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Three-Dimensional Structure of Connective Tissue Papillae in the Human Gingiva

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Three-dimensional structures of connective tissue papillae of the human gingiva were studied by scanning electron microscopy. Specimens were obtained from human cadavers fixed with 10% formaldehyde. The free gingiva, attached gingiva and alveolar mucosa were excised from the incisor, canine and molar regions. They were further fixed with 2.5% glutaraldehyde and treated with 2 N NaOH for 2 weeks at 20˚C to remove the epithelium followed by routine specimen preparation methods. Consequently, the connective tissue papillae were observed three-dimensionally and were classified into three types as follows. Type I was an elongated papilla with a pointed tip, which was usually observed in the free gingiva. Type II was a bud-like papilla whose tip was forked into several tips and usually found in the attached gingiva. Type III was a plate-like papilla chiefly located in the alveolar mucosa. In general, pointed elongated papillae (type I) in the free gingiva reduced in height changing into type II papillae towards the attached gingiva, and eventually sparsely distributed changing into type III papillae. These findings were commonly observed both in the upper and lower jaws. Lots of small holes, measuring 10 to 30 μm in diameter, were observed at the incisor and canine regions. Functional significance and origin of the holes remain unknown.

Key words: connective tissue papillae; human gingiva; scanning electron microscopy; NaOH maceration method

The gingiva, which covers the neck of the tooth and a part of the alveolar bone, is a soft tissue preventing the invasion of foreign materials and bacteria into underlying tissues. It is grossly divided into “free gingiva” and “attached gingiva”, which are separated from each other by a fine line running parallel to the margin of the gingiva (Orban, 1948; Bollinger and Riethe, 1973). Microscopically, it is composed of the stratified squamous epithelium, basal lamina and underlying lamina propria. The basal lamina is located at the epithelium-connective tissue interface which is undulated by connective tissue papillae.

The three-dimensional structures of the connective tissue papillae were reconstructed by wax modeling of serial sections using light microscopy (Karring and Löe, 1970; Löe and Karring, 1971). Since scanning electron microscopy (SEM) was introduced to examine the three-dimensional architecture of biological materials, it has been usefully applied to the observations of gingival surfaces (Matravers and Tyldesley, 1977, 1978; Cleaton-Jones et al., 1978; Hodgkins et al., 1978; Kullaa-Mikkonen 1986). With the progress of techniques for cleaving the interface between the epithelium and underlying lamina propria, connective tissue papillae have been studied three-dimensionally (Klein-Szanto and Schroeder, 1977; Ooya and Tooya, 1981). However, there have been no precise studies which describe the morphological differences in various regions of the gingiva.

Abbreviation: SEM, scanning electron microscopy

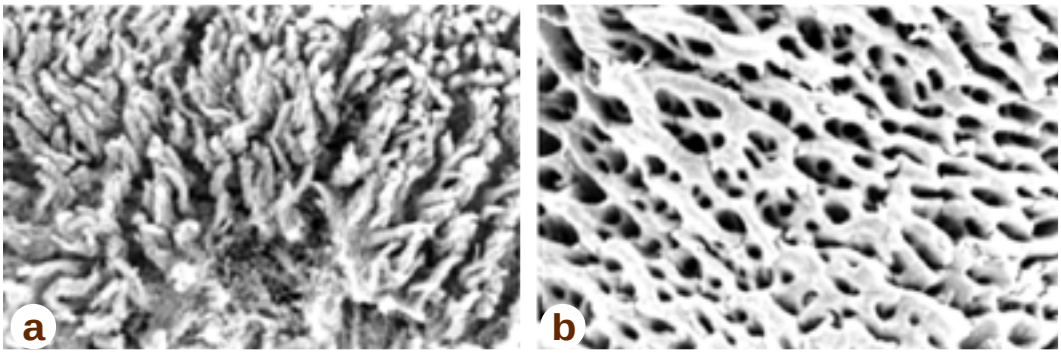

Fig. 1. Scanning electron micrographs showing the surface of the lamina propria (**a**) and basal surface of the epithelium (**b**). The interface between the epithelium and lamina propria are cleaved by the NaOH digestion method. Although the ultrastructures of the basal epithelial surface are well retained, the preservation of the connective tissue papillae is relatively poor. **a**: × 40, **b**: × 40.

In this study, we studied various regions of human connective tissue papillae in the maxillary and mandibular gingiva together with the alveolar mucosa, by SEM after removing the epithelium using some chemical digestion techniques.

