Relationships between Tooth Eruption, Occlusion and Alveolar Bone Resorption: Cytological and Cytochemical Studies of Bone Resorption on Rat Incisor Alveolar Bone Facing the Enamel

Kazuharu IRIE and Hidehiro OZAWA
Department of Oral Anatomy, Niigata University School of Dentistry, Niigata, Japan

Received January 16, 1990

Summary. The rat labial incisor alveolar bone facing the enamel and bearing the occlusal force was examined by electron microscopy after being compared with the lingual alveolar bone by histological and scanning electron microscopic (SEM) observations.

On the labial side, shallow resorptive lacunae were recognized all over the bone surface; these were mainly covered by osteoclasts and some mononuclear cells. The cement line was absent from the bone matrix. On the lingual side, residues of Sharpey's fibers, the bone formation surface and deep resorptive lacunae were observed by SEM. Histologically, bone remodeling areas showing both osteoclasts and active bone-forming osteoblasts on the bone surface, as well as many cement lines in bone matrix, were recognized.

Furthermore, electron microscopic and cytochemical studies demonstrated that mononuclear cells located close to osteoclasts displayed osteoblastic characteristics such as alkaline phosphatase activity, a developed Golgi apparatus, and a rough endoplasmic reticulum.

These findings indicate that continuous bone resorption occurs on the labial bone surface, while active bone remodeling occurs on the lingual surface. Even on the labial surface, osteoblastic cells close to osteoclasts seem to play an important role in the differentiation and/or activation of osteoclasts.

Bone is continually reconstructed by a coordination of osteoclastic bone resorption and osteoblastic bone formation.

In the remodeling of bone, osteoclastic bone resorption is always followed by osteoblastic bone formation, which is known as the coupling phenomenon between bone resorption and bone formation (Takahashi et al., 1964; Thompson et al., 1975). The phenomenon is thought to be controlled by the microenvironment as well as systemic conditions. Howard et al. (1981) suggested that coupling is mediated by a humoral factor which is released during bone resorption to stimulate osteoblasts to form bone. Oguro and Ozawa (1988) proposed in their morphological study that the newly formed cement line is responsible for the induction of osteoblasts in the resorbed lacunae. Furthermore, mechanical force is known to be an important local environmental factor, since mechanical support as well as mineral homeostasis are major functions of the bone.

Since one of the major functions of alveolar bones is to support the teeth with the periodontal ligaments (Liu and Baylink, 1984), mechanical force plays an important role in bone remodeling. Vignery and Baron (1980) reported a high turnover rate of remodeling on the alveolar bone. Furthermore, in orthodontic treatment, alveolar bone is resorbed on the compression side and formed on the tension side when a suitable force is applied to move the teeth. However, most of the reports on alveolar bone remodeling have dealt with the alveolar bone of molars, which bind to the cementum of the molar by the periodontal ligament. Reports about rodent incisor alveolar bone, which consists of two different parts, are scarce. Lingual alveolar bone binds to the tooth through periodontal ligaments. However, the labial alveolar bone faces the enamel; there are no periodontal ligaments, and the periodental space is occupied by the enamel organ, loose connective tissue and blood vessels. Therefore, labial periodental tissue is considered a buffer against mechanical compressive force, not only occlusal, but also the pressure.
produced by a growing, erupting incisor (Boyle, 1938; Matëna, 1972; Berkovitz and Shore, 1978).

In an attempt to clarify the characteristics of bone cells involved in bone remodeling under mechanical compressive forces, this study focuses on elucidating cytological and cytochemical characteristics of the bone cells of rat incisor alveolar bone facing the enamel.

**MATERIALS AND METHODS**

Mandibles of Wistar strain rats about 200 g in weight were used. After intracardiac perfusion with an aldehyde mixture, the mandibles were removed and immersed in the same fixative for one and a half hours before undergoing the following procedures.

**Light microscopic study**

A mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in a 0.065 M cacodylate buffer (pH 7.4) was used as fixative. After fixation, the mandibles were decalcified in 5% EDTA for 3 weeks at 4°C. Following dissection, the specimens were dehydrated and embedded in Technovit 7100. Semithin sections were obtained and stained with toluidine blue.

