The Mechanisms of Mineralized Tissue Resorption by Clast Cells in Relation to Orthodontic Tooth Movement and Root Resorption

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Abstract

Orthodontic tooth movement depends on favorable supporting tissues responding to the forces used during treatment. The tissues involved are the periodontal ligament (PDL), alveolar bone, cementum and dentin. Clast cells play an important role in the process of physiologic remodeling and mineralized tissue responses to external forces. They also play a significant role in unfavorable iatrogenically induced root resorption. Understanding the tissues and cells involved is essential for modulating the biology of orthodontic tooth movement and the prevention of the unwanted side effects of orthodontic treatment.

Keywords: Orthodontic tooth movement; Clast cells; periodontal ligament (PDL); Mineralized tissue resorption, Bone cells; Osteoblasts; Osteoclasts

Introduction

Orthodontic tooth movement is possible due to favorable supporting tissues responding to the forces used during treatment. The tissues involved are the periodontal ligament (PDL), alveolar bone, cementum and dentin. Next, we consider the two mineralized tissues, namely alveolar bone and cementum. Then, we discuss the details of the process of mineralized tissue resorption, emphasizing its involvement in alveolar bone remodeling and induced root resorption associated with orthodontic tooth movement.

Bone Cells

The alveolar bone is a specialized type of connective tissue. It is considered to be dense, hard connective tissue with a mineralized extracellular matrix [1]. Alveolar bone is a highly dynamic structure that responds to various physiological processes such as tooth eruption, physiological mesial migration of teeth and masticatory forces by remodeling and adjusting to the required external demands. Further, alveolar bone remodeling is required during orthodontic tooth movement. It allows for the movement of teeth within the jaws, and it maintains the teeth in the new, desired position.

Bone cells include osteoblasts, osteocytes, bone lining cells, and osteoclasts. Osteoblasts in the alveolar bone have an ectomesenchymal origin. They differentiate and secrete bone organic matrix (osteoid), and they play a role in mineralization. Osteoblasts also play a role in the activation of osteoclasts [2]. It has been shown that they respond to a variety of signaling molecules and to mechanical stimulation. They are cuboidal in shape and are considered to be post-mitotic cells. They line the alveolar bone surface in the PDL and marrow spaces. The major product of these cells is collagen type I. In addition, they synthesize the non-collagenous proteins that are involved in the mineralization of the osteoid and the adhesion of cells to bone. These non-collagenous proteins are either adhesion glycoprotein's, such as fibronectin and thrombospondin, or the acidic glycoprotein osteonectin (SPARC). SPARC is the major non-collagenous protein in bone. However, it is not specific to mineralized tissue. It is believed to mediate cell migration, cell shape, and proliferation in the extracellular matrix. It binds to collagen and calcium, and it has been shown to modulate hydroxyapatite crystal growth. The exact role of SPARC/osteonectin in bone has not yet been established [1,3]. It may enhance mineralization of the collagen matrix in bone [3].

Bone contains another group of acidic glycoprotein's known as phosphoproteins, namely osteocalcin, osteopontin, and bone sialoproteins [4]. These proteins are highly negatively charged

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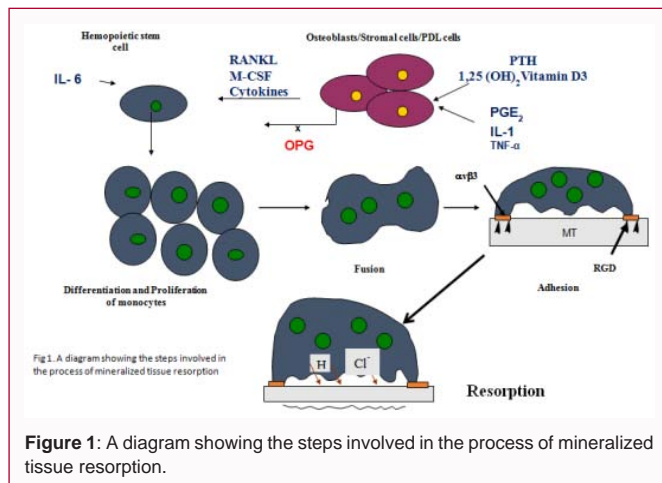**Figure 1**: A diagram showing the steps involved in the process of mineralized tissue resorption.

