ALVEOLAR BONE

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Medical and Research Publications

Alveolar Bone

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Dr. G. Shiva Shankar

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Introduction

The alveolar process can be defined as the portion of the maxilla and mandible that forms and supports the tooth sockets. It forms when the tooth erupts to provide the osseous attachment to the forming periodontal ligament and disappears gradually after the tooth is lost. [(1)] It is an arbitrary boundary at the level of the root apices of the teeth separating the alveolar processes from the body of the mandible or the maxilla.[(2)]

The alveolar bone has its embryological origin from the initial condensation of ectomesenchyme around the early tooth germ near the end of 2nd month of foetal life during intramembranous ossification. [(3)] It is formed during root development and is derived from cells (osteoblasts) originating in dental follicle. Its development is independent of other parts of alveolar bone and is associated with the development and presence of teeth, and subsequent development of attachment apparatus. Just before mineralization, osteoblasts start producing matrix vesicles. These vesicles contain enzymes such as alkaline phosphatase that help in starting of the nucleation of hydroxyapatite crystals. As these crystals grow and develop, they form bone nodules and coalesces with fast-growing non-oriented collagen fibers which are substructures of woven bone (the first bone formed in the alveolus). Later, mature lamellar bone is formed through bone deposition, remodelling, and the secretion of oriented collagen fibers in sheets.[(4)]

Primary and permanent teeth which do not have any precursor tooth develop alveolar bone around their roots during development and eruption.[(5)] Initially, the succedaneous tooth germs are present with in the same osseous cavities as their deciduous precursors but when deciduous teeth erupt, alveolar bone is deposited around the developing roots and serves to separate the erupting deciduous tooth from the crowns of underlying developing succedaneous tooth. Thus the developing permanent tooth is present enclosed within their own bony crypt, located lingual and apical to primary tooth in the basal bone of alveolar process.[(6)]

During the eruption of permanent teeth- walls of bony crypts, roots of primary tooth, and its alveolar bone housing are resorbed. Thus after losing of primary tooth succedaneous or permanent tooth occupies the vacant area. During this process permanent tooth forms its own alveolar bone from its own dental follicle. As the tooth root forms and the surrounding tissues develop and mature, alveolar bone merges with the separately developing basal bone and the two become one continuous structure.[(1)]

With this, the alveolar bone of maxilla and mandible also remodel leading to increase in facial length. Thus with the emergence of succadeneous or permanent teeth there is complete deposition and remodelling of whole alveolar process.(6)

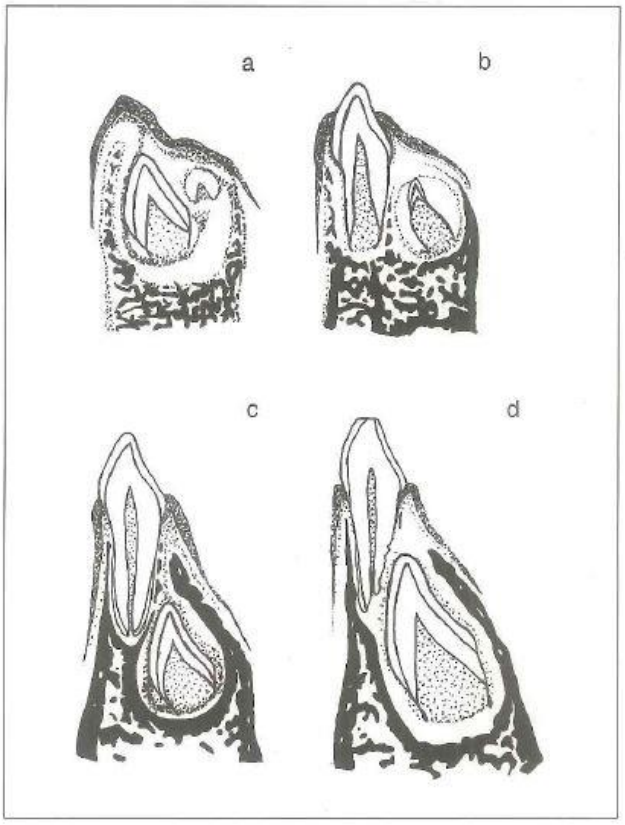


Fig 7-4 Schematic representation of the relationship between a deciduous tooth and its accompanying succedaneous tooth detailing the formation of the alveolar bone portion of the periodontium (adapted from Scott and Symons 1974). **a** At birth, both the deciduous incisor and the tooth germ for its permanent successor share the same alveolus and follicle. **b** At about 7 months, the deciduous incisor erupts into the oral cavity and a separate follicle begins to form around the associated permanent successor. **c** By 2½ years, the incisor is fully erupted and is encased in its own bony socket. The forming permanent successor is contained in a fully formed crypt. **d** By 7 years, the permanent successor begins to erupt and is accompanied by resorption of the bone forming the roof of its crypt and the root of the deciduous incisor.

(6) ***Bartold PM, Narayanan AS. Biology of the Periodontal Connective Tissues: Quintessence Publishing (IL); 1998***

Alveolar bone consists of following parts:

1. An external plate of cortical bone formed by Haversian bone and compacted bone lamellae

2. The inner socket wall of thin, compact bone called the alveolar bone proper, which is seen as the lamina dura in radiographs. Histologically, it contains a series of openings (cribri form plate) through which neurovascular bundles link the periodontal ligament with the central component of the alveolar bone, the cancellous bone.

3. Cancellous trabeculae, between these two compact layers, which act as supporting alveolar bone. The interdental septum consists of cancellous supporting bone enclosed within a compact border.(1)

In addition, jaw bones consist of basal bone which is portion of jaw located apically but not related to teeth. On anatomic basis, the alveolar process is divisible into separate areas but it functions as a unit with all parts interrelated in the support of teeth.

Socket Wall

The socket wall consists of dense lamellated bone which is arranged in Haversian systems, and bundle bone. Bundle bone is the term given to bone adjacent to the periodontal ligament that contains a great number of Sharpey's fibers.(7) It is characterized by thin lamellae arranged in layers parallel to the root, with intervening appositional lines. Bundle bone is localized within the alveolar bone proper. Some Sharpey's fibers are completely calcified, but most contain an uncalcified central core within a calcified outer layer.(8)

Formation of Alveolar Bone

Near the end of the second week of intrauterine life, the maxilla as well as the mandible forms a groove that is open towards the surface of the oral cavity - the tooth germs are contained in this groove, which also includes the alveolar nerves and vessels. Gradually bony septa develop between the adjacent tooth germs and much later, the primitive mandibular canal is separated from the dental crypts by a horizontal plate of bone. The word alveolar process in the strict sense is that it develops only during the eruption of the teeth.[9]

Although histologically one bone is no different from another, bone formation occurs by two main mechanisms: endochondral bone formation and intramembranous bone formation. Endochondral bone formation takes place when cartilage is replaced by bones (especially in long bones), whereas intramembranous bone formation occurs directly within mesenchyme. The development of the alveolar bone strictly follows the intramembranous type of bone formation, where the bone develops directly within the soft connective tissue. The mesenchymal cells differentiate into osteoblasts and start exhibiting alkaline phosphatase activity; this sequence of events occurs at multiple sites surrounding each tooth bud. Once begun, intramembranous bone formation proceeds rapidly. This first embryonic bone is termed woven bone. Initially this woven bone takes the form of radiating spicules and trabeculae, but progressively these fuse into thin bony plates. Early plates of intramembranous bone are structurally unsound because of poor fibre orientation, mineralization, and presence of many islands of soft connective tissue within the plates. Soon after plate formation in the mid shaft region during the establishment of intramembranous bone formation, the bone becomes polarized. The establishment and expansion of the marrow cavity turns the endosteal surface of the bone into primarily a resorbing surface, whereas the periosteum initiates the formation of most of the new bone. However, depending on the adjacent soft tissues and their growth, segments of the periosteal surface of an individual bone may contain focal sites of bone resorption. For instance, growth of the tongue; nasal cavity and lengthening of the body of the mandible all require focal resorption along the periosteal surface.[9]

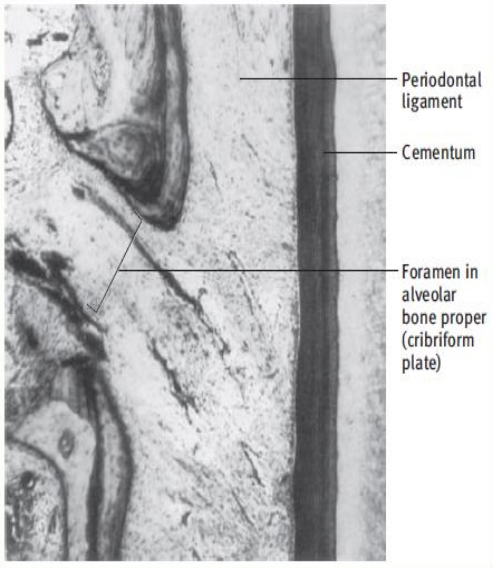
From early foetal development to full expression of the adult skeleton, a continual slow transition occurs from woven bone to lamellar bone. This transition is rapid during late foetal development and the first years of life and involves the formation of primary osteons deposited around a blood vessel in the connective tissue surrounded by trabecular or surface vessels. The primary osteon tends to be small,

with lamellae that are neither numerous or well delineated. As more osteons are formed at the periosteal surface, they become more tightly packed so that, eventually, a higher percentage of compact bone consists of osteons.

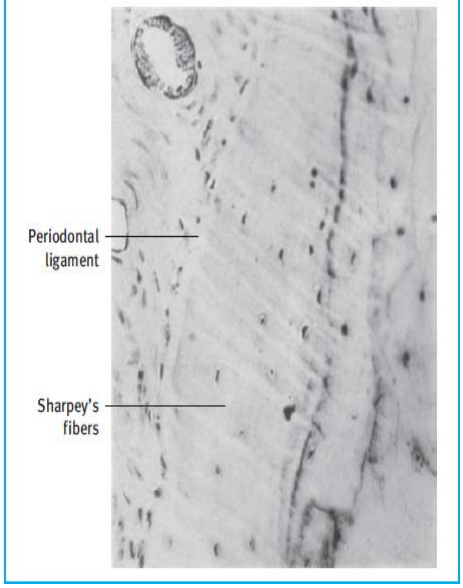
Woven bone is characterized by intertwined collagen fibrils oriented in many directions, showing wide inter fibrillar spaces. Collagen fibrils in lamellar bone however, are generally thicker and arranged in ordered sheets consisting of aligned and closely packed fibrils. It follows from these structural features that the widely spaced collagen meshwork of woven bone will accommodate more non-collagenous matrix proteins.

Just as in the case of calcified cartilage, matrix vesicles are believed to be implicated in the initiation of mineral deposition during intramembranous bone formation. The relative importance of matrix vesicles versus secreted non-collagenous matrix proteins in the control of initial events in mineralization remains unclear, and both may be implicated, independently or in succession. The sporadic observation of matrix vesicles may play a predominant role when mineralization needs to be intensely promoted.[10] During growth, part of the alveolar process is gradually incorporated into the maxillary or mandibular body while it grows at a fairly rapid rate at its free borders. During the period of rapid growth, a tissue may develop at the alveolar crest that combines characteristics of cartilage and bone-this is called chondroid bone. [9]The alveolar process forms with the development and the eruption of teeth, and conversely it gradually diminishes in height after the loss of teeth.[9]

Histologic section showing foramen in alveolar bone proper (cribriform plate) (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).



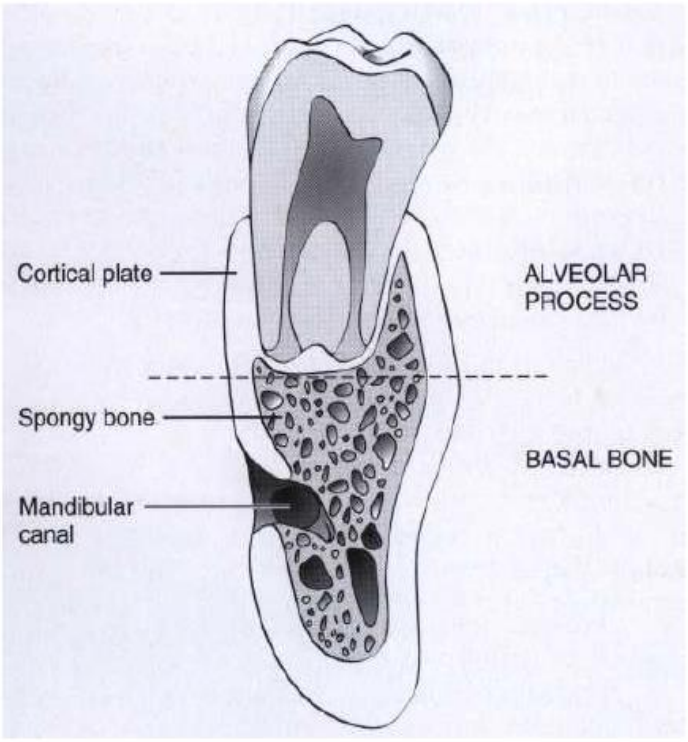
Histologic section showing Sharpey's fibers in alveolar bone proper (From Bhaskar SN: Synopsis of oral histology, St Louis, 1962, The CV Mosby Co).



(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014***.

Histologically, bundle bone generally is described as containing less intrinsic collagen fibrils than lamellar bone and exhibiting a coarse-fibered texture. Bundle bone is apposed to an outer layer of lamellar bone, but in some cases the alveolar bone can be made up almost completely of bundle bone.(9)

The cancellous portion of the alveolar bone consists of trabeculae that enclose irregularly shaped marrow spaces lined with a layer of thin, flattened endosteal cells. Wide variation occurs in the trabecular pattern of cancellous bone, which is affected by occlusal forces. The matrix of the cancellous trabeculae consists of irregularly arranged lamellae separated by deeply staining incremental and resorption lines indicative of previous bone activity, with an occasional haversian system. Cancellous bone is found predominantly in the interradicular and interdental spaces and in limited amounts facially or lingually, except in the palate. In the adult human, more cancellous bone exists in the maxilla than in the mandible.



(1) ***Newmann MG TH, Klokkevold PR, Carranza FA. . Clinical Periodontology. 10th ed ed. Philadelphia: saunders: Elsevier; 2007***

Alveolar Bone proper:

The alveolar bone proper consists partly of lamellated and partly of bundle bone which is about 0.1–0.4 mm thick. It surrounds the root of the tooth and gives attachment to principal fibers of the periodontal ligament.

Lamellated bone:

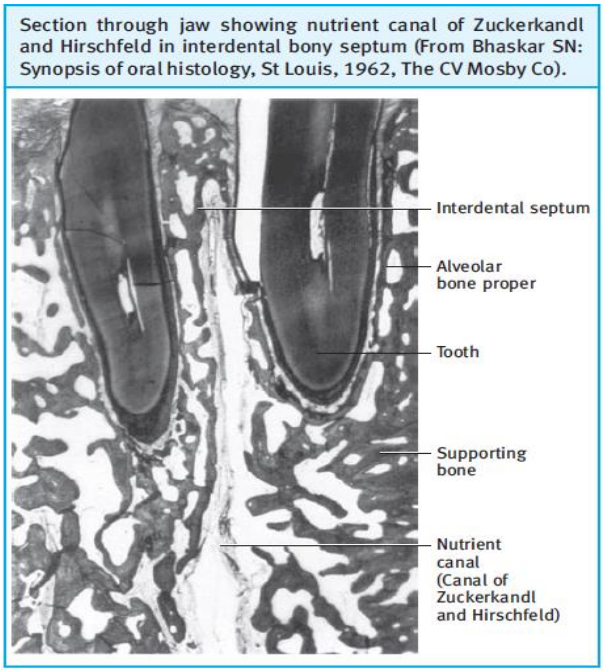
The lamellar bone contains osteons each of which has a blood vessel in a haversian canal. Blood vessel is surrounded by concentric lamellae to form osteon. Some lamellae of the lamellated bone are arranged roughly parallel to the surface of the adjacent marrow spaces, whereas others form haversian systems.

Bundle bone:

Bundle bone is that bone in which the principal fibers of the periodontal ligament are anchored. The term 'bundle' was chosen because, the bundles of the principal fibers continue into the bone as Sharpey's fibers. The bundle bone is characterized by the scarcity of the fibrils in the intercellular substance. These fibrils are arranged at right angles to Sharpey's fibers. The bundle bone contains fewer fibrils than lamellated bone and appears dark in routine haematoxylin and eosin stained sections whereas much lighter stained preparations are seen with silver than lamellated bone.[11] These fibers are mineralized at the periphery and have a larger diameter. These fibers are less numerous than the corresponding fiber bundles in the cementum on the opposite side of the periodontal ligament. The collagen adjacent to bone is less mature than adjacent to cementum. In some areas, the alveolar bone proper consists mainly of bundle bone and it is formed in areas of recent bone apposition. Lines of rest are seen in bundle bone.[9]

Radiographically, it is also referred to as the ***lamina dura***, because, of increased radiopacity, which is due to the presence of thick bone without trabeculations, that X-rays must penetrate and not to any increased mineral content.

The alveolar bone proper, which forms the inner wall of the socket, is perforated by many openings that carry branches of inter alveolar nerves and blood vessels into the periodontal ligament, and it is therefore called the cribriform plate. Bone between the teeth is called interdental septum and is composed entirely of cribriform plate. The interdental and interradicular septa contain the perforating canals of Zuckerkandl and Hirschfeld (nutrient canals) which house the interdental and interradicular arteries, veins, lymph vessels and nerves



(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014.***

The supporting alveolar bone consists of two parts:

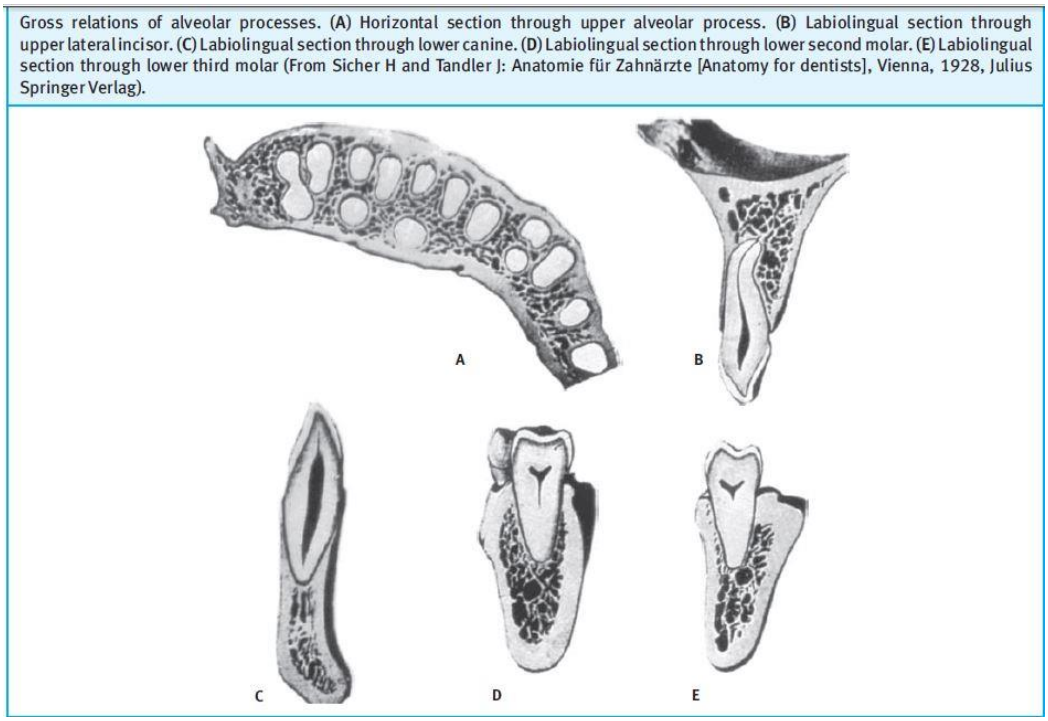
(a) Cortical plates

(b) Spongy bone

Cortical plates

Cortical plates consist of compact bone and form the outer and inner plates of the alveolar processes. The cortical plates continuous with the compact layers of the maxillary and mandibular body, are generally much thinner in the maxilla than in the mandible. They are thickest in the premolar and molar region of the lower jaw, especially on the buccal side. In the maxilla, the outer cortical plate is perforated by many small openings through which blood and lymph vessels pass. In the region of the anterior teeth of both jaws, the supporting bone usually is very thin. No spongy bone is found here and the cortical plate is fused with the alveolar bone proper. In such areas, notably in the premolar and molar regions of the maxilla, defects of the outer alveolar wall are fairly common. Such defects, where periodontal tissues and covering mucosa fuse, do not impair the firm attachment and function of the tooth.

Bone underlying the gingiva is the cortical plate. Both cribriform plate and cortical plate are compact bone separated by spongy bone.(9)



Gross relations of alveolar processes. (A) Horizontal section through upper alveolar process. (B) Labiolingual section through upper lateral incisor. (C) Labiolingual section through lower canine. (D) Labiolingual section through lower second molar. (E) Labiolingual section through lower third molar (From Sicher H and Tandler J: Anatomie für Zahnärzte [Anatomy for dentists], Vienna, 1928, Julius Springer Verlag).

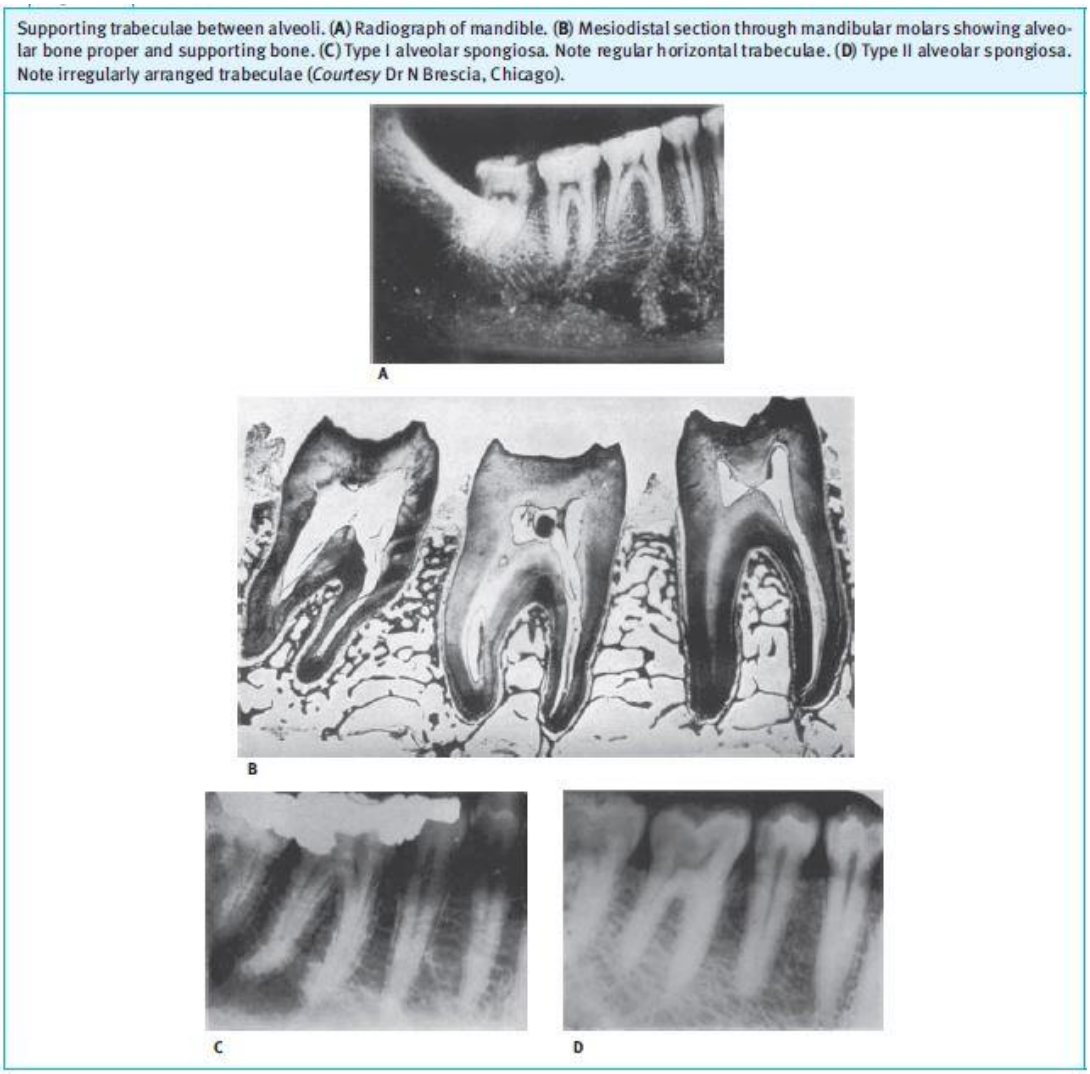
(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014.***

Spongy bone:

Spongy bone fills the area between the cortical plates and the alveolar bone proper. It contains trabeculae of lamellar bone. These are surrounded by marrow that is rich in adipocytes and pluripotent mesenchymal cells.

The trabeculae contain osteocytes in the interior and osteoblasts or osteoclasts on the surface. These trabeculae of the spongy bone buttress the functional forces to which alveolar bone proper is exposed. The cancellous component is more in maxilla than in the mandible. The study of radiographs permits the classification of the spongiosa of the alveolar process into two main types. In ***type I*** the interdental and interradicular trabeculae are regular and horizontal in a ladder like arrangement. ***Type II*** shows irregularly arranged, numerous, delicate interdental and interradicular trabeculae. Both types show a variation in thickness of trabeculae and size of marrow spaces. The architecture of ***type I*** is seen most often in the mandible and fit well into the general idea of a trajectory pattern of spongy bone. ***Type II***, although evidently functionally satisfactory, lacks a distinct trajectory pattern, which seems to be compensated for by the greater number of trabeculae in any given area. This arrangement is more common in the maxilla.

From the apical part of the socket of lower molars, trabeculae are sometimes seen radiating in a slightly distal direction. These trabeculae are less prominent in the upper jaw, because of the proximity of the nasal cavity and the maxillary sinus. In the condylar process, angle of the mandible, maxillary tuberosity, and in other isolated foci, hematopoietic cellular marrow is found.



Supporting trabeculae between alveoli. (**A**) Radiograph of mandible. (**B**) Mesiodistal section through mandibular molars showing alveolar bone proper and supporting bone. (**C**) Type I alveolar spongiosa. Note regular horizontal trabeculae. (**D**) Type II alveolar spongiosa. Note irregularly arranged trabeculae (*Courtesy* Dr N Brescia, Chicago).

(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014.***

Crest of the alveolar septa

The shape and outlines of the crest of the alveolar septa in the roentgenogram is dependent on the position of the adjacent teeth. In a healthy mouth the distance between the cementoenamel junction and the free border of the alveolar bone proper is fairly constant. If the neighbouring teeth are inclined, the alveolar crest is oblique. In the majority of individuals the inclination is most pronounced in the

premolar and molar regions, with the teeth being tipped mesially. Then the cementoenamel junction of the mesial tooth is situated in a more occlusal plane than that of the distal tooth, and the alveolar crest therefore slopes distally. Cortical bone and alveolar bone proper meet at the alveolar crest usually 1.5 to 2 mm below the level of the cementoenamel junction on the tooth it surrounds.

Histology of Bone

Osteoid is an un-mineralized bone matrix on the surface where active bone formation is taking place. Before the commencement of mineralization, osteoid is about 5-10µm thick and has linear mineralizing front. Osteoid contains type I collagen fibers, parallel to the bone surface, embedded in ground substance of proteoglycans, glycoproteins and other proteins.

All mature bones has dense outer sheet of compact bone and central medullary cavity that is filled with red or yellow bone marrow. This cavity shows a network of bony trabeculae. For this network Trabecular, spongy or cancellous bone terms are used.[9]

All bone surfaces are covered by layers of differentiated osteogenic connective tissue.[1] The tissue covering the outer surface of bone is termed periosteum, whereas the tissue lining the internal bone cavities is called endosteum. Periosteum consists of two layers- outer layer is irregular connective tissue called fibrous layer and inner osteogenic layer consisting of bone cells called cambium layer. The inner surfaces of compact and cancellous bone are covered by a thin cellular layer called endosteum**.** In resting adult bone, quiescent osteoblasts and osteoprogenitor cells are present on the endosteal surfaces. These cells act as reservoir of new bone forming cells for remodelling or repair.

At the periosteal and endosteal surfaces, the lamellae are arranged in parallel layers surrounding the bony surface and are called circumferential lamellae. Deep to the circumferential lamellae, the lamellae are arranged as small concentric layers around a central vascular canal. Haversian (vascular) canal (about 50 µ in diameter) and the concentric lamellae together are known as the osteon or Haversian system. There may be up to 20 concentric lamellae within each osteon.

Osteon is the basic metabolic unit of bone. A cement line of mineralized matrix delineates the Haversian system. This cement line contains little or no collagen and is strongly basophilic, because it has a high content of glycoproteins and proteoglycans. It marks the limit of bone erosion prior to the formation of osteon, and is therefore also known as reversal line. This line appears to be highly irregular as it is formed by the scalloped outline of the Howship's lacunae. This line has to be distinguished from the more regular appearance of the resting line, which denotes the period of rest during the formation of bone (Fig. 9.4). The collagen fibers within each lamella spiral along the length

of lamella, but have different orientations to those in adjacent lamella. This pattern is to withstand torsion stresses.

Adjacent haversian canals are interconnected by Volksmann's canals, channels that contain blood vessels, creating a rich vascular network, throughout the compact bone. Osteocytes are present in lacunae, at the junctions of the lamellae. Small canaliculi radiate from lacunae to haversian canal to provide a passage way through the hard matrix. The canaliculi connect all the osteocytes in an osteon together. This connecting system permits nutrients and wastes to be relayed from one osteocyte to the other. The adult bones, between the osteons, contain interstitial lamellae, which are remnants of osteons, left behind during remodelling.

Cells of Bone

Different cells are responsible for the formation, resorption, and maintenance of osteo architecture. Two cell lineages are present in bone, each with specific functions: (1) osteogenic cells, which form and maintain bone, and (2) osteoclasts, which resorb bone. Osteogenic cells have variable morphology (including osteoprogenitors, preosteoblasts, osteoblasts, osteocytes, and bone lining cells) representing different maturational stages.[10]

Osteogenic cells arise from primitive mesenchymal cells contained in the stroma of bone marrow and from pericytes adjacent to small blood vessels in connective tissue[12-14] Differentiation of osteogenic stem cells requires stimulation by transforming growth factor β (TGF- β), and bone morphogenetic protein 2 (BMP-2).[12] Differentiation markers include the expression of osteocalcin, osteonectin, alkaline phosphatase, and bone sialoprotein.

Preosteoblasts:

Periosteal and connective tissue preosteoblasts have the morphologic appearance of an inactive fibroblast, containing many free ribosomes, only a few profiles of rough endoplasmic reticulum (RER), and a small Golgi complex. During differentiation, pre-osteoblasts make contact with adjacent pre-osteoblasts or with previously differentiated osteoblasts, develop cytoplasmic polarity and greatly increase the amount of RER and Golgi cisternae.

Mesenchymal cell differentiation into the osteogenic cell line is preceded by the activation of the Osf2/Cbfa 1 gene, which appears to serve as a master gene to turn on the expression of osteocalcin,

osteopontin, bone sialoprotein, and collagen synthesis.[15, 16] The Osf2/Cbfa1 protein is induced by bone morphogenetic protein 7 and is decreased by vitamin D3.[15]

Osteoblasts:

Osteoblasts are mononucleated cells responsible for the synthesis and secretion of the macromolecular organic constituents of bone matrix. These cells are derived from osteoprogenitor cells of mesenchymal origin, which are present in the bone marrow and other connective tissues. Periosteum also serves as an important reservoir of osteoblasts, particularly during childhood growth, after skeletal fractures or with bone forming tumors.[9]

Although osteoblasts are differentiated cells, both pre-osteoblasts and osteoblasts can undergo mitosis during prenatal development and occasionally during postnatal growth. Both cell types exhibit high levels of alkaline phosphatase activity on the outer surface of their plasma membrane. The liberated phosphate contributes to the initiation and progressive growth of bone mineral crystals.[10] Alkaline phosphatase is expressed at high levels in osteoblasts and is preferentially distributed along the apical surface and on cytoplasmic processes extending into the osteoid layer.[17]

Osteoblasts secrete the collagenous and non-collagenous proteins and the proteoglycans of bone matrix. They secrete matrix metalloproteinases (MMPs) into the extracellular bone matrix in an inactive form , along with tissue inhibitors of metalloproteinases.[18, 19] Osteoblasts also contain plasma membrane calcium adenosine triphosphatase (ATPase), also known as the calcium pump, a transporter that actively pumps Ca" into the extracellular space using the energy of adenosine triphosphate (ATP) hydrolysis.[20] At the innermost surface of the tooth alveolus, the positional arrangement of alveolar bone osteoblasts must accommodate the interdigitating portions of the periodontal ligament collagen fibers known as Sharpey’s fibers that insert into the bone.[21] Thus in three dimensions these cells form an extensively perforated sheet of contiguous osteoblasts which produce alveolar bone matrix proper that embed continuously in the remodelling of periodontal ligament fibers in a precise manner.[22]

Osteoblasts have a lifespan of 1 month in which thirty percent of osteoblasts become embedded in the organic matrix as osteocytes, while remaining appear to undergo apoptosis.[2] Electron microscopic studies provide additional evidence that adjacent osteoblasts form gap junctions and adhesive contacts across narrow intercellular spaces. During mineralization of the bone matrix, the lateral intercellular spaces appear to be sealed by tight junctions thereby creating a bone compartment distinct from the general interstitial spaces.[23]

Ninety percent of bone matrix consists of type I collagen (with a minor fraction of type V collagen). The remaining 10% of bone matrix is composed of several non-collagenous proteins and small proteoglycans (decorin and biglycan). The osteoblast's Golgi complexes, their presecretory and secretory granules that arise in it resemble those observed in active fibroblasts and odontoblasts. Secretory granules are roughly 300 nm long, 30 nm wide and containing a moderately dense filamentous material. These secretory granules are present in the Golgi complex and in the apical cytoplasm.

An intact micro tubular network is required for the translocation of secretory granules into the secretory pole of the cell. Fusion of these granules to the cell membrane and the extrusion of their contents give rise to un-mineralized bone matrix, or the osteoid layer. Osteoid like pre-dentin undergo a period of "maturation" before it becomes mineralized. Thus, there is a band of osteoid approximately 10µm deep between osteoblasts and the mineralization front. Numerous cytoplasmic processes arising from the apical cell surface of the osteoblast penetrate the osteoid layer. These cytoplasmic processes make gap junctional contacts with cytoplasmic processes arising from osteocytes.

