, acid hydrolases, and defensins.[30] Lactoferrin, elastase, and lysozyme are found in the secondary granules.[30] (See chapter 4 for a complete discussion of GCF and its response to bacterial infection.)

Gingival tissues and age

All cells in the body and periodontium experience preprogrammed cell death (apoptosis). In particular, gingival cells and fibroblasts have a high rate of apoptosis in the normal turnover of the periodontium.[31,32] Apoptosis may play a role in different types of drug-induced gingival enlargement, particularly that caused by calcium channel blockers.[33] However, the mechanisms are multifactorial and not fully understood.[34,35] Increased collagen content of gingival connective tissue has been identified with increasing age in the dog, whereas no age-related gingival epithelial differences were found.[23] However, an earlier study found a thicker keratinized epithelial layer in the juvenile dog.[36] The most apical cells of the JE were at the CEJ in young dogs, and apical to the CEJ in older dogs with periodontal inflammation control. These findings suggest that teeth may be undergoing continued passive eruption.[23]

Periodontal ligament

The PDL is continuous with the gingival tissues, anchors the tooth in the jaw, acts as a shock absorber, and is active in periodontium maintenance (Figures 1.13 and 1.14). The PDL can be divided into a bone-related region rich in cells and vessels, a middle zone with fewer cells and thinner collagen fibrils, and a cementum-related region with dense collagen bundles.[6] Progenitor cells for fibroblasts, osteoblasts, and cementoblasts are found in the PDL.[7] The width of the buccal PDL was found to be 0.15–0.2 mm in dogs.[23] When PDL is destroyed, dentoalveolar ankylosis occurs and the adaptability of the periodontium is lost.

The principal collagenous fibers of the PDL are arranged in bundles that insert into the cementum and alveolar bone and are referred to as *Sharpey's fibers*. Type I collagen is the primary component of these principal fibers. The tensile

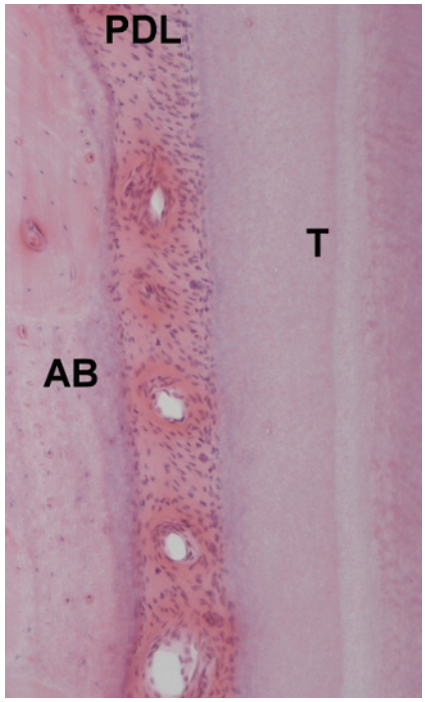


Figure 1.14 Histomicrograph of feline periodontal ligament. Alveolar bone (AB), periodontal ligament (PDL), tooth (T). Reprinted with permission, Roux P, et al. Observations of the periodontal ligament and cementum in cats with dental resorptive lesions. J Vet Dent. 22(2):74–85, 2005.