and acidic. They bind calcium and collagen with high affinity. Therefore, they may play a role in mineralization and may control the growth of apatite crystals [4]. Osteocalcin contains an unusual amino acid called gamma-carboxyglutamate (GLA); therefore, it is called bone GLA protein. Osteocalcin binds to calcium with high affinity. The exact role of osteocalcin in bone remains unclear. It is believed to play a role in mineral maturation [1]. Osteopontin and bone sialoproteins were initially known as bone sialoprotein I and II, respectively. They are similar and are highly phosphorylated and negatively charged. Osteopontin is enriched with aspartate, and bone sialoproteins are enriched with glutamate. They contain the ArgGlyAsp (RGD) amino acid sequence, which is required for the adhesion of osteoclast cells to bone during the resorption process [2]. Osteopontin is known to be produced by osteoblasts, osteoclasts and osteocytes. It plays an important role in bone formation and resorption [4,5]. It has been shown that during experimental tooth movement in rats, the osteopontin level of mRNA expression in osteocytes, in some osteoblasts, and in bone lining cells increased in resorption sites early during tooth movement [5]. Additionally, an *in vitro* osteoclast migration assay showed that osteopontin has chemotactic functions in recruiting osteoclasts to the site of resorption and it may play a role in their survival [5,6]. The fate of osteoblasts includes cell death, being trapped by the bone matrix to form osteocytes, or remaining on the bone surface as bone lining cells.

Osteocytes are surrounded by bone matrix, and they occupy spaces known as lacunae. Morphologically, they display less protein synthesis and secretory machinery than osteoblasts. Osteocytes usually communicate with each other through cellular processes extended in the canaliculi. Gap junctions have been shown at the extremities of cellular process extensions, making it possible for osteocytes in the lacunae to communicate with each other and/or with osteoblasts and to couple their functional activities. It has been shown that osteocytes can sense mechanical stresses, which lead to the secretion of paracrine factors such as insulin like growth factor (IGF-I). IGF can act as a coupling factor in the remodeling cycle of bone [7]. These factors play central roles in bone homeostasis by being the main target for parathormone and through their apoptosis due to bone remodeling, which can trigger osteocalastogenesis [8].

Bone lining cells display a flattened morphological appearance and show characteristic features that indicate less involvement in matrix production. However, they are involved with osteoclasts in the homeostasis of calcium and phosphate in the body [1]. Bone lining cells belong to the same lineage as osteoblasts because they express

alkaline phosphatase, respond to parathyroid hormone (PTH) and associate with bone surfaces [9]. Bone lining cells are usually in contact with each other via gap junctions, and they are found in close proximity to osteoclasts attached to bone. These cells do not synthesize collagen. However, they enwrap collagen fibrils and phagocytize these fibers in the resorption lacunae that are left behind by withdrawing osteoclasts [8].This process is believed to be essential prior to bone deposition at the sites of resorption during bone remodeling [9]. Additionally, it has been suggested that bone lining cells are involved in the elimination of non-mineralized collagen fibers extending from bone surfaces prior to clast cell attachment and resorption. This un-mineralized osteoid layer acts as a barrier to osteoclastic activity because contact with bone mineral acts as a signal that induces bone resorption [9,10]. Furthermore, studies have shown that synthetic inhibitors of mammalian collagenase almost completely inhibited the formation of resorption lacunae, emphasizing the importance of collagenase-mediated degradation of un-mineralized osteoid prior to bone resorption by osteoclasts [11]. However, other studies have shown that osteoclasts are capable of resorbing both un-mineralized and mineralized tissues [12].

The recruitment of osteoclasts into the pressure regions of the PDL during experimental tooth movement in rats was quantified at different time intervals [13]. Both mature osteoclasts and mononuclear clast cells were identified using a tartrate-resistant acid phosphatase (TRAP) marker. It was concluded that the number of osteoclasts, after 24 hours of tooth movement, was not significantly different between the experimental and control groups [13]. However, it was significant in the 3, 5, and 7 day groups. The number of mononuclear TRAP-positive cells was only significantly different on day 3 [13]. In addition, it was concluded that clast cell precursors were recruited from the hematopoietic tissue. These precursor cells developed into mature clast cells, which are involved in the process of bone resorption during tooth movement [13]. It was shown, using H^+-ATPase as a marker for identifying osteoclast and mononuclear clast cells in the PDL during experimental tooth movement in rats, that the numbers of osteoclasts and pre-osteoclast mononuclear cells were increased on the pressure side after one day of elastic band placement. There was also a marked increase after 4 days of elastic band placement [14]. There was no increase in the number of osteoclasts or mononuclear clast cells in the tension region after 24 hours of elastic band placement. However, after 4 days, there was a slight increase in osteoclasts and mononuclear clast cells in the tension region [14].