Functions of Osteoblasts

The main function of osteoblast is the formation of new bone via synthesis of various proteins and polysaccharides. Other functions include the regulation of bone remodelling and mineral metabolism. Osteoblasts also play a significant role in the mineralization of osteoid. Osteoblasts secrete type I collagen which is widely distributed and not unique to osteoblasts whereas, osteocalcin and cbfa-1 (osteoblast specific transcription factor) are specific to cells of osteoblast lineage. These provide useful markers of osteoblast phenotype. Osteoblasts also secrete small amounts of type V collagen, osteonectin, osteopontin, RANKL, osteoprotegerin, proteoglycans, latent proteases and growth factors including bone morphogenetic proteins.

These exhibit high levels of alkaline phosphatase on outer surface of plasma membrane which is used as a cytochemical marker to distinguish preosteoblasts from fibroblasts. Osteoblasts express receptors for various hormones including PTH,[24] vitamin D3[25], Oestrogen and glucocorticoids, which are involved in the regulation of osteoblast differentiation. The osteoblasts recognize the resorptive signal and transmit it to the osteoclast.[10]

Osteoblasts promote formation of new blood vessels through secretion of vascular endothelial growth factor (VEGF), a mitogen for endothelial cells. The development of new blood vessels is an essential component of new bone formation and the repair of bone defects.[26]

Osteocytes:

Osteocytes are the post mitotic cells lying within the bone itself and represent ‘entrapped’ osteoblasts. There are about 25000 osteocytes per cubic millimetre of bone. The number of osteoblasts that become osteocytes, depends on rapidity of bone formation In prepared ground sections of bone, the osteocytes themselves are lost but the spaces or lacunae which were previously occupied by these cells, seem to be filled with air or cell debris and appear black in routine transmitted light sections.[2] There are approximately ten times more osteocytes than osteoblasts in an individual bone. The life span of osteocytes exceeds that of active osteoblasts, which is estimated to be only three months in human bones.[9]

Fig. 13.15 Electron microscopic appearance of demineralized bone showing an osteocyte. The cell has few organelles and possesses cell processes (arrowed) extending into canaliculi. A pericellular space is seen between the cell membrane and the wall of the lacuna and is probably related to a shrinkage artefact (×5300).

(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014.***

The osteocyte develops many cytoplasmic processes that faces the direction of the overlying osteoblasts and bone-lining cells, where the nutrient supply is highest.(27) Within the bone matrix, the osteocyte reduces in size and creates a space around it which is called the osteocytic lacuna. Under the electron microscope, it has been observed that a thin layer of uncalcified tissue lines the lacuna which appears as ovoid or flattened. Narrow extensions of these lacunae form channels called canaliculi within which osteocytic processes are present. Canaliculi do not usually extend through and beyond the reversal line surrounding an osteon and will not communicate with neighbouring systems. These processes also contain bundles of microfilaments and some smooth endoplasmic reticulum. At the distal end, these processes contact the processes of adjacent osteocytes through gap junctions. They also maintain contact with osteoblasts and bone lining cells on the surface.

Osteocytes also sense the changes in environment and send signals that affect response of other cells involved in bone remodelling. This interconnecting system maintains the bone integrity and bone vitality. Failure of the interconnecting system between osteocytes and osteoblasts leads to sclerosis and death of bone.(9)

Mature inactive osteocytes possess an ellipsoid cell body with long axis parallel to the surrounding bony lamellae. The nucleus is oval with a narrow rim of faintly basophilic cytoplasm. The cell has very few organelles but contain sufficient rough endoplasmic reticulum and large Golgi complex (regions) which suggest that these cells are capable of keeping the bone matrix in a state of good repair. Osteocytes also secrete a few matrix proteins and older osteocytes have lysosomes.

Old osteocytes retract their processes from the canaliculi and may get plugged with debris when become dead. The death of these osteocytes leads to resorption of the matrix by osteoclasts.

Transformation of osteoblasts into osteocytes:

At the end of bone forming phase osteoblasts can have one of four different fates

(a) Become embedded in the bone as osteocytes

(b) Transform into inactive osteoblasts and become bone lining cells

(c) Undergo apoptosis

(d) Trans differentiate into cells that deposit chondroid or chondroid bone.

The transformation process is proposed to involve three cells, pre-osteoblasts which differentiate into osteoblasts which in turn get entrapped as osteocytes. Pre-osteoblasts are less cuboidal in shape and are located at a distance from the bone surface. These do not deposit bone matrix, but can still divide. These cells produce type I collagen precursor molecules which later assemble into collagen fibrils after post transitional modification.

Pre-osteoblasts differentiate into active bone matrix secreting osteoblasts, which are cuboidal in shape, and ultimately deposit the bone matrix. As the bone matrix deposition continues, osteoblasts become embedded in the secretory product, the osteoid. Cells at this early stage of osteoblast to osteocyte differentiation are called large osteocytes. These cells are large with a well-developed Golgi apparatus for collagen storage.

Four schemes have been proposed to explain how an osteoblast could get trapped within bone matrix. Osteoblasts are unpolarised and lay down bone in all directions, i.e. the cells become trapped in their own secretions. Individual osteoblasts are polarized but those within same generation are polarized differently to those in adjacent layers. As a result, bone is deposited in all directions and osteoblasts become trapped. Osteoblasts of each generation are polarized in the same direction and hence one generation buries the preceding one in bone matrix.

Within one generation, some osteoblasts slow down rate of bone deposition or stop laying down bone, so that they become trapped by the secretion of their neighbouring cells.[9]

At the ultrastructural level, the appearance of osteocytes varies according to their position in relation to the surface layer. Osteocytes which are newly incorporated into bone matrix from the osteoblast layer have high organelle content similar to osteoblasts. However, as they become more deeply situated

with continued bone formation, they appear to be less active. The cells are then seen to have a nucleus and thin ring of cytoplasm containing few organelles, reflecting the decreased cellular activity (Fig. 13.15). However, some secretions are likely to be necessary for osteocyte function as they are involved in the reception and transduction of mechanosensory information.[2]

In the development of bone trabeculae as the thickness of bone approaches its physiologic limit, the recruitment of new pre-osteoblasts to the bone surface is diminished. Under these conditions whenever a new osteocyte is formed, the remaining osteoblasts must spread over a greater area of the bone surface, then eventually bone formation ceases at that site and the resting bone surface is covered by extremely flattened bone lining cells.[28] Between the bone-lining cell and the mineralized bone surface there is no osteoid.

The reduction in osteoblastic production of osteoid is probably regulated by the inability of the deepest osteocytes to obtain adequate nourishment and/or by systemic or paracrine hormonal signals impinging on the osteoblastic layer. The ability of osteocytes to communicate via gap junctions with the osteoblasts, as well as with the bone-lining cells, is probably a key pathway for the transmission of factors regulating and coordinating these changes.

Bone-lining cells:

When bone surfaces are neither in the formative nor resorptive phase, the bone surface is lined by a layer of flattened cells termed bone-lining cells, with little or no osteoid being present. Like osteoblasts, the bone-lining cells are connected to underlying osteocytes. They show little sign of synthetic activity as evidenced by their reduced organelle content and thus may be regarded as post proliferative osteoblasts.[2]

It is estimated that 80% of the total bone surface is covered by bone-lining cells. Approximately 20 bone-lining cells line every linear millimeter of resting bone surface. Bone-lining cells act as gatekeepers, protecting the bone surface from osteoclasts, regulating the ionic composition of bone fluid, and regulating the initiation of new bone formation or bone resorption.[28, 29]

Bone-lining cells are not connected by zonula occludens junctions; thus there is no tight cytoplasmic barrier between bone and the general body fluids. Despite the lack of occluding junctions, differences

in ionic composition exist between bone fluid and the interstitial fluids.[30, 31] Bone-lining cells can be stimulated to incorporate thymidine, which in turn divide and give rise to osteoblasts. The osteoprogenitor capacity of bone-lining cells is important in responding to increased strain and in forming a fracture callus during bone repair[32]

Thus by covering the surface of bone, they may 1) play a role in calcium and phosphate metabolism, 2) protect the surface from any resorptive activity by osteoclasts, 3) Participate in initiating bone remodelling. Bone-lining cells could also be a source of osteoprogenitor cells and be reactivated to form osteoblasts.[2]

Osteoprogenitor cells:

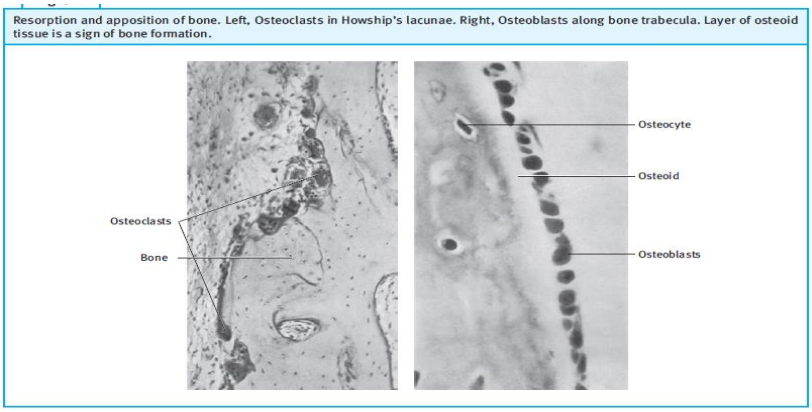
Stem cells have the ability to maintain their numbers throughout life. When a stem cell divides, one of the daughter cells remains as a stem cell, while the other can differentiate into another cell type. This property of self-renewal is a unique property of stem cells. In the case of alveolar bone, the cells derived from the initial stem cells and that eventually give rise to osteoblasts are termed osteoprogenitor cells. They reside in the layer of cells beneath the osteoblast layer in the periosteal region, in the periodontal ligament or in the marrow spaces.

The osteoprogenitor cells are fibroblast-like cells, with an elongated nucleus and few organelles. Their life cycle may involve up to about eight cell divisions before reaching the osteoblast stage. There is a gradual acquirement of osteoblast like features associated with an ordered increase in gene expression. Initially, genes related to cell growth are expressed (such as c-myc, c-fos and Cbfa1), followed by genes related to osteoblast products such as type I collagen, fibronectin, some growth factors and alkaline phosphatase. Finally, genes are expressed related to products associated with mineralization (such as osteocalcin, osteopontin and bone sialoprotein).[2]

Osteoclasts:

The word “osteoclast” is derived from the Greek words for “bone and broken”. Osteoclast is a type of bone cell that removes bone tissue by removing the mineralized matrix of bone.(9) Osteoclasts lie in resorption bays called Howship’s lacunae. Osteoclast is a large cell approximately 40–100 μm in diameter with 15 to 20 closely packed nuclei. Osteoclasts with many nuclei resorb more bone than osteoclasts with few nuclei. The different nuclei are proposed to be of different ages and there is evidence of apoptosis. These cells are variable in shape due to their motility. The cytoplasm of the osteoclast has presence of acid phosphatase that distinguishes the osteoclast from other multinucleated

giant cells. Mitochondria are extensive and distributed throughout the cytoplasm, except below the ruffled border. Rough endoplasmic reticulum is relatively sparse for the size of the cell. Golgi complex is extensive and arranged in stacks. The cytoplasm also contains microtubules, which transport vesicles between Golgi stacks and ruffled membrane. Cathepsin containing vesicles and presence of vacuoles close to the ruffled border indicate the resorptive activity of these cells.



(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014.***

Tissue culture studies indicate that osteoclasts are highly motile and the cells will resorb only when attached to bone, evidence from the presence of elongated 'snail track' resorption lacunae on bone surfaces suggest that osteoclasts also move across the bone in vivo.(33) Osteoclasts are recruited only when required and there is, consequently, no significant reservoir of inactive osteoclasts. The lifespan of osteoclasts is not known with any certainty, although it is thought to be at least 10–14 days after which the cells finally undergo apoptosis.(2)

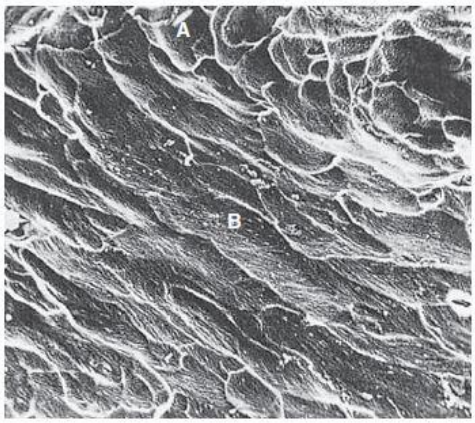


Fig. 13.32 SEM of periosteal surface of bone (lacking Sharpey fibres) undergoing resorption. In addition to pit-like resorption lacunae (A), elongated 'snail-track' resorption lacunae are evident (B) (Anorganic preparation; ×300). Courtesy of Professor S.J. Jones.

(9) ***Kumar G. Orban's Oral Histology & Embryology. 13th Ed ed: Elsevier Health Sciences; 2014.***

Zones of osteoclasts:

When actively resorbing, osteoclasts are highly polarized cells and exhibit four main different membrane domains namely the ruffled border and the sealing zone in contact with bone and the basolateral and functional secretory domains away from the bone. The ruffled border is that part of the cell that lies adjacent to bone and where resorption occurs. More than one ruffled border may be present at any one time.

At the light microscope level it often has a foamy or striated appearance. At the ultrastructural level, the ruffled border is composed of many tightly packed microvilli adjacent to the bone surface, providing a large surface area for the resorptive process. It has been postulated that products from the osteoclast (such as protons and proteases) are discharged (exocytosed) at the lateral aspects of the ruffled border and the resulting degraded matrix absorbed (endocytosed) in the central region of the ruffled border, thereby allowing for continuous activity.[(2)]

The sealing zone (also referred to as the annular or clear zone) at the periphery of the ruffled border separates the ruffled border from the basolateral membrane. Here, the plasma membrane tends to become smooth and the organelle-free cytoplasm beneath it contains numerous contractile actin microfilaments (surrounded by two vinculin rings). It has been suggested that this sealing zone may serve to attach the cell very closely to the surface of the bone thus creating an isolated microenvironment in which resorption of bone can take place without diffusion of the protons and proteases produced by the cell into adjacent soft tissue.[(34)] This isolated microenvironment can be considered as a specialized ‘extracellular’ lysosome.

The membrane regions of the osteoclast away from the bone are subdivided into functional secretory and basolateral domains. The functional secretory domain opposite the ruffled border is a collection site of vesicles. It is believed that bone matrix degraded at the ruffled border passes across the cell in these vesicles to be exocytose here (transcytosis). The basolateral surface may be a regulatory surface for receiving messages from neighbouring cells.[(2)]

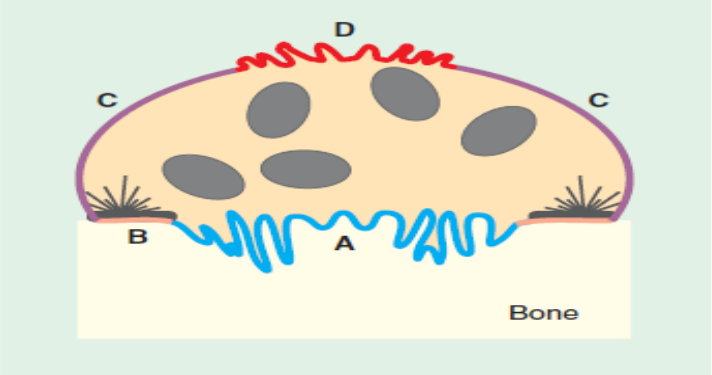


Fig. 13.19 Diagram showing four zones on osteoclast.
A = ruffled border; B = sealing zone; C = basolateral zone;
D = functional secretory domain.

(2) ***Berkovitz BK, Holland GR, Moxham BJ. Oral Anatomy, Histology and Embryology E-Book: Elsevier Health Sciences; 2017.***

Non collagenous proteins:

Non-collagenous proteins comprise the remaining 10% of the total organic content of bone matrix. Most are endogenous proteins produced by bone cells while others like albumin are derived from sources such as blood and become incorporated into bone matrix during osteosynthesis. The non-collagenous proteins function in bone matrix mineralization, cellular adhesion and regulation of bone cell activity during coupling of bone formation and resorption.

1) **Osteocalcin:** Osteocalcin is a low-molecular weight protein containing three alpha carboxyglutamic acid residues per molecule (also called Gla containing protein). Osteocalcin is one of the most abundant non-collagenous proteins of bone matrix. Vitamin K is required for the synthesis of the α-carboxy glutamic acid residues. These residues provide calcium-binding sites that are believed to play a role in bone matrix mineralization or in the regulation of crystal growth. The role of osteocalcin in bone mineralization is supported by the observation that osteocalcin messenger ribonucleic acid (mRNA) is localized in osteoblasts and simultaneously in the mineralized bone matrix. It has been localized over the mineralized portion of bone and in acellular cementum.(35, 36) Osteocalcin is a glycoprotein secreted by osteoblasts and is regulated by vitamin D3 and parathyroid hormone. The carboxy terminal segment of osteocalcin acts as a chemoattractant to osteoclast precursors, suggesting a role in bone resorption.(9) It is also believed to be involved in bone calcification as it is a calcium binding protein. It is used as a marker of new bone formation.

2) **Bonesialoprotein:** Osteopontin and bone sialoprotein were previously, termed as bone sialoproteins I and II respectively. These have been demonstrated in alveolar bone using immunohistochemistry. Both proteins are heavily glycosylated and phosphorylated, with high levels of acidic amino acids. Glutamic acid is predominant in bone sialoprotein and aspartate is predominant in osteopontin. Thus bone sialoprotein can bind tightly to hydroxyapatite as well as to cells. Immunocytochemical localization of bone sialoprotein showed that it is not found in osteoid but is restricted to the mineralized bone matrix.[17] Bone sialoprotein, which has a molecular mass of about 33,000 kDa, contains the RGD tripeptide sequence, a motif contained in attachment proteins that interact with cell surface integrins. These have been shown to inhibit mineral deposition when present in solution. However, when bound to a solid substrate they can act as promoters of mineral deposition.(37) It has been proposed that the association of osteocalcin and/or bone sialoprotein with collagen fibrils creates locally high concentrations of calcium, leading to precipitation of mineral.

3) **Osteopontin:-** osteopontin is a charged protein that is expressed by differentiating bone cells.[35, 38, 39] Osteopontin contains several serine phosphorylation sites and a stretch of nine negatively charged aspartic acid residues that bind calcium. Osteopontin also has an RGD sequence with specificity toward cell surface integrins (in this case to the vitronectin receptor, αvβ3). Osteopontin is concentrated in small globular deposits in bone matrix and in the lamina limitans at the bone surface, suggesting that it plays a role in bone mineralization and in the attachment of osteoblasts and osteoclasts to bone matrix. It is expressed by a variety of cell types and is found in many soft tissues, suggesting that it may have a role in soft tissue organization. Its significance in development may be related to its increased expression during mesenchymal cell migration.[39]

4) **Osteonectin:** Osteonectin comprises about 25% of non-collagenous proteins. It is bound to collagen and hydroxyapatite crystals. It is a secreted calcium binding glycoprotein that interacts with extracellular matrix molecules. It has been proposed that, it may play a role in the regulation of cell adhesion, proliferation, modulation of cytokine activity and in initiating hydroxyapatite crystal formation. It is expressed by osteoprogenitor cells, osteoblasts, and newly formed osteocytes. Osteonectin is a 32-kDa protein with calcium- and collagen-binding domains.[40] Numerous cells of soft tissues, such as periodontal ligament (PDL) fibroblasts and endothelial cells also produce osteonectin. It may have a

generalized function in a calcium-mediated organization of extracellular matrices because of its ability to bind to various collagens and substrate adhesion molecules.[37]

5) **Biglycan and Decorin:** Proteoglycans are also present in the bone matrix. A large chondroitin sulfate proteoglycan, has been extracted from the non-mineralized bone matrix, while two small proteoglycans, biglycan and decorin (chondroitin sulfate proteoglycan I and II respectively) have been found in EDTA extracts of bone. Decorin and biglycan comprise < 10% of the non-collagenous proteins in bone, but this decreases with maturation of bone. A third small proteoglycan (chondroitin sulfate proteoglycan) has been found entirely associated with mineral crystals. Biglycan is more prominent in developing bone and has been mineralized to pericellular areas. The precise function of biglycan is unknown, but similar to decorin, it can bind TGF-β and extracellular matrix macromolecules, including collagen and thereby regulate the fibrillogenesis. Decorin, as the name suggests, binds mainly within the gap region of collagen fibrils and decorates the fibril surface. The primary calcification in bones is reported to follow removal of the decorin and fusion of collagen fibrils.[41]

6) Lysyl oxidase and tyrosine rich acidic matrix proteins (TRAMP) are components of demineralized bone and dentin matrix. Lysyl oxidase is a critical enzyme for collagen crosslinking. TRAMP is also known as dermatopontin that binds decorin and TGF-β which (these proteins together) regulate the cellular response to TGF-β.[42]

Other proteins that are found in bone include procollagen peptides, thrombospondin, fibronectin and vitronectin. These are the proteins that modulate cell attachment and the enzyme alkaline phosphatase, which is important for mineralization to occur. Of the proteins that are not produced by osteoblasts but accumulate in bone, matrix gla protein and a2Hs-glycoprotein bovine fetuin are of particular interest with respect to regulation of mineralization. Although a clear bone phenotype was not evident in either MGP-null or a2Hs-glycoprotein-null mice, definitive effects on ectopic mineralization were evident, indicative of a regulatory role in bone similar to that observed with osteocalcin.[43]

Matrix components:

Although alveolar bone and the alveolar process have specialized features relating to their funtional properties, the composition of the extracellular matrix of alveolar bone appears to be similar to the bone tissues as indicated largely by immunohistochemical analysis. The bone matrix is formed

from a scaffold of interwoven collagen fibers within and between which small uniform plate-like crystals of carbonated hydroxyapatite are deposited. Other proteins including proteoglycans, acidic glycosylated and non-glycosylated proteins, associate with and regulate the formation of collagen fibrils and mineral crystals, or provide continuity between matrix components and between the matrix and cellular components. In addition, small amounts of carbohydrate and lipid contribute to the organic matrix, which comprises approximately one-third of the matrix while the inorganic components account for the remaining two-thirds. Calcium and phosphate in the form of poorly crystalline, carbonated apatite, also described as Dahllite, predominates the inorganic phase, largely replacing the water component of the soft dense connective tissues that include the periodontal ligament and gingiva.[43]

Collagen

Collagen comprises the major (80-90%) organic component in mineralized bone tissues.
Type-I collagen (>95%) is the principal collagen in mineralized bone, together with type V <5%) collagen. The type I collagen forms heterotypic fiber bundles that provide the basic structural integrity of connective tissues. In addition to the presence of type I and V collagen in alveolar bone, both type III and XII collagens are also present. The type III collagen is present as mixed fibers with type I collagen in Sharpey's fibers that insert from the periodontal ligament into the lamellar bone lining the alveolus to provide a stable connection with the tooth. The expression of type XII collagen is related to mechanical strain and the alignment of collagen fibres, as demonstrated in the maturation of the periodontal ligament. While the type I, V, XII collagen are expressed by osteoblasts; type III and some of the type XII collagen fibers appear to be produced by fibroblasts during the formation of the periodontal ligament.[44]

The collagen fibrils in bone are stabilized by intermolecular cross-linking involving lysines and modified lysines that form pyridinium ring structures (pyridinolines). These cross links are primarily responsible for the high tensile strength of collagen fibers, which are formed from fibrils as higher order structures laid down in a specific orientation by the bone forming osteoblasts. In rapidly forming (woven) bone that is produced during early development and repair sites, these fibers are extensively inter woven, leaving a substantial volume of interfibrillar that is largely occupied by mineral crystals and associated acidic proteins. In mature lamellar bone, the collagen fibers form highly organized sheets in which successive layers of fibers are oriented perpendicular to each other with little inter fibrillar space. In both woven and lamellar bone, the mineral crystals within the collagen fibrils are believed to form initially within the gap region between successive

collagen molecules such that their c-axes are aligned with the long axis of the collagen fibril. Additional formation of crystals and crystal growth occurs in the channels formed by the gap regions and in the spaces that exists between the collagen molecules, which have a characteristic intermolecular spacing.[43]

Cell Kinetics

Formation of Osteoblasts:

Stem cells for osteoblasts in the periodontal ligament may be derived from perivascular cells in the ligament as well as from the adjacent bone marrow. From a stem cell, intermediate progenitor cell forms have been described leading to post mitotic osteoblasts. These forms include osteo progenitors (immature and mature forms) and pre osteoblasts and involve about eight cell divisions. As cells generally increase in size during differentiation, the size of the cell has been used in an attempt to distinguish osteoblasts and their precursors. The process of bone formation requires 1) cell proliferation, 2) the synthesis and secretion of an extracellular matrix and 3) mineralization of the matrix. The process is characterized by a decreasing proliferative capacity and an increasing degree of differentiation of the cells.[2]

Among the earliest markers to indicate that a stem cell is progressing along an osteogenic phenotype are the expression of the nuclear transcription factor core binding factor 1 (Cbfa1, also called Runx2) which is responsible for regulating the production of a number of important protein products in bone matrix and specific cell surface markers (e.g. STRO-1).

Supporting the importance of Cbfa1 is the observation that knockout mice lacking this gene lack bone. The induction of Cbfa1 involves the action of growth factors such as TGFβ and BMP-2. Osteoprogenitor cells can be identified by the progressive expression of molecules such as type I collagen, alkaline phosphatase, osteopontin and by the appearance of specific receptors such as PTH1R. In the pre- osteoblast, the concentration of many of the osteogenic markers seen in the osteoprogenitor cells increases and there is still some limited proliferation. The cell is relatively undifferentiated and there is little roughened endoplasmic reticulum. In the post mitotic osteoblast, even more activity of the markers first seen in pre-osteoblasts is present and there is marked development of roughened endoplasmic reticulum and Golgi material. In addition, new molecules related to mineralization (e.g. osteocalcin) and cell adhesion molecules make their appearance.[43]

The differentiation of osteoblasts and their subsequent lifecycle is regulated by numerous factors, among which will be transcription factors (e.g. TAZ, Msx2, Dlx5 and Osterix), growth factors,

cytokines (e.g. BMP, TGFβ, IGF, IL-1) (Fig. 13.21), and hormones (e.g. glucocorticoids, parathyroid hormone). The actions of such molecules may differ according to the stage of differentiation and concentration whereas the final stage of the osteoblast concerns its entrapment in bone matrix, where it becomes the osteocyte.(45)

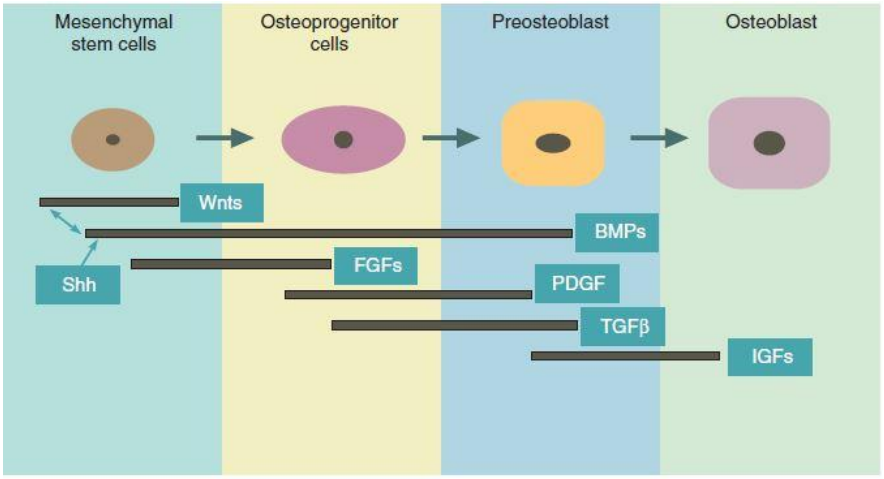


Fig. 13.21 The main stages of growth factor activity during osteoblast differentiation. BMPs = bone morphogenetic proteins; FGFs = fibroblast growth factors; IGFs = insulin-like growth factors; PDGF = platelet-derived growth factor; Shh = sonic hedgehog; TGFβ = transforming growth factor-β; Wnts = Wnt proteins. Redrawn after Hughes FJ et al 2000 *Periodontology 2000* 41: 48–72, with permission of Blackwell Publishing, a subsidiary of John Wiley & Sons Inc.

(2) ***Berkovitz BK, Holland GR, Moxham BJ. Oral Anatomy, Histology and Embryology E-Book: Elsevier Health Sciences; 2017.***

Formation of Osteoclasts:

Unlike the other cells associated with bone (e.g. osteoblasts, osteocytes, bone-lining cells), osteoclasts are not derived from stromal cells but from blood cells. The pluripotent stem cell is of the monocyte/macrophage lineage. Early important transcription factors indicative of its eventual fate are c-Fos and PU-1.(46) Differentiation from the myeloid progenitor to the mononuclear osteoclast precursor involves the activity of many factors, two of the most important factors are M-CSF and receptor activator of nuclear factor κ B ligand (RANKL), also known as ODF (osteoclast differentiation factor), OPGL (osteoprotegerin ligand) and TRANCE (TNF related activation-induced cytokine). These two factors are produced by osteoblast/stromal cells. As osteoclast progenitors have a receptor for MCSF (c-Fms) and a receptor (RANK) for RANKL, close association between the two cell types drives the differentiation of the osteoclast precursors into mononuclear pre-osteoclasts. As

a method of controlling the rate of formation of osteoclast precursors, the osteoblast also secretes osteoprotegerin (OPG), which acts as a soluble decoy molecule by binding with RANKL and thereby inhibiting osteoclast formation.[43] Pre-osteoclasts also contain receptors for calcitonin.

Fusion of mononuclear osteoclasts into multinucleated osteoclasts and their subsequent activation is also driven by the RANKL/RANK system. Many complex membrane interactions must occur when cells are undergoing fusion to become multinucleated. Initially the cell is non-polarized and it is only on attaching to bone by cell–matrix interactions (involving Trans membrane receptors such as integrins and matrix components such as collagen and osteopontin) that the osteoclast becomes polarized and develops the ruffled border, sealing zone and systems to successfully demineralize bone and degrade its organic matrix. Following its resorptive phase, osteoclasts are thought to be removed by apoptosis.

Two additional important factors involved in the activation of osteoclasts are acidification and hypoxia. RANKL is an important agent driving the development and activation of osteoclasts. However when added to osteoclasts in culture, RANKL by itself has little effect on bone resorption at physiological pH of blood (approximately pH 7.4) but, when combined with low pH there is a dramatic increase in resorption. In this aspect it is worth noting that the activity of a number of cytokines and growth factors results in the release of hydrogen ions from the affected cells.[47]

Hypoxia: There is evidence to suggest that a reduction in oxygen levels in the microenvironment of bone tissue provides a stimulus for osteoclastogenesis although the mechanism is poorly understood. The hypoxia may be associated with acidification, or it may cause the release. The importance of factors listed above in association with the formation and activity of osteoclasts has been deduced from studies designed to produce deficiencies or overexpression of the factor. Thus, mice lacking the ability to produce M-CSF, RANKL or RANK do not develop osteoclasts.[2] They are therefore unable to resorb bone and thick bone is produced (osteopetrosis). Their teeth may be prevented from erupting (because of the inability to resorb bone overlying the erupting teeth) but this can be corrected by restoring the missing factor. In contrast, mice lacking the ability to produce OPG (the osteoclast inhibitor) have increased numbers of osteoclasts and develop osteoporosis.[43]

Radiographic Appearance of Alveolar Bone in Health:

1. Lamina Dura:

A radiograph of sound teeth in a normal dental arch demonstrates that the tooth sockets are bounded by a thin radio opaque layer of dense bone termed "lamina dura", a name derived from its radiographic appearance. This layer is continuous with the shadow of the cortical bone at the alveolar crest. It is slightly thicker and less mineralized than the trabeculae of the cancellous bone. Its radiographic appearance is due by the fact that the x-ray beam passes tangentially through the thickness of the bony wall. On the tooth side, a thin dark shadow represents the space occupied by a periodontal membrane; and on the opposite aspect lies the cancellous bone of the alveolar process.(48)

The thickness and density of the lamina dura on the radiograph varies with the amount of occlusal stress to which the tooth is subjected. It is wider and denser around the roots of teeth in heavy occlusion and thinner and less dense around teeth that are not subjected to occlusal function.(49)

Normal radiographic appearance of Lamina Dura:

- In cases where the mesial or distal aspects of the tooth are flat, the adjacent lamina dura is also flat, and the rays will pass between the teeth in the direction of the bucco-lingual axis of the lamina dura and there will be a narrow shadow of good density.
- In cases where the mesial or distal aspects of the tooth are not flat (where the root is inclined even so slightly), the lamina dura will be slightly oblique to the rays and the shadow will be wider.

Rays are able to penetrate and so the shadow of the lamina dura in the second case is wider than in the first, it is also dense and less white, because the total amount of dense bone which the beam of rays must penetrate is small.

- Where the mesial or the distal surface of the root is sharply convex, only a very small fragment of the lamina dura at the extreme summit of the convexity will be portrayed in the radiograph and it will be relatively gray because the amount of bone penetrated is small.

- If the shape of a tooth is such that the two separate portions of the mesial or the distal surface lie one behind the other, there may be two lamina dura shadows on that aspect of the root. The mesial aspect of the mesial root of the lower first molar is a good example of this. The lower cuspid is another example: because the lingual portion of the root is narrower than the labial portion, four portions of the corresponding lamina dura are often visible in radiographs. It is this tooth, above all others, which causes the dentist difficulty when it comes explaining the multiplicity of lamina dura shadows. If one bears in mind the great variety of shapes of the roots of teeth and the physical factors which enter into the production of the shadow of the lamina dura, it is easy to understand why there must be great radiographic differences in the lamina dura. Differences in thickness, density, shape and number of shadows would be expected merely form the study of cross sections of sockets seen in the dried skulls. It is seen, therefore, that there must be variations in the width of normal lamina dura shadows, owing to differently shaped teeth, and that the lamina dura always confirms to the shape of the teeth.

For e.g the anterior root of the lower first molar. This root is often dumbbell–shaped and so the buccal portion obscures, in part at least, the lingual portion. The penetration of the rays is such that in some cases the lamina dura over the mesial aspect of the obscured root may be seen, if not clearly, at least to some extent. Similarly, when the surface of the root is a smooth single curve, the lamina dura at the extreme apex of the curve is visible. Part of lamina dura which is little below the summit of the curve may also enter into the composition of the lamina dura shadow, because the x-rays are able to penetrate the small amount of tooth substance which stands in the way.(50)

All these differences in the radiographic appearances of the lamina dura have no clinical-significance so long as the lamina dura is continuous around the root, with few exceptions; discontinuity is evidence of abnormality, usually disease.

The widely held belief that a broad shadow indicates sclerosis of the lamina dura, the result of some stress or infection, cannot be substantiated. In sclerosis of the bone with involvement of the lamina dura, it is inconceivable that the adjacent bone will not be affected also. The essential feature in radiographic interpretation is that the shadow of the lamina dura shall be continuous throughout its extent: any deviation from this – any slight deficiency or discontinuity is highly suggestive, if not quite indicative, of an abnormal condition.