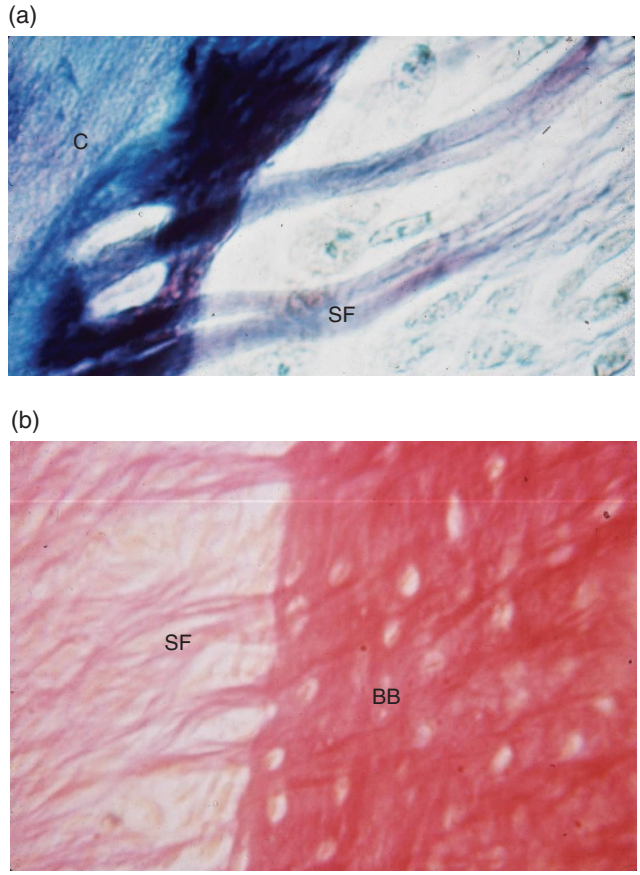


Figure 1.15 (a) Insertion of Sharpey's fibers (SF) into cementum (C). (b) Insertion of Sharpey's fibers (SF) into bundle bone (BB).

strength of these fibers has been reported to be greater than that of steel.[10] These fibers mineralize when embedded into the cementum and alveolar bone (Figure 1.15). The alveolar wall insertion of the Sharpey's fibers is the tension side of the PDL.[8] As Sharpey's fibers traverse deep into the bone, the length of penetration into the hard tissues can be much greater than the width of the PDL.[8]

The PDL acts as a shock absorber, and the viscoelastic system theory is used to explain the mechanism. As force is applied to the tooth, the fibers of the PDL tighten and blood is then forced from PDL vessels traversing the cribiform plate to the cancellous bone. The cribiform plate is most abundant in the cervical third of the tooth.[37] Additionally, mechanoreceptors, free nerve endings, pressure, and vibration sensors are found in the PDL.

Cells and connective tissue of the periodontal ligament

The cellular constituents of the PDL include epithelial rests of Malassez, immune cells, connective tissue cells, and neurovascular cells. The connective tissue cells include fibroblasts, osteoblasts, and cementoblasts. Undifferentiated cells that can differentiate into all of the above cells are also found in the PDL.[6,38] However, the exact source of the PDL is not fully understood. The fibroblasts in the PDL, similar to osteoblasts and cementoblasts, have an increased level of alkaline phosphatase activity and produce a mineralized matrix with osteopontin and bone sialoprotein.[6] It has been proposed that the maintenance of epidermal growth factor receptors on the fibroblasts of the PDL maintains these precursor cells as fibroblasts, whereas the loss of these growth factors allows differentiation into cementoblasts and osteoblasts.[6] Others have suggested HERS may give rise to cementoblasts and PDL fibroblasts.[7]

The fibroblast is the most abundant cell of the PDL and these cells are continually renewed.[8] Fibroblasts synthesize collagen and have phagocytic capacity. They remove collagen fibrils by phagocytosing the fibrils from the extracellular environment and degrading them with lysomal cysteine proteinases.[6] This lysosomal mechanism does not involve extracellular collagenase enzymes. Collagen degradation of the PDL is an intracellular phenomena following phagocytosis by periodontal fibroblasts,[8] whereas with disease, PDL breakdown is via extracellular mechanisms. The intracellular degradation allows a more specific and precise control for remodeling and replacement of the PDL under normal physiological

