Electron Microscope Study of Osteoclasts with Special Reference to the Three-Dimensional Structure of the Ruffled Border

Takanori Domon and Minoru Wakita

Department of Oral Anatomy (Prof. Wakita), Hokkaido University School of Dentistry, Sapporo, Japan

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Summary. Osteoclasts of rat mandibles were observed under the transmission electron microscope with the aim of understanding the three-dimensional structure of the ruffled border. After observation, the same block was remounted to obtain sections of the same osteoclasts at right angles to the first sectioning plane. The structure of the ruffled border of osteoclasts was observed in the two perpendicular directions.

The ruffled border of osteoclasts was found to consist of two areas: one being composed of finger-like processes and the other, of plate-like processes. The distribution of the two areas of processes in the ruffled border did not show any apparent regularity. Not all processes continued to the cell body; some processes (stem processes) did while others were interwoven branches of the stem processes. The finger-like processes were long and rod-shaped and a few of them branched directly from the stem processes. The plate-like processes were long and belt-like and showed complicated branching from the stem processes; some of these were possibly the long belt-like processes that were complicatedly folded up to make many secondary plates; they are arranged parallel to each other in a given area. The relationship between the structure and function of the ruffled border is discussed.

The part of the cells situated at the bone resorbing sites of osteoclasts was originally called the brush border by Kölliker (1873). Later in electron microscopy it was called the ruffled border by Scott and Pease (1956). These and many later investigators studied the ruffled border by electron microscopy, describing it as comprising infoldings or projections of the cell membrane (Gonzales and Karnovsky, 1961; Dudley and Spiro, 1961; Kallio et al., 1971; Fetter and Capen, 1971; Lucht, 1972). The morphology of the ruffled border has been expressed variously as villus-like, finger-like, foliate, slender, bulbous, short and broad, among others (Gonzales and Karnovsky, 1961; Schenk et al., 1967; Fetter and Capen, 1971; Kallio et al., 1971; Lucht, 1972; Luk et al., 1974).

Kallio et al. (1972) were the first to report that, after administration of calcitonin, osteoclasts had flattening processes with a few infoldings instead of finger-like processes. Holtrop et al. (1974) and Luk et al. (1974) classified the ruffled border into well developed and poorly developed forms based on the depth and number of membrane infoldings. Previous descriptions have reported two types of membranous structures forming the ruffled border, both finger-like or slender processes and foliate or bulbous processes (Lucht, 1972; Luk et al., 1974). In many cases, the ruffled border has been
observed to be formed of these two different elements, while in some cases it has appeared to consist of only one type of process. It is still not clear whether the profiles of these processes under the transmission electron microscope are direct projections of the cell membrane or secondary branches. The present study aims to clarify the three-dimensional structure of the ruffled border which fills the space between osteoclasts and the bone surface.

MATERIALS AND METHODS

Seven-day-old wistar strain rats were perfused with a 2% paraformaldehyde–2.5% glutaraldehyde mixture buffered at pH 7.4 with 0.05 M sodium cacodylate at room temperature for 10 min. After perfusion, the mandibular was removed and immersed in the same fixative at 4°C for 4 hrs. The specimens were decalcified in 2% ascorbic acid (WAKITA et al., 1983a, b) and postfixed in 1% osmium tetroxide for 3 hrs. The specimens were stained en bloc with 4% uranyl acetate. They were dehydrated with a graded series of ethanol and embedded in Epon 812.

After making osteoclast sections, the block was remounted to obtain sections of the same osteoclast at right angles to the former sectioning plane. Then ultrathin sections were stained with uranyl acetate and lead citrate and observed with a transmission electron microscope, Hitachi H-7000. Using this method, the structure of osteoclast at the resorbing sites was observed in two directions.

RESULTS

Osteoclasts observed at the bone resorbing sites are large multinuclear cells, and the cytoplasm facing the bone surface is found in the Howship's lacuna. Each nucleus was enclosed by a well developed Golgi apparatus. Abundant mitochondria were scattered in the cytoplasm, and the major part of the rough-surfaced endoplasmic reticulum was situated in the cytoplasm away from the bone surface. On the side facing the bone, the osteoclasts form the ruffled border, a relatively thick layer of cytoplasmic processes. The ruffled border fits in the resorptive lacuna close to the bone surface. On either lateral side of the ruffled border there is a special structure called the clear zone. The clear zone is not provided with the regular cytoplasmic processes seen in the ruffled border and is usually close to the bone surface. The clear zone can be classified into two areas: one is an area involving the electron dense components located near the ruffled border, called the dense area; the other is an organella-free area located external to the dense area, called the light area (MALKANI et al., 1973; KING and HOLTROP, 1975).