In almost every normal tooth, the lamina dura can be traced from the crest, around the root and into the bifurcation or trifurcation. There are, of course, some anatomic foramens in the lamina dura, but these are not apparent in radiographs.(51)

2. Alveolar Crest

The gingival margin of the alveolar process extends between the teeth. This is referred to as "alveolar crest". This appears as a radio opaque line on radiographs. The level of this alveolar crest is considered normal when it is not more than 1.5mm from the cemento enamel junction of the adjacent teeth. The length of the normal alveolar crest in a particular region depends on the distance between the teeth. In the anterior region, the crest is reduced to only a point of bone between the close-set incisors.

Posteriorly it is flat, aligned parallel and slightly below the line connecting the cemento enamel junctions of the adjacent teeth. The crest of the bone is continuous with the lamina dura and forms a sharp angle with it. Any rounding of these sharp junctions indicates the presence of periodontal disease.(52)

3 Cancellous Bone

The cancellous bone also known as the "trabecular bone or spongiosa" lies between the cortical plates in both jaws. It is composed of thin radio opaque plates and rods surrounding many small radiolucent pockets of marrow. The radiographic pattern of trabeculae shows considerable variation which is normal and not a manifestation of disease. The trabeculae in the anterior maxilla are typically thin and numerous. These form a fine granular dense pattern and the marrow spaces are small and relatively numerous. In posterior maxilla, the trabecular pattern is similar to that in anterior maxilla, but the marrow spaces are slightly larger. In mandible, the trabeculae are somewhat thicker that the maxilla resulting in a coarser pattern. If the trabeculae are apparently absent, it suggests the presence of disease.(49)

Radiographic Appearance of Alveolar Bone Loss:

The radiographic image shows less bone loss than that actually present. The amount of bone lost is determined to be the difference between the physiological bone levels and the height of the remaining bone. The distance from the cemento enamel junction to the alveolar crest has been investigated by several authors. Most of the studies have been conducted in adolescents and the distance was calculated as 2mm. However this distance may be greater in older patients.(49)

Formation, maintenance, and regeneration of alveolar bone:

Both the mandibular and maxillary jaw bones develop from the first branchial arch under the direction of homeobox genes that are expressed in a temporo-spatial manner and have a central role in skeletal pattern formation.[53] Alveolar bone comprises the alveolar process, which is an extension of the basal bone of the jaws. Paracrine factors including cytokines, chemokines and growth factors which have been implicated in the local control of mesenchymal condensations that occur at the onset of organogenesis are likely to have a prominent role in the development of the alveolar processes. While the growth and development of the jaw bones determines the position of the teeth, a certain degree of re-positioning of teeth can be accomplished through occlusal forces and in response to orthodontic procedures that rely on the adaptability of the alveolar bone and associated periodontal tissues. Consequently, understanding the molecular events that regulate the formation and remodelling of bony tissues is of fundamental importance in the development of rational treatment modalities to circumvent or correct structural and functional anomalies.

Physiological Remodelling of Alveolar Bone:

Complete remodelling of the alveolar bone occurs when the primary dentition is replaced by succedaneous teeth. The alveolar bone associated with the primary tooth is completely resorbed together with the roots of the tooth while new alveolar bone is formed to support the newly erupted tooth. Significant remodelling of the alveolar process also occurs as part of this process. The ability of the alveolar bone to remodel rapidly [54] also facilitates positional adaptation of teeth in response to functional forces and in the physiological drift of teeth that occurs with the development of jaw bones. From a clinical perspective, the rapid remodelling of the alveolar bone facilitates movement of teeth within the jaw bone by the application of orthodontic forces. However, the application of force on bone tissues can also influence the remodelling rate. Formation of alveolar bone is a prerequisite for the regeneration of tissues lost through periodontal disease and for osseointegration of implants used in restorative dentistry. Bone remodelling involves the co-ordination of activities of cells from two distinct lineages, the osteoblasts and the osteoclasts which form and resorb the mineralized connective tissues of bone respectively.

Regulation of bone remodelling is a complex process involving hormones and local factors acting in an autocrine and/or paracrine manner on the generation and activity of differentiated bone cells. While there is considerable knowledge of the kinetics of bone turnover at the cellular level, the regulation of bone remodelling at the molecular level is poorly understood.

Specific factors are believed to regulate each step in the remodelling process to integrate the development of osteoblasts and osteoclasts. Their activities are well modulated and exerted through the endocrine system. Notably, the cellular and molecular events involved in bone remodelling have a strong similarity to many aspects of inflammation; repair and the relationships between matrix molecules such as osteopontin, bone sialoprotein, Secreted Protein Acidic and Rich in Cysteine (SPARC), osteocalcin, blood clotting and wound healing are clearly evident. The associations between bone formation, remodelling and inflammatory response systems are further emphasized by the recent identification of ‘‘master genes’’ involved in the generation of osteoblasts and osteoclasts that belong to families of transcription factors with prominent roles in the development of immune responses. The regulated remodelling of alveolar bone is anticipated to follow the general principle of bone formation and resorption.[43]

Bone Formation:

Formation of bone which appears to be linked with bone resorption to maintain bone mass, involves the proliferation and differentiation of stromal stem cells along an osteogenic pathway that leads to the formation of osteoblasts. The process of cellular differentiation is controlled by a cascade of events that involves a combination of genetic programming and gene regulation by various hormones, cytokines and growth factors. While an understanding of the complexities of the differentiation process is still at an elementary stage, significant advances have been made in recent years in identifying regulatory genes and molecular markers that define specific stages of osteogenic cell development. Notably, matrix macromolecules to date have proven to be the best developmental markers particularly for the later stages of differentiation.[55, 56]

Although stromal stem cell has to be isolated from osteogenic tissues, a population of small, granular cells has been isolated from foetal rat calvaria & shown to be enriched with stem progenitor cells.[57] Following plating & attachment, these cells begin expressing collagens I, II, III as well as alkaline phosphatase and osteopontin. The alkaline phosphatase and collagen expression are characteristics of the osteogenic lineage and their synthesis continues to increase while the expression of type II collagen is lost and type III collagen progressively diminishes. At early stages of differentiation, the potential for entering into alternate pathways of differentiation is retained, as depicted for periodontal cell differentiation.[43]

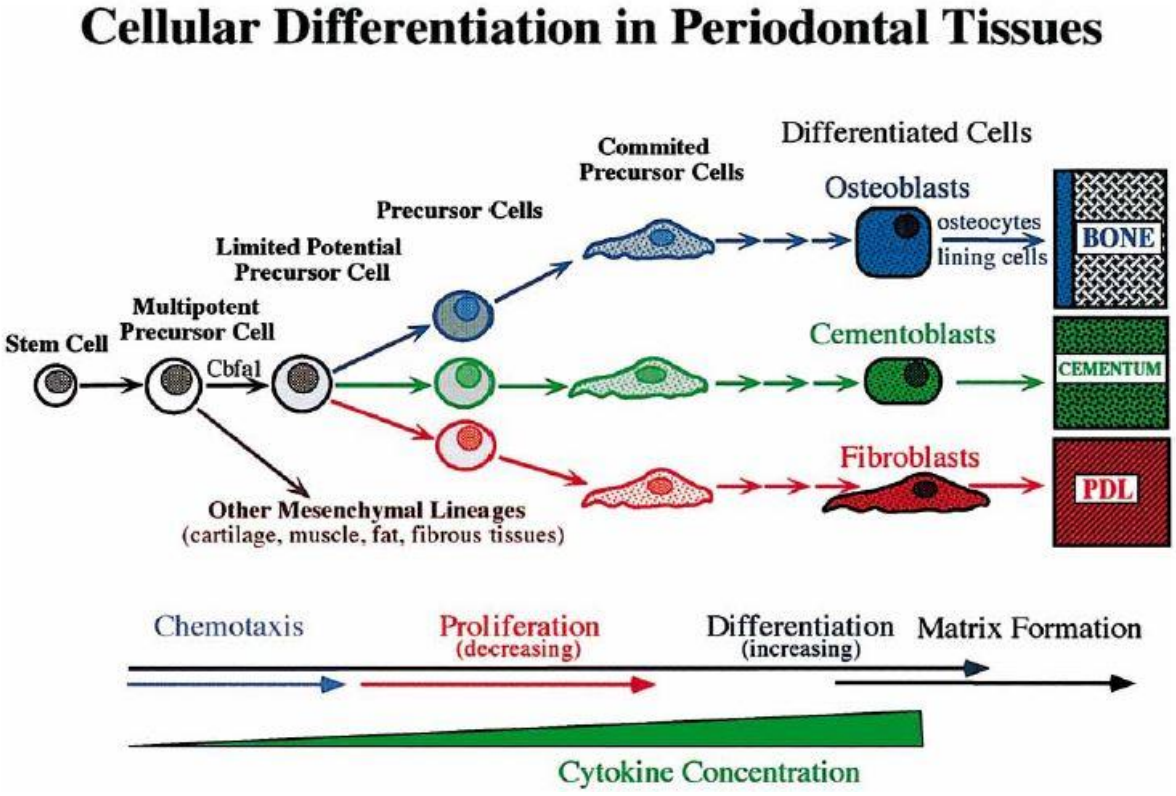


Fig. 7. Osteogenic differentiation. A schematic model of the events believed to be involved in osteogenic differentiation is shown in the context of a multipotent stem cells in the periodontium. Several lineages can potentially be generated including the osteogenic lineage. In each case a decrease in proliferation potential occurs as differentiation progresses. Expression of the "osteogenic master gene" core binding factor a1 (cbfa1) is likely a key event that directs multipotent cells into the osteoblast, cementoblast and periodontal ligament fibroblast pathways. The physical environment as well as growth factors and cytokines can influence the progression along each lineage until the fully differentiated cell is generated. PDL: peridontal ligament. (43)

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The formation of collagen substratum appears to trigger the differentiation of pre-osteoblastic cells into osteoblasts through interactions with the α2 β1 receptor. In vivo, this stage likely follows the condensation of mesenchymal cells. The subsequent emergences of osteoblasts are indicated by the expression of induced bone sialoprotein that correlates with the initiation of mineralization in vitro as well as in vivo. In comparison, the expression of osteopontin is variable and is complicated by its production by cement-forming cells. Nevertheless, osteopontin generally declines prior to osteoblastic differentiation while osteocalcin is expressed at high levels after mineralization has been initiated. With these high levels being maintained osteoblasts differentiate further into osteocytes and lining cells.

Expression of developmentally regulated genes and transcription factors that regulate the expression of differentiation associated genes appear to be the most useful for defining the early stages of osteo-differentiation. Many of the developmental genes including homeo box genes such as hoxa-2, hoxd-13 & hoax-13, dlx 5,msx-l& msx-2, are common to various forms of organogenesis. Similarly, different classes of transcription factors involved in osteogenesis have broad targets of regulation. However recent studies[58, 59] have identified a runt domain-related gene core binding factor al/PEBP2αA/ AML-3 as a bone restricted transcription factor that has been described as a potential "master gene" for osteogenic differentiation.[60] Expression in developing odontoblast, cementoblasts and ameloblasts indicate that core binding factor al [61] may also have a functional role in the differentiation of all mineralization tissue cells.[62]

Studies in vitro have shown that bone morphogenic proteins act upstream of core binding factor-αl.[40] Thus bone morphogenic protein-2 treatment of the myogenic cell line c2c12 transiently upregulates core binding factor-αl and Msx-2 leading to osteogenic differentiation. Notably transforming growth factor-β can also increase core binding factor-αl and suppresses myogenic differentiation in these cells. Even then osteogenic differentiation does not occur indicating that other factors induced by bone morphogenetic proteins are necessary for complete expression of the osteoblastic phenotype.[63] A potential factor is the homeo-box-containing gene dlx5 which regulates osteoblast differentiation and has been shown to be induced by bone morphogenetic protein l. Treatment of undifferentiated cells with bone morphogenetic protein-7/op-l, which has been shown to signal through smad 5, has also identified a non-translated RNA, bone morphogenetic protein lop-l responsive gene, and a novel zinc finger transcription factor, AJ-18, as immediate targets of bone morphogenetic protein-7, in osteogenic systems. However, the functional attributes of these target genes have yet to be determined.

Regulation of Bone Formation:

Bone formation is regulated by factors that affect either the production of osteoblastic cells or their activity. Many of these factors also affect bone resorption either directly or indirectly. Thus parathyroid hormone which regulates serum calcium levels by stimulating bone resorption, can also have anabolic effects in vivo that appear to be mediated through transforming growth factor-β and insulin-like growth factor-I[64] Such opposing effects of parathyroid hormone are consistent with the apparent coupling of bone formation and remodelling. The secosteroid (endocrine mechanism of action) vitamin D3 also has paradoxical effects in bone remodelling while stimulating bone resorption, it is essential for normal bone growth and mineralization and has a primary function in calcium absorption from the intestine. Vitamin D3 also strongly stimulates the synthesis of osteocalcin and osteopontin by osteoblastic cells,

while suppresses the collagen production. In contrast, insulin and growth hormone have anabolic effects on bone. Insulin targets osteoblasts directly stimulating bone matrix formation and mineralization and indirectly effecting bone formation through a stimulation of insulin-like growth factor-l produced in the liver. Growth hormone is required for attaining normal bone mass, the anabolic effects apparently being mediated through the local production of insulin - like growth factor-l produced in the liver. The anabolic effects of glucocorticoid are complicated by secondary effects initiated in response to the primary effects. Thus, the ability of glucocorticoids to promote differentiation of osteoblastic ells and to stimulate bone matrix formation has been well established in vitro. Thyroid hormone and the sex steroids are also necessary for normal growth and development of bones but they appear to act indirectly and the mechanisms are poorly defined. Thus, thyroid hormone affects endochondral bone formation through its action on cartilage formation, while the manner whereby oestrogens aid is maintaining bone mass through anabolic effects on bone is yet to be established.

Of the many growth and differentiation factors that influence bone formation, the bone morphogenic proteins have the most profound effect on bone formation. These cytokines belong to the transforming growth factor-β super family which induce chondrogenic & osteogenic differentiation in undifferentiated mesenchymal cells, their prolonged presence being required to generate endochondral bone in ectopic sites.[65] However, bone morphogenetic proteins do not have marked effects on bone matrix formation[66], In contrast, transforming growth factor-β can act as a potent inhibitor of osteogenic induction by bone morphogenetic protein, while strongly stimulating expression of matrix proteins by osteoblastic cells.[38, 67]

The anabolic effects of transforming growth factor-β are augmented by a suppression of matrix degradative activity through the inhibition of matrix metalloproteinase expression and the enhanced expression of tissue inhibitor of matrix metalloproteinases.[68] The insulin-like growth factors (I & II) are also potent anabolic agents in bone, having effects similar to transforming growth factor-β on matrix proteins and matrix metalloproteinases but insulin like growth factors also stimulate proliferation of osteoblast precursors.[69, 70] The acidic and particularly the basic fibroblast growth factors which are characteristically expressed early in skeletal growth development, exert their effects on bone formation primarily through increased proliferation of osteoprogenitor cells and promotion of osteogenic differentiation.[71]

Bone Resorption:

Resorption of mineralized tissues requires the recruitment of a specialized cell, the osteoclast which is produced by the monocytes/macrophage lineage of hematopoietic cells that are derived from bone marrow. The stages in the life cycle of the osteoclast are as follows:

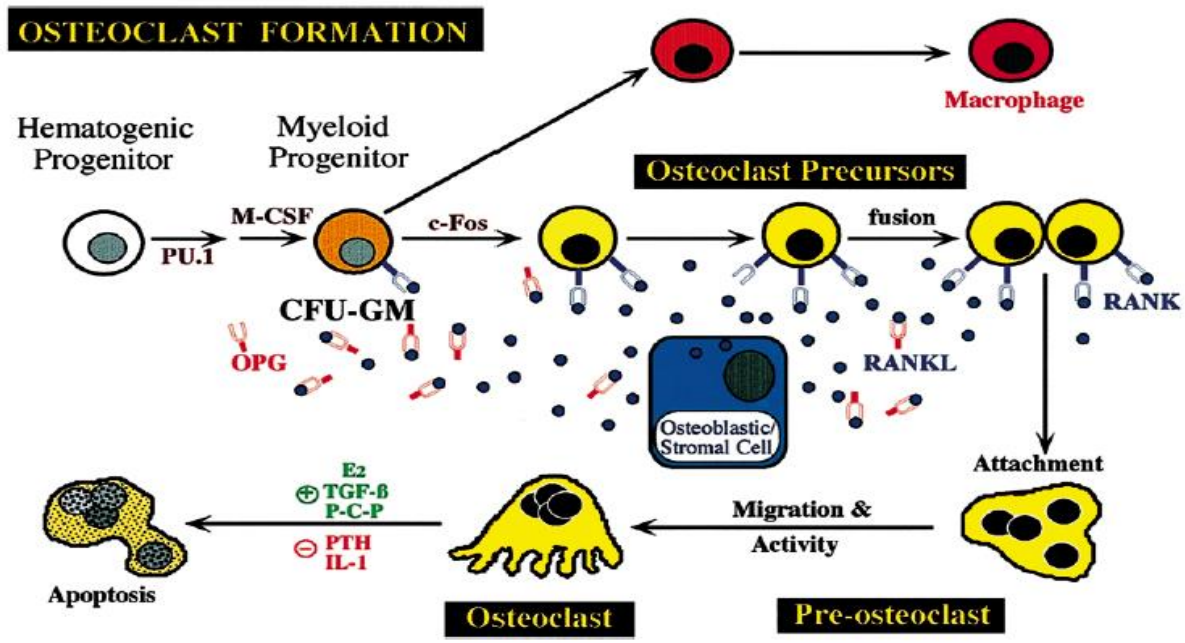

Fig. 8. Osteoclastic differentiation. Osteoclasts are formed from a haematogenic precursor cell which generates a progenitor of the granulocyte/macrophage (CFU-GM) lineage under the influence of the PU.1 gene and M-colony-stimulating factor. The myeloid progenitor requires a functional c-Fos gene to differentiate along the osteoclast pathway which is regulated by the receptor activator of nuclear factor κB (RANK)/receptor activator of nuclear factor κB ligand (RANKL) receptor/ligand system. Receptor activator of nuclear factor κB ligand, also known as osteoprotegerin ligand produced by stromal bone cells binds to receptor activator of nuclear factor κB receptors on the pre-osteoclasts and promotes osteoclast differentiation while the truncated, soluble form of receptor activator of nuclear factor κB, osteoprotegerin (OPG), can bind receptor activator of nuclear factor κB ligand and block signaling, thereby preventing osteoclast differentiation. Pre-osteoclasts form by fusion of precursors and following attachment to the bone surface become active osteoclasts. The survival and activity of the osteoclast is dependent upon factors such as estrogen (E), transforming growth factor-β (TGF-β) and bis-phosphonates (P-C-P) which promote osteoclast apoptosis, while parathyroid hormone (PTH) and interleukin-1 (IL-1) block osteoclast apoptosis.

(43)

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Osteoclasts develop from a pluripotent mononuclear precursor which is stimulated to proliferate and differentiate under the influence of monocytes-macrophage colony-stimulating factor. A variety of soluble and membrane bound factors play a critical role in regulating osteoclast formation, including growth factors, systemic hormones, and cells in the marrow microenvironment, such as osteoclasts and marrow stromal cells. Cell-to-cell interactions are important in both the formation and activity of the osteoclast. The identification of a novel receptor termed osteoprotegerin (OPG), has recently uncovered a key regulatory mechanism in osteoclast differentiation and activity. The osteoprotegerin

ligand (OPGL) has been identified as the putative osteoclast differentiation factor that is expressed on the surface of stromal/bone cells.[72, 73] It has been shown to signal osteoclast differentiation through a tumour necrosis factor receptor (TNFR), osteoclast differentiation and activation receptor (ODAR) or receptor activator of nuclear factor κ B (RANK). Osteoprotegerin itself acts as a decoy receptor that binds to ligand but is incapable of signaling. Thus, while ablation of the osteoprotegerin gene in transgenic mice results in excessive production of osteoclasts and osteoporosis, over expression osteoprotegerin leads to impaired osteoclast formation and osteopetrosis. A similar impairment in osteoclast development is observed in osteoprotegerin ligand knock-out mice[74] and nuclear factor K B knock-out mice.[75] From these observations it is evident that signaling by receptor activator of nuclear factor K B - ligand through the receptor activator of nuclear factor K receptor, which is regulated by colony-stimulating factor-I, is a crucial step in osteoclast formation. Moreover, the involvement of the osteoprotegerin-osteoprotegerin ligand regulatory pathway in the immune system and the calcification of arteries in osteoprotegerin deficient mice provide further links between the hematopoietic system and bone and provide a connection between vascular calcification and osteoporosis.

Typically, formation of osteoclasts involves fusion of monocytic precursors which occur at the site of bone resorption. The hyaluronan receptor CD44, the $\alpha_v\beta_3$ integrin is highly expressed in osteoclasts and osteoclast precursors; both receptors being a primary target for the bone matrix protein osteopontin in signalling, cell attachment and also possibly for osteoclast chemotaxis (haptotaxis) and migration.[76]

On the bone surface, osteoclasts become polarized and form a ruffled border beneath which bone resorption takes place[77], while the molecular mechanisms involved are not known. Demineralization of the bone matrix, which is a prerequisite for matrix degradation is achieved through the acidification of a protected environment beneath the ruffled border. A specific type of electrogenic adenosine triphosphatase[78] pumps protons generated by type II carbonic anhydrase activity into the resorption bay which also receives lysosomal enzymes and thereby acts as a functional secondary lysosome. Following the dissolution of the mineral phase in the acidic environment, the lysosomal enzymes can degrade matrix macromolecules, including collagen, in a manner similar to that described for the phagocytic degradation of the matrix. Matrix metalloproteinases, which can be activated under the acidic conditions, have also been observed in resorption lacunae and contribute to matrix degradation.[79] Following resorption, osteoclasts may undergo apoptosis, which provides a mechanism for limiting resorptive activity while other factors such as transforming growth factor-β, oestrogen and acid bis-phosphonates promote apoptosis, parathyroid hormone and interleukin-l act as

suppressors, prolonging osteoclast activity. Thus the formation, activity and survival of osteoclasts are all potential targets for regulation of osteoclast-mediated bone-resorptive activity.[80]

Regulation of Osteoclastic Activity:

The primary factors that stimulate bone resorption through osteoclasts include parathyroid hormone, vitamin D3, interleukin-1, interleukin-6, tumor necrosis factor α, and transforming growth factor-α, whereas calcitonin, transforming growth factor-β, oestrogen and interferon-γ inhibit osteoclastic bone resorption. Osteoclasts have receptors for calcitonin and oestrogen, as well as for most cytokines. Vitamin D3[81, 82] and parathyroid hormone[83] affect osteoclasts indirectly through receptors on pre-osteoblasts, osteoblasts and lining cells. Parathyroid hormone, parathyroid hormone– related protein, vitamin D3, transforming growth factor- α and pro-inflammatory cytokines, such as interleukin- 1 and tumor necrosis factor a, all promote differentiation of osteoclasts.[83] The pro-inflammatory cytokines can act through the OPG/ OPGL/RANK regulatory pathway[84, 85] which may be a key target of factors that affect osteoclast generation and activity. Similarly, parathyroid hormone and vitamin D3 have recently been shown to regulate osteoclast development through the osteoprotegerin/ Osteoprotegerin ligand/receptor activator of nuclear factor k B pathway.

Interleukin-6 is also produced by osteoblastic cells in response to parathyroid hormone and vitamin D3; it is a prominent cytokine produced by osteoclasts. Although interleukin-6 has important effects on bone remodelling and has been implicated in bone resorption associated with oestrogen deficiency, it is a much less potent stimulator of osteoclast generation than interleukin-1 and tumor necrosis factor α.[86]

Arachidonic metabolites are also important modulators of bone cell function. In particular, prostaglandins of E-series can act as powerful mediators of bone resorption and can also influence bone formation.[87] The prostaglandins exert a local effect on osteoclasts and their precursors, often mediating the effects of growth factors and transforming growth factor prostaglandins can also stimulate bone formation when administered systemically and the local infusion of prostaglandins E2 has been used to stimulate alveolar bone formation in vivo.[88] When this prostaglandin E2 applied directly also caused bone resorption.[89]

Oestrogen is believed to suppress the production of bone resorbing cytokines, including interleukin-l and interleukin-6, while transforming growth factor-B and interferon-γ inhibit proliferation and differentiation of osteoclast precursors.[90] Calcitonin is a particularly potent inhibitor of osteoclast

activity, but its effects are transient, likely due to the downregulation of calcitonin receptors on osteoclasts in the sustained presence of hormone. Calcitonin inhibits proliferation and differentiation of osteoclast precursors and causes cytoplasmic contraction of the cell membrane in mature osteoclasts and their dissociation into monocytic cells.

In addition to their effects on osteoclast development, interleukin-I, tumour necrosis factor-α and the functionally related lymphotoxin also stimulate osteoclastic activity. Although the mechanism is possibly indirect involving cells of the osteoblastic lineage, which mediate the effects of parathyroid hormone and vitamin D3 on osteoclastic activity, these cytokines and osteotropic hormones may also involve the OPG/OPGL/RANK pathway.[74] Notably, effects of vitamin D3 can also be mediated by the bone matrix protein osteopontin, which is strongly up regulated by the secosteroid. Another bone matrix protein, bone sialoprotein, can regulate osteoclast activity through the αvβ3 integrin[91, 92] which was originally characterized as a vitronectin receptor. Binding of ligand to the β component of the αvβ3 integrin activates the focal adhesion kinase related protein tyrosine kinase 2 (pYK2) through C-Src, which binds PYK2 through the SH_2 domains. Upon activation, PYK2 translocate to the Triton X-IOO insoluble cytoskeleton compartment and together with p 130^{cas} is found in the sealing zone required for osteoc1astic bone resorption.[43]

Influence of Cytokines and Growth Factors on Bone cells:

The regulatory role of growth factors and cytokines on bone cells is highly complex. Much of the information available is derived from in vitro studies on defined osteogenic or osteoclastic cells and their precursors. The effects produced by specific agonists and/or the antagonists on bone cultures can be direct or indirect; can involve several cell types and can result from the secondary production of additional cytokines. It is also true that hormones and growth factors have different effects, depending on the stage of differentiation of the specific target cells and their species of origin. A given cytokine or growth factor may stimulate both osteoblasts and osteoclasts. Furthermore, a cytokine, depending on its concentration, may exert an opposite effect on the same cell type. Consequently, it is often difficult to define with certainty what role a given cytokine and/or growth factor has on bone formation and resorption in-vitro and even more so in-vivo. Numerous interacting mediators within the local milieu undoubtedly modify the effect in vivo of a given factor on a cell type. The following is the action of those agents in general that regulate bone cells.

Bone Morphogenetic Protein:

Bone morphogenetic proteins and TGF-β are members of a superfamily of morphogenetic proteins that perform essential functions in embryonic development and bone cell differentiatlon.[93] Great progress has been made in isolating the members of this super family of morphogens, which now total about 40 proteins (Reddi et al 1998).[94]

Bone morphogenetic protein1 differs from the other BMPs in that it does not resemble TGF-β rather; it has been shown to be identical to procollagen C-proteinase, which processes procollagen to collagen fibrils (Kessler et al 1996) [95]. Bone morphogenetic protein 2, 3, 4, 6, & 7 has bone-inductive activity (Amedeed[96] and Wang et al.;[97] 1990, 1997). Bone morphogenetic protein 2 is a chemo attractant for osteoblasts (Lind M et al.;[98] 1996). As the structure of the BMPs was revealed, it became clear that BMP-3 was identical to osteogenin and BMP-7 to osteogenic protein1. Bone morphogenetic protein 7 and IGF-1 act synergistically to stimulate bone cell proliferation and differentiation.

Bone morphogenetic proteins are expressed in bone cells as well as in a wide number of soft tissues. They were first discovered as the active ingredient of demineralized bone matrix, responsible for endochondral bone induction. The expression of BMP-2, BMP-4 and BMP-7 and the presence of BMP receptors are increased in chondrogenic and osteogenic cells in sites of bone fracture repair (Sakou and Urist et al.;[99] 1965,1990). Bone morphogenetic proteins trigger increased proliferation and differentiation of chondrogenic and osteogenic cells. Osteoblastic cells respond to BMPs by increasing the number of PTH receptors, alkaline phosphatase activity, and the synthesis of collagen, osteocalcin, and other non-collagenous proteins (Bareille et al 1996). [99]

Bone morphogenic protein 7 has been shown to activate the cbfa 1 transcription factor regulating the genes that code for bone matrix proteins. The bone - inductive actions of BMP-2 and BMP-7 have been used clinically to accelerate bone healing and to create new bone in osseous defects (Sakou et al.; 1998)[100]. To activate bone differentiation, BMPs are best administered immobilized in a collagenous matrix (although synthetic polymers work as stabilizers). In addition BMP-2 enhances the expression of IL-6 and TGF-β in osteoblast cells. Both factors may have autocrine and paracrine mediated regulatory effects on adjacent bone cells.

Basic Fibroblast Growth Factor:

Basic fibroblast growth factor (b-FGF) increases the proliferation and differentiation of osteogenic cells. Systemic and local administration of b-FGF enhances endosteal bone formation in experimental

animals (Nakamura et al.; 1995)[101]. As b-FGF increases the expression of TGF-β in osteogenic cells, it has been proposed that the osteogenic effect of FGF may be mediated by TGF-β. Basic Fibroblast growth factor (FGF) up regulates the expression of IL-6, a cytokine- activating factor for pre-osteoclasts, and promotes osteoclast formation (Hurley et al.; 1998)[102].

Colony Stimulating Factor:

Colony-stimulating factors control haematopoiesis and in so doing contribute to an increase the pool of osteoclast precursors. Monocyte colony-stimulating factor (also known as CSF-l) regulates the proliferation of monocytes and promotes pre-osteoclast differentiation (Sarma et al.; 1996)[103]. Colony-stimulating factor 1 is produced by osteoblasts and is inserted in the plasma membrane and/or secreted into the bone matrix. Granulocyte/macrophage colony-stimulating factor is an autocrine growth factor for osteoblastic cells (Modrowski et al.; 1997)[104].

Glucocorticoids:

Glucocorticoids decreases bone formation and promote osteoclastic bone resorption in-vitro (Delany and Ishida et al.; 1996)[105, 106]. Prolonged exposure to increased levels of glucocorticoids leads to osteoporosis (Lukert et al.; 1990)[107]. Glucocorticoids depress osteoblastic activity by decreasing the expression of integrins and IGF (Gohel, Gronowicz et al.; 1995)[108, 109]. They also stimulate the secretion of collagenase by osteoblasts which in turn degrades osteoid matrix, thereby releasing factors that activate osteoclastic activity. Other studies have demonstrated that in contrast to its catabolic effects noted earlier glucocorticoids at physiologic levels may regulate bone matrix synthesis and induce osteoclastic apoptosis (Lutton et a1.; 1996)[110].

Hepatocyte Growth Factor and Macrophage - Stimulating Protein:

Both hepatocyte growth factor and a related serum protein, macrophage stimulating protein, activate bone resorption.[111]

Immunoregulatory Cytokines:

The interleukins, a family of cytokines produced by many cell types but in high levels by activated lymphocytes and macrophages, regulate the differentiation of effector cells of the immune system. Many cells that do not belong to the immune system such as fibroblast and keratinocytes are also capable of secreting interleukins. In addition to their regulatory effects on cells of the immune system, the interleukins influence the activity of a wide variety of cells, including those of skeletal system.

Bone resorption observed in regions of inflammation is likely to be caused by locally produced interleukins and prostaglandins acting on the expression of OPG and ODF/RANKL, there by altering the balance in favour of osteoclastogenesis (Teterbaum et a1.;1998)[112].

Interleukin 1 is a potent stimulator of osteoclastic bone resorption (Rifas et al.; 1999)[113]. Activated monocytes, macrophages, T cells, neutrophils, fibroblasts and epithelial cells produce IL-1 during inflammation. The bone resorbing activity of IL-l may occur indirectly through stimulation of PGE2 production (Amano et al.; and Harrison et al.;1996, 1999)[114, 115]. The local production PGE2 and IL-l in inflamed gingival and periodontal connective tissue is believed to be responsible for stimulating alveolar bone resorption. Interleukin 6 and PTH stimulate osteoclastic activity indirectly by increasing osteoblastic expression of monocyte CSF (CSF-l) and IL-6 (Pollock et al.; 1994)[116]. In contrast IL-4, IL-I0 and IL-13 decreases bone resorption (Onoe et al.; 1996)[117]. Interleukin 10 inhibits bone resorption by decreasing the proliferation of pre-osteoclasts. It has been reported that IL-I0 and IL-8 stimulate osteoclastic activity by activating nitric oxide synthase in mature osteoclasts. IL-4 stimulates the expression of alkaline phosphatase and collagen type I in osteoblasts (Nohtomi et al.; 1994)[118].

Insulin Like Growth Factors:

Insulin like growth factors (IGF-l and IGF-II) are produced by several cell- types, including fibroblast and osteoclasts. Both factors are deposited in bone matrix, where they are stored in association with IGF-binding proteins. During bone resorption, IGFs are released from bone matrix and undergo disassociation from IGF-binding protein to act in a delayed paracrine mode, along with TGF-β, to increase osteoblastic activity and new bone formation. Furthermore, it has been reported that TGF-β decreases the expression of IGF-binding protein, thereby making more IGF available (Gabbitas B et al.; 1989)[119]. Insulin growth factors stimulate osteogenic cell proliferation and increase the synthesis of collagen, alkaline phosphatase, osteocalcin, and integrins in osteogenic cells (Me Carthy et al.; 1989)[120]. Because of the ability of IGFs to increase the proliferation and differentiation of osteogenic cells, they are regarded along with TGF-β as significant components of the coupling mechanism linking bone formation to prior osteoclastic bone resorption. Insulin-like growth factors has also been shown to increase osteoclastic simulating effect of IGF (Hill et al.; 1995)[121]. Because of this dual action IGFs are thought to be regulators of bone remodelling.

Leptin:

A small polypeptide hormone produced by fat cells, leptin has been shown to act as a potent inhibitor of bone formation. Leptin does not act directly on osteoblasts but instead exerts its effects through the central nervous system to regulate bone mass in a pathway that has yet to be defined (Ducy et al.; 2000)[122]. Hormonal (systemic) control of bone mass is coordinated by leptin, PTH and sex steroids (Ducy et al.; 2000)[123].