The fate of osteoclasts includes withdrawal and apoptosis. It has been shown that during experimental tooth movement, stimulated bone lining cells will be induced to differentiate into mature osteoblasts and to begin synthesizing factors such as transforming growth factor-β (TGF-β) and osteoprotegrin (OPG). TGF-β can recruit osteoblast precursors to the site of bone formation, and it has an effect on osteoblast differentiation leading to bone matrix production [15]. In addition, TGF-β and OPG inhibit bone resorption, inhibit the recruitment of clast cell precursors, and promote osteoclast apoptosis [15]. The rate of apoptosis of osteoclasts during experimental tooth movement in rats was quantified using morphological changes, TdT-mediated dUTP-biotin nick 3 end labeling and TRAP markers to identify osteoclasts in the PDL [15]. There was a significant increase in the number of osteoclasts showing apoptosis in the 3 day group. The highest percentage of apoptosis was found in the 7-day group [16].
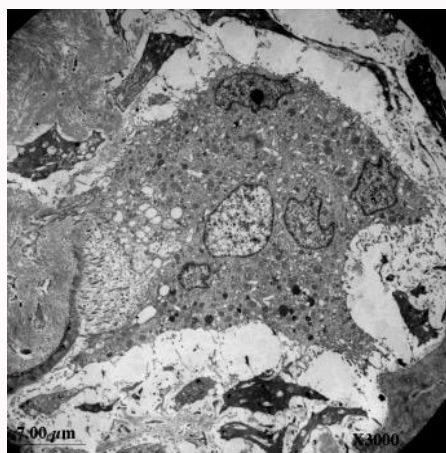
Figure 2A: A low magnification (X3000) electron microscope photomicrograph showing an osteoclast adhered to a multiple surfaces of bone. Note the multiple nuclei in the cell.
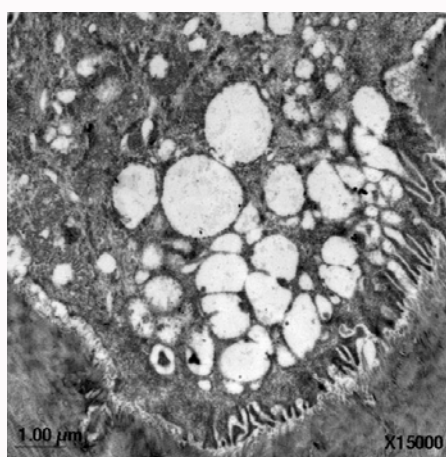

Figure 2C: A high magnification electron microscope (X15000) photomicrograph showing the tight seal area.

from that of collagen type I [19]. It is believed that CAP promotes the adhesion and spread of mesenchymal cells, and it promotes the adhesion of mineralized tissue, forming cells preferentially [19]. Further, cementum contains enamel matrix proteins such as amelogenin [19].

Osteopontin, through its binding domains to mineralized crystals, collagen, and osteocalcin, promotes cohesion between dentin and cementum at the dentinocemental junction. Furthermore, osteopontin present on the root surface through its RGD amino acid sequence promotes the adhesion of cementoblasts during the regeneration of cementum and odontoclasts during root resorption. Bone sialoproteins also contains the RGD sequence that promotes cell adhesion during cementum development and regeneration. It also plays a role in the regulation of mineral formation. Osteopontin and bone sialoproteins are mainly expressed in a cellular extrinsic fibrous cementum and a cellular afibrillar cementum, and they remain bound to collagen fibers [19]. Additionally, cementum contains adhesion glycoproteins such as fibronectin and proteoglycans such as tenascin [18].

Figure 2B: A higher magnification (X15000) electron microscope photomicrograph showing the resorption front at the cell-mineralized tissue and the intra-cellular vacuoles of phagocytosis.
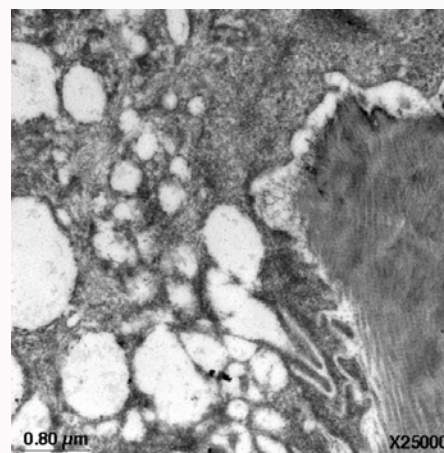
Cementum

As reviewed by Diekwisch [17] and Saygin [18], cementum is a specialized mineralized connective tissue that covers the root dentin surface. Cementum has several functions. It acts as an anchor for the PDL fibers and protects the underlying dentin. In addition, cementum plays an important role in the maturation of the PDL during dental development by providing anchorage for the developing PDL fibers [18]. The cementum composition is very similar to that of bone, yet its structure is different because it is a vascular and has no nerve supply. It is the PDL that provides nutrition to cementum. Additionally, cementum does not undergo remodeling; however, cementum undergoes regeneration and repair, especially following physiological and/or induced root resorption associated with orthodontic tooth movement.