In the case where an osteoclast resorbs two trabeculae (Fig. 1), the ruffled border is located on each trabecula and the clear zones are seen on both sides of each of the ruffled borders. The light area of the clear zone has irregular processes in various directions, between osteocytes and the bone surface, osteoblasts and the bone surface, and also on the exposed bone surface.

The processes of the ruffled border were classified into two types based on the structure of the processes. One was an aggregation of many processes which appeared round or oval in cross sections, with a few thin processes sectioned longitudinally between them. Therefore, the structure of these processes was assumed to be finger-like
Fig. 1. Electron micrograph showing an osteoclast (Ocl) resorbing two trabeculae (*) formed by an osteocyte (Oc). The ruffled borders (arrow) are situated on the ends of the trabeculae and the clear zones (arrow head) are formed on both sides of these ruffled borders. bl Blood capillary. ×3,400
or rod-like (Fig. 2). The other was formed by processes of a few infoldings and various thickness, and some of these were often observed as broad cytoplasmic processes. These processes were assumed to be narrow plate-like or lamellar in structure (Fig. 3).

There were few observations of processes forming the ruffled border continuing to the cytoplasm; in many of the sections they appeared separated from the cell body. It was seen noted that processes connected to the cell body were stem processes, and many complicated branches extended from one stem process to form secondary processes. This indicated that most of the processes forming the ruffled border were branches from the stem processes and very long branches, finger-like or plate-like, in shape, were intertwined (Fig. 2, 3).

Observation of the same osteoclast from two directions

Figure 4 shows a typical osteoclast with the characteristic structure described above and a well developed ruffled border. The processes of the ruffled border are divided into finger-like and plate-like processes in those structures. The sectioning plane shown in Figure 4 is called the first plane in this study. After picking up this section, the block was remounted and ultrathin sections of the same osteoclast were made at the two sites indicated, at right angles to the first plane. Thus the osteoclast in the first plane is also observed in the second plane, and it becomes possible to observe this cell three-dimensionally. Figures 5A and 6A in the second plane show that the mononuclear osteoclast appearing in the first plane is apparently a multinuclear cell.
The finger-like processes of the ruffled border in the first plane (Fig. 5B) are shown as an accumulation of similarly shaped processes in the second plane, too (Fig. 5A). This means that, three-dimensionally, cytoplasmic processes in this area are long finger-like forms and in this case, rarely branch (Fig. 7A). On the other hand, the processes of various thicknesses and with unclear contours in the first plane (Fig. 6B) are observed as elongated thin structures arranged parallel to each other in the second plane (Fig. 6A). These processes rarely continue to the cell body, and most of them appeared as secondary branches of stem processes. This means that the cytoplasmic processes in this area are three-dimensionally plate-like in shape and usually branch to form secondary processes with the same shape, and then these processes arrange themselves parallel to each other. This also suggests that a long belt-like process is bent many times to make flat parts, these parts of processes being arranged parallel to each other, that is, a long belt-like process is intricately folded up in this region (Fig. 7B).

Only occasionally was the ruffled border of osteoclasts was formed only of finger-like processes or of plate-like processes. Usually the ruffled border of osteoclasts was composed of both finger-like and plate-like processes. There was no apparent regularity in the distribution of areas composed of finger-like processes and plate-like processes in one ruffled border. Some parts of the ruffled border showed processes which could not be identified either as finger-like or plate-like processes (Fig. 4). Microfilament bundles were seen in the cytoplasm near the plate-like processes in the first plane (Fig. 6B), but not in the second plane (Fig. 6A).
Fig. 4. Electron micrograph showing an osteoclast in the lacuna. The ruffled border is composed of areas of finger-like (arrow) and plate-like (double arrow) processes. The osteoclast appears mononuclear in the first plane. The sections of the second plane were made at the sites indicated by the lines (line 1, line 2). Line 1 is the area composed of finger-like processes. Line 2 is area composed of plate-like processes. ×4,200

Fig. 5. A and B. Electron micrographs showing the area composed of finger-like processes cut along line 1 in the first plane (Fig. 4). The processes in the second plane (A) are also shown as an accumulation of similar shaped processes observed in the first plane (B). The mononuclear osteoclast observed in the first plane (Fig. 4) appears multinuclear. The structures shown by arrows in Figure A respectively correspond to the structures in Figure B. The lack of correspondence in distances among the structures in upper parts of the Figure A, B has been caused by the distortion of ultrathin sections during sectioning. ×7,300
DISCUSSION