Platelet Derived Growth Factor:

Platelet derived growth factor (PDGF) acts as a chemotactic and mitogenic factor on osteoblastic cells. It increases the production of bone matrix proteins. Because PDGF is synthesized by osteoblasts in response to TGF-β stimulation, it could act like PGE2 in an autocrine pathway to mediate the anabolic effects of TGF-β on bone formation.[3]

Prostaglandins:

Prostaglandins EI E2and F2 (PGF2) are potent stimulators of new bone formation (Miller et al.; 1995)[124]. Prostaglandins E2 and F2 stimulate bone cell proliferation by activating phospholipase C and by increasing calcium influx through plasma membrane calcium channels (Baylink et al.; 1996)[125]. Increased cAMP has also been implicated in regulating osteoblastic cell proliferation in response to PGE2. Recent animal studies indicate that prostaglandins can be administered locally to restore bone defects.[26] In addition PGE1 and PGE2 stimulate osteoblastic cells to produce TGF, a mitogen for endodontic cells. The role of osteogenic cells in coordinating vascular proliferation by VEGF is one mechanism for ensuring an adequate blood supply for new bone formation. It should be noted that PGE2 also stimulates osteoclastic activity with both the number and size of osteoclasts increasing under the influence of PGE2.[126] In vitro studies have shown that the osteoclast-stimulating effect of PGE2 is mediated by osteoblasts. Osteoblasts regulated by PGE2 contract and thereby expose the bone surface to pre-osteoclasts. [127] Prostaglandin E2 also stimulates bone resorption in calvaria organ cultures. Resorption is blocked by antibodies directed against IL-l, suggesting that osteoclastic bone resorption stimulated by PGE2 may be caused by increased production of IL-l. Because prostaglandins and interleukins have short half-lives (2-3 minutes), their effects are local and short acting.[114]

Sex Steroids:

Sex steroids exert an overall anabolic effect on bones by stimulating the proliferation and differentiation of osteoblasts. They also decrease the transcription of the IL-6 gene. The combination

of osteoclastic bone resorption and decreased osteoblast proliferation, caused by oestrogen levels is a common cause of osteoporosis in post-menopausal women (Jilka et al.; 1998)[128].

Transforming Growth Factor α:

Transforming growth factor α is closely homologous in structure and action to epidermal growth factor. It is produced by malignant cells and activated macrophages. The mitogenic effects of TGF- α and EGF on fibroblasts and osteogenic cells are exerted via the EGF receptor (tyrosine kinase mechanism). In general, TGF-α stimulates proliferation of pre-osteoblasts while decreasing the differentiated state. It also stimulates osteoclastic bone resorption. The production of TGF-α by cancer cells is in part responsible for the bone resorption associated with certain neoplasms.[129]

Transforming Growth Factor β:

Transforming growth factor-β exerts an anabolic effect on osteogenic cells. It is a product of bone-forming cells that is stored in bone matrix. On its release during bone resorption, TGF-β exerts a paracrine effect to increase the proliferation of preosteoblasts.[130, 131] TGF-β also acts as an autocrine factor to increase the synthesis of collagen, alkaline phosphatase, and osteopontin in osteoblasts. As TGF-β increases the synthesis of PGE2 and PDGF in osteoblastic cells, it has been suggested that the local anabolic effect of TGF- β on bone might in part be mediated by PGE2 and PDGF. TGF-β also inhibits matrix degradation by autocrine negative regulation of osteoclasts and by down regulation of ODF/RANKL.[132] TGF- β increases the expression of connexin 43 and cell-cell communication in osteogenic cells.[133]

Recent evidence points to direct action of TGF- β in controlling osteogenic cell growth by activating key members of a signalling pathway involved in regulating gene transcription. The osteogenic potential of TGF-β has been demonstrated by its ability to act synergistically with BMP-7 to induce ectopic bone formation when implanted along with a collagen matrix.[134, 135]

Tumor Necrosis Factor:

Produced by many cancer cells, as well as bone cells, TNF increases osteoclastic activity, either by direct action (ODF/TNFS-II) or by increasing the expression of IL-6. Recently, a member of the TNT receptor family, osteoprotegerin has been found to block osteoclast formation.[136] Osteoprotegerin has no transmembrane domain and is secreted as a soluble protein by osteoclasts in response to vitamin D and BMP-2. In contrast the secretion of TNFSF-II by osteoblasts in response to stimulation by PTH and IL-l increases osteoclastic activity.[3]

Pathologies Affecting the Alveolar Bone:

In a healthy individual alveolar bone constantly undergoes bone formation by osteoblasts and bone resorption by osteoclasts. But certain disorders of the endocrine system, bone metabolism and other systemic diseases are known to disturb this balance and may alter the form and function of alveolar bone. Systemic influences on bone resorption may be exerted by several mediators, including parathyroid hormone (PTH), interleukin-1, tumour necrosis factor (TNF), transforming growth factor (TGF), and 1,25-dihydroxy vitamin D3. This increased bone resorption modifies the quality and quantity of alveolar bone which provides an easier pathway for the spread of inflammation.[137]

Mechanism of bone resorption: Bone resorption is considered as normal destruction process in the bony remodelling phenomenon which is mediated by osteoclasts. These occur usually at random sites or are specific to the areas that require repair. Bony remodelling comprises of six phases:[138-140]

1. **Quiescent phase:** In this phase the bone is at rest. Factors that initiate this are still unknown.
2. **Activation phase:** This phase starts with the activation of bone surface through dissolution of endosteal surface by collagenases and retraction of osteoblasts on the endosteal surface. This leads to activation of osteoclast precursors from blood circulation which in turn results in differentiation, migration and fusion of multinucleated osteoclast cells. Furthermore, osteoclast cells orient towards the bone surface.

3. **Resorption phase:** Upon orientation of osteoclast cells over the site of resorption cathepsin - k, reactive oxygen species produced by Tartrate resistant acid phosphatase (TRAP) are secreted at ruffled border into the resorptive pit. The effectiveness of the secretion by osteoclast cell depends on sealing zone formed on the resorption compartment. Within this sealing zone pH reduces and results in degradation of bone matrix. This resorption produces irregular scalloped cavities on bone surface known as ‘Howship lacunae’. Bone resorption usually takes 2-4 weeks in each remodelling cycle.

4. **Reversal phase:** This phase is characterized by transition from bone resorption to bone formation under the influence of coupling signals like TGF-/3, IGF-1, IGF-2, bone morphogenetic proteins, PDGF, or fibroblast growth factor.

5. Formation phase: under the influence of these coupling signals osteoblast are formed from pre-osteoblastic precursors. The osteoblasts then lay down the osteoid matrix.

6. Mineralization phase: This phase occurs 30 days after the osteoid formation. Once mineralization is complete again quiescent phase starts.[141]

In various pathologic conditions, this remodelling cycle is characterized by prolonged resorption phase and an impaired reversal phase which leads to significant bone loss without bone formation.

Alveolar Bone in Periodontitis:

Various authors like Saglie et al.; (1987)[142] have postulated that the connective tissue invasion by various bacterial species elicits an abnormal host response leading to rapid bone resorption. Similarly, Newman et al.; (1979)[143] also associated rapid bone loss with increased presence of loose, unattached and motile gram negative bacterial species in pocket.[144] The initial response to bacterial invasion is the localized inflammatory reaction that activates the innate immune system. Amplification of this initial localized inflammatory reaction causes the release of an array of cytokines and other mediators through which the inflammation propagates in the gingival tissues. The failure which regulates this ‘‘inflammatory front’ ’within gingival tissue will result in the expansion of the response in alveolar bone. The inflammatory process thus results in the destruction of connective tissue and alveolar bone that may lead to tooth loss.[145]

This pathway of inflammation becomes a crucial factor in determining the type of alveolar defect that is seen in periodontal disease.

Factors Determining Bone Morphology in Periodontal Disease

Considerable normal variation exists in the morphologic features of alveolar bone, which affects the osseous contours produced by periodontal disease. The anatomic features that substantially affect the bone destructive pattern in periodontal disease include the following:

- The thickness, width, and crestal angulation of the interdental septa.
- The thickness of the facial and lingual alveolar plates.
- The presence of fenestrations and dehiscences.
- The alignment of the teeth.
- Root and root trunk anatomy.
- Root position within the alveolar process.
- Proximity with another tooth surface.[1]
-

Alveolar bone defects: Glickman (1964)[146] classified the alveolar defects into osseous craters, hemiseptal defects, Infrabony defects, Bulbous bone contours, Reversed architecture, Inconsistent margins and Ledges. Whereas Prichard (1967)[147] has expanded the Glickman's classification of these alveolar bone defects by including the furcation involvement, anatomic aberrations of alveolar process, exostoses and tori, dehiscence and fenestrations.

Goldman and Cohen (1958)[148] classified alveolar defects into:

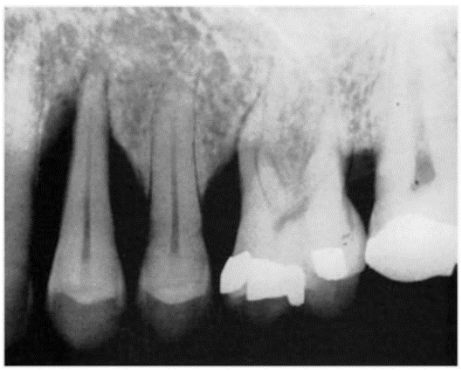
1. Suprabony defects: Where the base of pocket is located coronal to the alveolar crest.
2. Infrabony defects: Where the base of the pocket lies apical to the alveolar crest.
3. Intrabony defects: Bony defects whose infrabony component affects primarily one tooth.
4. Craters: The defect affects two adjacent root surfaces to a similar extent[149, 150]

Types of alveolar bone defect

1. **Horizontal Defects:** It is the commonest pattern of bone loss. In this defect, the bone is reduced in such a way that the bone margin is approximately perpendicular to the teeth surface. Interdental septa, facial and lingual plates of bones are affected, but to an equal degree around the same tooth.[150]

(151) Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.

2. Vertical /Angular Defects: It occurs in oblique direction usually. The base of the defect is situated apical to the surrounding bone. Vertical defects are seen adjacent to a tooth and form a triangular area of missing bone, known as triangulation. In most instances, angular defects are accompanied by an infra bony pocket.(150)



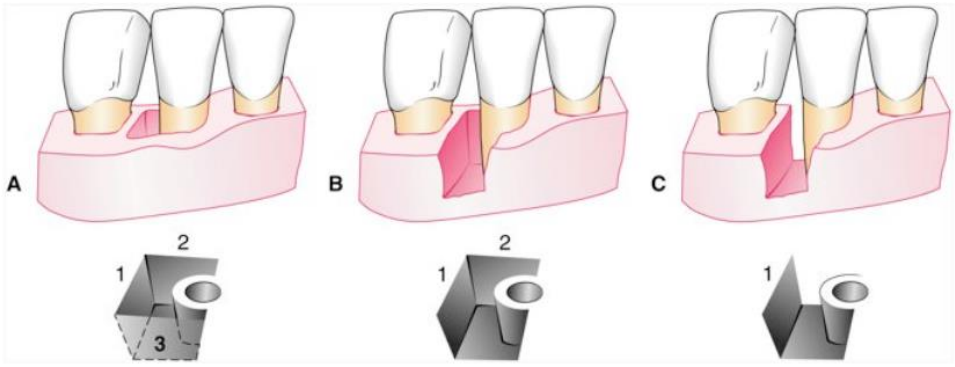
(151) Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.

Angular defects are classified by Goldman and Cohen(148) on basis of number of walls involved:

i. One wall defect/Hemiseptum: Only one wall of the interseptal wall remains and there is complete destruction of mesial and distal portion of interseptal bone which is visible on radiographs.

ii. Two walled defects: Characterized by saucer shaped cavity in interdental bone, facial and lingual walls intact; most commonly found in posterior segment of maxilla/mandible which is visible on radiograph.

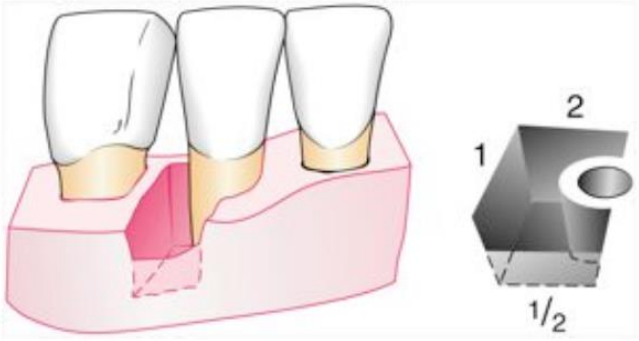
iii. Three walled defects: In this the bony walls are present on three sides and tooth forms the fourth wall.



One-, two-, and three-walled vertical defects on right lateral incisor. **A,** Three bony walls: distal *(1),* lingual *(2),* and facial *(3).* **B,** Two-wall defect: distal *(1)* and lingual *(2).* **C,** One-wall defect: distal wall only *(1).*

(1) ***Newmann MG TH, Klokkevold PR, Carranza FA. . Clinical Periodontology. 10th ed ed. Philadelphia: saunders: Elsevier; 2007.***

iv. Combined defect: Number of walls in apical portion of defect are greater than its occlusal portion.(149)



Combined type of osseous defect. Because the facial wall is half the height of the distal *(1)* and lingual *(2)* walls, this is an osseous defect with three walls in its apical half and two walls in the occlusal half.

(1) ***Newmann MG TH, Klokkevold PR, Carranza FA. . Clinical Periodontology. 10th ed ed. Philadelphia: saunders: Elsevier; 2007.***

3. Fenestrations: Are the isolated areas in which the root is denuded of bone and the root surface is covered only by periosteum and overlying gingiva. Predisposing factors includes prominent root contours, malposition, labial protrusion of the root combined with a thin bony plate. It is seen more often on facial bone than on lingual bone more common on anteriorly than posteriorly. It occur bilaterally.(152)

4. Dehiscences: When the denuded areas extend through the marginal bone then the defect is called a dehiscence. Alveolar bone dehiscences are classified into the following types, based on the dehiscence

height along with other accompanying alveolar bony defects. These classifications are based on the measurements obtained in the sagittal planes.[153, 154]

Class I: This includes dehiscence's which are present on either buccal or lingual side of the tooth, without any other alveolar bone defects. Then tooth root is divided into three equal parts, from the cemento enamel junction to the apex of root for further classifying into subdivisions.

- Division I: Dehiscence's of the coronal one-third of the root.
- Division II: Dehiscence's of the middle one-third of the root.
- Division III: Dehiscence's of the apical one-third of the root, without involving the apical foramen.

Class II: This includes dehiscences with other alveolar bone defects which are present periapically either in buccal or lingual side of the tooth.

- Division I: Dehiscences of the whole root involving the apical foramen.
- Division II: Dehiscences with periapical lesions. Wherein periapical lesion occurred as radiolucency in the apical part of root that exceeded twice the width of the periodontal ligament space.[155]
- Division III: Dehiscences with fenestrations surrounding the apex of the root. Though fenestration is an alveolar bone defect without involving the alveolar margin.[156]

Class III: Dehiscences are located on both sides (buccal or lingual) of the tooth. Further classification is done referring to the divisions of Class I and Class II whichever is more severe.

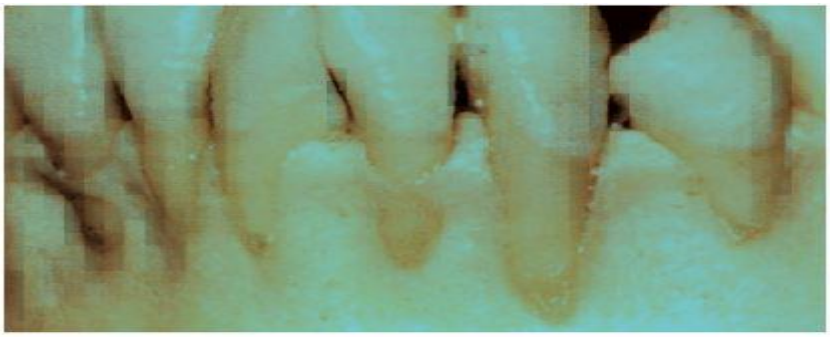
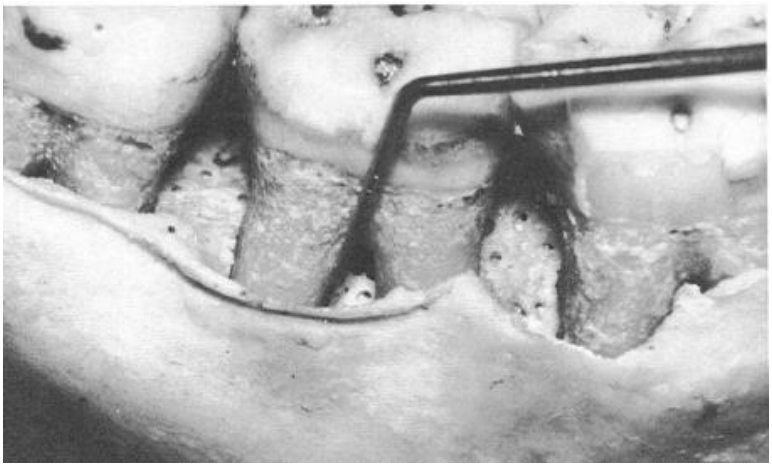


Figure 1.
Multiple fenestration and dehiscence defects identified in a mandible from the Robert J. Terry Collection of skeletons at the National Museum of Natural History, Washington, DC.

(155)

Rupprecht RD, Horning GM, Nicoll BK, Cohen ME. Prevalence of dehiscences and fenestrations in modern American skulls. J Periodontol. 2001;72(6):722-9.

5. Osseous Crater: Osseous craters are characterized by the concavities in the crest of the interdental bone confined within the facial and lingual walls. Craters have been found to make up to one third (35.2%) of all defects and about two thirds (62%) of all mandibular defects. They are twice as common in posterior segments as in anterior segments.[145]



coronal view revealing the defect

[151] ***Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.***

6. Bulbous Bone Contours: Bulbous bone contours are bony enlargements that are caused by exostosis, adaptation to function, or even buttressing bone formation. They are found more frequently in the maxilla than in the mandible.

7. Reversed Architecture: Reversed architecture defects are produced by loss of interdental bone including the facial plates, lingual plates, or both without concomitant loss of radicular bone, thereby reversing the normal architecture. Such defects are more common in the maxilla.(157)

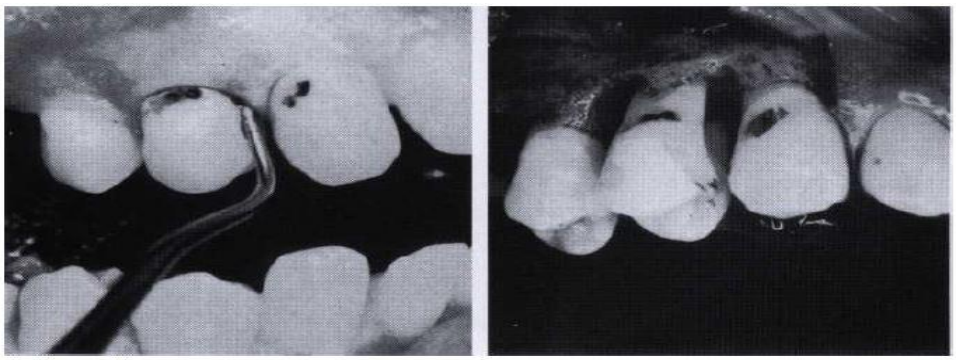


Fig. 23-21 Reversed architecture. *Left,* Probe in the deep infrabony pocket on the mesial surface of the maxillary premolar. *Right,* Elevated flap shows irregular bone margin with notching of interdental bone.

(1) ***Newmann MG TH, Klokkevold PR, Carranza FA. . Clinical Periodontology. 10th ed ed. Philadelphia: saunders: Elsevier; 2007.***

8. Ledges: Ledges occur as the defects with plateau-like bone margins resulting from loss of thickened bony plates.(149)

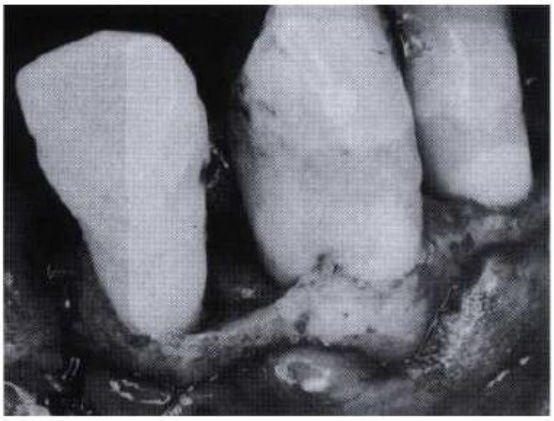


Fig. 23-22 Labial ledge produced by interproximal resorption.

(1) ***Newmann MG TH, Klokkevold PR, Carranza FA. . Clinical Periodontology. 10th ed ed. Philadelphia: saunders: Elsevier; 2007.***

9. Furcation Involvement: The furcation involvement is defined as the invasion of the bifurcation and trifurcation of multi rooted teeth by periodontal disease. The prevalence of furcation involved molars is not clear. Though mandibular first molars are most commonly involved, and the maxillary premolars are the least common. The number of furcation involvements increases with the age.(155) Furcation involvement is classified based on the amount of tissue destruction by ***Glickman*** in 1953.(158, 159)

i. Grade I is associated with incipient bone loss.
ii. Grade II is associated with partial bone loss.
iii. Grade III is associated with total bone loss leading to through-and- through passage from the furcation.
iv. Grade IV is same as grade III, but with gingival recession resulting in the exposure of furcation.
Apart from the above classification some additional classification systems of furcation involvement are:(160)

***Goldman*[161]** 1958-

Grade I: incipient

Grade II: cul-de-sac (pouch)

Grade III: through and through

***Staffileno*[162]** 1969-

Grade I: soft tissue lesion extending to the entrance of the furcation with minor degree of bone loss

Grade II: loss of furcal bone but not through and through

Grade III: through and through

***Easley and Drennan*[163]** 1969-

Class I: incipient involvement, entrance of the furcation detectable with no horizontal bone loss

Class II, Type 1: horizontal bone loss but no vertical component

Class II, Type 2: horizontal bone loss and vertical bone loss

Class III, Type 1: through-and-through bone loss with no vertical component

Class III, Type 2: through-and-through bone loss with vertical component

***Hamp et al.;*[164]** 1975-

Degree/Class I: horizontal loss of periodontal tissue support <3 mm

Degree/Class II: horizontal loss of periodontal tissue support >3 mm but not through and through

Degree/Class III: through-and-through defect

***Ramfjord*[165]** 1979-

Degree 1: horizontal penetration <2 mm

Degree 2: horizontal penetration >2 mm but not through and through

Degree 3: through and through

***Richietti, P.A.*[166]** 1982-

Class I: 1 mm of horizontal invasion.

Class Ia: 1–2 mm of horizontal invasion.

Class II: 2–4 mm of horizontal invasion.

Class IIa: 4–6 mm of horizontal invasion.

Class III: >6 mm of horizontal invasion.

Tal, H. et al.;[167] 1982

Furction involvement index (FII) scores:

Furcal rating 1: Depth of the furcation is 0 mm.

Furcal rating 2: Depth of the furcation is 1–2 mm.

Furcal rating 3: Depth of the furcation is 3 mm.

Furcal rating 4: Depth of the furcation is 4 mm or more.

Eskow, R.N. et al.;[168] 1984

Furcation involvement is classified as grade I subclasses A, B, and C (vertical involvement):

Subclass A: Vertical destruction > 1/3.

Subclass B: Vertical destruction of 2/3.

Subclass C: Vertical destruction beyond apical third of interradicular height.

Tarnow and Fletcher[169] 1984-

Uses Grades I, II, III proposed previously by Glickman20 with an additional sub classification based on vertical invasion from the furcation fornix:

A: VPD, 1 to 3 mm

B: VPD, 4 to 6 mm

C: VPD, >7 mm

Fedi, P.F.et al.;[160] 1985

Glickman[158, 159] + Hamp [164] classifications

Grades are the same as Glickman's classification (I–IV).

Grade II is subdivided into degrees I and II.

Degree I: Vertical bone loss 1–3 mm.

Degree II. Vertical bone loss > 3 mm, but not communicate through-and-through.

Rosemberg, M.M[170] 1986-

Horizontal

Degree I: Probing < 4 mm.

Degree II: Probing > 4 mm.

Degree III: Two or three furcations classified as degree II are found.

Vertical

Shallow: Slight lateral extension of an interradicular defect, from the center of the trifurcation in a horizontal direction.

Deep: Internal furcation involvement but not penetrating the adjacent furcation.

Hou et al.; [171] 1998-

Three classes (Class I, II, and III):

Classes are the same as grades in the classification by Hamp et al.;[164]

Two subclasses (Subclass a and b):

a: for suprabony defects

b: for infrabony defects

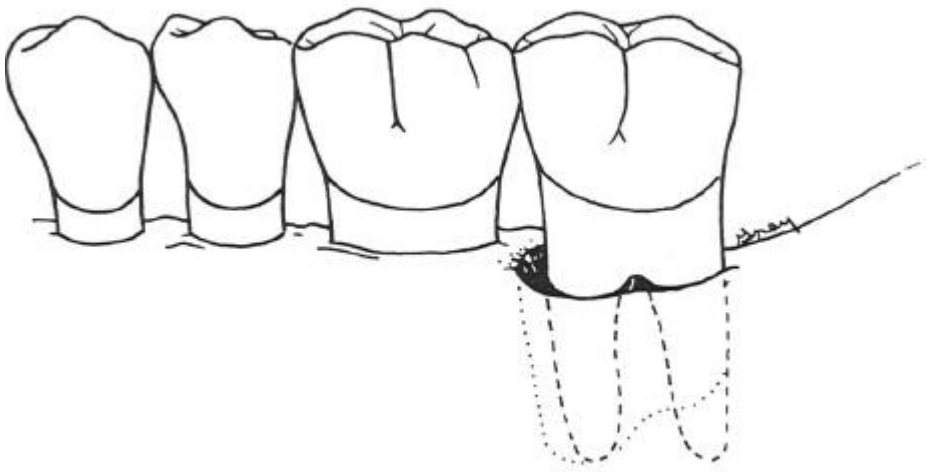
Three types (A, B, and C):

A: root trunk represents the cervical one-third of the root complex

B: root trunk represents half of the root complex

C: root trunk represents the cervical two-thirds of the root complex

10. Trench shaped defect: Trench defect occurs when such bone loss affects two or three confluent surfaces of the same tooth. Trenches can be similarly identified by the tooth surfaces involved (e.g., mesiofacial, mesio-lingual-distal, etc.). Hence, there are eight possible combinations seen in this defect i.e. mesio-facial, mesio-lingual, disto-facial, disto-lingual, mesial-facial-distal, mesial-lingual-distal, facial-mesial-lingual, facial-distal-lingual. [151]

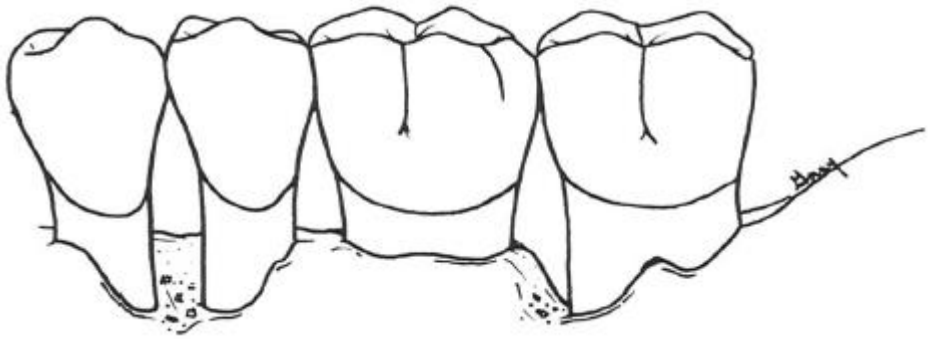


Facial view of mesiofacial trench #18. [151] ***Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.***

11. Moat shaped defects: Moat shaped defects occurs circumferentially around the teeth i.e. when bone loss deformity involves all the four surfaces of a tooth.(151)

12. Ramp shaped defects: Ramp shaped defects occurs when both alveolar bone and its supporting bone are lost such that the margins of the defect lie at different levels.(151) Ramps are named for the tooth surface aspect from which the greatest bone loss has occurred and the teeth involved. Visualization and classification of ramps become more complex when a ramp-type deformity demonstrates loss of bone from more than one aspect. Facial and lingual ramps, in addition to being found inter proximally, may also be seen facial and lingual to the teeth.

This is usually the case when thick alveolar bone existed before the bone loss occurred and is almost always in combination with interproximal ramps. Therefore, such ramps are named for the alveolar process aspect (rather than the tooth surface aspect) from which bone has been lost.



Facial view of mesial ramp #18 and facial ramp #20–21. (151) ***Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.***

Cratered Ramp: If only the most coronal rim of the deformity were considered, it would represent a ramp. However, a crater presents apical to the entire extent of the ramp and hence the term "cratered ramp." It is basically a crater with a portion of its facial and/or lingual wall missing. Cratered ramps are named for the teeth involved, the aspect of the alveolar process from which bone has been lost in the ramp portion and the tooth.

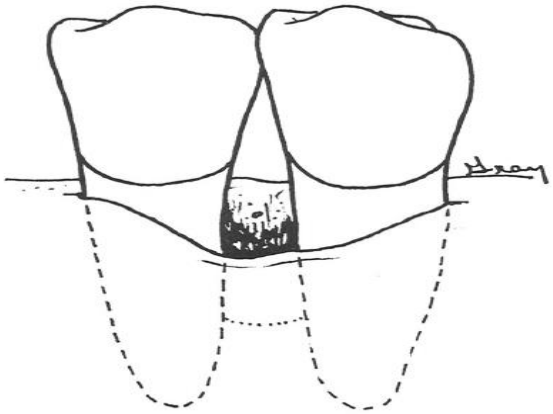


Figure 8. *Facial view of a facial cratered ramp between #20 and #21. Note the lingual crest of the crater is more coronal than the facial crest.*

(151) ***Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.***

13. Plane defect: Plane defect occurs when both alveolar bone and supporting bone is lost such that the margins of the defect lie at the same level.(151)

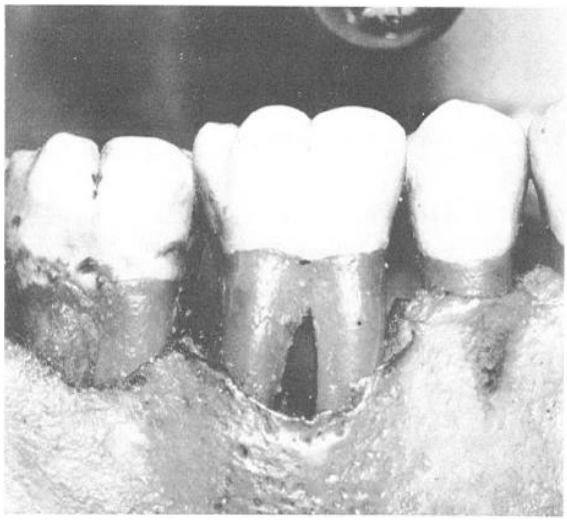


Figure 17. *Facial view of a plane with Class III furcation on Tooth #30.* **(151)** ***Karn KW, Shockett HP, Moffitt WC, Gray JL. Topographic classification of deformities of the alveolar process. J Periodontol. 1984;55(6):336-40.***

Trauma from Occlusion:

Trauma from occlusion may be considered a factor in determining the dimension and shape of bone deformities. It may result in the thickening of the cervical margin of alveolar bone or a change in the morphology of the bone on which inflammatory changes might get superimposed later.(172)

Sometimes bone formation occurs in an attempt to buttress weakened bony trabeculae by resorption. For example:

i. Central buttressing bone formation which occurs within the jaw.

ii. Peripheral buttressing bone formation when it occurs on the external surface. Peripheral buttressing may cause bulge in the bone contour, called lipping, which is accompanied by the production of osseous craters and angular defects.[149, 173]

Molecular Biology of Bone Destruction in Periodontal Disease

Although periodontitis is an infectious disease of the gingival tissue, changes that occur in the bone are crucial because the destruction of the bone is responsible for the tooth loss. The height & density of the bone are normally maintained in equilibrium, regulated by local & systemic influences [174-176] between bone formation and bone resorption. When resorption exceeds formation, both bone height and bone density may be reduced. The level of bone is the consequence of past pathologic experiences, whereas changes in the soft tissue of the pocket wall reflect the present inflammatory condition. Therefore the degree of the bone loss is not necessarily correlated with the depth of the periodontal pockets, severity of the ulceration of the pocket wall, or presence or absence of pus.[1]

Bone Destruction Caused by the Extension of Gingival Inflammation

The most common cause of bone destruction in periodontal disease is the extension of inflammation from the marginal gingiva into the supporting periodontal tissues. The inflammatory invasion of the bone surface & the initial bone loss that follows, mark the transition from gingivitis to periodontitis. The transition from gingivitis to periodontitis is associated with changes in the composition of the bacterial plaque. In the advanced stages of the disease, the number of coccoid rods & straight rods decreases.[177] Fibroblasts and lymphocytes predominate in stage I gingivitis, where as the number of plasma cells & blast cells increases gradually as the disease progresses. Seymour et al.;[178, 179] have postulated a stage of contained gingivitis in which T lymphocytes are preponderant and believed that as the lesion becomes a B lymphocyte predominant lesion, it becomes progressively destructive.

Heijl L et al.;[180] were able to convert a confined naturally occurring chronic gingivitis into a progressive periodontitis in experimental animals by placing a silk ligature in to the sulcus & tying it around the neck of the tooth. This induced ulceration of the sulcular epithelium, a shift in the connective tissue population from predominantly plasma cells to predominantly polymorphonuclear leukocytes, & osteoclastic resorption of the alveolar crest. Acute episodes of destruction are one of the mechanisms leading to bone loss in marginal periodontitis.[1]

The pathway of the spread of inflammation is critical because it affects the pattern of bone destruction in periodontal disease. Considerable controversy exists about the possible changes in the pathway of

gingival inflammation caused by trauma from occlusion. The suggested change in the pathway of inflammation, going forward into the periodontal ligament rather than to the bone has not been confirmed. The dense transeptal fibers are of clinical importance when surgical procedures are used to eradicate periodontal pockets as they form a firm covering over the bone, which is encountered after the superficial granulation tissue is removed.(181)

Radius of Action:

Garant and Cho (182) suggested that locally produced bone resorption factors may need to be present in the proximity of the bone surface to exert this action. Page and Schroeder(183), on the basis of ***Waerhaug's measurements***(175, 176) made on human autopsy specimens, postulated a range of effectiveness of about 1.5 to 2.5mm within which bacterial plaque can induce loss of bone.

Beyond 2.5mm there is no effect; interproximal angular defects can appear only in spaces that are wider than 2.5mm because narrower spaces would be destroyed entirely tall corroborated this with measurements in human patients.(184, 185) Large defects greatly exceeding a distance of 2.5mm from the tooth surface may be caused by the presence of bacteria in the tissues.(1)

Rate of bone loss:

In a study of Srilankan tea labourers with no oral hygiene and no dental care, Loe et al.;(149) found the rate of bone loss to average about O.2mm a year for facial surfaces and about 0.3mm a year for proximal surfaces when periodontal disease was allowed to progress untreated. However, the rate of bone loss may vary, depending on the type of disease present. **Loe et al.;** (149), identified the following three sub groups of patients with periodontal disease based on interproximal loss of attachment and tooth mortality.