**SEM study of the bone surface**

Four percent paraformaldehyde in a 0.07 M phosphate buffer (pH 7.4) was used as fixative. After fixation, sockets including the incisor were cut mediolaterally or labiolingually. The segments were immersed in 5% sodium hypochloride for 1 to 2 min. to remove organic materials, and incisors were simultaneously removed, and washed with distilled water. The bones were postfixed with 1% osmium tetroxide, dehydrated, critical point dried and coated with gold using an ion-coater (Eiko IB-3), and observed by SEM (Hitachi S-570).

**Electron microscopic study**

Two point five percent glutaraldehyde and 2.0% paraformaldehyde mixture in a 0.065 M cacodylate buffer (pH 7.4) containing 0.05% calcium chloride was used as fixative. After fixation, the mandibles were decalcified in 5% EDTA for 3 weeks and dissected. Following postfixation in 1% osmium tetroxide, the specimens were dehydrated and embedded in Epon 812. Ultrathin sections were obtained and stained with uranyl acetate and lead citrate or tannic acid, uranyl acetate and lead citrate. The sections for cytochemical studies were stained with lead citrate, and then observed by TEM (Hitachi 11-DS or Hitachi H-500).

**Cytochemical studies**

Some decalcified specimens from the electron microscopic study were prepared for cytochemical studies. After dissection, the specimens were sectioned at about 50 μm thickness with a microslicer (D.S.K. DTK-1000).

1) **Alkaline phosphatase (ALPase):** Reactivation of the enzyme was performed by immersing the sections in 0.1 M Tris-maleate buffer (pH 7.4) containing 50 mM magnesium chloride (Yoshiki et al., 1972). After reactivation, the sections were incubated at room temperature for 15 to 20 min in a medium containing β-glycerophosphate as substrate at pH 9.2 (Mayahara et al., 1967).

2) **Acid phosphatase (ACPase):** The microsliced sections were incubated at 37°C in a medium containing cytidine 5'-monophosphate as substrate at pH 5.0 (Smith, 1980).

Following incubation, in procedures 1) and 2), the sections were rinsed, postfixed with 1% osmium tetroxide containing 1.5% potassium ferrocyanide and embedded in 812 for electron microscopic observation.

**RESULTS**

**Light microscopic observations**

On the labial side, the periodental space was divided into two distinct zones: a zone of loose connective tissue near the enamel organ, and a zone of large sinusoidal blood vessels near the alveolar bone. Osteoclasts were prominent on the inner surface of the alveolar bone facing the enamel and some mononuclear cells were observed among osteoclasts, while active osteoblasts lined up on the outer surface. No cement line related to bone remodeling was observed in the bone matrix (Fig. 1a).

On the lingual side, the periodontium consisted of prominent connective tissue fibers. Some osteoclasts were found on the bone surface and active osteoblasts lined up on the newly formed cement line. Many cement lines were observed in the bone matrix near the surface (Fig. 1b).

**SEM observations**

On the labial side, resorptive lacunae — shallow concavities oval, polygonal or irregular in shape — were
Fig. 1. Light micrographs of the incisor alveolar bone. a. Labial side. Osteoclasts (Oc) are prominent on the alveolar bone surface facing the enamel, while osteoblasts (Ob) line up on the outer surface. No cement line seen in the bone matrix. EO enamel organ, BV blood vessel. b. Lingual (lateral) side. Cement lines (arrowheads) seen in the bone matrix and both osteoblasts (Ob) and osteoclasts (Oc) seen on the bone surface. D dentine, PDL periodontal ligament. a, b: ×250
found all over the surface facing the enamel. The borders between adjacent lacunae appeared low, though their ridges were distinct, and the bottom of each lacuna was mostly smooth. Branching vascular canals were exposed, and round or oval cavities, which were exposed osteocyte lacunae, were scattered over the surface (Fig. 2).

The lingual (lateral) side of the incisor alveolar bone surface was composed of bone formation areas and deep resorptive lacunae. Residues of Sharpey's fibers were sporadically found (Fig. 3).

**Electron microscopic observations**

On the labial side facing the enamel, osteoclasts were prominent. However, a number of mononuclear cell types were also observed. Between the osteoclasts and the blood vessels, intercellular fibers were scarce and a few mononuclear cells were found (Fig. 4). The mononuclear cells were characterized by both a flattened cell body and their extending long cytoplasmic processes which were often in close contact with osteoclasts (Figs. 4, 5). Some mononuclear cells, in close contact with osteoclasts, possessed a well-developed rough endoplasmic reticulum (rER) (Fig. 5b). On the bone surface, osteoblastic cells with a well-developed Golgi apparatus were often in proximity to osteoclasts (Fig. 6). Preosteoclasts were also observed near flattened mononuclear cells (Fig. 7). Among the osteoclasts on the bone surface, at least three types of mononuclear cells were observed: osteoblastic cells with developed cell organelles (Fig. 8a); excavated osteocytes extending cytoplasmic processes into bone canaliculi (Figs. 8a, b); and flattened bone lining cell-like cells (Fig. 8c).