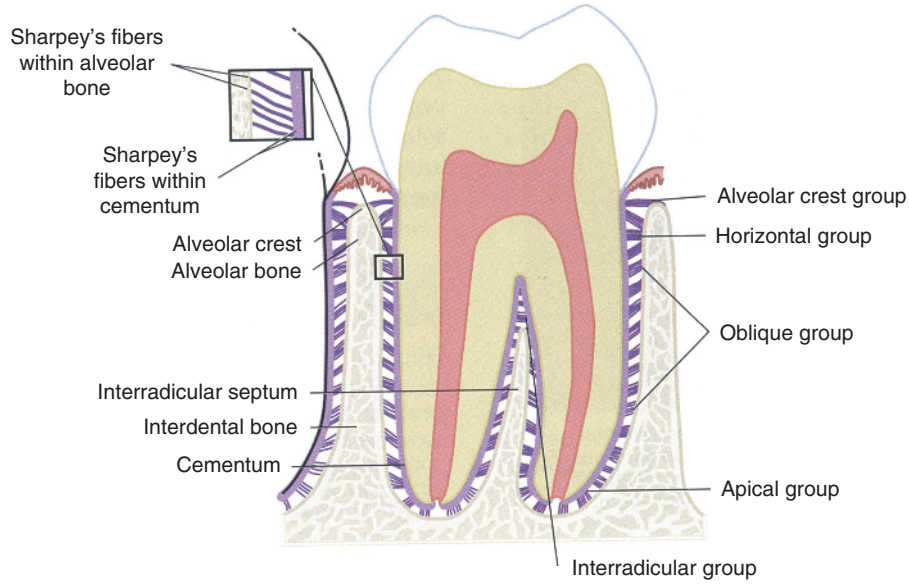


Figure 1.16 The alveolodental fiber groups of the periodontal ligament. Reprinted with permission, chapter 14, Periodontium: Cementum, alveolar bone, periodontal ligament. In: Dental Embryology, Histology, and Anatomy (Bath-Balogh M, Fehrenback MJ). 2nd ed. Copyright Elsevier Inc., 2006, p. 226.

conditions. New fibroblasts in repair of the periodontium arise from the perivascular cells in the PDL as well as from progenitor cells in the adjacent alveolar bone.[6]

Ground substance containing glycosaminoglycans (hyaluronic acid and proteoglycans), glycoproteins (fibronectin and laminin), and water fill the spaces between the cells and fibers in the PDL.[37] The extracellular components of the PDL connective tissue have a high turnover rate. Collagen has a very short half-life (several days) in the PDL, but collagenases have not been detected in the normal tissues.[8] However, the matrix metalloproteinase (MMP) enzymes are synthesized in a latent, nonactive form that becomes activated by tissue and plasma proteinases, bacterial proteinases, and oxidative stress.[14] MMPs play a major role in connective tissue breakdown during disease, whereas phagocytosis degrades collagen for normal replacement, remodeling, and repair.[14]

The epithelial rests of Malassez are identified as isolated clusters of cells or interlacing strands in prepared tooth specimens.[37] The ERMS are most numerous in the apical and coronal regions and have been reported more on the mesial side of human molars compared to the distal side.[8] The exact function ERMS is not known but they do not appear to maintain the width of the PDL, prevent ankylosis, or prevent root resorption.[8]

Periodontal ligament fiber groups

Principal fiber bundles insert into bone and cementum as Sharpey's fibers. The primary collagens of the PDL are type I, III, and XII.[14] These fibers reconstruct and reinsert following periodontal damage from periodontitis, trauma, and orthodontic tooth movement. The PDL fiber groups respond to the physiological needs of the tooth.

The alveolodental fibers anchor between cementum and bone and include the following (Figure 1.16):[6]

1. *Alveolar crest fiber group* inserts in an apico-oblique direction beneath the JE and functions to resist extrusion, horizontal, rotational, and lateral tooth movement.
2. *Horizontal fiber group* inserts between cementum and bone at right angles and functions to resist horizontal and rotational tooth movement. They are located just apical to the alveolar margin.
3. *Oblique fiber group* inserts in a coronal-oblique direction (the bone insertion is positioned coronal to the cementum insertion) and functions to resist intrusive forces associated with occlusal stress and rotational tooth movement. The forces are transferred into tension of the alveolar bone. This fiber group is the largest, making up two-thirds of the fibers in the PDL.[6]
4. *Apical fiber group* inserts from the apex to the alveolar bone and functions to resist extrusion and rotational tooth movement.
5. *Interradicular fiber group* is found in multirooted teeth and connects cementum to marginal bone (Figure 1.17).
6. *Transseptal fiber group* anchors the cementum of two adjacent teeth (Figure 1.18). This group of fibers has an important role necessitating retention in orthodontics.



Figure 1.17 Intact interradicular periodontal fibers traverse the intraseptal bone and insert into the roots of a molar tooth despite extensive periodontitis in furcation region.

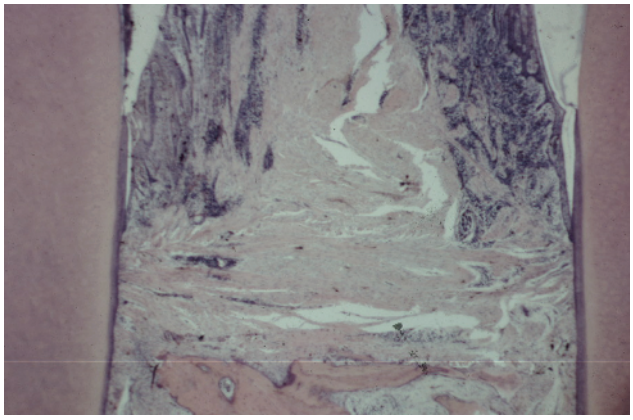


Figure 1.18 Horizontally oriented transseptal periodontal fibers lay coronal to the alveolar bone and insert into proximal surfaces of adjacent teeth.