In addition to collagen, mainly type I with some of types III and XII, cementum contains a group of non-collagenous proteins similar to those present in alveolar bone and some specific to cementum. The specific non-collagenous proteins include cementum attachment protein and insulin like growth factor-1 or cementum growth factor. Cementum attachment protein (CAP) is very similar to collagen type I structurally, but its expression pattern in cementum is different

Mineralized Tissue Resorption by Clast Cells

The process of mineralized tissue resorption involves several tightly regulated steps. These steps are: 1. the proliferation of hemopoietic clast cell precursors; 2. differentiation into mononuclear clast cells; 3. fusion to form multinucleated clast cells.; 4. clast cell activation and migration to the site to be resorbed; 5. adhesion of clast cells to the mineralized surface and forming a cytoplasmic tight seal; and 6. actual resorption of the mineralized tissue (Figure 1) [2].

Clast cells were first described and named by Kolliker in 1873 due to their function of resorbing bone (Figure 2) [20]. These diverse-shaped cells could be found lining bone surfaces or wrapping bone spicules. Clast cells are multinucleated, terminally differentiated cells that resorb mineralized tissues, e.g., bone, cementum, and dentin. They are formed by the fusion of mononuclear progenitors of the monocyte family [21]. For a long time, the origin of these cells was unclear. It was believed that they develop from mesenchymal cells. Walker DG, et al. [22,23] showed the reversal and curing of skeletal sclerosis characteristic of osteopetrosis (diminished bone resorption) and the presence of normal osteoclasts in mice after parabiotic union with normal siblings. Additionally, Walker DG, et al. [24,25] in 1975 showed through the transplantation of bone marrow and spleen cells

into mutant mice following total body irradiation that circulating osteoclast precursor cells were of hematopoietic origin because the transplants cured and reversed the induced osteopetrosis. Then subsequent studies using nuclear and cytoplasmic markers revealed beyond a doubt that the osteoclasts responsible for the reversal of the disease developed from transplanted bone marrow cells [26]. In addition, *in vitro* osteoclast-free bone organs, when co-cultured with non-adherent bone marrow cells, allowed for osteoclast formation, invasion and resorption of the bone organs. Additionally, human osteopetrotic patients were treated with marrow transplants, and sex chromatin was used as a marker to identify the cells in the recipient of the transplant. In 1985, Schneider [27] showed that isolated pluripotent hematopoietic stem cells cured osteopetrosis, but populations of mature macrophages did not, indicating that osteoclasts and macrophages have a common stem cell lineage but different cell lines. Furthermore, some osteoclast cell precursors do circulate in the blood, but evidence has shown that the circulating precursor cells are rare among blood leukocytes [27].

Hematopoietic stem cells become determined and committed to differentiate into clast cells or macrophages. PU.1 is a transcription factor expressed in hematopoietic stem cells, and it is necessary for the commitment and determination of these cells to become a macrophage or a clast cell [28]. Osteoblasts play a central role in mediating bone resorption. The major hormones and cytokine factors, such as PTH, 1, 25-dihydroxyvitamin D3 and IL-1, which induce bone resorption, do not act on clast cells directly, but they act on osteoblasts, causing them to synthesize factors stimulating clast cell formation [26]. PTH and cytokines stimulate osteoblasts and stromal cells to secrete a number of factors that induce clast cell differentiation, proliferation and subsequent bone resorption. These factors include macrophage-colony stimulating factor (M-CSF), IL-1, TNF-α, and RANKL [2,10,21]. RANK and RANKL belong to the TNF super family, so TNF is potentially a significant factor in the maturation and development of multinucleated clast cells [2,29]. RANKL deficient mice showed signs of osteopetrosis and inhibition of osteoclastogenesis [30]. C-fos is an early response gene necessary for the differentiation of monocytes into clast cells. RANKL and RANK are believed to be responsible for the expression of this gene in monocytes [31]. RANKL is expressed by osteoblasts and is also expressed in the perivascular regions of the PDL. Thus, cells of the PDL may be involved in inducing the differentiation of monocytes into clast cells during tooth movement [30]. Additionally, it has been shown in cell culture that periodontal cells contribute to the process of osteoclastogenesis through the expression of RANKL [32]. Further, RANKL expression was seen in PDL osteoblasts and fibroblasts, supporting osteoclastogenesis during experimental tooth movement [33]. RANKL was detected during the process of dental root resorption [30]. Furthermore, osteoblasts negatively regulate osteoclastogenesis by secreting OPG, a dimeric protein that is a member of the TNF receptor super family [30]. Its N-terminal is a ligand-binding domain, and its C-terminal domain resembles the death domain. It has been shown the cells isolated from the PDL express OPG may regulate the process of clastogenesis in the PDL [32]. OPG inhibits the differentiation of clast cell precursors into mature clast cells. OPG deficiency results in severe bone loss, both in humans and in animals. Further, OPG can reverse bone loss associated with ovariectomy in mice by altering the fine structure of osteoclasts and decreasing their expression of H^+-ATPase. OPG administration did not affect the number of osteoclasts [30].