1. Approximately 8% of persons had rapid progression of periodontal disease, characterized by a yearly loss of attachment of 0.1 to l.0mm.
2. Approximately 81% of individuals had moderately progressive periodontal disease, with a yearly loss of attachment of 0.05 to 0.5mm.
3. The remaining 11% of persons had minimal or no progression of destructive disease (0.05-0.09mm yearly).

Period of Destruction:

Periodontal destruction occurs in an episodic, intermittent manner, with periods of inactivity or quiescence. The destructive periods result in loss of collagen and alveolar bone with deepening of the periodontal pocket. The reasons for the onset of destructive periods have not been totally elucidated, although the following theories have been offered:

1. Bursts of destructive activity are associated with subgingival ulceration and an acute inflammatory reaction, resulting in rapid loss of alveolar bone.[183, 186]
2. Bursts of destructive activity coincide with the conversion of predominantly T-lymphocyte lesion to one with a predominantly B-lymphocyte plasma cell infiltrate.[179]
3. Periods of exacerbation are associated with an increase of the loose, motile, gram negative anaerobic pocket flora, and periods of remission coincide with the formation of a dense, unattached, gram-positive flora with a tendency to mineralize.[187]
4. Tissue invasions by one or several bacterial species are followed by an advanced local host defense that controls the attack.[1]

Mechanisms of Bone Destruction

The factors involved in bone destruction in periodontal disease are bacterial and host mediated. Bacterial plaque products induce the differentiation of bone progenitor cells into osteoclasts and stimulate gingival cells to release mediators that have the same effect. Plaque products and inflammatory mediators can also act directly on osteoblasts or their progenitors, inhibiting their actions and reducing their numbers.

In addition, in rapidly progressing disease such as aggressive periodontitis, bacterial micro colonies or single bacterial cells may also be present between collagen fibers and over the bone surface, suggesting a direct effect.[188, 189]

Several host factors released by inflammatory cells are capable of inducing bone resorption in vitro and can play a role in periodontal disease. These include host produced prostaglandins and their precursors, interleukin-l α and IL-lβ, and tumor necrosis factor α. When injected intradermally, prostaglandin E2 induces the vascular changes seen in inflammation; when injected over a bone surface, PGF2 induces bone resorption in the absence of inflammatory cells and with few multinucleated osteoclasts.[89, 190]

Bone formation in periodontal disease:

Areas of bone formation are also found immediately adjacent to sites of active bone resorption and along trabecular surfaces at a distance from the inflammation, in an apparent effort to reinforce the remaining bone. This osteogenic response is clearly found in experimentally produced periodontal bone loss in animals. In humans, it is less obvious but has been confirmed by histometric and histologic studies.

The response of alveolar bone to inflammation includes bone formation and resorption. Thus, bone loss in periodontal disease is not simply a destructive process but results from the predominance of resorption over formation. New bone formation impairs the rate of bone loss, compensating in some degree for the bone destroyed by inflammation.

Autopsy specimens from individuals with untreated disease occasionally show areas where bone resorption has ceased and new bone is being formed on previously involved bone margins. This confirms the intermittent character of bone resorption in periodontal disease and is consistent with the varied rates of progression observed clinically in untreated periodontal disease.

These periods of remission and exacerbation appear to coincide with the quiescence or exacerbation of gingival inflammation, manifested by changes in the extent of bleeding, amount of exudates and composition of bacterial plaque.

The presence of bone formation in response to inflammation, even in active periodontal disease, has an effect on the outcome of treatment. The basic aim of periodontal therapy is the elimination of inflammation to remove the stimulus for bone resorption and therefore allow the inherent constructive tendencies to predominate.

Bone Destruction Caused by Trauma from Occlusion:

Another cause of periodontal destruction is trauma from occlusion, which can produce bone destruction in the absence or presence of inflammation.

In the absence of inflammation, the changes caused by trauma from occlusion vary from increased compression and tension of the periodontal ligament and increase osteoclastogenesis of alveolar bone to necrosis of the periodontal ligament and bone and the resorption of bone and tooth structure. These changes are reversible so that they can be repaired if the offending forces are removed. However persistent trauma from occlusion results in funnel-shaped widening of the crestal portion of the

periodontal tissues aimed at "cushioning" increased occlusal forces, but the modified bone shape may weaken tooth support and cause tooth mobility.

When combined with inflammation, trauma from occlusion aggravates the bone destruction caused by inflammation and result in bizarre bone patterns.[1]

This can be explained by two concepts

a) Glickman's Concept
b) Waerhaug's Concept

Glickman's Concept:

Glickman (1965, 1967) [191, 192] claimed that the pathway of the spread of a plaque-associated gingival lesion can be changed if forces of an abnormal magnitude are acting on the contaminated tooth. This implies that the character of the progressive tissue destruction of the periodontium at a "traumatized tooth" may be different from that characterizing a "non-traumatized" tooth. According to this concept periodontal structures can be divided into two zones

1. Zone of irritation
2. Zone of co-destruction.

The ***zone of irritation*** includes the marginal and interdental gingiva. The soft tissue of this zone is bordered by hard tissue (the tooth) only on one side and cannot therefore be affected by forces of occlusion. Thus, the gingival lesion is the tissue response to products from microbial plaque. This gingival lesion at a "non-traumatized" tooth propagates, according to Glickman, in the apical direction by first involving the alveolar bone and only later the periodontal ligament area. The progression of this lesion results in an even (horizontal) bone destruction.

The ***zone of co-destruction*** includes the root cementum (mineralized tissue), the periodontal ligament and the alveolar bone (mineralized tissue), and is coronally demarcated by the trans-septal (interdental) and the dento alveolar collagen fiber bundles The tissues in this zone may become the seat of a lesion caused by trauma from occlusion.

It was claimed that the fiber bundles which separate the zone of co-destruction from the zone of irritation can be affected from two different directions:

1. From the inflammatory gingival lesion maintained in the ***zone of irritation***
2. From trauma-induced changes in the ***zone of co-destruction***.

Through this exposure from two different directions, the fiber bundles may become dissolved and/or orientated in a direction parallel to the root surface.

The gingival lesion in the ***zone of irritation*** will spread directly into the "trauma-exposed" periodontal ligament. Through this exposure from two different directions, the fiber bundles may become dissolved and/or orientated in a direction parallel to the root surface.

The gingival lesion in the ***zone of irritation*** will spread directly into the "trauma-exposed" periodontal ligament.[193]

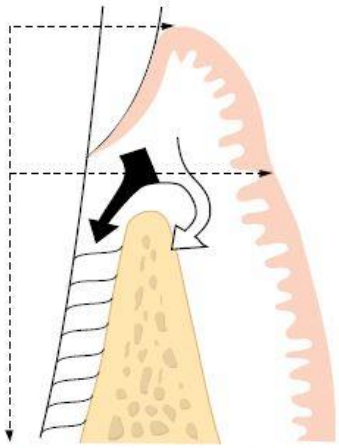


Fig. 16-2 Inflammatory lesion in the zone of irritation can, in teeth not subjected to trauma, propagate into the alveolar bone (open arrow), while in teeth also subjected to trauma from occlusion, the inflammatory infiltrate spreads directly into the periodontal ligament (filled arrow).

[193] ***Lang NP, Lindhe J. Clinical periodontology and implant dentistry, 2 Volume Set: John Wiley & Sons; 2015.***

Waerhaug's Concept:

According to Waerhaug, the loss of connective attachment and bone around teeth is, exclusively the result of inflammatory lesions associated with subgingival plaque. Waerhaug concluded that angular bony defects and infrabony pockets occur when the subgingival plaque of one tooth has reached a more apical level than the plaque on the neighbouring tooth, and when the volume of the alveolar bone surrounding the roots is comparatively large.

Waerhaug's observations support findings presented by Prichard (1965)[147] and Manson[194] (1976), which imply that the pattern of loss of supporting structures is the result of an interplay between the form and volume of the alveolar bone and the apical extension of the microbial plaque on the adjacent root surfaces.[193]

Systemic Diseases

Systemic Diseases affecting the bone architecture include Osteoporosis, Vitamin D deficiency, Diabetes, Hyperparathyroidism, Haematological disorders, Paget's disease, Fibrous dysplasia etc.

Osteoporosis is a systemic bone disease characterized by reduced bone strength, low bone mineral density (BMD), and altered macroscopic and microscopic architecture, and is associated with increased risk of fractures. A reduced buccolingual width in the dentate alveolar process may occur as a result of periosteal resorption. Osteoporosis could also influence the rate of tooth movement. Persistent trabeculae are found along the planes of bone stress. Trabeculae may be arranged radially; in between wide spaces are present. Radiographically, there is reduced density accompanied by thinning of cortical boundaries.[195]

Vitamin D at standard level maintains the calcium balance in the body. This calcium can be used for mineralization of bone. In hypo-calcaemic, alveolar bone showed hypomineralization and demonstrated a cellular and matrix organization, similar to the immature woven bone. Vitamin D deficiency results in osteoporotic bone with thinned dental crypts. Also, Vitamin D excess can affect nuclear receptors in the osteoblasts resulting in bone resorption. The trabeculae become reduced in number. In severe cases, jaws appear completely radiolucent, so that teeth appear to be suspended in air. Thus, it can be concluded that deficiency as well as excess of Vitamin D can lead to osteopenia and bone resorption.[196]

Diabetes results in a poor bone quality because of the formation of advanced glycation end products, eventually resulting in fractures. Taylor et al.;[197] postulated that inadequate glycaemic control can result in increased alveolar bone loss. It alters the response of the periodontal lesion to local irritants, hastening bone loss and retarding postsurgical healing of the periodontal lesions. This disease exhibits a fulminating periodontitis with periodontal abscess formation. This gives rise to mobility of teeth. There is severe and rapid alveolar bone resorption seen. Diabetes also increases apoptosis and decreases the number of bone-lining cells, osteoblasts, and periodontal ligament fibroblasts. Thus, diabetes causes a more persistent inflammatory response, greater loss of attachment and more alveolar bone resorption, as well as impaired new bone formation.[198]

In leukaemia, the infiltrate fills the marrow spaces and the periodontal ligament which results in osteoporosis of alveolar bone and the supporting bone along with disappearance of periodontal fibers. Destruction of alveolar bone is the most common manifestation of leukaemia. Bone loss may be in the

form of transverse lines of increased radiolucency or irregular areas and bone loss produced which gives the so called, ***moth eaten appearance*** [199]

Paget's disease of bone/ Osteitis deformans is associated with abnormal remodelling of bone that results in the weakening of the affected bone with pain, fractures and arthritis in the joints near the affected bones. In this disease coarse and sparse trabecula are seen that tend to converge towards the midline of mandible. In the skull, early lytic lesion may be seen as discrete radiolucent areas termed as ***osteoporosis circumscripta***. The margins are somewhat irregular. Bone scan may demonstrate marked uptake throughout the entire mandible which is seen radiographically as ***Lincoln's sign or black beard***. In later stages, rounded radiopaque patches of abnormal bone are often seen giving a ***cotton wool appearance.*** Also, there is increase in ***alveolar width*** associated with flattening of palate when maxilla is involved.[200]

Fibrous dysplasia is a disorder where fibrous tissue replaces normal bone and marrow that makes the bone weak. Fibrous dysplasia appears as areas of whorled amorphous calcified materials that are well circumscribed. Radiographically, this gives a characteristic ***ground glass appearance***. As the lesions grow, dysplastic bony trabeculae increase in size and number and give an appearance of smoky mottled radiopacities. The replaced structure of bone resembles the ring of orange which is called as ***'orange peel'***. Enlarging deformities of alveolar process mainly in buccal and labial cortical plates are also seen.[201]

In Osteomalacia affecting adults, there is incomplete mineralization of osteoid that leads to pseudo fractures. There is decrease in Ca/PO_4 ratio, increase in alkaline phosphatase and decrease in calcium excretion. Osteoid tissue is formed in the defect but there is no calcium available to be deposited in the osteoid and the zone is called as ***Looser's zone***. A poorly calcified ribbon like zone extending into bone at approximately right angles to the periosteal margin is also seen radiographically. There is increased tendency towards fracture, peculiar waddling or penguin gait, tetany and green stick bone fractures.[202]

Potential Mechanisms of Association of Bone Loss and Osteoporosis:

Several potential mechanisms by which osteoporosis or systemic bone loss may be associated with loss of alveolar bone height and tooth loss have been proposed - 1) low bone density in the oral bone associated with low systemic bone density, which leads to more rapid resorption of alveolar bone following insult by periodontal bacteria; 2) modification of local tissue response to periodontal infections due to systemic factors affecting the bone remodelling - persons with systemic bone loss are known to have increased systemic production of cytokines IL-1 & IL-6) that may have an effect on the bone throughout the body including the bone of the oral cavity. Periodontal infections have been shown to increase local cytokine production that in turn increases local osteoclast activity resulting in increased bone resorption; 3) environmental factors such as cigarette smoking and sub optimal calcium intake may put individuals at a risk for development of both osteopenia and periodontal disease. However, most of the studies consider low systemic bone density as the primary factor for the rapid resorption of alveolar bone. Elders et al.; (1992)[203] assessed the association between alveolar bone height, spinal bone mineral density (BMD) and metacarpal cortical thickness (MeT) in 286 women, aged 46-55 years and 21% of whom were edentulous. In dentate subjects, mean alveolar bone height was significantly correlated with spinal BMD, MeT, age and years since menopause. However, umbar BMD and MeT were not found to significantly correlate with alveolar bone height. The fact that no association was detected may be due to the selection of the subjects, younger individuals (40-65 yrs) and lower prevalence of osteoporosis thus limiting the association observed.[204]

Ward and Manson (1973)[205] were unable to show a significant relationship between alveolar bone loss & bone density of hand using metacarpal bone index. However, rapidity (a measure of alveolar bone loss divided by age) was found but not in males, potentially suggesting some role for osteoporosis in the loss of oral bone, based on gender and with ageing. In a cross sectional study of mandibular bone density by Kribs P.J[206] in osteoporotic women, tooth loss and edentulism were found to be significantly more attributed to osteoporosis. Certainly one clinical implication is to advise patients to have a good diet, exercise, and explain regarding hormone placement therapy (HRT). Several longitudinal cohort studies have examined the effects of hormonal replacement therapy on tooth loss - each of the studies demonstrated that long term oestrogen replacement as a part of post-menopausal replacement therapy exerted a protective effect limiting tooth loss, after correcting the data for

confounding variables such as age, smoking and education. Estrogen repletion is associated with less bleeding on probing and a tendency for less frequent clinical attachment loss.

Paganini - Hill (1995)[207] examined the relationship between post-menopausal oestrogen replacement and number of missing teeth in 3921 women. They found that age adjusted risk of edentulism was approximately half that of non-users. However HRT is not acceptable to, nor is it appropriate, for all humans. Therefore some attention has been focused on identifying nutritional factors that will reduce bone loss, either alone or in combination with osteoporosis medication. On average, osteoporotic women had lost 6.9 mandibular teeth compared to 4.5 teeth in women with normal bone density.

Taguchi et al.; (1995)[208] studied the relation between tooth loss and oral bone density in 269 subjects, including 99 men and 170 women. No relationship was seen between mandibular cortical width and tooth loss in males. However, in female subjects, a decrease in mandibular cortical bone width was positively correlated with tooth loss. The association was not apparent in women past their 7th decade of life

Management:

Combination of lack of exercise, decreased nutritional intake of calcium and lack of hormone replacement after menopause may explain upto 50% of loss of bone mineral density. Medications and strategies for prevention and treatment of osteoporosis also include anti resorptive drugs. It includes selective oestrogen receptor modulators & bis-phosphonates. Bis-phosphonates bind avidly to apatite crystals, mainly on remodelling surfaces & inhibits their growth, aggregation and dissolution. The more potent nitrogen containing member of this drug class includes alendronate, risedronate, ibandronate and zoledronic acid. Alendronate has been shown to reduce active bone resorption significantly without interfering with bone mineralization and quality. Clinical trials have shown that bis-phosphonates also decrease bone turnover and increase bone mass strength.

Weinreb et al.; (1994)[209] tested the efficacy of alendronate in reducing alveolar bone loss caused by experimental periodontitis in cynolomolgus monkeys. Alendronate was found to be significantly effective in reducing bone loss associated with experimental periodontitis. Reddy et al.; (1995)[210] also evaluated alendronate for inhibition of alveolar bone loss in naturally occurring periodontitis in beagle dogs. They concluded that the group receiving alendronate exhibited statistically significant increase in bone mass and density.

There have been few human studies to date on the oral effect of medication for osteoporosis and therefore carefully designed studies and randomized controlled trials with adequate statistical power are required to determine the clinical significance of osteoporosis therapies on risk of tooth loss and other oral health outcomes.

Parathormone and Calcitonin:

Parathyroid hormone/ Parathormone (PTH) or parathyrin, is secreted by the chief cells of the parathyroid gland as a polypeptide containing 84 amino acids. It acts to increase the concentration of calcium in the blood, whereas calcitonin (a hormone produced by parafollicular cells of the thyroid gland) acts to decrease calcium concentration. PTH acts to increase the concentration of calcium in the blood by acting upon the parathyroid hormone receptor (high levels in bone and kidney) and the parathyroid hormone 2 receptor (high levels in the central nervous system, pancrease, testis and placenta). PTH half-life is approximately 4 minutes. It has a molecular mass of 9.4 kDa.

Role of osteoblasts in hormonal control of bone resorption - A hypothesis:[(211)]

It has been shown to have a large number of effects on osteoblasts/ "osteoclast- like" cells including; 1. Stimulation of adenylate cyclase activity results in a cyclic AMP (cAMP) surge. Rapid activation of this cyclic AMP- dependant protein kinase causes Inhibition of collagen synthesis and Inhibition of alkaline phosphatase activity. 2. Later on there is a stimulation of calcium uptake. 3. Production of cell shape changes resulting in less tight packing of cells, observed both in calvaria and in culture.

On the other hand, there is little evidence so far that osteoclasts possess PTH receptors or respond to PTH directly. There is accumulating evidence that circulating mononuclear cells (monocytes) are osteoclast precursors and can resorb devitalized bone in culture, but PTH has no effects on the chemotactic migration or the resorbing activity of these cells. Moreover, PTH does not seem to be essential for normal osteoclastic activity and bone remodelling since these functions are retained in parathyroidectomized new born rats. Furthermore, no PTH-related defect can be implicated in osteoclast malfunction associated with osteoporosis.

Morphologically, it was shown in calvaria that the osteoblasts form a contiguous layer that covers the matrix and separates it from inactive (non-resorbing) osteoclasts. These 'idle' osteoclasts have long projections that screen the surface and may start resorbing bone if the bone matrix becomes exposed within their reach. The osteoclasts might also migrate toward uncovered matrix areas. Bone resorption products specifically osteocalcin, were indeed shown to be chemotactic to mononuclear cells, presumed to be precursors of osteoclasts. This information led to the following hypothesis for

osteoblastic involvement in hormonal control of bone resorption. On the one hand, hormone actions remove the osteoblastic barrier. Removal of inhibition is a common feature of biological control. Resorbing agents such as PTH and prostanoids, induce (probably via cAMP or calcium) a shape change in osteoblasts, which uncovers matrix, exposing it to osteoclasts or osteoclast projections. The resulting matrix digestion further enhances resorption by releasing collagen and osteocalcin, which attract monocytic osteoclasts. The second aspect of this hypothesis is the potential direct activation of osteoclasts by products of hormone action on the osteoblast. These could contribute to chemotaxis, as osteocalcin and some products of arachidonic acid metabolism do, or could directly accelerate another rate-limiting step in osteoclastic resorption. The third aspect of osteoblast involvement, effective on a longer time scale, is hormone inhibition of anabolic functions, such as collagen synthesis and alkaline phosphatase activity.

This effect would reduce the drain on extracellular fluid calcium and sequence of events also fits calcium-kinetics data. Following PTH administration, there is an early increase in the calcium exchangeable pool that can be attributed to the uncovered matrix, and a later elevation in calcium levels that may be due to newly mobilized resorbing cells and reduction in mineral deposition. The hypothesis postulated above, considers resorption and formation as opposing processes, the rate of which is determined by the competition of osteoblasts and osteoclasts for the bone matrix surface, as has long been recognized in morphometric studies. Various factors that can affect this balance including mechanical or electrical stimulation, inflammation products, osteoclast activating factors and many others- contribute to the dynamic balance of bone mass. Many concepts of this hypothesis can be tested experimentally. It may also suggest new strategies for therapeutic approaches to pathological bone loss.[211]

Pharmacological Agents Affecting Alveolar Bone

Bone Sparing Agents

Bisphosphonates:

Bisphosphonates are bone sparing agents used in the management of metabolic bone diseases that are associated with excessive bone resorption, including osteoporosis, Paget's disease, multiple myeloma and cancer related diseases secondary to breast cancer and prostate cancer.

Structurally, bisphosphonates are analogues of pyrophosphate in which the carbon atom replaces the linking oxygen atom in the pyrophosphate molecule. They possess a P-C-P back bone, to which two side chains (R1 and R2) are attached. It has been reported that the pharmacological characteristics of bisphosphonates vary, depending on the nature of the side chain. Bisosphonates are completely resistant to enzymatic hydrolysis and are extremely stable. They bind to the hydroxyapatite crystals of bone, preventing their growth and dissolution.

Based on their structure, bisphosphonates have been classified as:
1st generation drugs - with alkyl side chain: Etidronate
2nd generation drugs - with amino side chain: Alendronate, Pamidronate, Zoledronate
3rd generation drugs - with cyclic side chain: Risedronate
The anti resorptive properties of bisphosphonates are said to increase approximately 10-fold between the drug generations.

On the basis of their effects on macrophages, bisphosphonates can be grouped as:
Amino bisphosphonates: sensitize macrophage to an inflammatory stimulus, including an acute - phase response.

Non amino bisphosphonates: they can be metabolized by macrophages and may inhibit the inflammatory response of macrophages.

Amino bisphosphonates stimulate the release of pro-inflammatory cytokines while non-amino bisphosphonates seem to have anti-inflammatory activity caused by an inhibition of the release of inflammatory mediators from activated macrophages, such as IL-6,TNF-a and IL-β. This activity

enables the use of non – amino bisphosphonates in several inflammatory diseases characterized by phagocyte-mediated production of acute phase cytokines, such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, myelofibrosis and Hypertropic Pulmonary Osteoarthropathy. The effect of bis-phosphonates on bone metabolism is mediated through the suppression of the interactions between the RANK and RANKL as well as osteoprotegerin. These are the final effector molecules of bone resorption whose activation is responsible for inflammatory and metabolic bone disease.(212)

Given their known affinity to bone and their ability to decrease osteoclastic differentiation, inhibit osteoclast recruitment and activity, and interfere with secretion of lysosomal enzymes, there exists a possible use for bisphosphonates in the management of periodontal disease. Bisphosphonates down regulate levels of several matrix metalloproteinases including MMP-3, MMP-8 and MMP-13 from human periodontal ligament cells. These bone-specific properties also provide an interesting management strategy to stimulate osteogenesis in conjugation with regenerative materials around osseous defects and may result in the promotion of bone formation around endosseous implants.(213)

Disodium dihydrogen-4 methane bisphosphonate (TRK-530) is a novel synthetic bisphosphonate suggested to have both anti-resorbing and anti-inflammatory effects. It has been found to dose-dependently prevent most of the stimulators of bone resorption, including lipopolysaccharides, PGE2, IL-lβ and TNF-α. The expression of cyclooxygenase COX-2 mRNA and COX-2 protein were also prevented. At the molecular level, bis-phosphonates have been found to inhibit the mevalonate pathway and decrease the post-transitional phrenylation of GTP-binding proteins. (214)
For bisphosphonates to be effective, chronic dosage for long periods are required. An important adverse effect with IV bisphosphonate delivery in the treatment of malignant bone disease is the resorption-remodelling cycle. Bisphosphonates are also contraindicated in patients with gastro-intestinal disorders and sensitivity to phosphates.

Tetracyclines

Tetracyclines were first introduced as broad spectrum antibiotics in 1948 and have had a wide therapeutic usage in addition to its antimicrobial activity. This group of compounds has the capability of inhibiting the activities of neutrophils, osteoclasts and MMPs (specifically MMP-8), there by working as an anti-inflammatory agent that inhibits bone destruction. A germ-free rat model of connective tissue break down and a series of *in vitro* studies identified an unexpected non-antimicrobial property of tetracyclines. This ability of tetracyclines to inhibit MMPs is found to reflect

multiple direct and indirect mechanisms of actions, and to be therapeutically useful in adult periodontitis as well as medical diseases such as arthritis, osteoporosis and cancer.[213]

The mechanisms by which tetracycline inhibits connective tissue breakdown include:

a) Mediated by extracellular mechanism:

i) Inhibition of active MMPs depends mainly on the calcium and zinc binding properties of tetracycline.

ii) Inhibition of oxidative activation of pro-MMPs, independent of cation binding properties of tetracycline.

iii) Promoting excessive proteolysis of pro-MMPs into enzymatically inactive fragments, dependant on cation binding of tetracycline.

iv) Inhibition of MMPs protects α-1 proteinase inhibitors and thus indirectly decreases serine protein (such as PMN elastase activity).

b) Mediated by cellular regulation:

i) **Tetracycline inhibits inflammatory cytokines:**

The lipopolysaccharide component of Gram-negative bacteria (endotoxin) induces the production of pro-inflammatory cytokines, TNFα and IL-1β, which can mediate septic shock systemically and connective tissue breakdown, including bone resorption, locally. The local effect is likely a host-mediated pathogenic pathway for microbially induced periodontal disease. In this regard, endotoxin-induced bone resorption and the excess collagenase activity secreted by endotoxin-stimulated macrophages were both inhibited by TCs when added to the culture media (Golub et al.; 1984, 1994b) [215, 216] The inhibition of cytokine secretion by TCs appears to reflect a post-transcriptional effect (cytokine mRNA was not reduced by these drugs), the previously recognized ability of these drugs to inhibit MMP activity may play a role for the following reason: MMPs have been shown to convert membrane-bound pro-TNFα into its biologically active soluble form, a secretory process that can be inhibited by anti-collagenase agents such as hydroxamic acid derivatives (Mohler et al.;[217], 1994). Clearly, the ability of TCs to inhibit cytokine production, by whatever pathway, provides another mechanism for these drugs to inhibit MMP activity in the extra cellular matrix, thus protecting the latter from pathologic destruction.

ii) **Inducible nitric oxide synthase:**

One example of the complex interaction of these various cell regulators in tissue destruction was described by Ralston and Grabowski[218] (1996), who stated that cytokines likely stimulate osteoclast-mediated bone resorption by altering "the balance between levels of NO and PGE2". Nitric oxide (NO) is a short-lived messenger molecule with numerous functions, including (but not limited to) smooth muscle relaxation, neurotransmission, and tumor cell killing, as well as participation in the inflammatory process and tissue breakdown, and is produced from L-arginine by nitric oxide synthase, or NOS (Nathan and Xie, 1994)[219]. NOS1 and NOS3are constitutive isoforms of the enzyme found in neuronal and endothelial cells, respectively, while the inducible form (NOS2 or iNOS) is expressed by various cells such as macrophages and fibroblasts (Trachtman et al.;[220], 1996). Amin et al.;[221] (1996) found that minocycline and doxycycline blocked NOS activity in IL-lβ-stimulated human osteoarthritic cartilage explants and inhibited endotoxin-stimulated iNOS in murine macrophages in culture. These effects were not due to a direct inhibition by these TCs of NOS activity in vitro, but were found to reflect suppressed expression and translation of these enzymes.

iii) **Phospholipase A2 and Prostaglandin E2 synthesis:**

TCs have been found to have an impact at several steps in this pathway. Phospholipase A2 (PLA2) is a pro-inflammatory enzyme that participates early in the arachidonic acid cascade (it hydrolyses glycerophospholipids in cell membranes to AA) and its levels in tissue fluids are correlated to connective tissue destructive activity during diseases such as rheumatoid arthritis.

ElAttar et al.;[222] (1988) reported that relatively high concentrations (—50 (jug/mL) of minocycline were required to inhibit prostaglandin E2 (PGE2) synthesis by fibroblasts in culture. An intriguing mechanism is suggested by the ability of TCs to dampen reactive nitrogen as well as reactive oxygen species (Landino et al.;[223] 1996): By inhibiting iNOS (and nitric oxide production) and scavenging ROS, TCs could suppress peroxynitrite levels (see previous section) which, in turn, could suppress cyclo-oxygenase activity and prostaglandin biosynthesis. PGE2 and other arachidonic acid metabolites have long been recognized as mediators of connective tissue breakdown, including bone and cartilage destruction, in part by inducing MMP expression and activity (Golub et al.; 1984; Zhang et al.; 1997).[215, 224]

iii) **Inhibition of protein kinase C:**

Webster et al.;[225] (1994) demonstrated that TCs can inhibit protein kinase C (PKC). PKC mediates the transcriptional activation of several MMPs, such as stromelysin and collagenase. One possibility involves the activating proteinfactor-1 complex (AP-1); the latter binds to the MMP promoter, thus

stimulating transcription of these enzyme proteins. However, Jonat et al.;[226] (1996) proposed that the inhibition of MMP transcription by TC was not due to a block of AP-1 activity, since this effect of the drug occurred upstream of the AP-1 binding site. To date, it is not clear which signalling pathway for MMP expression is down-regulated by TCs.

c) Mediated by pro-anabolic effects

i) Increases collagen production

ii) Increases osteoblastic activity and bone formation

Schneir et al.;[227] (1990) suggested that TCs could increase the rate of collagen production in connective tissues during experimental (and human) diabetes by increasing the production and/or biologic activity of insulin-like growth factor (IGF-1). In this regard, this proanabolic activity of TCs might reflect, at least in part, the earlier-recognized anti-catabolic {i.e., MMP-inhibitory) properties of these drugs for the following reason: The increased proteinase activity in skin (and other tissues) of diabetic rats was recently associated with an increase in low-molecular-weight IGF-binding proteins—the latter suppress the biologic activity of this growth factor (Cechowska-Pasko et al.;[228] 1996). Thus, the previously discussed ability of TCs/CMTs to inhibit proteinase activity in the diabetic rat could reduce the level of these low-molecular-weight "inactivating" binding proteins, thus "normalizing" IGF-1 activity.

Chemically Modified Tetracyclines

Identification of the site on the tetracycline molecule responsible for its matrix metalloproteinase - activity led to the development of a series of chemically modified non- anti-microbial analogues, called chemically modified tetracyclines. They are devoid of antimicrobial activity (10% antimicrobial action and 90% anti-collagenolytic; Golub et al.;[229]) and hence have therapeutic potential but do not appear to induce antibiotic side-effects.

Mechanism of action:

i) Prevent the oxidative activation of latent pro-MMPs.

ii) Decrease the level of pro-inflammatory cytokines.

iii) Prevent the formation of multinucleated osteoclast-like cells from tartarate resistant acid phosphatase - stained cells of the osteoclast lineage.

iv) Binds to osteoclast sensor (ryanodine receptor on its plasma membrane) and diminishes cell functions; i.e. matrix adhesion, cell spreading enzyme secretion and bone resorption.

v) CMTs also bind to the ryanodine receptor on the nuclear membrane, alter the nucleoplasmic calcium influx and consequently affect osteoclast gene expression and apoptosis.
vi)Increases the level of IL-10, an anti-inflammatory cytokine, resulting in reduced osteogenesis and bone resorption.[213]

Osteoprotegrin/Anti-Rankl

The RANK-RANKL interaction is responsible for differentiation and maturation of osteoclast precursor cells to osteoclast. Osteoprotegerin, a member of TNF receptor super family is expressed by gingival fibroblasts and osteoblasts acts as a decoy receptor which binds to RANKL and inhibits RANK-RANKL interactions, thus preventing osteoclast development. Osteoprotegerin is a basic glycoprotein comprising 401 amino acid residues arranged into 7 structural domains. It is found as either a 60-kDa monomer or 120-kDa dimer linked by disulphide bonds.[230] Osteoprotegerin, also known as osteoclastogenesis inhibitory factor (OCIF) is encoded in humans by the TNFRs /IB gene. By binding to RANKL, OPG inhibits nuclear factor kappa-B which is a central and rapid acting transcription factor for immune-related genes, and a key regulator of inflammation, innate immunity and cell survival and differentiation. OPG levels are influenced by voltage - dependant calcium channel Cav 1.2. OPG can reduce the production of osteoclasts by inhibiting the differentiation of osteoclast precursors into osteoclasts and also regulating the resorption of osteoclasts *in vitro* and *in vivo*. OPG binding to RANKL on osteoblast cells block the RANKL - RANK interaction between osteoblast/stromal cells and osteoclast precursors. This has the effect of inhibiting the differentiation of the osteoclast precursor into a mature osteoclast. Recombinant human osteoprotegerin specifically acts on bone, increasing bone mineral density and bone volume.

The use of osteoprotegerin as a therapeutic agent for regulation of bone density was first evaluated by Simonet et al.;[231] where they treated overiectomized rats with murine osteoprotegerin-Fc protein and protected them against losses of bone volume associated with deficiencies of oestrogen. Other preclinical studies [232-234] demonstrated a potential therapeutic role of osteoprotegerin in the prevention and reduction of lytic bone lesions associated with skeletal tumour, prostatic carcinoma metastases, hypercalcemia of malignancy and breast cancer. Gene therapy for the lifelong delivery of osteoprotegerin has also been proposed as a more practical therapy for chronic inflammatory diseases. OPG-expressing adenoviral vectors provided sustained and efficacious levels of circulating osteoprotegerin that enhances bone mineral density and reduced osteoclast numbers for an extended period of time (18 months) in overiectomized mice.[235] A recombinant adeno-associated virus vector expressing OPG, and administered in a single injection, demonstrated complete inhibition of osteolysis

in a periprosthetic bone resorption model in mice and reversed osteopenia in overiectomized mice without liver toxicity.[231, 236]

The use of osteoprotegerin as an inhibitor of alveolar bone destruction in periodontal disease was investigated in mice orally infected with A. actinomycetemcommitans (Mahamed et al.;[237], Teng et al.;[238]). Inhibition of RANKL function with OPG treatment significantly reduced the number of osteoclasts and the alveolar bone destruction in both studies. Studies have also been done by Liu et al.;[239] 2003, Valderve et a1.; [240]2004, Belibaskis et al.;[230] 2005 on the role of RANKL-OPG in periodontitis. In summary the osteoprotegerin/RANK/RANKL axis presents a new target for the treatment of destructive periodontal disease and other bone- resorption related diseases. Further studies are necessary to determine the most efficacious therapeutic approach based on molecular interactions.[230]

Non-Steroidal Anti-Inflammatory Drugs

One of the earliest pharmacological strategies described to block the inflammatory processes in periodontal tissues as well as elsewhere in the body was the use of non-steroidal anti-inflammatory drugs (NSAIDs), to lock the arachidonic acid metabolites that are pro-inflammatory Mediators implicated in a variety of bone resorptive and tissue degrading processes.[213]

Arachidonic acid, a 20-carbon eicosanoid, is liberated from plasma membrane phospholipids via the enzyme, phospholipase A2. Free arachidonic acid can then be metabolized via cyclooxygenase or lipoxygenase pathways. Two isoforms of cyclooxygenase are recognized.