In addition to the principal fiber groups, there is an indifferent fiber plexus of collagen inserting in all directions.[10] The PDL does not contain mature elastin but does contain two immature forms known as oxytalan and elunain.[10] *Oxytalan* fibers, similar to elastin, are found oriented in an apico-coronal direction and attach to the coronal third of the tooth.[8] It has been speculated that the function of these fibers is to provide elastic properties to the PDL, facilitate fibroblast attachment and migration, and may be involved in vascular flow.[8]

Vascular network of the periodontal ligament

The PDL has a good blood supply for a connective tissue. Branches of the maxillary and mandibular alveolar artery provide blood to the terminal branches.[39] The blood supply to the PDL arrives via (1) the alveolar bone blood supply through the Volkmann's canals (cribiform plate), (2) anastomosis with the gingival vessels, and (3) vessels arriving apically to the tooth.[18] The PDL has a polygonal mesh vascular network.[18] The movement of blood from the PDL to the alveolar bone allows the PDL to act as a shock absorber of occlusal forces. In large molars, the apical region of the vascular meshwork has a vertically longer, more elliptical shape, and adjacent capillary venules with anastomosing branches.[18] Blood vessels of the PDL provide nourishment to the cells of the PDL and osteoblasts of the adjacent alveolar bone. Lymphatics of the PDL drain into the alveolar bone in the coronal two-thirds and the apical lymphatics in the apical one-third.[20] Lymphatics were identified in the alveolar bone traversing to adjacent periodontal ligaments.[20]

The *rete venosum* in the alveolar bone may act as the reservoir for the displaced blood from the PDL vessels during occlusal force loading.[18] Additionally, there are very few valves in the venules of the PDL that traverse the Volkmann's canals.[18] Valves normally prevent backflow of blood in veins and venules. In the case of the PDL, the absence of valves can allow blood to flow back to the PDL from the alveolar bone when the occlusal forces are removed.

Cementum

Cementum, an avascular tissue with no innervations, is approximately 45–50% mineralized [hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$], and 55–50% connective tissue. The organic matrix of cementum is mostly composed of types I (90%) and III (5%) collagen.[14,38,40] The cementum receives nourishment from the PDL.

Cementum must attach to the dentin surface of the root. In cementum, bone sialoprotein may act as an adhesion molecule to maintain cells on the root surface and initiate mineral formation.[38] It is proposed that there is a specific cementum attachment protein (a cementum-specific collagen) and a cementum-derived growth factor unique to cementum.[38]

Cementum is thinnest at the cementoenamel junction and thickest apically. Root cementum was found to be 5–10 times greater in older dogs and cementum thickness increased apically, whereas the width of the PDL remained constant.[23] In humans, approximately 60–65% of teeth have cementum overlapping enamel, 30% ending

Box 1.1 Development of the cementum

The development of the cementum is not fully understood or characterized. Although basic periodontium embryology was previously discussed, it is worthwhile to focus on the cementum more closely, as there are consequences when evaluating periodontal "repair" and "regeneration." The cementoblasts arise from the dental follicle. The disintegrating HERS does not have a secretory role in acellular cementum formation, despite some theories suggesting otherwise.[38,40] Amelogenin, an enamel matrix protein, was not found in cells of HERS.[38] Enamel matrix proteins (EMPs) have been debated as an important molecule in regeneration. However, detection of EMP expression along the root is sparse and its presence in bone and cementum is unclear.[7] Detection in some studies could be the result of dentin contamination during extraction of the EMP. Although enamel matrix protein extracts have been used for periodontal regeneration, and there is some evidence of successful "regeneration," there is no evidence they play a role during normal cementum development.[40]

EMP may promote the cementoblast activities of proliferation, migration, adhesion, and differentiation and may stimulate periodontal attachment structures during repair, but this does not necessarily result in regeneration.[38]

at the junction between enamel and cementum, and 5–10% have a space between enamel and cementum. Clinical conditions such as cemental aplasia, hypoplasia, cemental hyperplasia, and hypercementosis can occur. When cementum and the PDL are lost, the tooth can become ankylosed to the alveolar bone with resulting loss of proprioception and resistance of occlusal forces.