Certain cytokines have been shown to induce clastogenesis, namely IL-1, IL-6, IL-11, IL-15, and IL17. These cytokines increase the expression of RANKL by osteoblasts, which induce clast cell differentiation and maturation. IL-6 may also act on clast cells directly [28,34]. Using immunohistochemistry, it was shown that IL-1α and IL-1β are present in many cell types during physiological root resorption in kittens. It was concluded that IL-1 is an important endogenous regulator of physiological root resorption and tooth eruption [35]. Further, it has been shown, using in situ hybridization, that the levels of mRNAs of IL-1β and IL-6 were increased during experimental tooth movement in rats [36]. It has also been shown that IL-1β, IL-6 and TNFα levels were significantly increased as measured in the gingival crevicular fluid after 24 hours of tooth movement in humans [37]. The committed monocytes proliferate and survive under the influence of M-CSF. It has been shown that M-CSF gene mutations result in decreased numbers of macrophages and clast cells [37].

After the proliferation of the immature clast cells and their migration, the attachment of the clast cells to mineralized tissue occurs whether it is bone or mineralized dental tissues: cementum and dentin in the case of root resorption. Following clast cell attachment to the mineralized surface and differentiation into mature clast cells, they form a sealing zone at the periphery of the area to be resorbed [7]. This sealing or clear zone is essential for cell polarization and the formation of two distinct plasma membrane domains: the apical domain facing the mineralized tissue; and the basolateral domain away from the mineralized tissue. Further, it restricts the movement of lipids and proteins between the apical and basolateral plasma membrane domains. It also maintains an acidic pH in the microenvironment formed between the clast cells and the mineralized tissue surface called resorbing compartments or resorption lacunae [7]. The molecular basis of this tight adhesion at the sealing zone is mediated by cell surface receptors, namely integrins. The plasma membrane at the sealing zone follows the mineralized tissue surface with less than 10 nm of space between the membrane and the surface [38]. The permeability of the tight seal formed by clast cells was measured using fluorescent markers with various molecular weights ranging between 480 and 70,000 kDa [39]. Lucifer yellow, a dye with a molecular weight of 480 kDa that is known to pass through gap junctions but not tight junctions in epithelial cells, was used; it passed the tight seal formed by clast cells and accumulated beneath the cells. FITC-labeled dextran with a molecular weight of 10,000 kDa also passed across the tight seal. However, increasing the molecular weight of the marker to 40,000 kDa showed that the sealing zone did not allow its passage. It was concluded that the tight seal is a dynamic structure able to differentiate between molecules by excluding those molecules with a molecular weight greater than 10,000 kDa, and the rate of marker accumulation is inversely related to the molecular mass, suggesting that molecular movement across the sealing zone occurs by diffusion [40]. Additionally, these investigators compared clast cells obtained from neonatal rabbits and cultured on dentin slices to those cultured on bovine bone slices. The properties of the sealing zones formed on different substrates were similar [40].

Studies have shown that clast cells utilize a specific integrin αvβ3 in mineralized tissue recognition and attachment [41,42], the formation of the clear zone, and the transmission of the bone matrix-derived signals necessary for cytoskeletal organization to form the ruffled border [2]. Clast cell polarization is important for microtubule cytoskeleton organization and for the transport of vesicles toward