The mononuclear cell located close to the osteoclasts and contacting its cytoplasmic process possessed a well-developed rER, as osteoblastic cells

![Fig. 2. SEM image of the labial alveolar bone surface. Shallow resorptive lacunae are visible all over the surface. ×450](image-url)
have (Fig. 9a). Osteoblastic cells located close to the osteoclast reached their cytoplasmic ends to the bone surface. The cytochemical study revealed ACPase activity in the lysosomes (Fig. 9b) and ALPase activity in the plasma membrane of the above-described osteoblastic cells (Fig. 9c).

DISCUSSION

Mineral homeostasis and mechanical support are two of the major functions of the bone; in the case of the alveolar bone, mechanical support of the tooth seems to be the major function.

In rodent incisor sockets, two distinct parts bear occlusal force in their respective ways: the lingual periodontium suspends the incisor by prominent periodontal fibers, and labial tissues cushion and buffer the force by large sinusoidal blood vessels and loose connective tissue (BOYLE, 1938).

In this study, histological and SEM observations revealed two distinct parts of the alveolar bone. Osteoclasts were prominent while no active bone-forming osteoblast was observed on the shallow resorptive lacunae of the labial bone surface. Moreover, cement lines which reflect bone remodeling were absent from this bone matrix. Meanwhile, on the lingual side, osteoclasts and osteoblasts on bone surface as well as cement lines in the bone matrix were seen, and both resorptive lacunae and bone formation surfaces were observed under SEM. These findings reveal that continuous bone resorption occurs on the labial side of the alveolar bone surface. The active bone remodeling that occurred on the lingual side of alveolar bone surface supports the findings by VIGNERY and BARON (1980).

On the labial side of the alveolar bone, compressive force produced by occlusion and by an increase in

![Fig. 3. SEM image of the lingual alveolar bone surface. Among the deep resorptive lacunae (left upper part) and formation area (right lower part), residues of Sharpey’s fibers (*) are scattered. ×450](image-url)
Fig. 4 a and b. Electron micrographs of the labial alveolar bone surface facing the enamel. The bone surface is covered by osteoclasts (Oc) and some mononuclear cells (a, b). Intercellular fibers are scarce (b). BV blood vessel, POC preosteoclast. a: ×3,300, b: ×2,800
Fig. 5 a and b. Electron micrographs of mononuclear cells (⋆) in close contact (arrowheads) with osteoclasts (Oc). a: ×5,500, b: ×8,000
incisor curvature radius during incisor development (HERZBERG and SCHOUR, 1941) and eruption may cause continuous resorption of the bone. OZAWA et al. (1989) have reported that the compressive force by itself suppressed the osteoblast differentiation and promoted osteoclast formation. In this study, we observed osteoblastic cells with developed cell organelles on alveolar bone facing the enamel; however, they did not seem to secrete bone matrix, suggesting their being depressed by compressive force, and then inhibited to differentiate into active osteoblasts.

In the mediation of the mechanical forces to bone resorption, prostaglandins (PGs) must be considered as an important substance, since mechanical force stimulates the synthesis of PGs (SOMJEN et al., 1980), and PGs stimulate the formation of osteoclasts (AKATSU et al., 1989). Other bone resorptive factors such as the epidermal growth factor (EGF) (TASHJIAN and LEVINE, 1978; RAISZ et al., 1980) and interleukin-1 (DEWHIRST et al., 1985) may also be involved in the resorption as well.

In this study, osteoclasts were often observed near blood vessels. Blood vessels are important in bone resorption since their elasticity itself may mediate the force to the bone cell microenvironment, and since they may control local oxygen tension, nutritional supply and the elimination of waste matters. Moreover, the blood vessels serve as pathways for osteoclast progenitor supply.