Types of cementum

Cementum is classified based on cells and the source of collagen fibers.[38] Unlike bone, cementum is avascular, lacks innervation, and shows little or no remodeling in the canine and feline.[38] Cementum is divided into acellular (referred to as primary) and cellular (referred to as secondary) and then further divided based on the origins of the collagen fibers from cementoblasts (intrinsic fiber cementum) and fibroblasts (extrinsic fiber cementum).[6] It is possible that acellular and cellular cementum are distinct types of cementum developing from different embryonic origins.[2]

Both acellular and cellular cementum are arranged in lamellae parallel to the long axis of the tooth representing rest periods. Acellular cementum is more mineralized than cellular, whereas cellular contains cementocytes in lacunae.[6] Cementocytes have a physiological adaptive role in tooth movement and repair of periodontal tissue.

The intrinsic fibers are arranged parallel to the root surface. Sharpey's fibers (extrinsic fibers) insert at approximately 90 degrees to the root surface, are fully mineralized in acellular cementum, and are only partially mineralized (mineralized periphery with unmineralized core) in cellular cementum. Acellular cementum forms before cellular cementum. However, cellular cementum forms at a faster rate.

1. *Acellular afibrillar cementum* (ACC): No cementocytes, extrinsic, or intrinsic fibers are present. It is a mineralized ground substance located over the enamel and dentin at the CEJ and has no role in attachment.[6,7] The precise cellular origin and function is unknown.
2. *Acellular extrinsic fiber cementum* (AEFC): Densely packed with Sharpey's fibers and lacking cementocytes. It is located on the cervical and middle third of the tooth, covering 40–70% of the root surface, and functions to anchor the PDL to the tooth root.[6] It is estimated there are 30,000 fibers/mm^2 inserting into the cementum.[7]
3. *Cellular mixed stratified cementum* (CMSC): Extrinsic and intrinsic fibers are present and it may contain cementocytes. It is found on the apical one-third of the root and in the furcation areas.[6,7] It contains layers of acellular extrinsic fiber cementum and cellular/acellular intrinsic fiber cementum.[6] The purpose is to compensate for physiological forces acting on the tooth in the alveolus.
4. *Cellular intrinsic fiber cementum* (CIFC): Cementocytes and intrinsic fibers are present with no extrinsic fibers. It is found filling resorption lacunae and root fracture sites.[7] The intrinsic fibers are not found within the PDL. CIFC has no role in tooth attachment with the fibers arranged parallel to the root surface while circling around the root.[6]
5. *Acellular intrinsic fiber cementum* (AIFC): There are no cementocytes and intrinsic fibers are only present. It is a component of the stratified layers of AIFC and CIFC that contributes to the CMSC.[7]
6. *Intermediate cementum*: This cementum is found near the cementodental junction and remnants of HERS are embedded in the calcified ground substance.

Alveolar bone

Bone is comprised of 67% inorganic material (hydroxyapatite) and 33% organic material. Collagen makes up the majority (80–90%) of the organic component of bone with greater than 95% being type I collagen and less than

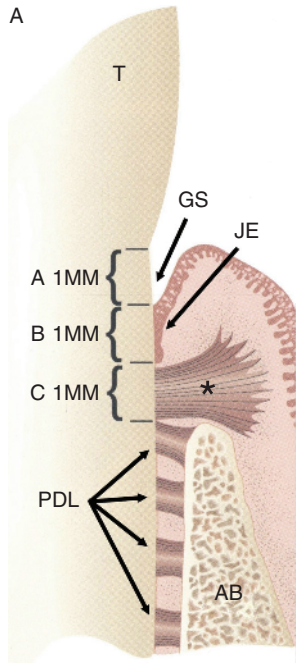


Figure 1.19 A drawing of the dentogingival interface demonstrating the biological width measured from the apical aspect of the gingival sulcus to the alveolar margin (B + C). This space is occupied by the junctional epithelium (JE) and the gingival connective tissue and gingival fibers (*). The tooth (T) is anchored in the alveolar bone (AB) by the periodontal ligament (PDL). Reprinted with permission, J Vet Dent. 25(2):86–95, 2008.