the resorption front. Cell attachment to the mineralized tissue is important for clast cell polarization [43]. The low pH in the resorbing compartment develops through the action of H^+-ATPase pumps, which pump protons to the extracellular environment. These vacuolar proton pumps are delivered to the ruffled border via microtubules and microfilaments [44]. Protons are formed by the hydration of carbon dioxide to carbonic acid, which dissociates into bicarbonate ions and protons. These protons are formed by the action of the enzyme carbonic anhydrase II, which is located in the cytoplasm next to the ruffled border [7]. Protons pumped into the resorption lacunae result in an electrical potential across the plasma membrane. Therefore, chloride ions are also pumped across the ruffled borders of clast cells via chloride channels, namely CIC-7, which localizes to the ruffled border. The end result is the secretion of hydrochloric acid into the microenvironment. To maintain a neutral intracellular pH, bicarbonate ions are exchanged for chloride ions through an HCO_3^-/Cl^- exchanger located at the basolateral domain of clast cells [39,44]. It is believed that protons released to decrease the pH immediately associate with hydroxyapatite crystals at the site of secretion, decreasing their mobility and creating a pH gradient in the resorbing compartment. This process explains the different pH requirements for lysosomal enzymes [45]. Recently, it was shown the H^+-ATPase pumps bind directly to actin microfilaments through an actin-binding domain present in the amino terminal of the protein. This interaction is believed to be important in the transport of the H^+-ATPase from the cytoplasm, specifically from cytosolic vacuoles, to the plasma membrane of the ruffled border of the clast cell [31]. Furthermore, at the ruffled borders of clast cells, there is uptake of bone degradation products. It has been shown that the ruffled border has two sub domains: one for the release of protons and enzymes and one for the uptake of degradation products. Vacuolar membrane fusion with the ruffled border for the delivery of proton pumps and the degrading of enzymes occurs at the periphery of the ruffled border, while the uptake of degradation products occurs at the central region of the ruffled border [39]. This phenomenon was revealed by the localization of endocytosis machinery, such as clathrin, adaptor proteins (AP-1), and dynamin II at the central region of the ruffled border. Further, iron-loaded transferrin taken up by clast cells was delivered to the peripheral regions of the ruffled border. Cathepsins and proton pumps are mainly localized to the periphery of the ruffled border [38,39]. This demineralization causes a surge in Ca^{2+} concentrations in the microenvironment. Therefore, Ca^{2+} diffusion across the plasma membrane of resorbing clast cells cannot be avoided. However, constant Ca^{2+} concentration intracellularly is important; otherwise, it triggers an unfavorable reaction, such as apoptosis. Thus, the cell has a mechanism to maintain a constant Ca^{2+} concentration by active extrusion of Ca^{2+} via plasma membrane Ca^{2+}-ATPase pumps [45]. These pumps were identified on the plasma membrane opposite from the ruffled border [46]. The inorganic component of the mineralized tissue released by acid action is further degraded intracellularly, as indicated by observations of apatite crystals in endocytic vacuoles.

Collagen, which is a major component of the organic matrix in bone and root mineralized tissues, is denatured by acids and is further degraded by proteinases released by clast cells. There are at least two groups of enzymes involved in the extracellular degradation of collagen, namely lysosomal cysteine proteinases, such as cathepsin B, L, and K, and MMPs, such as MMP-9 [2,7,38,44,47-49]. It is believed that cathepsins and MMPs in clast cells act in the process of degradation of the organic components in stages, and they are not parallel to each other [50]. Cathepsin K enzyme is important for clast cell function, as revealed by knockout and over expression studies. The level of expression of cathepsin K is much higher than those of the other types of cathepsins. Cathepsin K cleaves the native triple helix of collagen at multiple sites, which leads to the unwinding of the helical structure of collagen, which then becomes susceptible to degradation by any enzyme with gelatinolytic activity [50]. Cathepsin K-knockout studies revealed that MMPs play compensating roles for the absence of cathepsin K. Several MMPs were identified to be present in bone resorption areas such as MMP-2, MMP-9 (gelatinase), MMP-13 and MMP-14 (interstitial collagenase), [50] as well as other MMPs such as MMP-12. Some of these MMPs, such as MMP-13, which are involved in bone resorption, originate from adjacent cells, such as osteocytes or mononuclear cells surrounding the osteoclasts. MMP-13 is believed to be involved in the removal of collagen remaining after completion of the resorption process [50]. In general, cathepsin K plays the prevailing role in the degradation of collagen in resorption lacunae, and the other MMPs play less significant roles. Further, it is believed that MMPs play important roles in the recruitment and migration of clast cells [50].

References

1. Sodek J, Mckee MD. Molecular and cellular biology of alveolar bone. Periodontol. 2000; 24: 99–126.

2. Teitelbaum SL. Bone Resorption by Osteoclasts. Science. 2000; 289: 1504-1508.

3. Rosset EM, Bradshaw AD. SPARC/osteonectin in mineralized tissue. Matrix Biol. 2016; 52-54: 78-87.

4. Robey P, Fedarko N, Hefferan T, Bianco P, Vetter U, Grzesik W, et al. Structure and molecular regulation of bone matrix proteins. J Bone Miner Res. 1993; 8 Suppl. 2: S483-S487.

5. Tarai K, Takano-Yamamoto T, Ohba Y, Hiura K, Sugimoto M, Sato M, et al. Role of osteopontin in bone remodeling caused by mechanical stress. J Bone Miner Res. 1999; 14: 839-849.