Since Scott and Pease (1956) reported that the ruffled border was the complex infoldings of cell membrane, the structures of the ruffled border have been variously termed as villus-like, finger-like, slender, foliate, bulbous, short and broad (Gonzales and
KARNOVSKY, 1961; SCHENK et al., 1967; FETTER and CAPEN, 1971; KALLIO et al., 1971; LUCHT, 1972; LUK et al., 1974). Many investigators believed that all processes forming the ruffled border were infoldings or projections of cell membrane, and that each process continued to the cell body. The present study showed that finger-like and plate-like processes are intricately interwoven between cell body and bone surface, and that these processes do not all continue individually to the cell body, but many are originated from stem processes which continue to the cell body. By observation of osteoclasts from two directions, it was shown three-dimensionally that finger-like processes always appear as finger-like (Fig. 7A) and that plate-like processes are arranged parallel to each other. This observation also suggests that a plate-like process branches to form the secondary processes with the same shape and these processes are gathered parallel to each other, or that a long belt-like process is rather awkward folded up in a complicated way to make secondary plates and they are gathered parallel to each other in a given area (Fig. 7B).

It is remarkable that bundles of microfilaments are observed in the cytoplasm near those plate-like processes and run parallel to the folded up processes. The functional significance of these filamentous structures can not be completely explained, but it may relate to the arrangement of processes forming the ruffled border (Fig. 7B).

KALLIO et al. (1972) and HOLTROP et al. (1974) reported that plate-like processes, the term used in the present study, were poorly developed structure. This was because osteoclasts inactivated after the administration of calcitonin showed this type of flattened processes instead of the finger-like processes, and later even completely lacked the ruffled border. Some investigators classified the ruffled border into well developed and poorly developed forms based on the depth and number of the membrane infoldings (HOLTROP et al., 1974; LUK et al., 1974). According to this classification, the finger-like processes are well developed forms and plate-like ones are poorly developed forms. JONES et al. (1985) suggested that the formation of the ruffled border in vitro began at the central region of the cytoplasm facing the bone surface and extended to the peripheral regions. Accordingly, there should be an established tendency in the location of processes, i.e., the well developed finger-like processes should be predominant at the central region of the ruffled border and the poorly developed plate-like processes, at the peripheral regions.

The present study did not find such a tendency; we observed that two types of regions with finger-like processes or plate-like processes were intermingled in one ruffled border. This suggests that the morphological differences between finger-like and plate-like processes do not directly indicate the developmental stage of the processes.

ALI et al. (1984) and JONES et al. (1985) cultured osteoclasts on dentine slabs, and demonstrated that hard tissue resorption in vitro occurred by both disintegration of the mineral components and digestion of the organic matrix in one ruffled border, i.e., a mineral resorptive phase and organic resorptive phase occur in the same ruffled border. Although many investigators have suggested that two-phase bone resorption occurs in one ruffled border, it is difficult morphologically to distinguish the two resorptive phases (KALLIO et al., 1972; LUCHT, 1972; BONUCCI, 1974). It is certain, however, that functional change should show the morphological change in some way; we presume that if the ruffled border of one osteoclast changes from one resorptive phase to the other resorptive phase, the processes of the ruffled border must also show structural differences depending on the functional change by alternation of resorptive phases. From these considerations, it is possible to postulate that the morphological differences in the two
types of processes forming the ruffled border indicate functionally different roles of the processes during bone resorption. Therefore, it is possible to consider that each finger-like or plate-like process corresponds to one of the mineral or organic resorptive phases. The space between the ruffled border and bone surface may be further divided into smaller regions in either the mineral or organic resorptive phase, and after a resorptive phase where one process' structure has progressed to a degree, this resorptive phase may be replaced by the other phase with a morphological change. Processes showing an intermediate structure between the finger-like and plate-like forms may then be explained to be in the process of phase change. This is a possible explanation as to how one ruffled border can be composed of finger-like and plate-like processes and why there is no apparent regularity in the distribution of the two types of areas, and that morphological differences do not indicate the developmental stage of the processes. It also supports the view that the ruffled border performs resorption of both mineral and organic matrix in one lacuna (Ali et al., 1984; Jones et al., 1985). However, further study is needed to explain the relationship between the resorptive function and the structure of the ruffled border of osteoclasts. It seems necessary to observe undecalcified sections and to perform more detailed cytochemical studies.

REFERENCES

Malkani, K., M. M. Luxembourger and A. Rebel: Cytoplasmic modifications at the contact zone


土門卓文
〒060 札幌市北区北13条西7丁目
北海道大学医学部
口腔解剖学第二講座

Dr. Takanori Domon
Department of Oral Anatomy
Hokkaido University School of Dentistry
Kita-13, Nishi-7, Kita-ku
Sapporo, 060 Japan