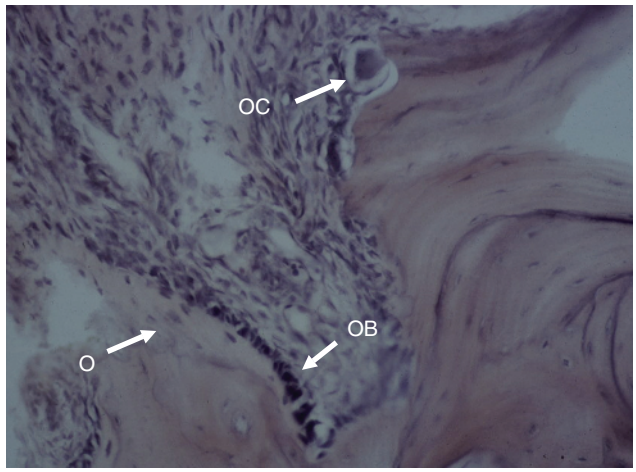


Figure 1.20 Remodeling of alveolar bone depicted by a line of osteoblasts (OB—lower center) forming new bone (O—osteoid) with entrapped osteocytes (dark nuclei). A resorption bay is noted in top center with one multinucleated osteoclast (OC).

5% type III, V, VI, XII collagen.[7,41] Osteopontin (OPN) and bone sialoprotein (BSP) are two non-collagenous proteins of bone and cementum that are present in the interfibrillar spaces.[7] The collagenous matrix called osteoid, secreted by osteoblasts, provides the scaffolding for apatite mineral deposition as well as other non-collagenous proteins.

Different types of bone are found in the maxillofacial region. Woven bone arises from a connective tissue template and a process of intramembranous ossification. The maxilla and mandible contain alveolar processes that house roots of the teeth.[6] Alveolar bone supports the tooth structure, helps distribute occlusal forces, and is continually remodeled as it responds to the forces of mastication. The alveolar bone is composed of plates of cortical (compact) bone with spongy cancellous (trabecular) bone in between. Cancellous bone is also found in the interradicular and interdental spaces between cortical bone and the alveolar wall.[42] Bone lining the alveolus, where the Sharpey's fibers insert, is referred to as *bundle bone*. Bundle bone, irregularly arranged and less dense, is produced by osteoblasts between the Sharpey's fibers.[42] The bone layers are arranged parallel to the apico-coronal direction of the tooth.[41] The inner cortical bone is radiographically dense and is radiographically referred to as the *lamina dura*. Cortical bone meets coronally at the alveolar margin and is usually 1.5–2.0 mm below the CEJ (Figure 1.19).[43]

Alveolar bone consists of lamellated and bundle bone.[6] The osteon is the structural unit of bone with a central Haversian canal and interconnecting Volkmann's canals. Histologically, a cement line can be seen between old and newly formed bone. The apical and coronal aspects of the alveolar bone (cribiform plate) have openings connecting the bone marrow to the PDL via Volkmann's canals.[6]

Alveolar bone remodeling

Bone is remodeled while under constant force. There is an interdependency of osteoblasts and osteoclasts called coupling.[37] Osteoclasts and osteoblasts interact in a paracrine and autocrine fashion. Osteoblasts and newly formed osteoid (Figure 1.20) line the region of recently resorbed bone vacated by osteoclasts. Cancellous bone trabeculae increase in number and thickness and cortical bone may be added when increased forces are placed on the jaws.[37]

Osteoclasts are responsible for resorption of bone by removing the hydroxyapatite and organic matrix. They arise from hematopoietic cells of the monocyte/macrophage lineage of the bone marrow. Osteoclasts are large multinucleated cells that produce a variety of hydrolytic enzymes that are secreted into an acidic environment. Osteoclasts are found in *Howship's lacunae* (Figure 1.21). The ruffled border of a multinucleated osteoclast creates a microenvironment for bone resorption.

Osteoblasts produce bone matrix (both collagenous and non-collagenous), which is termed osteoid. Rapidly forming bone (embryonic and juvenile growing bone, as

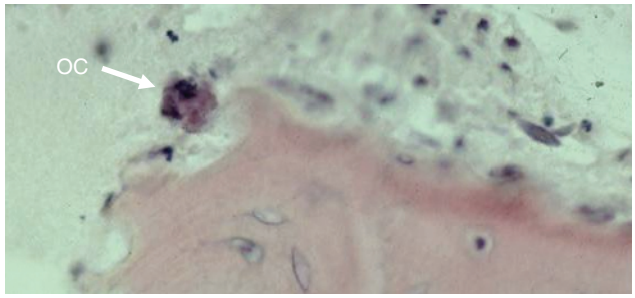


Figure 1.21 A multinucleated osteoclast (OC) in Howship's lacunae bay of resorption.