6. Tanabe N, Wheal BD, Kwon J, Chen HH, Shugg RP, Sims SM, et al. Osteopontin signals through calcium and nuclear factor of activated T cells (NFAT) in osteoclasts: a novel RGD-dependent pathway promoting cell survival. J Biol Chem. 2011; 286: 39871-39881.

7. Hill PA. Bone remodeling. Br J Orthod. 1989; 25: 101-107.

8. Szczęsny G, Brodzikowska A, Galus R, Włodarski P, Włodarski KH. Regulation of Bone Homeostasis by Osteocytes. Ortop Traumatol Rehabil. 2015; 17: 567-675.

9. Everts V, Delaisse J, Jansen K, Tigchelaar-Gutter W, Saftig P, Beertsen W. The bone lining cell: Its role in cleaning Howship's lacunae and initiating bone formation. J Bone Miner Res. 2002; 17: 77-90.

10. Chambers T. Resorption of bone. In: Davidovitch Z, editor. The Biological Mechanisms of Tooth Eruption and Root Resorption. Birmingham, AL, EBSCO Media. 1988; 93-100.

11. Heersche J, Kanehisa J. The role of osteoblasts in the initiation of osteoclastic resorption and the degradation of the organic matrix. In: Davidovitch Z, editor. The Biologicl Mechanisms of Tooth Eruption and Root Resorption. Birmingham, AL, EBSCO Media. 1988; 101-106.

12. Jones S. and Boyde A. The resorption of dentine and cementum. In: Davidovitch Z, editor. Biological Mechanisms of Tooth Eruption and Root Resoption. Burmingham, AL, EBSCO Media. 1988; 335-354.

13. Rody WJ Jr., King GJ, Gu G. Osteoclast recruitment to sites of compression in orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2001; 120: 477-489.

14. Yokoya K, Sasaki T, Shibasaki Y. Distributional changes of osteoclasts and pre-osteoclastic cells in periodontal tissues during experimental tooth movement as revealed by quantitative immunohistochemistry of H(+)-ATPase. J Dent Res. 1997; 76; 580-587.

15. Kobayashi Y, Hashimoto H, Miyamoto H, Kanaoka K, Miyazaki-Kawashita Y, Nakashima T, et al. Force induced osteoclast apoptosis *in vivo* is accompanied by elevation in transforming growth factor β and osteoprotegrin expression. J Bone Miner Res. 2000; 15: 1924-1934.

16. Noxon SJ, King G, Gu G, Huang G. Osteoclast clearance from periodontal tissues during orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 2001; 120: 466-476.

17. Diekwisch TG. The developmental biology of cementum. Int J Dev Biol. 2001; 45: 695-706.

18. Saygin NE, Giannobile WV, Somerman MJ. Molecular and cell biology of cementum. Periodontol. 2000; 24: 73-98.

19. Grzesik WJ, Narayanan AS. Cementum and periodontal wound healing and regeneration. Crit Rev Oral Biol Med. 2002; 13: 474-484.

20. Holtrop M. Light and electromicroscopic structure of osteoclasts. In Brian K Hall, editor. The Osteoclast. Boca Raton, FL, CRC Press, Inc., 1991; 1-29.

21. Fujikawa, Y, Quinn JM, Sabokbar A, McGee, JO, Athanasou NA. The human osteoclasts precursor circulates in the monocyte fraction. Endocrinology. 1996; 137: 4058-4060.

22. Walker DG. Congenital osteopetrosis in mice cured by parabiotic union with normal siblings. Endocrinology. 1972; 91: 916-920.

23. Walker DG. Osteopetrosis cured by temporary parabiosis. Science. 1973; 80: 875.

24. Walker DG. Control of bone resorption by hematopoietic tissue. The induction and reversal of congenital osteopetrosis in mice through use of bone marrow and splenic transplants. J Exp Med. 1975; 142: 651-663.

25. Walker DG. Bone resorption restored in osteopetrotic mice by transplants of normal bone marrow and spleen cells. Science. 1975; 190: 784-785.

26. Burger E, Nijweide P. Cellular origin and theories of osteoclast differentiation. In Brian K. Hall, editor. The osteoclast. CRC Press, Inc. Boca Raton, FL, 1991; 32-59.

27. Schneider GB. Cellular specificity of the cure for osteopetrosis: Isolation of and treatment with pluripotent hemopoietc stem cells. Bone. 1985; 6: 241-247.

28. Zaidi M, Blair HC, Moonga BS, Abe E, Huang CL. Osteoclastogenesis, bone resorption, and osteoclast-based therapeutics. J Bone Miner Res. 2003; 18: 599-609.

