Three dimensional structure of multinucleated giant cell "Osteoclast"

Koji Noda, Ayao Hirashita and Yosuke Kuwabara

*Department of Orthodontics, School of Dental Medicine, Tsurumi University,
2-1-3 Tsurumi, Yokohama 230, Japan*

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Osteoclasts are multinucleated giant cells with a characteristic appearance. Generally, these cells contain from one or two nuclei in the smallest to 100 nuclei in the largest. The size of the cells is about 20–100 μm in diameter. The structures of osteoclasts have been reported in detail light- and electron-microscopically by a number of investigators^{1,3,6,10}). However, these findings have been found mainly from the study of static sections. In addition, if these cells can be observed in three dimensional structures, it would enable us to understand more accurately the structures of osteoclasts and the relationships between the cells and surrounding tissues incident to experimental tooth movement. The purpose of this study is to make the three dimensional structures of osteoclasts incident to experimental tooth movement light-microscopically.

Material and Methods

The upper molars of adult Wistar rats (5 rats, each weighing 300–308 g) were moved experimentally by wire-spring method⁸) for 9 days. Anesthetized rats were perfused with 2.0% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) through their ascending aortae for 30 min. The upper jaws were subsequently excised and cut into a few small blocks with dental disks. After these blocks were fixed in freshly prepared 2.0% glutaraldehyde for 90 min, they were rinsed with 0.1 M cacodylate buffer (pH 7.3) containing 0.25 M sucrose and then decalcified with a cold 5.0% EDTA- Na_2 solution for about 6 weeks. After decalcification,

they were post-fixed in 2.0% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.3, 0°C–4°C) for 90 min. After dehydration with a grade ethanol series, they were embedded in Epon 812. Series of cross sections (about 2–2.5 μm in thickness) were cut on the ultramicrotome with glass knives and several specimens on a glass slide were stained with toluidine blue in 0.1 M phosphate buffer. In order to make up the three dimensional structure of a osteoclast, each appearance of osteoclasts photographed with 200 magnifications by light microscope was projected on an Audioviewer projector (Kodak, Ektagraphic Model 260) and traced on tracing papers with standard magnifications. Then, outlines of the cell were drawn by superimposing the dental paraffin waxes (about 3 mm in thickness) on the papers. A cavity of each piled up waxes was made by removing the waxes of the outlined cells; it is the three dimensional form of a cell⁹). Then, quick self-curing acrylic resins were used to fill the cavity of a cell by a direct resin filling method.

Results

The selection of 9 days' experimental duration seems to be favorable for observations of osteoclasts with the most striking characteristic features incident to experimental tooth movement^{4,8,9}). Figs. 1a, 1b, 2a and 2b show a series of osteoclasts obtained from serial cross sections incident to tooth movement. These osteoclasts are located in areas of bone resorption and the features typical types of undermining resorption. The

osteoclasts seen in Figs. 1a and 1b were obtained from 53 specimens of serial cross sections—an osteoclast (arrow 1) is about 106–133 μm in maximal length, about 21 μm in maximal width; an osteoclast (arrow 2) is about 58–73 μm , about 16 μm , and an osteoclast (arrow 3) is about 68–85 μm , about 12 μm respectively. Other osteoclasts seen in Figs. 2a and 2b were obtained from 22 specimens of serial cross sections and the giant cell is about 44–55 μm in length and about 33 μm in width. As revealed by the light microscope, the plasma-membranes are markedly varied in appearance. These numerous processes protrude from the cell surface to terminate on the bone surface or hyalinized tissues as seen in Fig. 1b. Also, as observed in Fig. 1 (arrow 3), there is a fusion of the membrane of capillary and osteoclast with borders stained strongly by toluidine blue.

The number of nuclei contained in an osteoclast sectioned in maximal width show from several to more than ten. The sizes are varied and the distribution is scattered in the cytoplasm.

In the cytoplasmic membrane of osteoclasts closely adjacent to the surface of the alveolar bone or hyalinized tissues, they are varied; parts containing numerous vesicles and vacuoles beneath ruffled-border like structures and a part of membrane or only processes without containing vesicles and vacuoles were seen in the cytoplasm of a cell. On the other hand, Figs. 3a, 3b and 3c show a modified osteoclast of three dimensional structures made up by acrylic resin from serial cross sections. Figs. 3a are stereo-pair photographs⁷⁾ of a model of an osteoclast and Fig. 3c is the osteoclast closely adjacent to the surface of the surrounding tissues (alveolar bone and hyalinized tissues). During the observation of the model, the appearance of a giant cell showed most striking characteristics which differed from the other bone cells.

Discussion

Rusmussen and Bordier¹⁰⁾ reported that in people over 70 the mean number of

nuclei per osteoclast section is 2.55 ± 1.3 , which is similar to the number found in bone sections from patients with post-menopausal osteoporosis (2.6 ± 1.1). In primary hyperparathyroidism the mean number is 4.8 ± 2.2 and in Paget's disease the mean number is 11 ± 6.4 nuclei per cell. Though the accurate number of nuclei could not be counted in an osteoclast incident to the tooth movement of 9 days' duration, the number estimated by counting the same nuclei more than once showed more than 70 nuclei per osteoclast. This fact may suggest that active osteoclasts have more active organelles under unfavorable environment of tooth movement than under favorable physiological condition. Also, from the striking features of osteoclasts with a variety of the prominent cytoplasmic membranes, it seems to be more active to resorption of alveolar bone or hyalinized tissues (Figs. 1b, 2b, 3b and 3c). On the other hand, close contact between the cytoplasmic membranes of osteoclast and the plasma membranes of capillary were observed in bone marrow sides exposed by undermining resorption (Fig. 1b, arrow 3). However, the membrane structure of the areas of contact was blurred. It is not clear that the contacts involve the origin of osteoclast from vascularity²⁾ or a supplement of nutriment from capillary to osteoclast. In this study, it could not be elucidated with regard to the fusion of mononuclear cells or mononuclear macrophages.

In summary, it is concluded that active functions of osteoclasts under unfavorable environment of periodontium incident to experimental tooth movement involve not only the prominent characteristic organelles, but also variable changes of cytoplasmic membranes for objects to be resorbed or dissolved. If a more accurate representation of the giant cells' ultrastructures were to be studied by a series of osteoclasts in serial ultra-thin sections in vivo, the functional mechanism of a cell may be more clearly exposed.

Fig. 1a

Fig. 1b

Fig. 2a

Fig. 2b

Fig. 3a

Fig. 3b

Fig. 3c

Explanation of figures

- Fig. 1a: A typical feature of osteoclast seen in Fig. 1b was obtained from 53 specimens. Cement (C), Bone (B), Hyalinized-Tissue (HY). $\times 400$
- 1b: Series of osteoclasts (arrow 1, 2, 3) are shown. $\times 400$
- Fig. 2a: The other osteoclast seen in figs. 2b was obtained from 22 specimens. $\times 400$
- 2b: Series of osteoclast (arrow) are shown. $\times 400$
- Fig. 3a: Stereo-pair photographs of a model of the osteoclast observed in Figs. 2a and 2b.
- 3b: This shows the area of osteoclast closely adjacent to the surface of alveolar bone or hyalinized tissues.
- 3c: This photograph shows the relationship between osteoclast and alveolar bone (B) or hyalinized tissues (HY).

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