COX-l is constitutively expressed and serves physiologic 'house-keeping' functions (such as gastric cytoprotection and vascular and renal homeostasis). In contrast, cycloxygenase-2 is induced by cytokines and other signal molecules at the site of inflammation and thought to be involved in mitogenesis, inflammation and cellular differentiation. COX-l and COX-2 produce prostaglandins, prostacyclin and thromboxane whereas lipoxygenase enzyme produces Leukotrienes and other hydroxyl eicosatetraenoic acids. These metabolites have been implicated as principal catabolic mediators in periodontal diseases since they are potent stimulators of bone resorption, are present in gingival tissues and are elevated in diseased individuals.[241]

Following Vane's[242] landmark discovery in 1971 that aspirin and some non-stroidal anti - inflammatory drugs blocked cyclooxygenase, Goldhaber et al.;[243] 1973 observed that an NSAID, Indomethacin, inhibited the *in vitro* bone resorption stimulated by media from gingival fragments.

Following this, several animal studies were undertaken to test the effect of indomethacin on alveolar bone loss by Torabinejad et al.;[244] (1979), Nyman et al.;[245] (1979), Nicholas et al,; (1979), Weaks - Dybig et al.;[246] (1982), Lasfargues and Saffar [247](1983), Vogel et al.;[248] (1986) and Williams et al.; (1987). The data obtained from these studies suggested that indomethacin, an indole derivative NSAID, could reduce alveolar bone destruction in animal models at dosages varying from 2 to 10 mg/kg.[241]

William et al.;[249, 250] (1984, 1985), Jeffcoat et al.;[251] (1986) and Offenbacher et al.;[252] (1987) found that flurbiprofen reduces the rate of alveolar bone loss in naturally occurring periodontal disease in Beagles and rhesus monkeys. In studying the NSAID ibuprofen, Williams et al.;[253] (1988) and Kornman et al.;[254] (1990) found that ibuprofen reduces the rate of alveolar bone loss significantly in beagles and monkeys respectively. Studies have also been done on the effect of perioral naproxen by Howell et al.; [255], Johnson et al.; and Jeffcoat et al.; on piroxicam gel/liquid by Howell et al.; [241]1991 and on aspirin by Flemming et al.; [256] 1996. Li et al.; [257] and Paquette et al.; [258] evaluated the topical effects of s-Ketoprofen. It (Ketoprofen) is a propionic acid derivative which can block both COX and a lipoxygenase pathway has recently received considerable attention. Its administration as a racemic cream (1%), (s)- enantiomer dentifrice (0.3%, 3%) or (s)-enantiomer capsule (10mg)was observed to prevent the progression of alveolar bone loss in periodontitis. The use of enantio seIective NSAID s-Ketoprofen (benefits restricted to s-enantiomer) may provide greater efficacy at lower doses and with fewer side effects than other NSAIDS.

Finally, it must be noted that bone resorptive processes are more sensitive to arachidonic acid metabolites than are processes involved with bone formation or that different eicosanoids are involved with two of these processes.[241]

Anti-cytokines

Cytokines are literally "cell proteins" in aetiology, transmit information from one cell to another via autocrine or paracrine mechanisms. (241) Following specific binding to their complimentary receptors, pro-inflammatory cytokines like interleukin-I and TNF-α trigger intracellular signalling events and catabolic cell behaviours. The effects of soluble receptors and receptor antagonists of IL-1 and TNF-α have been studied during experimentally induced Periodontitis in a non-human primate model - Macaca fascicularis (Assuma et al.; (259), Graves et al.;[260]

Delima et al.;[261] Oates et al.; [262]). The clinical, radiographic and biochemical findings of these experiments showed that IL-I and TNF-α antagonists blocked:

I) The progression of inflammatory cell infiltrate toward the alveolar crest

2) The recruitment of osteoclasts and

3) Periodontal attachment and bone loss [185]

Under natural conditions, uncontrolled inflammatory responses with rapid tissue destruction due to activities of IL-I and TNF-α are reversed by the production of anti-cytokines such as IL- 4, IL-10 and IL-11. The potential to down regulate mediators of inflammation associated with periodontal tissue destruction was investigated during experimental periodontitis in beagle dogs over an 8-week period. The findings indicated that subcutaneous injection of recombinant human IL-11 was able to alter periodontal disease progression measured by changes in attachment level and radiographic bone height. (Martuscelli et al 2000).[263]

In conclusion, despite the expanding use of drugs blocking pro-inflammatory cytokine production, their precise mechanisms of action remains unclear. The development of novel approaches of cytokine blockade that are based on the characterization of intracellular signalling pathways regulate expression (e.g. nuclear factor kappa - B and p38 mitogen activated protein kinase/MAPK) and the use of small molecule inhibitors are being studied. Whether these approaches will live up to their early promise and become a major and widespread treatment for several devastating autoimmune diseases will depend on specificity, safety, durability of the benefit and pharmaco economic issues.

Oestrogen and Selective Estrogen Receptor Modulators (Serm)

Oestrogen deficiency is associated with a large increase in bone resorption, with osteoclast formation and reduced osteoclast apoptosis. Treatment with oestrogens clearly inhibits bone loss and increase bone mineral density. Oestrogens inhibit osteoclast activity and differentiation by regulating cytokine production. The effect of steroid hormone as metabolic mediators of the expression of cytokines may be a plausible explanation for the protective effect of oestrogen supplementation against periodontal disease. The discovery of the agents exerting full/partial oestrogen effects on various tissues led to the development of a new class of drugs known as Selective Estrogen Receptor Modulators (SERMs), which have fewer adverse effects than oestrogen. Examples of these drugs are:

1) Raloxifen (Benzothiophene): first SERM approved for treatment of post-menopausal osteoporosis
2) Tamoxifen (Triphenylethylene): follow up treatment of women with breast cancer

Hormone Replacement Therapy and Its Effects on Bone

During menopause, women go through biological changes particularly in their hormone secretions. The number of women using hormone replacement therapy (HRT) to cope with the hormonal changes is increasing. Although HRT in postmenopausal women is still controversial, HRT has been indicated to treat menopausal symptoms and to reduce the risk of osteoporosis. Some studies observed that postmenopausal women using HRT have increased tooth retention and decreased periodontal destruction.

HRT exerts its effects by various mechanisms and through different pathways. It can be anticipated to have beneficial effects in the oral cavity also by a variety of mechanisms. These include inhibition of MMPs, inhibition of osteoclasts, and other anti-inflammatory mechanisms. In the study of Pilgram et al.;[264] on healthy post-menopausal women, no relationship was found between radiographic alveolar bone height and probing attachment level. However, Goodstein et al.;[265] in their prospective study on 42,171 postmenopausal women have observed that HRT reduced the risk for tooth loss by 24%, although little effect was found in this regard in women who had stopped taking hormones. Hence, HRT may have an effect on periodontal health but more investigations are needed before a final conclusion can be drawn on the interaction.[266]

Vitamin D

The main function of vitamin D is to support calcium homeostasis, but it also plays an important role in immunity, the cardiovascular system, diabetes and chronic illness (Adams and Hewison 2010)[267]. The primary source of vitamin D are dietary intake and sunlight exposure in the form of vitamin D2 and D3, which are metabolized to 25-hydroxyvitamin D [25(OH)D in the liver. Further metabolism in the kidneys produces the active form of vitamin D, 1,25-dihydroxy Vitamin D (Holick 2007)[268]. Periodontitis is characterized by alveolar bone loss induced by the host response to bacterial insult. Because vitamin D plays a crucial role in bone maintenance and immunity, there is a biologic rationale to suspect that a vitamin D deficiency could negatively affect the periodontium.[269]

The normal range of serum 25(OH) D levels is 20-74 ng/mI. No absolute threshold for deficiency status has been universally accepted, although most authorities agree that levels below 0-30 ng/ml

constitute at least a mild deficiency, with severe vitamin D deficiency beginning at a level of 12 ng/ml (Malabanan et al.; [270] 1998). Calcium, phosphorus, and parathyroid hormone levels all influence the rate of conversion of 25(OH) D to its active form (De Luca et al.;[271] 2004). Parathyroid hormone (PTH) is an endogenous hormone with both catabolic and anabolic properties in bone, depending on the concentration and dosing regimen (Khosla et al.;[272] 2008). Recently, it was determined that a minimum 25(OH) D serum concentration of 28ng/ml was required to stabilize PTH levels and maintain normal calcium availability (Okazaki et al,; [269] 2011). Consequently, low vitamin D levels may result in high catabolic PTH levels that could negatively affect bone health.[269] Krall EA, Wehler C,[273] 2001 in their study found that lower serum 1,25(OH)D3 concentration were associated with higher attachment loss, which may be explained by anti-inflammatory effects of vitamin D. Krall[273] 2001, considered two studies in which one study showed no association between vitamin D intake from foods and supplements and the number of teeth with progression of periodontal bone loss. The other study, which was a systematic review, stated that although the numbers of studies on the effect of calcium or vitamin D intake on oral health outcomes are limited, the available studies suggest that higher intake levels are associated with reduced prevalence of clinical attachment loss and low risk of tooth loss. Data from a prospective study[274] of oral health in men show that a similar association between higher calcium intakes on oral outcomes is limited; the available studies suggest that higher intake levels are associated with reduced prevalence of clinical attachment loss and low risk of tooth loss. Data from a prospective study [273] of oral health in men shows a similar association between higher calcium intake and reduced alveolar bone loss. Jabbar et al.;[275] 2011 in their study found that periodontal disease is more common in women with osteoporosis and is associated with lower vitamin D and higher RANKL and osteoprotegerin levels.[276]

Mora et al.;[277] in 2008 suggested that vitamin D acts to regulate the inflammatory response by modulating cells of the innate and adaptive immune systems. The reasons for vitamin D deficiency causing peri-radicular radiolucency's are unclear [278] Praveen Sharma and Paul Weston[279] in 2012 proposed two main mechanisms: first, vitamin D deficiency may directly affect alveolar bone mineral density (by reducing serum calcium levels) and results in localized bone loss in areas of inflammation, and second, localized inflammatory episodes may go unchecked because of a lack of vitamin D, this may result in localized bone loss.[279]

Civitelli et al.;[280] 2002, in their study found that periodontally healthy women who received only calcium and vitamin D supplementation for 3 years experienced a mean 0.l mm increase in crest height. [280] Baxter and Renner et al.;[281, 282] 1984, in their cross sectional study of 11 healthy postmenopausal

women who required complete-denture replacements found that the mean dietary intake of calcium was 18.8 ng/ml. The investigators suggested that these low levels contributed low mandibular bone densities in these subjects. This suggests that vitamin D may be of benefit in the treatment of periodontitis, not only because of its direct effects on bone metabolism, but also because of its direct effects on periodontopathogens and inhibition of inflammatory mediators that contribute to periodontal destruction.(283)

Significance of Vitamin-D in Immune Response of Periodontium:

Liu et al. discovered that, during an inflammatory process, dental pulp fibroblasts and periodontal cells produce 25-hydroxylase, which stimulates the production of 25(OH)D3 (284). As pathological microorganisms affect the cell membrane receptors, 1α-hydroxylase synthesis is activated, during which 1,25(OH)D3 is formed from 25(OH)D3(285). The resulting molecule binds with the VDR in the immune and epithelial cells and participates in the epithelium defense mechanism against the pathogen (285, 286). 1,25(OH)D3 activates the synthesis of proteins which are required in the tight, gap and desmosome junctions of epithelial cells (285). The junctional epithelium connects to the tooth through loose junctions, thus, creating favourable conditions for a bacterial invasion from dental plaque, which initially causes inflammation of the periodontal tissue (PT), and, as the process advances, resorption and tooth loss occurs.(287)

1, 25(OH) D3 regulates the non-specific immune response, activates hydrogen peroxide secretion in monocytes, stimulates the synthesis of antimicrobial peptides, e.g.,β-defensin and cathelicidinLL-37. Cathelicidin LL-37 plays a role in chemotaxis, production of cytokines and chemokines, cellular reproduction, vascular permeability, wound healing, and neutralization of bacterial endotoxins.(285) McMahon et al.; studied human gingival cell cultures and the effect of vitamin-D on the expression of non-specific immune system of these cells. After affecting gingival cell cultures with 1,25(OH)D3, Cathelicidin, LL-37 secretion increased, and its antimicrobial effect against Actinobacillus actinomycetemcomitans lasted for 24 h (288).

1,25(OH)D3 takes place in specific immune system by affecting B-lymphocytes and T-lymphocytes (285). These cells emit cytokines and immunoglobulins, and they specifically destroy bacterial pathogens which are transferred by macrophages and dendritic cells. Such immune processes harm the PT and aggravate the course of PD. Vitamin D suppresses the proliferation of T-lymphocytes, secretion of immunoglobulins, transformation of B-lymphocytes into plasma cells, it inhibits the unwanted process, and protects the organism from excessive specific immune response by decreasing the

secretion of IL-1, IL-6, IL-8, IL-12, TNF-α cytokines . These cytokines are released in PD pathogenesis during a bacterial invasion. They cause lymphocyte infiltration, bone resorption, deterioration of extracellular matrix.[283, 284] Tang et al.; [289] studied the human periodontal tissue cell cultures trying to discern the anti-inflammatory effect of vitamin D on the cells. Less IL-8 was discovered in the cell cultures affected by Porphyromonas gingivalis and 1, 25(OH) D3 than in cell cultures affected only by Porphyromonas gingivalis. Thus, the hypothesis that vitamin D is effective in PD prophylaxis and treatment was confirmed. Teles et al.;[290] confirmed anti-inflammatory properties of vitamin D by determining that higher concentrations of vitamin D in blood serum contain less IL-6 and leptin and more adiponectin, which regulates the immune response. An increase in leptin signifies the presence of an infectious process and inflammation (proliferation and activation of T-lymphocytes, production of cytokines), adiponectin suppresses the production and activity of cytokines.[291]

Calcium

Skeletal bone mass increases throughout infancy, childhood and adolescence to achieve a genetically determined peak bone mass in early adulthood. Thereafter the skeleton loses bone mass. The rate of both increase and loss is dependent upon heredity and the availability of calcium. At different stages in human life, even if the levels of calcium are sufficient, there are threshold values of calcium intake above which increased intakes are not utilized. If calcium takes are not at or above threshold values, skeletal calcium is resorbed to maintain the body's calcium homeostasis, which is essential for life sustaining processes such as blood clotting, muscle contraction and nerve excitability. More specifically, extracellular fluid levels of calcium are tightly controlled within a narrow range required for normal physiological function. This control is mediated by two calcitropic hormones - parathyroid hormone (PTH) and Vitamin D.

The current recommendation for calcium intake is 1,200 mg/day for ages over 50.(292)The bone reserve of calcium is large enough to support normal physiologic function for months to years. The result of bone resorption however can be osteoporosis, which for women are 2.5 standard deviations below those for young adult women (aged between 20-40 years). Vitamin D and calcium supplementation counteract deficiencies and reduce bone resorption and fracture rates (Tilyard MW[293], Dawson-Hughes B[294] 1992, 1997). Vitamin D supplementation (l0/mg -400 IU/day) and adjusted calcium intakes of 1,000 mg/day increased vertebral bone density and total body calcium in post-menopausal women (Gallagher et al.;[295] 1990). Cumming et al.;[296] 1997 found that calcium

supplementation results in either greater gain in bone during growth, less loss of bone with age and/or reduced fracture risk, relative to un-supplemented individuals.

Moreover, vitamin D has its greatest effect when combined with calcium supplementation (Lips et al.;[297] 2001). Krall EA et al.;[273] in 2001, demonstrated that subjects (N=82) who received 500 mg of calcium and 700 IU of vitamin D per day for 3 years had a 60% lower risk of tooth loss than did those who took placebos. Tooth loss was studied in a double-masked, randomized, placebo controlled trial that considered the effects of calcium and vitamin D supplementation on bone loss from the hip. The same patients were followed for an additional 2 years during which time it was seen that subjects who consumed at least 1000 mg/day of calcium also had a 60% lower risk of tooth loss than subjects who took less calcium. [273]

Wical et al.;[298] 1974 conducted a cross sectional study in which they noticed that low consumption of milk and milk products were attributed to more severe residual alveolar ridge resorption in 44 edentulous subjects who completed 14 day dietary surveys. The 14 subjects who had minimal ridge resorption on panoramic radiographs had a mean intake of calcium of 933 mg/day where as the 30 subjects who had severe resorption had a mean intake of 533 mg/day.

In a 1960 cross-sectional study done by Groen et al.;[299] two groups of 24 subjects each were followed. One group had periodontal disease and the other group did not have periodontal disease. The severe radiographic alveolar bone resorption in the subjects with periodontal disease was attributed to past histories of low calcium intakes (350 to 555 mg/day), which the investigators suggested was caused by low milk ingestion.[299] In an examination of data on 12000 adults who took part in the Third National Health and nutrition Examination Survey (NHANES III)[300], it was found that lower dietary intake of calcium increased attachment loss in a dose-dependent fashion. Nishida M[301] 2000 suggested that the increased risk of periodontal disease could be related to decrease alveolar bone density associated with inadequate calcium intake.[301]

Finally, numerous articles indicate that Vitamin D and calcium deficiencies result in bone loss and increased inflammation, which are well recognized symptoms of periodontal disease. It has been suggested that calcium deficiency may be a risk factor for periodontal disease (Nishida et al.;[301]). Further research is needed to clearly define the health risks (including periodontal disease) associated with inadequate levels of calcium intake.[300, 302]

B) Bone Resorbing Agents:

Corticosteroids:

Corticosteroids act as anti-inflammatory drugs by inhibiting the enzyme phospholipase and thus preventing the subsequent production of archidonic acid and their metabolites like prostanoids and leukotreines. Me Guire et al.;[303] 1989 suggested that steroids inhibit phospholipase by stimulating the production of annexins/lipocortins. Steroids like dexamethasone causes degradation of pre-existing mRNAs for lL-lα and TNF-α, there by dampening secondary PGE2 release.

Corticosteroids may mask the inflammatory manifestation of periodontitis but the underlying disease continues to progress. Fisher et al.;[304, 305] (1971, 1972) and Cruess et al.;[306] 1975 reported that, in comparison to normal controls, patients who have been on systemic corticosteroid therapy following kidney transplantation appeared to have a diminished number of osteocytes in femoral heads and showed signs of avascular necrosis in the bone. Increased serum levels of corticosteroid hormones have also been associated with osteoporosis in man and in laboratory animals (Applebaum and Seelig [307]1955, Ceniggia and Gennari [308]1973).

From experiments in animals and observations in humans, it has been suggested that the administration of corticosteroids may result in an increased osteoporosis of alveolar bone (Glickman et al.;[309] 1953, Glickman and Schklar[310] 1955, Dreizen et al.;[311] 1971), a reduced number of osteoblasts and decreased bone tissue formation. (Labelle and Schaffer 1967)[312]

According to a study conducted by the American College of Rheumatology[303] in 1996, 20% of osteoporosis patients in the USA (about 2 million) had glucocorticoid-induced osteoporosis and 25% of patients on long term steroid therapy had bone fractures. Compared with primary osteoporosis, the risk of fracture for steroid induced osteoporosis is clearly higher, even at comparable bone mineral density. In other words, corticosteroids appear not only to decrease the mineral density, but also to markedly damage bone quality.[313]

Weinstein et al.; [314] (1998) reported that steroids suppress bone formation, resorption and act to lower the entire bone metabolism. In other words, these findings suggest that steroid treatment caused a low bone turnover in the mandible, suppressing the growth of cortical bone and thus lowering the bone mineral content and bone strength. [314]

However, little information is available on the use of steroids in periodontal therapy. Corticosteroids causes suppression of cell mediated immunity and favour spread of infections. They also interfere with healing and scar formations and can lead to Cushing's face, osteoporosis, suppression of hypothalamus - pituitary - adrenal axis and muscle weakness. Hence, the harmful effects of steroids may out-weigh their benefits. Further research is needed in the application of steroids in periodontal therapy and its effects on bone

Immunosuppressants:

These are medications capable of affecting bone metabolism and the rate of tooth movement. Some of these medications are the immunosuppressants-inhibitors of calcineurin phosphatase (cyclosporine and tacrolimus), which are partly responsible for increased survival rates of patients who have undergone organ transplants and for the reduction in the glucocorticoids doses. Similar to the glucocorticoids, ca1cineurin - phosphatase inhibitors also cause reduction in bone mass, with the greatest bone loss occurring in the first 6 months after transplantation, when immunosuppressant therapy is most aggressive. (Cipriani et a1.;[315] 2005). Even though the present tendency is to use a lower total dose of immunosuppressants, many transplanted patients continue to develop fractures as a complication (Canalis et al.;[316] 2001). Immunosuppressant drugs may be grouped into biologic and chemical categories, according to the location of their action and their effects on lymphocytes. The most frequently used immunosuppressants nowadays are those that affect cytokine synthesis (glucocorticoids, cyc1oporin-CSA, tacrolimus-1=K506) and those that affect nuelecotide synthesis (azathioprine, mycophenolate - mefetil).[315, 317]

These immunosuppressants are found to inhibit the intracellular biochemical pathways dependent on the presence of the calcium ion (Ca") and of its interactions with cytoplasmic receptors of the family of immunophilines. Recently, Kirino et al.;[318] (2004) investigated the effects of tacrolimus on bone metabolism. In this case control study; the authors administered tacrolimus to test subjects for 6 weeks and verified that after the initial increase in serum osteocalcin concentration, tacrolimus caused its reduction to levels lower than the basal levels. The calcemia remained constant throughout the study in spite of the significant increase in calciuria; in the third week, the serum level of PTH was already significantly higher in the test subjects submitted to the immunosuppressants; and when compared with the control group, the test group presented thinner bone trabeculae, and wider medullary cavities in some regions, due to the increase in osteoclastic number and activity Kirino et a1.;[318] 2004).

It has been demonstrated that treatment with cyclosporine affects alveolar bone, and that the deleterious periodontal effects may be due to the reduction in bone volume, decrease in the number of

osteoblasts and increase in osteoclasts (Spolidorio et al.;[319] 2007). In spite of the contradictory results studies have demonstrated that cyclosporine and tacrolimus may induce bone loss both in human beings and experimental animal models, through interleukin gene expression cytokines (lL-l, lL-6 and TNF) that participate in bone resorption (Lee et al.;[320] 2004).

In vitro, the experimental data obtained from animal models have suggested that tacrolimus is an osteopenic agent, although less osteotoxic than cyclosporine (Guimaaes et al.;[321] 2007). In another rat model Holstein et al.;[322] 2008 utilized, Rapamycin (RAPA) a macrolide and Immunosuppressive drug. It was found to have strong anti angiogenic activity linked to reduction of vascular endothelial growth factor (VEGF) , which causes less proliferation of osteoblasts, endothelial cells and periosteal cells, thus inhibiting cell proliferation and neovascularization.

The use of these immunosuppressants for long periods or in high doses increases bone remodelling and inhibits longitudinal bone growth, reducing growth speed by approximately 30- 50%, in addition to inhibiting different cell types, including smooth muscle cells; vascular cells and fibroblasts (Waller et al.;[323] 2005).

Thus the use of immunosuppressants in transplant patients and those with auto-immune disease are partly responsible for their longer survival; however the use of immunosuppressants may influence bone metabolism by significantly altering the trabecular bone. Further studies are needed to know the mechanism by which the immunosuppressants show their effect on bone.[324]

Anti-Diabetic Drugs

Thiazolidinedione's and biguanides are anti-diabetic drugs used for diabetes mellitus or as Pre-diabetes treatment. Clinical evidence that thiazolidinedione's increase the risk of fractures in women with type 2 diabetes was first demonstrated in a diabetes outcome progression trial (ADOPT) (Kahn et al.;[325] 2006). ADOPT was a randomized, double blind, prospective controlled clinical trial comparing the effect of the thiazolidinedione - rosiglitazone, biguanide - metformin, and sulfonylurea - glyburide on glucose control in recently diagnosed (< 3yr), drug-native patients with type-2 diabetes. A review of the adverse event reported found an increased occurrence of bone fractures in the upper and lower limbs, but not in the hip or vertebra, in women treated with rosiglitazone (Kahn et al.; [325] 2006).

In vitro, thiazolidinediones promote the differentiation of mesenchymal progenitor cells into adipocytes rather than osteoblasts (Ali[326] and Lecka - Czernic et al.;[327] 2005, 1999) and may suppress osteoblast formation by reducing insulin like growth factor (IGF-l) levels in bone (leeka - Czemik et al.;[328] 2007). Both rosiglitazone and pioglitazone have been shown to cause bone loss in rodents in most studies accompanied by decreased osteoblast activity and bone formation (Grey et al.;[329] and Lecka-Czernie1c et al.;[330] 2008, 2006) but in some by increased bone resorption (Lazarenko et al.;[331] 2007, Sottile et al.;[332]2004).

However Adami et al.;[333], Vestergaard et al.;[334] 2009, 2008) reported in their studies that use of metformin was associated with a reduced risk of fractures in patients with diabetes. It was also demonstrated that metformin exerts an osteogenic effect on osteoblasts (Cortizo et al.;[335] 2006), suggesting that metformin has a beneficial effect on alveolar bone.[336]

Thus controversies exist regarding the characteristics of anti-diabetic drugs on prevention of bone degradation. Moreover the mechanisms which determine the association between the hypoglycaemic drugs and bone needs further research to elucidate the effects of drugs on the bone.

Alveolar Bone in Regeneration

The regeneration of the periodontal tissues is dependent on four basic components. The appropriate signals, cells, blood supply and scaffold need to target the tissue defect. Each of these elements plays a fundamental role on the healing process in a simultaneous and temporal timeframe that is interconnected into the generation of new tissues. Cells provide the machinery for new tissue growth and differentiation. Growth factors or morphogens modulate the cellular activity and provide stimuli to cells to differentiate and produce matrix toward the developing tissue. New vascular networks promoted by angiogenic signals provide the nutritional base for tissue growth and homeostasis. Finally, scaffolds guide and create a template structure three-dimensionally to facilitate the above processes critical for tissue regeneration. A major complication and limiting factor in the achievement of periodontal regeneration is the presence of microbial pathogens that contaminate periodontal wounds and reside on tooth surfaces as plaque-associated biofilms.(337)

With bone graft materials:

In most cases, the goal of placing a bone graft is to regenerate lost tissue or simply to repair or fill the defect. Graft materials should ideally transfer an optimal quality of viable osteopotent cells-including osteoblasts and cancellous marrow stem cells - to the host site. For the osseointegration of the graft to proceed successfully, the host tissue must have sufficient vascularity to diffuse nutrients to the cells before revascularization occurs and to bud new capillaries into the graft to create a more permanent vascular network. Thus, depending on the amount of new bone that must be formed, donor sites are selected based on their osteocompetent cell density. The graft also consists of islands of mineralized cancellous bone, fibrin from blood clotting and platelets within the clot.

In descending order of available cancellous bone, autogenous donor sites include the posterior and anterior ilium, tibial plateau, femoral head, mandibular symphysis, calvaria, rib and fibula.(338)Other intraoral sites may also be good choices for autogenous bone harvest, and non-autogenous material may be used in some cases. Placement of a graft that consists of endosteal osteoblasts, marrow stem cells surrounded by a vascular and cellular tissue bed creates a recipient site with a biochemistry that is hypoxic (02 tension of 3-10 mm Hg), acidotic (PH of 4 to 6) and rich in lactate.(339)The osteoblasts and stem cells survive the first 3 to 5 days after transplant to the host site largely because of their surface position and ability to absorb nutrients from the recipient tissues. The osteocytes within the mineralized cancellous bone die as a result of their encasement in mineral, which acts as a nutritional

barrier. Within the graft, the platelets trapped in the clot degranulate within hours of graft placement, releasing platelet derived growth factor. Thus, the inherent properties of the wound, particularly the oxygen gradient phenomenon and PDGF, initiate early angiogenesis from the surrounding capillaries and mitogenesis of the transferred osteocompetent cells.[340]By day three, buds from existing capillaries outside the graft can be seen. These buds penetrate the graft and proliferate between the graft and the cancellous bone network to form a complete network in 10 to 14 days. As these capillaries respond to the oxygen gradient, MDAF messengers effectively reduce the oxygen gradient as they perfuse the graft, thus creating shutoff mechanisms that prevent over angiogenesis.

Although PDGF seems to be the earliest messenger to stimulate early osteoid formation, it is probably replaced by MDGF and other mesenchymal tissue stimulators from the TGF-β family. During the first 3 to 7 days after graft placement, the stem cells and endosteal osteoblasts produce only a small amount of osteoid. Over the next few days, osteoid production accelerates after the vascular network is established, presumably because of the availability of oxygen and nutrients.

The new osteoid initially forms on the surface of the mineralized cancellous trabeculae from the endosteal osteoblasts. Shortly thereafter, individual osteoid islands develop between the cancellous bone trabeculae, presumably from the stem cells transferred with the graft material. A third source of osteoid production is circulating stem cells, which are attracted to the wound and are believed to seed into the graft and proliferate.[341]

During the first 3-4 weeks, this biochemical and cellular phase of bone regeneration coalesces individual osteoid islands, surface osteoid on the cancellous trabeculae, and host bone to clinically consolidate the graft. This process uses the graft's fibrin network as a framework to build upon a process referred to as osteoconduction. Normally non-motile cells such as osteoblasts may be somewhat motile via the process of endocytosis. The cell membrane is transferred from the retreating edge of the cell, through the cytoplasm, to the advancing edge to reform a cell membrane. During this process, the cell slowly advances and secretes osteoid onto the fibrin network. This cellular regeneration phase is often referred to as phase I regeneration. It produces disorganized woven bone, similar to fracture callus that is structurally sound, but not as strong as mature bone.[342]

Applied Biomaterials used in the Fabrication of 3d Scaffolds for Alveolar Bone Regeneration:

Biodegradable Natural Polymers:

Natural polymers, which include proteins and polysaccharides, are the first biomaterials to be recruited in different clinical applications because of their high biocompatibility, good cell recognition, enhanced cellular interactions in the surrounding environment and hydrophilicity[343]. Due to these properties, they have been thoroughly investigated as hydrogels in the earliest work of cell encapsulation in tissue engineering, demonstrating successful results.[344-346]

Collagen is one of the most widely expressed proteins in the human body, providing strength and structural stability to many tissues from skin to bone. Being the major organic component of the ECM in native bone, makes collagen an attractive biomaterial for Bone Tissue Engineering (BTE) applications. It is well documented that collagen matrices promote cell adhesion, proliferation, and osteogenic differentiation of bone marrow stromal cells, in vitro[347]. Similarly, the denatured form of collagen termed **gelatin**[348] enhances osteoblast adhesion, migration, and mineralization as it contains several biological and functional groups that promote such activities.[349]

Chitosan is a popular biomaterial in bone tissue engineering due to its appealing characteristics; it displays antibacterial and antifungal activities, rapid blood clot formation, and analgesic properties, all of which render chitosan useful in wound healing acceleration that would minimize the risk of scaffold contamination and postoperative infections, thus preventing eventual exposure and failure of the scaffold.[350]

Despite their good biological properties, the previously mentioned natural polymers lack bioactivity[351], which is the key factor in promoting hard tissue formation. They also share weak mechanical characteristics and somewhat rapid degradation rate, through enzymatic reaction.[352]
To overcome such undesired properties, scaffolds based on natural polymers are usually combined with bioactive materials (e.g., bioceramics) or mechanically strong ones (e.g., synthetic polymers or metals), depending on the area of application (e.g., load-bearing or not). Interestingly, although bioceramics are mechanically weak as well, they tend to increase the overall compressive strength of natural polymer based scaffolds.[353]

Biodegradable Synthetic Polymers:

Biodegradable synthetic polymers have generated interest in BTE because of their relatively low cost and ability to be produced in large quantities with long shelf life in comparison to their natural counterparts. The most investigated biomaterials of this group are *aliphatic polyesters* which include polycaprolactone (PCL), polylactic acid (PLA), polyglycolic acid (PGA), and their copolymer poly (lactic-co-glycolic) acid (PLGA).

Polycaprolactone (PCL): is one of the most popular aliphatic polyester in medical applications; it has been used in medical devices for the last 30 years[354] and has been investigated in craniofacial repair.[355] PCL is an excellent candidate for BTE applications due to its biocompatibility, suitability for various scaffold fabrication techniques, remarkably slow degradation rate, and mechanical stability. It is suggested that the latter two traits might allow for a better maintenance of generated bone volume and its contour over time. However, PCL is hydrophobic in nature which is also responsible for the inferior cell affinity and poor cellular responses and interactions to the surface.[356] Similar to PCL, polylactic acid (PLA) and poly(lactic-co-glycolic acid) (PLGA) are hydrophobic while polyglycolic acid (PGA) is hydrophilic, keeping in mind that these polymers still have higher rates of degradation in comparison to PCL. But, in general, aliphatic polyesters display a slow degradation rate in correlation to natural polymers and bioceramics.[357] Synthetic polymers degrade by hydrolysis which can be in the form of bulk degradation or surface erosion.[358, 359]

Most of the available polyesters degrade by hydrolysis within the interior part of the biomaterial, resulting in an empty shell formation, while the size is maintained for a considerable amount of time.[360]

This feature is considered appealing for scaffold utilization as a bone graft substitute and less suitable for drug-delivery purposes. Still, aliphatic polyesters release acidic by-products upon degradation, which can result in tissue necrosis and subsequent scaffold failure with chronic exposure. Therefore, they are usually combined with bioceramics that enhance the bioactivity of a construct and tend to neutralize the acidic by-products by elevating the overall pH value for the scaffold[361] to maintain tissue health.[349]

Bioceramics:

Bioceramics are inorganic biomaterials constituting different categories, among which are calcium phosphate bioceramics and bioactive glass with very well documented applications as bone fillers in

the dental field.[362] Calcium phosphate bioceramics enclose hydroxyapatite (HAp), tri calcium phosphate (α-TCP and β-TCP), and biphasic calcium phosphate (BCP), all of which can also be in the form of injectable cement materials (pastes) that are moldable and easy to handle and harden when left in situ. Moldable calcium phosphate materials allow for intimate adaptation to complex defects, which is difficult to accomplish with conventional bone grafting materials.[363] Bioceramics are attracting more attention in bone reconstruction due to their unlimited availability, bioactivity, excellent biocompatibility, hydrophilicity, similarity to native bone inorganic components, osteoconductivity, and reported potential osteoinductivity[364], which is the ability to induce ectopic bone formation by instructing the surrounding *in vivo* environment to do so This potential activity can be attributed either to the surface of bioceramics which absorbs and exhibits osteoinductive factors or to the gradual release of calcium and phosphate ions into the surrounding environment, subsequently stimulating the differentiation of osteoprogenitor cells into osteoblasts. Still, both theories are yet to be confirmed.[365]

The most investigated calcium phosphate ceramic in BTE is hydroxyapatite (HAp) because it shares the same chemical composition of native bone minerals, which positively influences adhesion and proliferation of osteoblasts. Despite this important feature, HAp takes a long time to degrade when in the "crystalline form" in vivo, causing the remaining particles to impede complete bone formation and increase the risk for infection and exposure in oral and maxillofacial regions.[366]Consequently, applications of crystalline Hap are being eventually substituted by amorphous hydroxyapatite, which has a faster degradation rate.[367] Modification of HAp degradation rate can also be achieved by its combination with other biomaterials of faster kinetics, such as natural polymers.[368]

The second most widely studied calcium phosphate ceramic is β-tri calcium phosphate (β-TCP), because of its ability to form a strong bone-calcium phosphate bond and its faster degradation rate. Interestingly, when tri calcium phosphate is combined with HAp, a mixture termed biphasic calcium phosphate (BCP) is produced. In comparison to other calcium phosphate ceramics, BCP has significant advantages in terms of controlled bioactivity, stability, while promoting bone ingrowth especially in large bone defects and controllable degradation rate as BCP has a higher degradation rate than HAp, yet slower than that of β-TCP.