well as bone involved in repair) is called woven bone. There are large areas of interfibrillar spaces occupied by mineral crystals and large numbers of osteocytes.[7] Alkaline phosphatase, secreted by osteoblasts, starts the nucleation of hydroxyapatite crystals. More mature lamellar bone has organized collagen fiber sheets perpendicular to one another with very little interfibrillar space.[41] Osteopontin and bone sialoprotein are expressed in alveolar bone.[41] Osteopontin is located at cement lines in bones and may play a role in integrating new and old bone together.[41] Osteoblasts that do not undergo apoptosis or remain on the bone surface can be trapped in bone lacunae as osteocytes.[41]

Bone formation and resorption are closely coordinated. Osteoclasts are stimulated to resorb bone by parathyroid hormone, parathyroid-related peptide, vitamin D_3, interleukin-1, interleukin-6, tumor necrosis factor-α, transforming growth factor-α, and prostaglandin-E_2 (PGE_2).[7,41] Parathyroid hormone and vitamin D_3 influence osteoclasts indirectly by acting on osteoblasts. Osteoclasts are inhibited by calcitonin, transforming growth factor-β, estrogen, and interferon-γ.[41] Prostaglandin E_2 is a potent stimulator of osteoclasts and bone resorption.

An interesting new area of bone regulation is the RANK (receptor-activated nuclear factor κβ)/RANKL(receptor-activated nuclear factor κβ ligand)/OPG (osteoprotegrin) system in relation to osteoclast regulation.[7] It is also necessary for macrophage-colony stimulating factor to be present along with osteoblasts and cell-to-cell contact for osteoclast regulation. RANKL/OPG balance in the bone microenvironment is crucial for osteoclast control. The stimulators of osteoclasts and subsequent bone resorption work via this system.

RANK is expressed on the plasma cell membranes of osteoclast precursor cells, whereas RANKL is expressed on the plasma cell membranes of osteoblasts. OPG produced by osteoblasts acts as a decoy receptor by binding RANKL, thereby preventing activation of RANK. The suppression of this system decreases development, formation, and activity of osteoclasts, whereas an induction of the system increases them.[7] Understanding this functional system of the alveolar bone can allow development of targeted therapies for alveolar bone loss.

It should be noted that the RANK/RANKL/OPG system is not the direct target of bisphosphonate compounds used in medicine. Bisphosphonates inhibit bone resorption by inducing osteoclast and macrophage apoptosis.[31,44] There has been bisphosphonate-related osteonecrosis of the jaws due to inhibition of osteoclasts in human patients. Yet at controlled local doses they may provide treatment of periodontitis due to control of osteoclasts.[45]

Conclusion

The periodontium is a dynamic system responding to attack from oral bacteria. Despite meticulous periodontal homecare and annual dental cleanings, periodontium is lost over time. There is increased attachment loss of the periodontium as the patient ages.[46,47]

Understanding the structure and function of the periodontium is necessary for understanding periodontal defense mechanisms, periodontal disease pathophysiology, periodontal treatments, and periodontal regeneration and repair. Regeneration implies reproduction or reconstitution of the tissues so they are completely restored (as in embryological tooth development) versus repair, which implies healing without restoration.[48]

Acknowledgment

Schematic diagrams depicted in Figures 1.5a and 1.11 were illustrated by Ms. Sarah L. Mann, University of Minnesota student.

Box 1.2 Key points

- The lining of the gingival sulcus is non-keratinized and is therefore more fragile than the outer coating of the attached gingiva. Care must be taken during subgingival cleaning.
- Dogs do not have proper interdental papillae.
- The normal gingival sulcus depths are $< 3\,mm$ in dogs and $< 0.5\,mm$ in cats.
- However, in plaque-free dogs and/or when plaque is well controlled, the gingival sulcus has a minimal probing depth.
- The periodontal ligament is continuous with the gingival tissues, anchors the tooth in jaw, acts as a shock absorber, and is active in maintenance of the periodontium.
- Alveolar bone supports the tooth structure, helps distribute occlusal forces, and is continually remodeled as it responds to the forces of mastication.
- The alveolar bone is composed of plates of cortical (compact) bone with spongy cancellous (trabecular) bone in between.

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