Materials and Methods

Specimens were obtained from human cadavers (two males: 63 and 80 years old, one female: 50 years old). These cadavers were initially fixed with a 10% formalin solution through the right femoral artery. The gingiva was excised from the incisor, canine and molar regions together with the alveolar mucosa in the left upper and lower jaws. The specimens were further cut into three parts: free gingiva, attached gingiva and alveolar mucosa. The size of each specimen was approximately 5 × 10 mm. They were fixed again for an additional 24 h in 2.5% glutaraldehyde buffered with 0.15 mol/L phosphate buffer (pH 7.4) and rinsed for 1 h in the same buffer. Some specimens were digested in 6 N NaOH at 60˚C for 10 min to cleave the epithelium-connective tissue interface (Inoué and Gabella, 1992). Other specimens were macerated in 2 N NaOH for 2 weeks at 20˚C (Ohtani et al., 1988). After rinsing in the same buffer, they were postfixed in 1% osmium tetroxide for 1 h followed by conductive-staining with 2% tannic acid and 1% osmium tetroxide (Murakami, 1974). The specimens were then dehydrated in a graded series of ethanol, and finally dried in t-butyl alcohol (Inoué and Osatake, 1988). After the dried specimens were mounted on metal specimen stubs with conductive-glue, they were sputter-coated with platinum at a thickness of approximately 5 nm. Specimens were observed and photographed by scanning electron microscope (HFS-2ST or S-430S, Hitachi, Tokyo, Japan) at 25 kV.

Results

When specimens were digested with 6 N NaOH, the epithelium-connective tissue interface was cleaved. The superficial surface of the lamina propria was characterized by lots of villous protrusions of connective tissue papillae (Fig. 1a), but their preservation was relatively poor. The corresponding basal surface of the epithelium had lots of large holes which had housed the connective tissue papillae (Fig. 1b).

When specimens were macerated with 2 N NaOH, the epithelium was effectively removed and the underlying connective tissue papillae were demonstrated in three dimensions by SEM (Figs. 2 to 4).

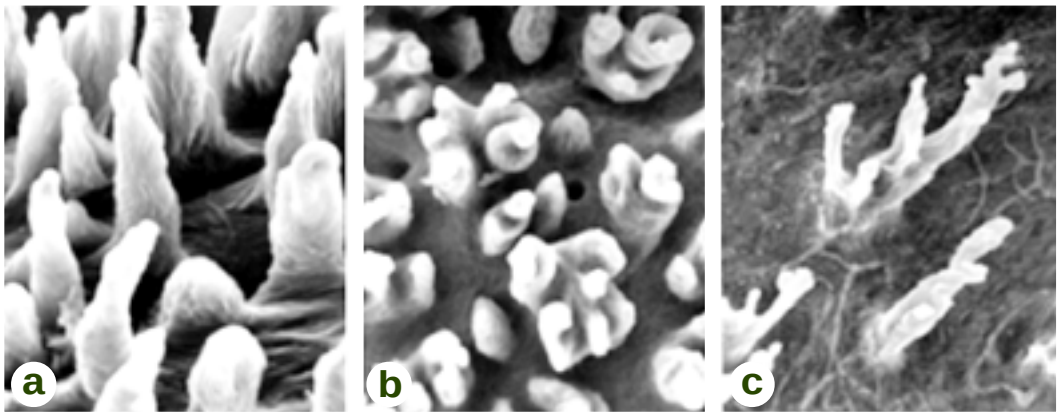

Fig. 2. Scanning electron micrographs showing the three types of connective tissue papillae of the human gingiva prepared by the NaOH maceration method. The ultrastructure of the connective tissue papillae is well preserved. **a**: Elongated papilla with pointed tip (type I). **b**: Bud-like papillae whose tips are forked into several tips (type II). **c**: Plate-like papillae whose tips are serrated (type III). **a**: × 160, **b**: × 95, **c**: × 90.

The connective tissue papillae showed different shapes and sizes in their locations. They were classified into types I, II and III as follows (Fig. 2). Type I papillae were elongated papillae whose tips were usually pointed, but occasionally bifurcated or three-forked (Fig. 2a). Type II papillae were lower than type I. They appeared as bud-like configurations because their tips were usually forked into two to five sections (Fig. 2b). Type III papillae exhibited plate-like elevations (Fig. 2c). Their free margins were usually serrated, showing fork-like configurations. They were sometimes highly serrated, changing into several solitary rod-like papillae.

Both in the upper and lower jaw, a pointed configuration (type I) in the free gingiva, reduced in height, could be seen showing the bud-like configuration of type II the farther they were from the gingival margin. The plate-like papillae of type III were usually found in the alveolar mucosa.

Connective tissue papillae in the upper jaw

In the free gingiva, connective tissue papillae were slender, and pointed (type I) (Figs. 3c, f and i). Their width and height measured 30 to 60 μm and 200 to 250 μm, respectively. In the area far from the free gingival margin, their height tended to be reduced and their tips became bifurcated and twisted. In the molar region, the papillae were arranged in parallel rows along the gingival margin. They were distributed at a density of 250 to 270/mm^2 and no significant differences in density were noted between the incisor, canine and molar regions.

In the attached gingiva, the papillae were mostly short (type II), showing a bud-like appearance (Figs. 3b, e and h). Their width and height measured 30 to 80 μm and 50 to 100 μm, respectively. They were distributed at the density of 90 to 150/mm^2. In the incisor region, the papillae tended to be arranged in rows perpendicular to the gingival margin (Fig. 3b). In the canine region, a cluster of type I papillae was intercalated among the type II papillae (Fig. 3e).