Another biomaterial that belongs to bioceramics and is investigated in BTE is bioactive glass (BG), which is a silicon oxide with substituted calcium. When exposed to body fluids, a layer of calcium phosphate forms on the surface of bioactive glass, which chemically binds to bone. The specific type

of Bioglass used as a synthetic graft in intraoral applications (termed 45S5 Bioglass) has a very slow degradation rate because it converts to a HAp-like material in the physiologic environment. These are available as granules or porous sintered blocks, fibers and woven structures. They are osteopromotive and chemically bind with on growing new bone. They are found to be superior to calcium phosphates owing to the relatively quick rate of reaction with host cells, ability to bond to hard and soft tissue. These resorb through chemical dissolution and inhibit bacterial growth in vitro depending on chemical composition.[349]

Metals:

Metallic biomaterials are extensively applied in dental and orthopaedic fields to support the replacement of lost bone structures because of their excellent mechanical properties; they display high strength, toughness, and hardness, in comparison to polymers and ceramics, making them suitable for applications in loadbearing areas. It is reported that metals enhance the mechanical properties of a scaffold by decreasing the pore size.

Within this group of biomaterials, titanium and titanium alloys are encouraged in bone regeneration due to their high biocompatibility, appropriate mechanical properties, and elasticity. Different studies reported that titanium-based 3D scaffolds display good hydrophilicity, which enhances mineral deposition and encourages cell attachment and proliferation in vitro and new bone formation without any signs of inflammation or necrosis *in vivo*.

Nonetheless, lack of biodegradability of titanium and titanium alloys is a major disadvantage and might discourage its (titanium alloys) applications in bone regeneration due to the need of a second surgery for removal, which can compromise patient satisfaction and increase health care costs.

In the past decade, magnesium and magnesium alloys have been thoroughly researched and found to be extremely appealing materials for orthopaedic applications with great potential in BTE; they have mechanical properties close to native bone and are completely biodegradable.[349]

Magnesium and its alloys are osteoconductive, play a role in cell attachment, and tend to increase the expression of osteogenic markers in vitro (Yoshizawa et al.;[369] 2014). Although pure magnesium has a rapid rate of degradation in vivo (J.E.Gray et al.;[370] 2002)

Thus the literature confirms that various composite scaffolds support attachment, proliferation, and differentiation of osteoblasts while maintaining the final shape of newly formed bone.[349]

Mechanism of bone formation and healing from various bone graft materials:

Calcium Phosphate based graft materials:

Various parameters of calcium phosphate change the cellular functions such as dissolution, composition, topography and surface energy. After the recruitment of monocytes/ macrophages in the substrate, osteoclasts are responsible for bone resorption. Calcium Phosphate ceramics also get degraded in similar manner to that of bone mineral. Osteoclasts bind firmly to sealing zone of substrate. In the center of this zone H^+ ions are secreted to a local pH of 4.5. In vivo osteoclasts participate in the degradation of calcium phosphate ceramics into minerals available for bone regeneration by providing space required for bone formation.

In mini pigs when defects are grafted with β-TCP new bone formation is seen at 4 weeks but the maturity of bone is much less when compared to auto grafts.[371] Particles of graft almost vanished and is substituted by bone. Complete trabecular bone filling is seen at 8 weeks and β- TCP particles were almost resorbed by dissolution rather than cellular resorption.[372]

Bioactive glasses:

Undifferentiated mesenchymal cells surround the microspheres of bioactive glass during the initial 2 weeks of primary bone formation and differentiate into an immature bone (Woven) formation. Histomorphometrically bioactive glass filling induce a constant time related increase in new bone. Silicon rich layer formation is a crucial stage in bone bonding as it acts as precipitate for precipitation of calcium phosphate. By the absorption of proteins calcium phosphate directs for new bone formation. These proteins of extracellular origin attract macrophages and mesenchymal stem cells and osteoprogenitor cells. Study done by Mahesh et al.;[373] stated that ridge preservation using calcium phosphate putty demonstrates more timely bone formation than ABB xenograft.[372]

With Growth Factors:

Adding certain growth factors to the material may also increase the amount of phase I bone that forms. In laboratory studies and some early human trials involving graft enhancement, BMPs (particularly recombinant DNA produced BMP), TGF-β, PDGF and IGF have shown promise in their ability to increase the speed and quantity of bone regeneration. Clinical studies on adding PRP to graft material have demonstrated its ability to induce early consolidation and graft mineralization in half the time

with a 15% to 30% improvement in trabecular bone density. This material, an enhanced fibrin clot rich in platelets that releases PDGF, will initiate osteocompetent cell activity more completely than that which inherently occurs in the graft and clot milieu alone. The enhanced fibrin network created by PRP may also enhance osteoconduction throughout the graft, supporting consolidation.[337]

Phase I bone undergoes resorption and remodelling until it is eventually replaced by phase II bone which is less cellular, more mineralized and more structurally organized. Phase II is initiated by osteoclasts that arrive at the graft site through the newly developed vascular network. BMP is released during resorption of both the newly formed phase I bone and the nonviable cancellous trabecular graft. As with normal bone remodelling, BMP acts as the link or couple between bone resorption and new bone apposition. Stem cells in the graft and from the local tissues and the circulatory system respond by osteoblast differentiation and new bone function. New bone forms while the jaw and graft are in function, developing in response to the demands placed on it.[337]

This bone develops into mature Haversian systems and lamellar bone that can withstand normal shear forces from the jaw and impact compressive forces that are typical of dentures and implant-supported prosthesis. Histologically, grafts undergo long-term remodelling that is consistent with normal skeletal turnover. A periosteum and endosteum develop as part of this cycle. Although the graft cortex never grows as thick as a normal jaw cortex, the graft itself retains a dense cancellous trabecular pattern that is beneficial for placing dental implants because its density promotes osseointegration of the implants. It can also be beneficial for placing conventional dentures because the dense trabecular bone can easily adapt to a variety of functional stresses. Radiographically, the graft takes on the morphology and cortical outlines of the mandible or maxilla over several years. Pre-prosthetic procedures such as soft tissue grafts can be performed at four months when a functional periosteum has formed. Osteointegrated dental implants can also be placed at this point.[337]

Resorption rates of various bone grafts:[337]

SNO	TYPE OF BONE GRAFT	RESORPTION RATE
1	ILIAC CREST	3-6 MONTHS
2	TIBIAL PLATEAU	3-6 MONTHS
3	MANDIBULAR SYMPHISIS	4-8 MONTHS
4	MAXILLARY TUBEROSITY	3-6 MONTHS
5	BONE SHAVINGS	3-7 MONTHS
6	BONE SUCTION TRAPS	1-3 MONTHS
7	FDBA	6-15 MONTHS
8	DFDBA	2-4 MONTHS
9	POP	1-2 WEEKS
10	Pep Gen P-15 (PEPTIDE BONE GRAFT SUBSTITUTE)	18-36 MONTHS
11	ANORGANIC BOVINE BONE	15-30 MONTHS
12	HYDROXY APPATITE (HA)	18-36MONTHS/ NON-RESORBABLE
13	β-TCP	4-12 MONTHS / PARTIAL
14	CORAL	5-7 YEARS
15	$CaSO_4$	1-2 MONTHS
16	HTR	10-15 YEARS/ NON-RESORBABLE
17	PERIOGLASS	18-24 MONTHS

Growth Factors Sequencing the Formation of New Bone

Growth factors may be delivered to bone wounds by 2 routes: 1) via platelets and plasma. 2) Local production of cells at injured site. PDGF and TGF-β are detected in early haematoma following fracture healing. TGF-β, aFGF and bFGF are produced locally adjacent to long bone fractures. Moderate levels of PDGF expression are also present in the fracture callus. Both TGF-β and FGF's are produced in the area of cartilage and bone formation in the healing of long bone fracture. PDGF is a more potent stimulator of cellular proliferation within the fracture callus than either TGF-β or bFGF; PDGF increases collagen expression when added to the fracture callus (Joyce et al.;[374] 1990a). The growth factors aFGF, bFGF and PDGF increase a cell population of the osteoblast lineage capable of synthesizing collagen but do not directly increase collagen production by differentiate osteoblasts. Both TGF-β and IGF-1can increase the proliferation of osteoblasts (or their precursors) and collagen production by differentiated osteoblasts. Along with these GF BMP's show effect on bone formation or resorption.[375]

- BMP's2,4 (by Osteoblasts) stimulates mesenchymal cell proliferation
- BMP-7 (by Osteoblasts), FGF-2 (by Macrophages and Endothelial cells) stimulates osteoblast and chondroblast differentiation
- IGF-II (by Macrophages and Fibroblasts) stimulates osteoblast proliferation and bone matrix synthesis
- PDGF (by Macrophages) stimulates differentiation of fibroblasts into fibroblasts, stimulates proliferation of mesenchymal progenitor cells.
- TGF-β (by Fibroblasts and Osteoblasts) induces endothelial cell and fibroblast apoptosis, it also induces differentiation of fibroblasts into myofibroblasts, stimulates chemotaxis and survival of osteoblasts.
- VEGF (by Macrophages) causes chemotaxis of mesenchymal stem cells, antiapoptotic effect on bone forming cells, angiogenesis promotion.[376-380]

Alveolar Bone in Edentulism

Topography of the Alveolar Process:

The alveolar process that houses the roots of the teeth extends from the basal bone of the maxilla and the mandible. The shape and dimensions (height and width) of the basal bone vary considerably from subject to subject and from site to site in the same individual. There is no distinct boundary between the alveolar process and the basal bone of the jaws. At sites of the jaws where the teeth erupt in "normal" orientation in the developing alveolar process, hard tissue will be present on the facial (buccal) as well as on the lingual (palatal) aspect of the roots. However, at sites where the teeth erupt with a facial orientation, the facial (buccal) bone of the alveolar process will become thin and at times even disappear (dehiscence, fenestration).[193]

The outer walls of the alveolar process – facial (buccal), marginal, and lingual (palatal) aspects – are continuous with the outer walls of the basal bone. The walls are comprised of dense cortical bone, while more central portions harbor trabecular bone (radiographic term; spongy bone, anatomic term; cancellous bone, histologic term) that contains bone trabeculae within the bone marrow. The cortical walls (plates) of the alveolar process are continuous with the bone that lines the sockets, that is the alveolar bone proper or the bundle bone. The cortical plates (the outer walls) of the alveolar process meet the alveolar bone proper at the crest of the interdental septum. In subjects (sites) with healthy periodontium, the crest of the septum is located 1–2 mm apical of the cementoenamel junction.

In some portions of the dentition (such as in the symphysis region of the mandible), the trabecular bone component of the alveolar process may be absent.[193]

From an Alveolar Process to an Edentulous Ridge:

The alterations that occur in the alveolar process following the extraction of a single tooth for didactic reasons can be divided in two interrelated series of events, namely intra-alveolar alveolar processes and extra-alveolar processes.[193]

Intra Alveolar Alveolar Processes:

The healing of extraction sockets in human volunteers was studied by Amler[381] (1969) and Evian et al.;[382] (1982). Although the biopsy technique used by Amler[381] only allowed the study of healing in the marginal portions of the empty socket, his findings are often referred. Amler[381]stated that

following tooth extraction, the first 24 hours are characterized by the formation of a blood clot in the socket. Within 2–3 days the blood clot is gradually replaced with granulation tissue. After 4–5 days, the epithelium from the margins of the soft tissue starts to proliferate to cover the granulation tissue in the socket. One week after extraction, the socket contains granulation tissue, young connective tissue and osteoid formation is ongoing in the apical portion of the socket. After 3 weeks, the socket contains connective tissue and there are signs of mineralization of the osteoid. The epithelium covers the wound. After 6 weeks of healing, bone formation in the socket is pronounced and trabeculae of newly formed bone can be seen.

Amler's[381]study was of short duration, so it could only evaluate events that took place in the marginal portion of the healing socket. His experimental data did not include the important later phase of socket healing that involves the processes of modelling and remodelling of the newly formed tissue in various parts of the alveolus. Thus, the tissue composition of the fully healed extraction site was not documented in the study.

In a later and longer-term study, Trombelli et al.;[383] (2008) examined socket healing in biopsies sampled during a 6-month period from human volunteers. They confirmed most of Amler's[381] findings and reported that in the early healing phase (tissue modelling), the socket was filled with granulation tissue that was subsequently replaced with a provisional connective tissue and woven bone. In biopsies sampled in later phases of healing, it was observed that the process by which woven bone was replaced by lamellar bone and marrow, that is remodelling, was slow and exhibited great individual variation. In only a limited number of specimens representing 6 months of healing had woven bone been replaced with bone marrow and trabeculae of lamellar bone. It can be assumed therefore that tissue modelling following tooth extraction in humans is a rather rapid process, while the subsequent remodelling is slow and may take years to be completed.[193]

Overall Pattern of Socket Healing:

Figure 3-12 shows a mesio distal section of a fresh extraction socket bordered by adjacent roots. The socket walls are continuous with the alveolar bone proper of the neighbouring teeth. The tissue inside the interdental (inter-radicular) septa is made up of cancellous bone and includes trabeculae of lamellar bone within bone marrow.

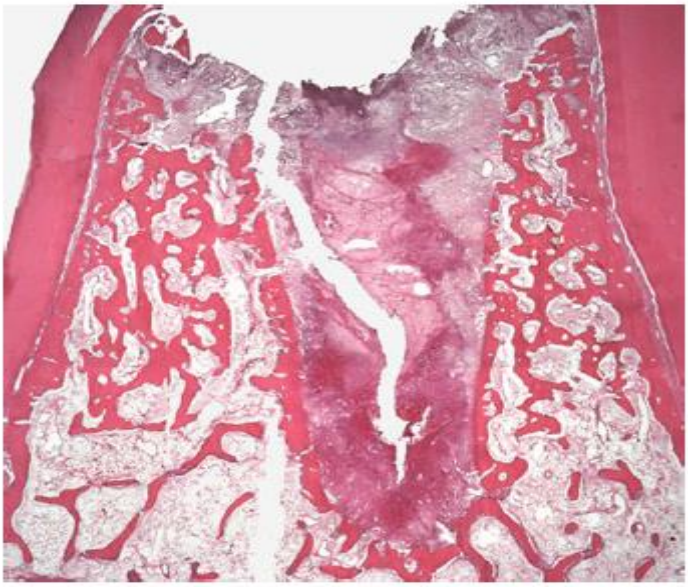


Fig. 3-12 Histologic section showing the mesiodistal aspect of a fresh extraction socket bordered by two neighboring roots. Note that the alveolar bone from the tooth sites is continuous with the walls of the empty socket. The interdental septum contains cancellous bone including trabeculae of lamellar bone and marrow.

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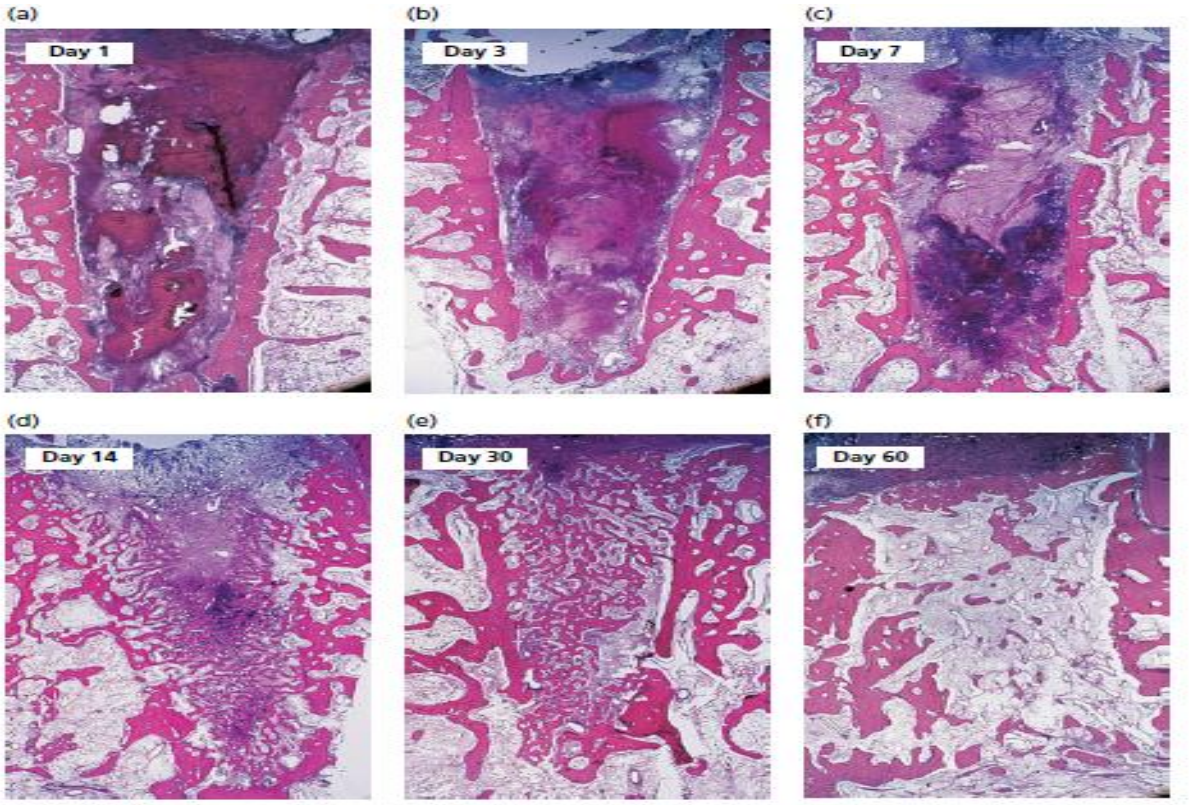
The empty socket is first filled with blood and a coagulum (clot) forms (Fig. 3-13a). Inflammatory cells (polymorphonuclear leukocytes and monocytes/macrophages) migrate into the coagulum and start to phagocytose elements of necrotic tissue. The process of wound cleansing is initiated (Fig. 3-13b). Sprouts of newly formed vessels and mesenchymal cells (from the severed periodontal ligament) enter the coagulum and granulation tissue is formed. The granulation tissue is gradually replaced with provisional connective tissue (Fig. 3-13c) and subsequently immature bone (woven bone) is laid down (Fig. 3-13d). The hard tissue walls of the socket – the alveolar bone proper or the bundle bone – are gradually resorbed and the socket becomes filled with immature woven bone (Fig. 3-13e). The initial phase of the healing process (tissue modelling) is now complete. In subsequent phases, the woven bone in the socket will be gradually remodelled into lamellar bone and marrow (Fig. 3-13f–h).



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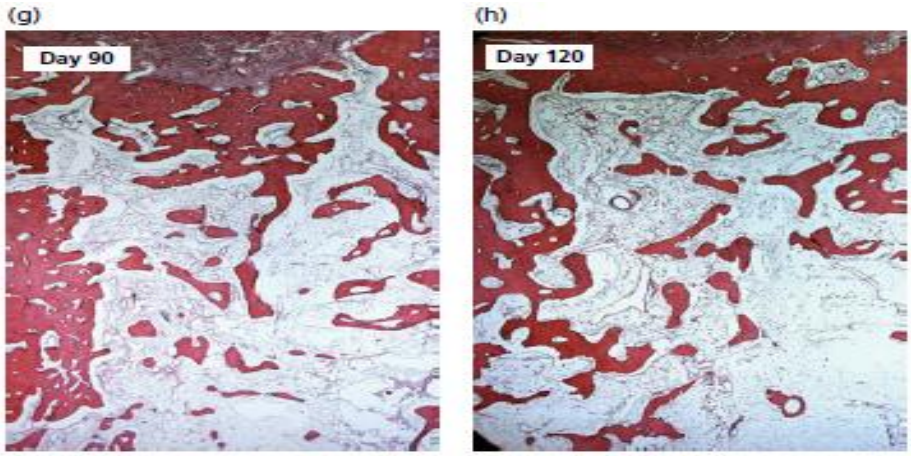

Fig. 3-13 (a–h) Overall pattern of bone formation in an extraction socket. For details see text. (193)

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Important Events in Socket Healing

Blood Clotting:

Immediately after tooth extraction, blood from the severed vessels will fill the socket. Proteins derived from vessels and damaged cells initiate a series of events that lead to the formation of a fibrin network (Fig. 3-14). Platelets form aggregates and interact with the fibrin network to produce a coagulum (a blood clot) that effectively plugs the severed blood vessels and stops the bleeding. The blood clot acts as a physical matrix that directs cellular movements and it contains substances that are of importance for the forthcoming healing process. Thus, the clot contains substances (i.e. growth factors) that (1) influence mesenchymal cells and (2) enhance the activity of inflammatory cells. Such substances will thus induce and amplify the migration of various types of cells into the socket wound, as well as their proliferation, differentiation, and synthetic activity within the coagulum.

Although the blood clot is crucial in the initial phase of wound healing, its removal is mandatory to allow the formation of new tissue. Thus, within a few days after the tooth extraction, the blood clot will start to break down, that is the process of "fibrinolysis" is initiated. Fig 3.15

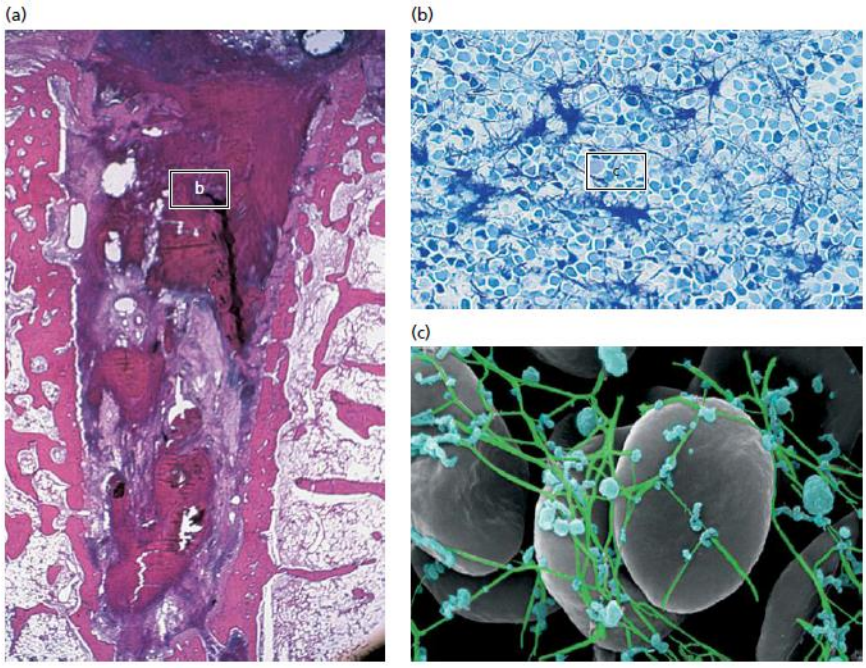

Fig. 3-14 Histologic section (mesiodistal aspect) representing 1 day of healing (a). The socket is occupied with a blood clot that contains large numbers of erythrocytes (b) entrapped in a fibrin network, as well as platelets [blue in (c)].

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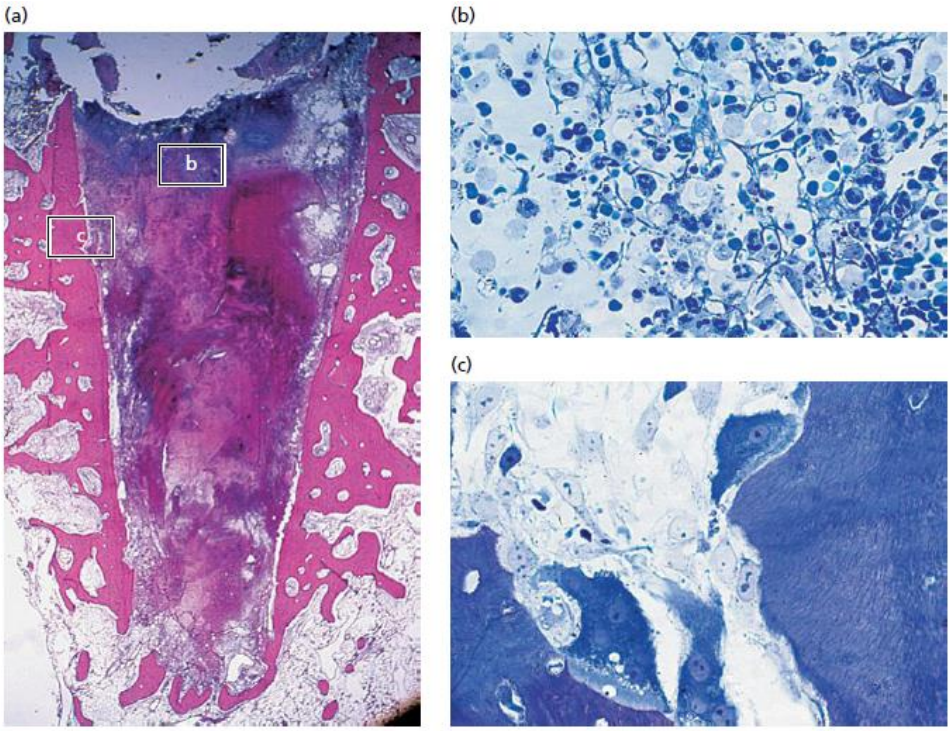

Fig. 3-15 (a) Histologic section (mesiodistal aspect) representing 3 days of healing. (b) Note the presence of neutrophils and macrophages that are engaged in wound cleansing and the breakdown of the blood clot. (c) Osteoclastic activity occurs on the surface of the old bone in the socket walls.

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Wound Cleansing:

Neutrophils and macrophages migrate into the wound, engulf bacteria and damaged tissue, and clean the site before the formation of new tissue can start. The neutrophils enter the wound early, while macrophages appear somewhat later. The macrophages are not only involved in the cleaning of the wound but they also release growth factors and cytokines that further promote the migration, proliferation, and differentiation of mesenchymal cells. Once the debris has been removed and the wound has been "sterilized", the neutrophils undergo a programmed cell death (apoptosis) and are removed from the site through the action of macrophages. The macrophages subsequently withdraw from the wound.(193)

Tissue Formation:

Sprouts of vascular structures (from the severed periodontal ligament) as well as mesenchymal, fibroblast- like cells (from the periodontal ligament and from adjacent bone marrow regions) enter the socket. The mesenchymal cells start to proliferate and deposit matrix components in an extracellular location (Fig. 3-16); granulation tissue will gradually replace the blood clot. This granulation tissue eventually contains macrophages and a large number of fibroblast- like cells, as well as numerous newly formed blood vessels. The fibroblast-like cells continue to (1) release growth factors, (2) proliferate, and (3) deposit a new extracellular matrix that guides the ingrowth of additional cells and allows the further differentiation of the tissue. The newly formed vessels provide the oxygen and nutrients that are needed for the increasing number of cells that occur in the new tissue. The intense synthesis of matrix components exhibited by the mesenchymal cells is called fibroplasia, while the formation of new vessels is called angiogenesis. A provisional connective tissue is established through the combination of fibroplasia and angiogenesis (Fig. 3-17).

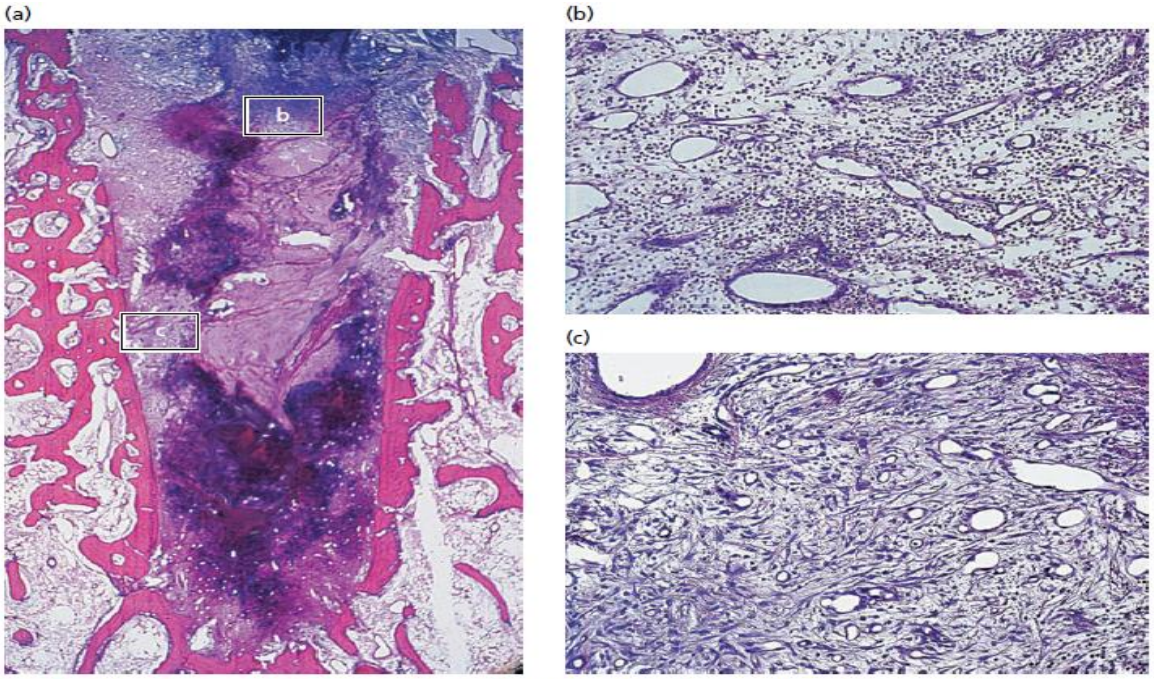

Fig. 3-16 (a) Histologic section (mesiodistal aspect) representing 7 days of healing. (b) Note the presence of a richly vascularized early granulation tissue with large numbers of inflammatory cells in the upper portion of the socket. (c) In more apical areas, a tissue including large numbers of fibroblast-like cells is present (late granulation tissue). (193)

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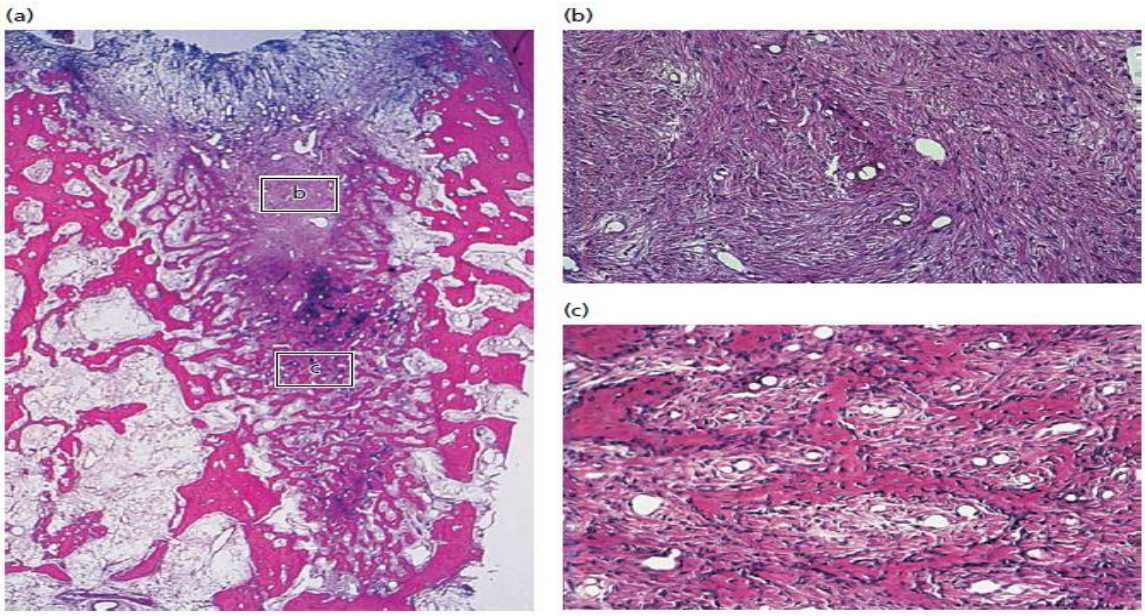

Fig. 3-17 (a) Histologic section (mesiodistal aspect) representing 14 days of healing. (b) In the marginal portion of the wound, a provisional connective tissue rich in fibroblast-like cells is present. (c) The formation of woven bone has at this time interval already begun in the apical and lateral regions of the socket. (193)

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The transition of the provisional connective tissue into bone tissue occurs along the vascular structures. Thus, osteoprogenitor cells (e.g. pericytes) migrate and gather in the vicinity of the vessels. They differentiate into osteoblasts that produce a matrix of collagen fibers, which takes on a woven pattern. The osteoid is formed. The process of mineralization is initiated within the osteoid. The osteoblasts continue to lay down osteoid and occasionally such cells are trapped in the matrix and become osteocytes. This newly formed bone is called woven bone (Figs. 3-17, 3-18).