29. Martin TJ, Ng KW. Mechanisms by which cells of the osteoblasts lineage control osteoclasts formation and activity. J Cell Biochem. 1994; 56: 357-366.

30. Sasaki T. Differentiation and functions of osteoclasts and odontoclasts inmineralized tissue resorption. Microsc. Res. Tech. 2003; 61: 483-495.

31. Kawamoto S, Ejiri S, Nagaoka E, Ozawa H. Effects of oestrogen deficiency on osteoclastogenesis in the rat periodontium. Arch Oral Biol. 2002; 47: 67-73.

32. Kanazaki H, Chiba M, Shimizu Y, Mitani H. Dual regulation of osteoclast differentiation by periodontal ligament cells through RANKL stimulation and OPG inhibition. J Dent Res. 2001; 80: 887-891.

33. Oshiro T, Shiotani A, Shibasaki Y, Sasaki T. Osteoclast induction in periodontal tissue during experimental movement of incisors in osteoprotegrin-deficient mice. Anat Rec. 2002; 266: 218-225.

34. Sims NA. Cell-specific paracrine actions of IL-6 family cytokines from bone, marrow and muscle that control bone formation and resorption. Int J Biochem Cell Biol. 2016; 79: 14-23.

35. Davidovitch Z, Lynch P, Shanfeld J. Immunohistochemical localization of interleukins in dental and paradental cells during tooth eruption and root resorption in kittens. In Z. Davidovitch, editor. Biological Mechanism of Tooth Eruption and Root Resorption. Birmingham, AL, EBSCO Media, 1988; 355-364.

36. Alhashimi N, Frithiof L, Brudvik P, Bakhiet M. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. Am J Orthod Dentofacial Orthop. 2001; 119: 307-312.

37. Uematsu S, Mogi M, Deguchi T. Interleukin (IL)-1β, IL-6, tumor necrosis factor-α, epidermal growth factor, and β2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. J Dent Res. 1996; 75: 562-567.

38. Mulari M, Vääräniemi J, Väänänen HK. Intracellular membrane trafficking in bone resorbing osteoclasts. Microsc Res Tech. 2003; 61: 496-503.

39. Cipriano DJ, Wang Y, Bond S, Hinton A, Jefferies KC, Qi J, Forgac M. Structure and regulation of the vacuolar ATPases. Biochim Biophys Acta. 2008; 1777: 599-604.

40. Stenbeck G, Horton M. A new specialized cell-matrix interaction in actively resorbing osteoclasts. J. Cell Science 2000; 113: 1577-1587.

41. Talic N, Evans CA, Daniel JC, George A, Zaki AM. Immunohistochemical localization of alphavbeta3 integrin receptor during experimental tooth movement. Am J Orthod Dentofacial Orthop. 2004 ; 125: 178-184.

42. Talic NF, Evans C, Zaki AM. Inhibition of orthodontically induced root resorption with echistatin, an RGD-containing peptide. Am J Orthod Dentofacial Orthop. 2006; 129: 252-560.

43. Teitelbaum S, Tanaka H, Mimura H, Inoue M, Shima M, Shioi A, et al. Integrins and osteoclast polarization. Osteoporos Int. 1997; 7: s54-s56.

44. Stenbeck G, Horton MA. A new specialized cell-matrix interaction in actively resorbing osteoclasts. J Cell Science. 2000; 113: 1577-1587.

45. Bekker P, Gay C. Characterization of Ca^{2+}-ATPase in osteoclast plasma membrane. J Bone Miner Res. 1990; 5: 557-567.

46. Sasaki T, Ueno-Matsuda E. Immunocytochemical localization of cathepsins B and G in odontoclasts of human deciduous teeth. J Dent Res. 1992; 1: 1881-1884.

47. Sasaki T, Motegi N, Suzuki H, Watanabe C, Tadokoro K, Yanagisawa T, et al. Dentin resorption mediated by odontoclasts in physiological root resorption of human deciduous teeth. Am J Anat. 1988; 183: 303-315.

48. Sun B, Sun J, Han X, Liu H, Li J, Du J, et al. Immunolocalization of MMP 2, 9 and 13 in prednisolone induced osteoporosis in mice. Histol Histopathol. 2016; 31: 647-656.

49. Delaisse JM, Andersen TL, Engsig MT, Henrisken K, Troen T, Blavier L. Matrix metalloprotinases (MMP) and cathepsin K contribute differently to osteoclastic activities. Microsc Res Tech. 2003; 61: 504-513.

50. Leonardi R, Talic NF, Loreto C. MMP-13 (collagenase 3) immunolocalisation during initial orthodontic tooth movement in rats. Acta Histochem. 2007; 109: 215-220.