Furthermore, the components between tooth and alveolar bone on the enamel side such as the enamel organ, loose connective tissue and blood vessels resemble the coronal aspect of erupting molar dental follicles. On the alveolar bone of erupting molars, osteoclasts mainly occupy the bone surface (MARKS

Fig. 6. Electron micrograph of an osteoblastic cell (Obl) in close contact with an osteoclast (Oc). Note the well-developed Golgi apparatus (Go). ×6,500
et al., 1983; Wise et al., 1985), and the bone surface shows resorptive lacunae (Marks and Cahill, 1986). In addition to our observations, in alveolar bone resorption on the enamel aspect of rat incisors and in erupting molars, continuous osteoclastic bone resorption may be activated by a similar mechanism, i.e., compressive force mediated by the tooth. However, the influence of the enamel organ remains a potent factor to promote osteoclastic bone resorption.

Many reports have mentioned mononuclear cells as being involved in bone remodeling. Kurihara (1977) reported two types of mononuclear cells, one being a preosteoclast and the other functioning in originating cells for osteoblasts, fibroblasts, reticulum cells or phagocytes. Since Heersche (1978) had suggested that mononuclear cells phagocytosed the exposed collagen fibrils after osteoclastic bone resorption, these cells were thought to be related to organic matrix degradation. Baron et al. (1980) indicated three types of mononuclear cells at the site in either bone resorption or the reversal phase: partially released osteocytes, mononuclear phagocytic cells and preosteoblasts. TranVan et al. (1982) suggested that these mononuclear phagocytic cells might play an important role during all processes of bone remodeling. Furthermore, Oguro and Ozawa (1989) suggested that macrophage-like mononuclear cells on the resorbed bone surface might principally derive from the excavated osteocytes, and might be identical with mononuclear phagocytic cells in the reversal phase as postulated by Baron et al. (1980). In our study, we demonstrated mononuclear cells in close contact with osteoclasts as having more characteristics attributable to osteoblastic cells than phagocytes, i.e., ALPase activity on their plasma membrane and developed cell organelles such as rER.
Fig. 8. Mononuclear cells on the bone surface. a. Osteoblastic cells (Obl) with developed cell organelles and an excavated osteocyte (*) extending cytoplasmic process into a bone canalicle. ×5,500. b. Excavated osteocyte (*) extending cytoplasmic processes along the bone surface. ×11,000. c. Flattened bone lining cell-like cell extending cytoplasmic processes on the bone matrix side. ×11,000
Fig. 9. Cytochemistry of mononuclear cells adjacent to osteoclasts (Oc). a. ACPase activity in a mononuclear cell (*) close to osteoclasts (Oc). $\times 6,500$. b. Osteoblastic cell locating closely to osteoclasts (Oc) shows ACPase activity in the lysosome. $\times 7,200$. c. ALPase activity on the plasma membrane of osteoblastic cell. $BV$ blood vessel. $\times 6,500$
and a Golgi apparatus. Moreover, some osteocytes excavated by osteoclasts were released from the osteocytic lacunae, and then seemed to occupy the bone surface. Since osteocytes are generally considered a part of the lining cell system and may produce osteoclast activating factors (Miller and Jee, 1987; Huffer, 1988), they may also be involved in the control of osteoclastic activity. Thus, osteoclasts and their precursor cells seemed to locate in the osteoblastic cell network, suggesting that their differentiation and activation may be controlled by direct contact and by other means, such as the secretion of chemical factors.

Ejiri (1983) suggested that osteoblasts influence the cytodifferentiation process of preosteoclasts in the bone tissue. Burger et al. (1984) reported that live bone-forming cells are required for osteoclast formation. Recently, Takahashi et al. (1988) reported that direct contact of progenitors with osteoblasts or a close microenvironment between them is required for osteoclast development.

The presence of osteoblastic cells in the proximity of osteoclasts, even at the site of continuous bone resorption, suggests that the osteoblastic cells have an influence on the activation of osteoclasts.

Recent papers have revealed that the osteoblastic cells have receptors of some bone resorptive factors such as parathyroid hormone and EGF (Rouleau et al., 1988; Martineau-Doizé et al., 1988; Cho et al., 1988). It is therefore reasonable to think of osteoblastic cells as being involved in the control of osteoclastic activity, even on the continuously resorbing bone surface.

In conclusion, our study demonstrates a continuous bone resorption on the labial side of the incisor alveolar bone facing the enamel, and the osteoblastic cells in close contact with osteoclasts at this site are suggested to exert an influence upon the differentiation and/or activation of osteoclasts.

Acknowledgments. We are grateful to Associate Prof. S. Ejiri, Department of Oral Anatomy, Niigata University School of Dentistry, for support and advice; and the staff of Department of Oral Anatomy for their assistance.

REFERENCES