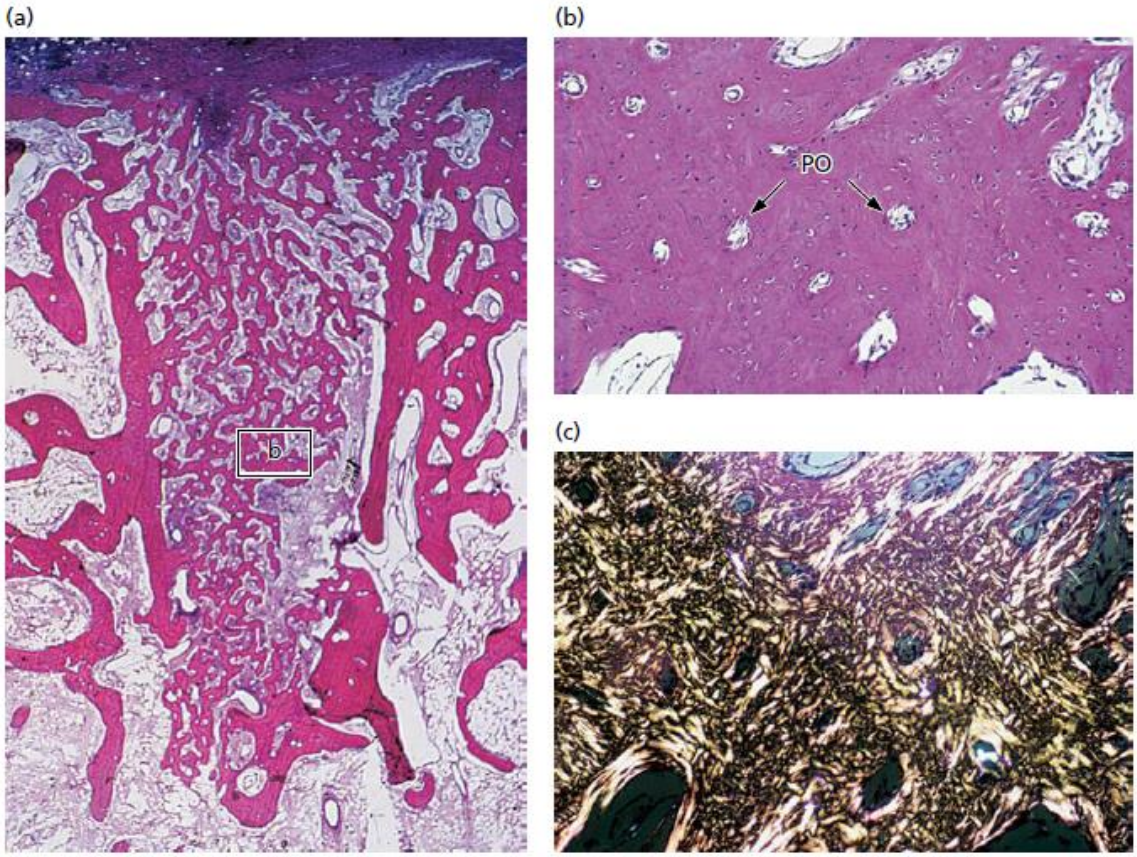

Fig. 3-18 (a) Histologic section (mesiodistal aspect) representing 30 days of healing. The socket is filled with woven bone. (b) Woven bone contains a large number of cells and primary osteons (PO). (c) The woven pattern of the collagen fibers of this type of bone is illustrated (polarized light). (193)

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The woven bone is the first type of the transition of the provisional connective tissue into bone tissue that occurs along the vascular structures. Thus, osteoprogenitor cells (e.g. pericytes) migrate and gather in the vicinity of the vessels. They differentiate into osteoblasts that produce a matrix of collagen fibers, which takes on a woven pattern. The osteoid is formed.(193)

The process of mineralization is initiated within the osteoid. The osteoblasts continue to lay down osteoid and occasionally such cells are trapped in the matrix and become osteocytes. This newly formed bone is called ***woven bone*** (Figs. 3-17, 3-18). Bone to be formed and newly formed woven bone are characterized by (1) its rapid deposition as finger- like projections along the route of vessels, (2) the poorly organized collagen matrix, (3) the large number of osteoblasts that are trapped in its mineralized matrix, and (4) its low load-bearing capacity. Trabeculae of woven bone are shaped around and encircle the vessel. The trabeculae become thicker through the deposition of additional woven bone. Cells (osteocytes) become entrapped in the bone tissue and the first set of osteons called the primary osteons is well organized. The woven bone is occasionally reinforced by the deposition of so-

called parallel-fibered bone (collagen fibers organized not in a woven but in a concentric pattern). It is important to realize that during this early phase of healing most of the bone tissue in the walls of the socket (the bundle bone) is removed.

Tissue Modelling and Remodelling:

The initial bone formation explained here is a dog model[384] which is a fast process. Within a few weeks, the entire extraction socket is filled with woven bone or, as this tissue is also called, primary bone spongiosa. The woven bone offers (1) a stable scaffold, (2) a solid surface, (3) a source of osteoprogenitor cells, and (4) an ample blood supply for cell function and matrix mineralization. The woven bone with its primary osteons is gradually replaced with lamellar bone and bone marrow (Fig. 3-19). In this process, the primary osteons are replaced with secondary osteons. The woven bone is first resorbed to a certain level. The level of the resorption front will establish a so-called reversal line, which is also the level from which new bone with secondary osteons will form (Fig. 3-20). Although this remodelling may start early during socket healing, it will take several months until all woven bone in the extraction socket has been replaced with lamellar bone and marrow.[193]

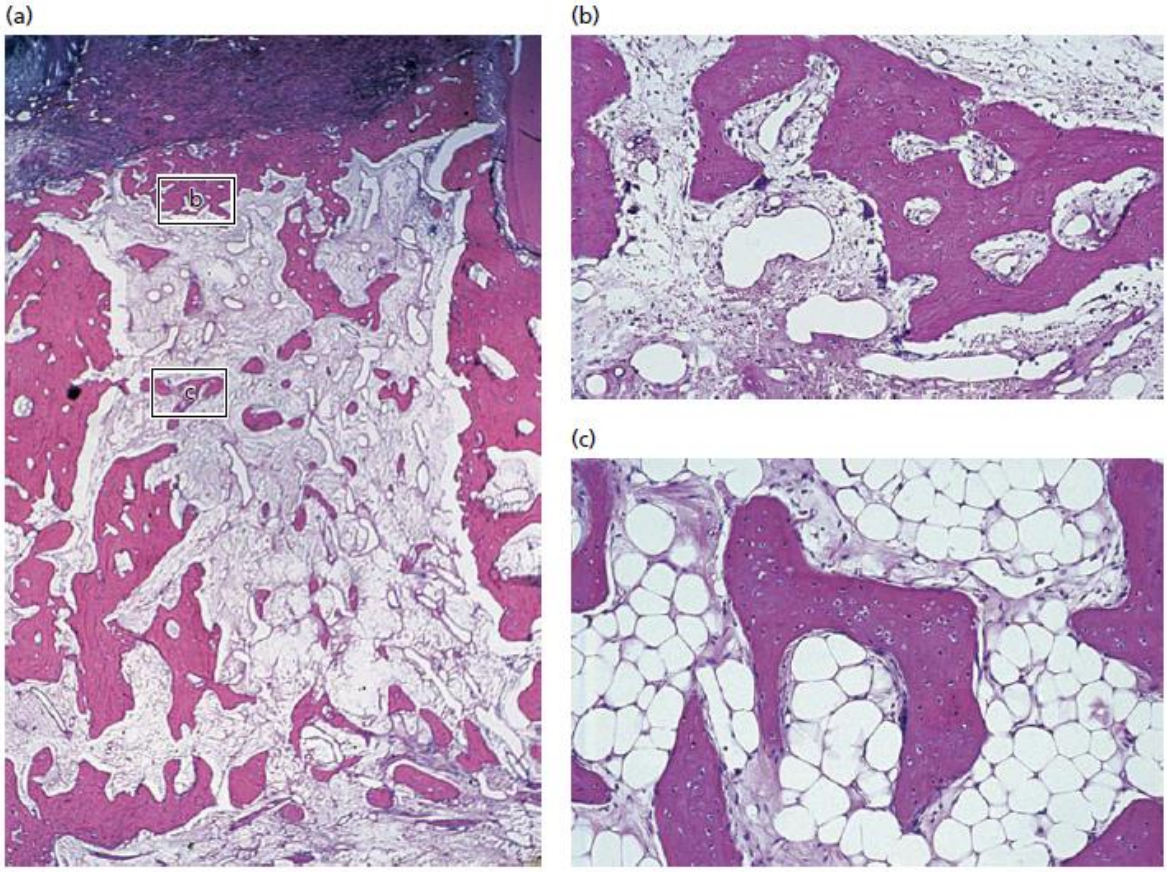

Fig. 3-19 (a) Histologic section (mesiodistal aspect) representing 60 days of healing. (b) A large portion of the woven bone has been replaced with bone marrow. (c) Note the presence of a large number of adipocytes residing in a tissue that still contains woven bone. (193)

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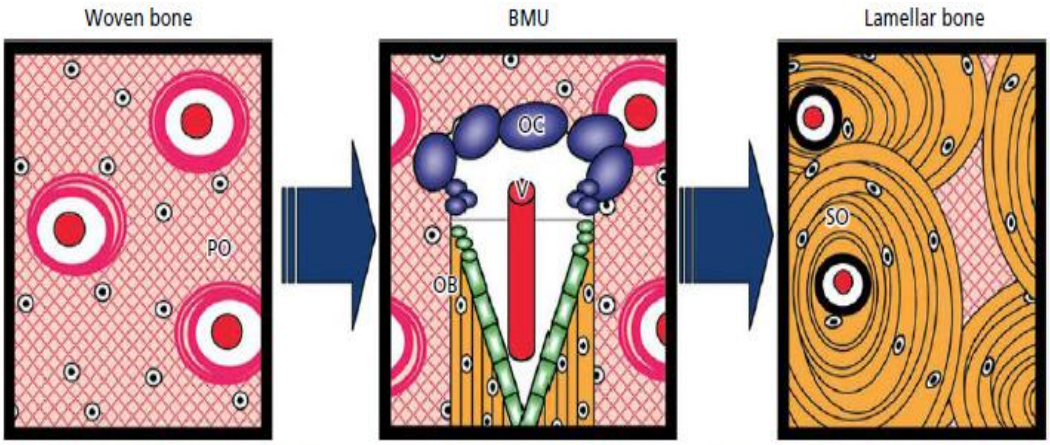

Fig. 3-20 Schematic drawing showing how woven bone is replaced by lamellar bone. Woven bone with primary osteons (PO) is substituted by lamellar bone in a process that involves the presence of bone multicellular units (BMU). The BMU contains osteoclasts (OC), as well as vascular structures (V) and osteoblasts (OB). Thus, the osteoblasts in the BMU produce bone tissue in a concentric fashion around the vessel, and lamellar bone with secondary osteons (SO) is formed.

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An important part of socket healing involves the formation of a hard tissue cap that will close the marginal entrance to the socket. This cap is initially comprised of woven bone (Fig. 3 21a), but is subsequently remodelled and replaced with lamellar bone that becomes continuous with the cortical plate at the periphery of the edentulous site (Fig. 3-21b). This process is called corticalization.[384]

The wound is now healed, but the tissues in the site will continue to adapt to functional demands. Since there is no stress from forces elicited during mastication and other occlusal contacts, there is no demand on the mineralized bone in the areas previously occupied by the tooth. Thus, in this model the socket apical of the hard tissue cap will remodel mainly into marrow

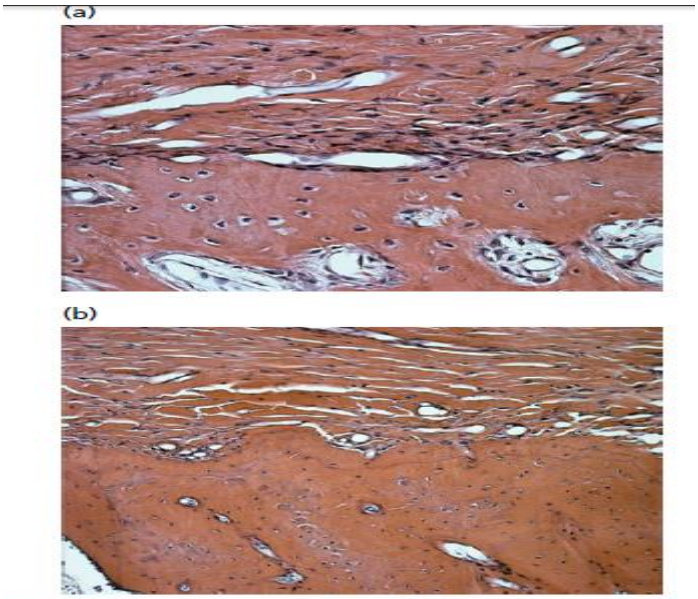

Fig. 3-21 Histologic sections (mesiodistal aspect) describing the hard tissue that has formed at the entrance of a healing extraction socket and the process of corticalization. (a) Woven bone with primary osteons occupies the socket entrance after 60 days of healing. (b) After 180 days, the woven bone has mainly been replaced with lamellar bone.

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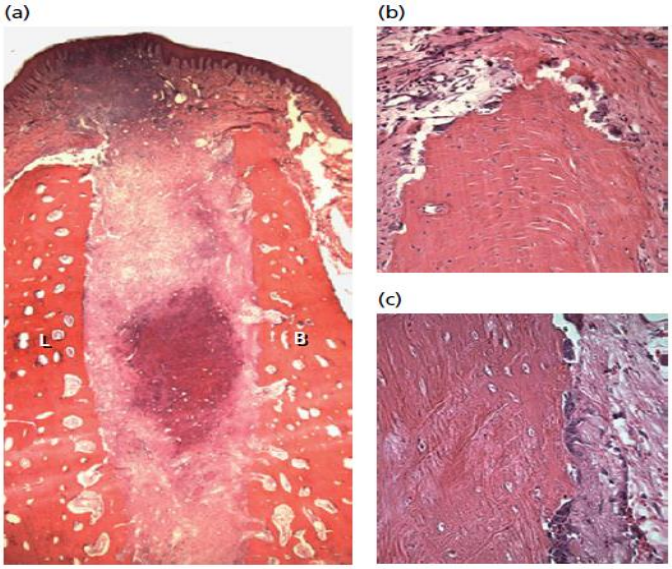
Extra -Alveolar Processes:

Alterations in the profile of the edentulous ridge that occurred following tooth extraction were carefully examined. In an experiment using the dog model (Araujo & Lindhe,[385] 2005), the third and fourth mandibular premolars were hemi-sected. Buccal and lingual full-thickness flaps were raised; the distal roots were carefully removed. The flaps were replaced and sutured to cover the fresh extraction socket. Biopsy specimens, including an individual extraction socket and adjacent roots, were obtained after 1, 2, 4, and 8 weeks of healing. The blocks were sectioned in the buccolingual plane.

1 week after tooth extraction (Fig. 3-22). At this interval the socket is occupied by a coagulum. Furthermore, a large number of osteoclasts can be seen on the Outside as well as on the inside of the buccal and lingual bone walls. The presence of osteoclasts on the inner surface of the socket walls indicates that the bundle bone is being resorbed.



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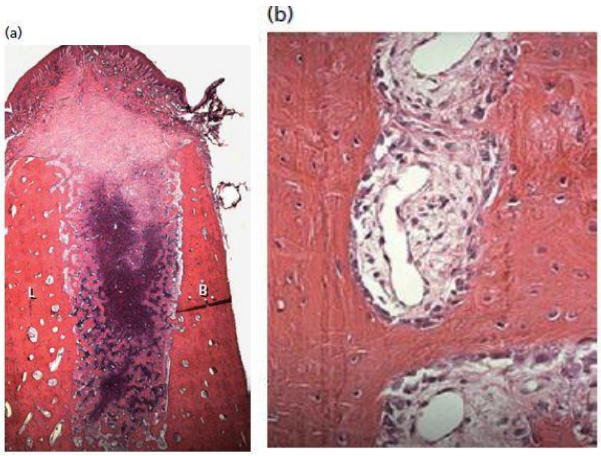
(a) Histologic section (buccolingual aspect) of the socket after 1 week of healing. Note the presence of a large number of osteoclasts on the crestal portion (b) and inner portion (c) of the buccal wall. (B- buccal bone; L- lingual bone.)

2 weeks after tooth extraction (Fig. 3-23). Newly formed immature bone (woven bone) resides in the apical and lateral parts of the socket, while more central and marginal portions are occupied by a provisional connective tissue. In the marginal and outer portions of the socket walls, numerous osteoclasts can be seen. In several parts of the socket walls the bundle bone has been replaced with woven bone.



(a) Histologic section (buccolingual aspect) of the socket after 2 weeks of healing. (b) Note that the bundle bone in the lingual aspect of the socket is being replaced with woven bone. (B-buccal bone; L-lingual bone.)

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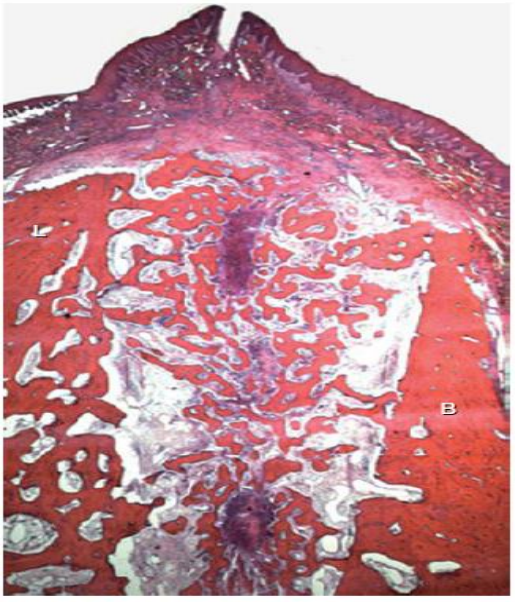
4 weeks after tooth extraction (Fig. 3-24). The entire socket is occupied with woven bone at this stage of healing. Large numbers of osteoclasts are present in the outer and marginal portions of the hard tissue walls. Osteoclasts also line the trabeculae of woven bone present in the central and lateral aspects of the socket. In other words, the newly formed woven bone is being replaced with a more mature type of bone.(193)



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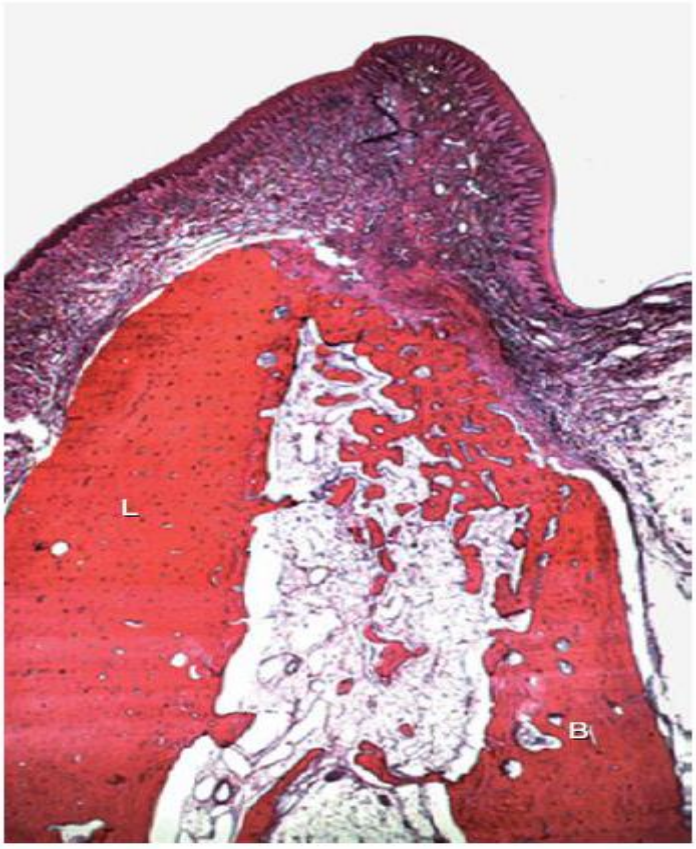
Histologic section (buccolingual aspect) of the socket after 4 weeks of healing. The extraction socket is filled with woven bone. On the top of the buccal wall, the old bone in the crest region is being resorbed and replaced with either connective tissue or woven bone. (B-buccal bone; L-lingual bone.)

8 weeks after tooth extraction (Fig. 3-25). A layer of cortical bone covers the entrance to the extraction site. Corticalization has occurred. The woven bone that was present in the socket at the 4-week interval is replaced with bone marrow and some trabeculae of lamellar bone in the 8-week specimens. On the outside and on the top of the buccal and lingual bone wall there are signs of ongoing hard tissue resorption. The crest of the buccal bone wall is located apical of its lingual counterpart.



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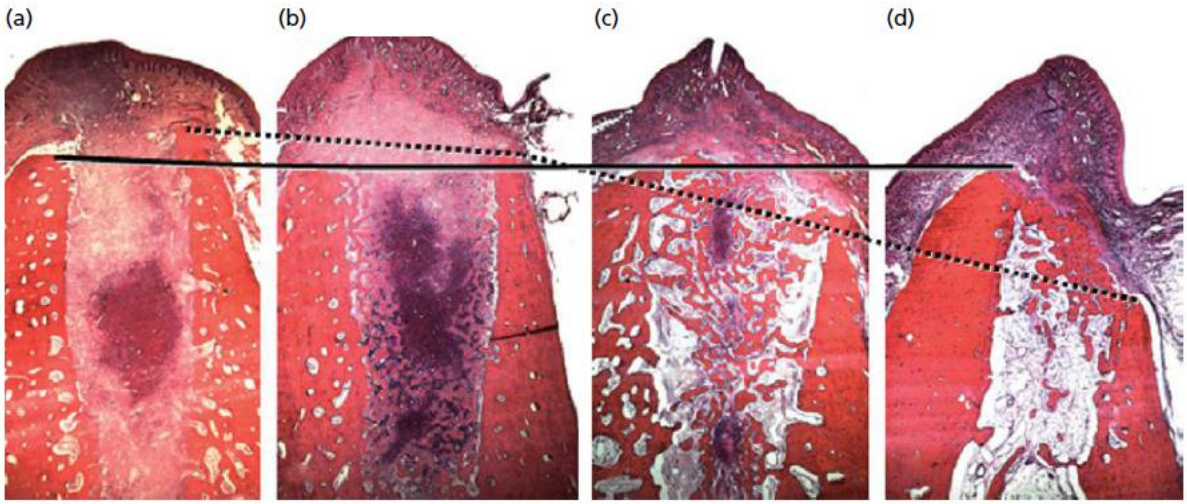
Histologic section (buccolingual aspect) of the socket after 8 weeks of healing. The entrance of the socket is sealed with a cap of newly formed mineralized bone. Note that the crest of the buccal wall is located apical of the crest of the lingual wall. (B-buccal bone; L-lingual bone.)

There is a relative change in the location of the crest of the buccal and lingual bone walls that took place during the 8 weeks of healing. While the level of the margin of the lingual wall remained reasonably unchanged, the margin of the buccal wall shifted several millimetres in an apical direction. The reason why more bone loss occurred in the buccal than in the lingual wall during socket healing in this animal model is not completely understood. Prior to tooth extraction, the marginal 1–2 mm of the crest of the thin buccal bone wall was occupied by bundle bone. Only a minor fraction of the crest of the wider lingual wall contained bundle bone. Bundle bone, as stated above, is a tooth-dependent tissue and will gradually disappear after tooth extraction. Thus, since there is relatively more bundle bone in the crest region of the buccal than of the lingual wall, hard tissue loss may become most pronounced in the buccal wall.



(193) ***Lang NP, Lindhe J. Clinical periodontology and implant dentistry, 2 Volume Set: John Wiley & Sons; 2015.***

Histologic sections (buccolingual aspects) show the profile of the edentulous region in the dog after (a) 1, (b) 2, (c) 4, and (d) 8 weeks of healing following tooth extraction. While the marginal level of the lingual wall was maintained during the process of healing (solid line), the crest of the buccal wall was displaced >2 mm in the apical direction (dotted line).(193)

Thus the processes of modelling and remodelling that occur, following tooth extraction (loss) result in resorption of the various components of the previous alveolar process. The amount of tissue loss that occurs in these processes varies considerably from subject to subject and from site to site in the same individual.

As a rule, the resorption of the buccal bone wall is more pronounced than the resorption of the lingual/ palatal wall and hence the center of the ridge will move in a lingual/palatal direction. In the extreme case, the entire alveolar process may be lost following tooth removal and then only the basal bone of the mandible and the maxilla may remain to constitute the ridge.

The outer (cortical) walls of the remaining portion of the alveolar ridge (basal bone and residues of the alveolar process) are comprised of lamellar bone. The cortical plates of the ridge often enclose the cancellous bone that harbours trabeculae of lamellar bone and marrow. The bone marrow contains numerous vascular structures as well as adipocytes and pluripotent mesenchymal cells. Depending on factors such as the type of jaw (maxilla or mandible), location (anterior, posterior) in the jaw, depth of the buccal and lingual vestibule, and amount of hard tissue resorption, the edentulous ridge may be lined with masticatory, keratinized mucosa, or lining, non-keratinized mucosa.[193]

Alveolar Bone and Implants

The healing and remodelling of tissues around an implant involves a complex array of events. Osseointegration refers to direct bone anchorage to the implant body, which can provide a foundation to support prosthesis and can transmit occlusal forces directly to the bone. This concept was developed and the term coined by Per-Ingvar Branemark, inventor of the well-known Branemark implant system. During animal studies of microcirculation in bone repair during the 1950s, Branemark discovered a strong bond between bone and titanium. It is now known that fully anchored prosthesis can provide patients with restored masticatory functions that are similar to the natural dentition.[337]

Several key factors influence successful implant osseointegration.[386, 387]These include the following:
1. The characteristics of the implant material (some appear to chemically bond to bone better than others)[388] and maintenance of implant sterility prior to placement.
2. Implant design, shape, and macro and micro surface topography.
3. Prevention of excessive heat generation during bone drilling.

The long term osseointegration of dental implants also relies on placement of the implant within bone that has adequate trabecular density, ridge height and width, and vascularity.[389] When the recipient bone or graft is deficient in height, the portion of the implant prosthesis that is above the bone is greater than the length of the implant within it there by possibly creating a destructive lever arm that will loosen the implant over time. A ridge that is too narrow, i.e., less than *5mm*, to accommodate standard 3.75 mm diameter implant placed outside the bone will force the clinician to use less desirable small diameter implants to gain necessary osseointegrated surface area. Like -wise, trabecular bone that is not sufficiently dense will either fail to osseointegrate or will lose its osseointegration over time. Ideally, the marginal and apical parts of the implant should be fully engaged in cortical bone or in cancellous bone that has a high proportion of bony trabeculae to support it. The in growth of fibrous tissue between the bone and implant also decreases the chances of long term success and the ability to withstand mechanical and microbial insults. In some cases this can be prevented by protecting against micro mobility and by using protective barrier membranes during healing. It is crucial to achieve initial stability and osseointegration because, a clinically mobile implant has never been observed to become re-osseointegrated.[387] Once stability is lost; the implant can only be removed."[337]

Implant Osseointegration

The healing process around an implant is the same as that which occurs in normal primary bone. Research with titanium dental implants suggests the following three-stage process: [389]

Osteophyllic phase

When a rough-surface implant is placed into the cancellous marrow space of the mandible or maxilla, blood is initially present between the implant and bone, and a clot subsequently forms. Only a small amount of bone is in contact with the implant surface; the rest is exposed to extracellular fluids and cells. During the initial implant-host interaction, numerous cytokines are released that have a variety of functions, from regulating adhesion molecule production and altering cellular proliferation, to enhancing collagen synthesis and regulating bone metabolism. These events also correspond to the beginning of the generalized inflammatory response to the surgical insult. By the end of the first week, inflammatory cells respond to foreign antigens introduced by the surgical procedures.

While the inflammatory phase is still active, vascular ingrowth from the surrounding vital tissues begins by about day three, developing into a more mature vascular network during the first three weeks following implant placement.[388] In addition, cellular differentiation, proliferation, and activation begin. Ossification also begins during the first week, and the initial response observed is the migration of osteoblasts from the endosteal surface of the trabecular bone and the inner surface of the buccal and lingual cortex to the implant surface. This migration is possibly a response to the release of BMP during implant placement and the initial resorption of bone crushed against the metal surface. The osteophyllic phase lasts about 1 month."[337]

Osteocondutive phase

Once the bone cells reach the implant, they spread along the metal surface (osteoconduction), laying down the osteoid. Initially this is an immature connective tissue matrix, and the bone deposited is a thin layer of woven bone called a foot plate (basis stapedis).The fibro- cartilaginous callus eventually remodels into bone callus (woven and later, lamellar bone) in a process similar to endochrondal ossification. This process occurs during the next 3 months (peaking between the third and fourth weeks) as more bone is added to the total surface area of the implant. Four months after implant placement, the maximum surface area is covered by bone. By this point, a relatively steady state has been reached and no further bone is deposited on the implant surface.[388]

Osteoadaptive phase

The final or osteoadaptive phase begins approximately 4 months after implant placement.
A balanced remodelling sequence begins and continues even after the implants are exposed and loaded. Once loaded, the implants generally do not gain or lose bone contact, but the foot plates thicken in response to the load transmitted through the implant to the surrounding bone, and some reorientation of the vascular pattern may be seen. To achieve optimal results, an osseointegration period of 4 months prior to loading is recommended for implants placed in grafted bone, and 4-8 months prior to loading for implants placed in normal bone, depending on its density.[337]

A thorough knowledge of bone physiology, biology and mass is essential for understanding bone behaviour during oral bone grafting, implant placement, osseointegration and long term bone maintenance. When rehabilitating jaws that have been reconstructed, it is even preferable to place implants in grafted bone rather than in normal bone, although each type of bone is acceptable.

Timing of Implant Placement:

Although several procedures [(1) sites with ridge defects of various dimensions, (2) fresh extraction sockets, (3) the area of the maxillary sinus, etc.] have been described in literature regarding the implant placement, there application has become relatively common. One issue of primary interest in current clinical and animal research in implant dentistry includes the study of tissue alterations that occur following tooth loss and the proper timing thereafter for implant placement.

In the optimal case, the clinician will have time to plan for the restorative therapy (including the use of implants) prior to the extraction of one or several teeth. In this planning, a decision must be made whether the implant(s) should be placed immediately after the tooth extraction(s) or if a certain number of weeks (or months) of healing of the soft and hard tissues of the alveolar process should be allowed prior to implant installation. The decision regarding the timing for implant placement, in relation to tooth extraction must be based on a proper understanding of the structural changes that occur in the alveolar process following the loss of the tooth (teeth).[193]

The removal of single or multiple teeth will result in a series of alterations within the edentulous segment of the alveolar process. Hence, during socket healing, the hard tissue walls of the alveolus will resorb, the center of the socket will become filled with cancellous bone, and the overall volume of the site will become markedly reduced. In particular, the buccal wall of the edentulous site will be

diminished not only in the buccolingual/palatal direction but also with respect to its apico coronal dimension (Pietrokovski & Massler[390] 1967; Schropp *et al.*;[391] 2003).

In addition to hard tissue alterations, the soft tissue in the extraction site will undergo marked adaptive changes. Immediately following tooth extraction, there is a lack of mucosa and the socket entrance is thus open. During the first weeks following the removal of a tooth, cell proliferation within the mucosa will result in an increase of its connective tissue volume. Eventually, the soft tissue wound will become epithelialized and a keratinized mucosa will cover the extraction site. The contour of the mucosa will subsequently adapt to follow the changes that occur in the external profile of the hard tissue of the alveolar process. Thus, the contraction of the ridge is the net result of bone loss as well as loss of connective tissue. It is obvious that no ideal time point exists following the removal of a tooth, when the extraction site has (1) maximum bone fill in the socket and (2) voluminous mature covering mucosa.

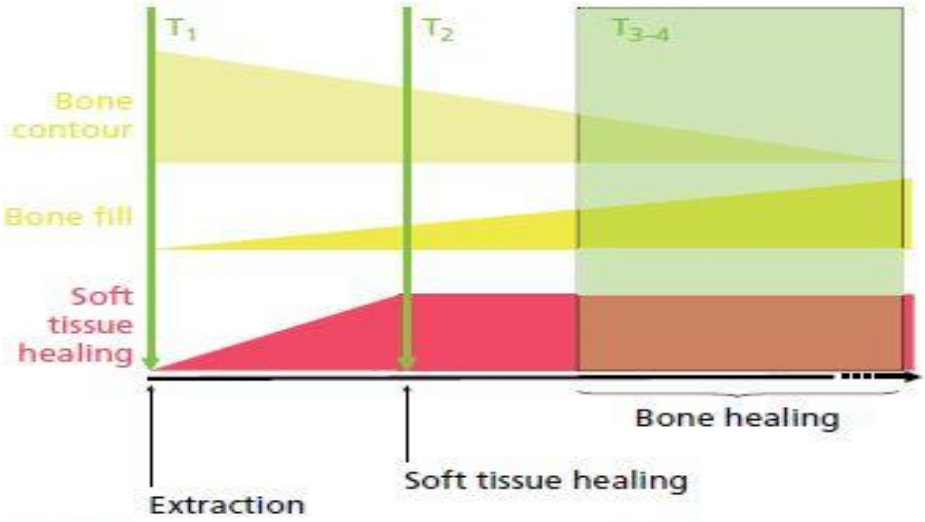


Fig. 49-1 Schematic drawing depicting the changes in the soft and hard tissues following tooth extraction over time. T_{1-4} represent the four different time points for implant placement.

[392] ***Chen ST, Buser D. Clinical and esthetic outcomes of implants placed in postextraction sites. Int J Oral Maxillofac Implants. 2009;24 Suppl:186-217***.

Hammerle[393] and co-workers considered it necessary to develop a new concept (classification) that incorporated the growing knowledge in this field of implant dentistry. This new classification took into consideration data describing structural alterations that occur following tooth extraction as well as knowledge derived from clinical observations.

Table 49-1 Classification of types 1–4 implant placements, and advantages and disadvantages of each type.

Classification	Definition	Advantages	Disadvantages
Type 1	Implant placement as part of the same surgical procedure as and immediately following tooth extraction	Reduced number of surgical procedures Reduced overall treatment time Optimal availability of existing bone	Site morphology may complicate optimal placement and anchorage Thin tissue biotype may compromise optimal outcome Potential lack of keratinized mucosa for flap adaptation Adjunctive surgical procedures may be required Technique-sensitive procedure
Type 2	Complete soft tissue coverage of the socket (typically 4–8 weeks)	Increased soft tissue area and volume facilitates soft tissue flap management Allows resolution of local pathology to be assessed	Site morphology may complicate optimal placement and anchorage Increased treatment time Varying amounts of resorption of the socket walls Adjunctive surgical procedures may be required Technique-sensitive procedure
Type 3	Substantial clinical and/or radiographic bone fill of the socket (typically 12–16 weeks)	Substantial bone fill of the socket facilitates implant placement Mature soft tissues facilitate flap management	Increased treatment time Adjunctive surgical procedures may be required Varying amounts of resorption of the socket walls
Type 4	Healed site (typically >16 weeks)	Clinically healed ridge Mature soft tissues facilitate flap management	Increased treatment time Adjunctive surgical procedures may be required Large variation in available bone volume

(392) ***Chen ST, Buser D. Clinical and esthetic outcomes of implants placed in postextraction sites. Int J Oral Maxillofac Implants. 2009;24 Suppl:186-217***.

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