In the alveolar mucosa, connective tissue papillae were poorly developed. Type III papillae were scattered on a relatively flat superficial surface of the lamina propria (Figs. 3a, d and g). In the incisor region, typical connective tissue papillae were not visible. Instead, ridge-like elevations of the superficial layer of the lamina propria were observed.

Small openings were sometimes visible among the connective tissue papillae. They usually occurred in the attached gingiva of the

incisor and canine region and in the alveolar mucosa of the incisor region. They measured 10 to 30 μm in diameter. They were most frequently observed in the attached gingiva and alveolar mucosa of the incisor region (40–50/mm^2) (Figs. 3a and b).

Connective tissue papillae in the lower jaw

In the lower jaw, the general morphological features of the connective tissue papillae were almost the same as those in the upper jaw.

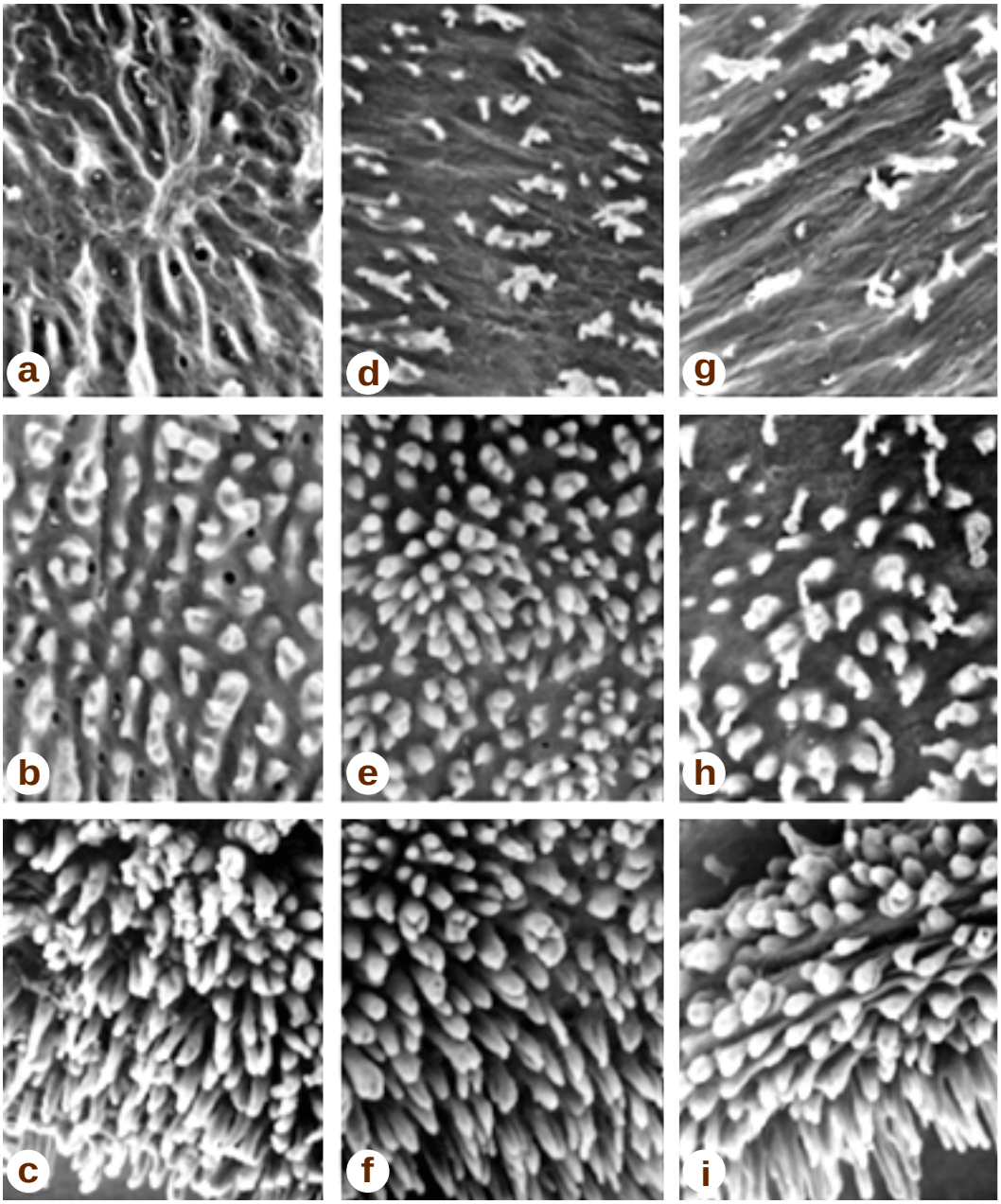

Fig. 3. Connective tissue papillae of the gingiva of the upper jaw prepared by the NaOH maceration method. **a**–**c**: incisor region, **d**–**f**: canine region, **g**–**i**: molar region. **a**, **d**, **g**: alveolar mucosa, **b**, **e**, **h**: attached gingiva, **c**, **f**, **i**: free gingiva. **a**–**i**: × 60.

In the free gingiva, connective tissue papillae were slender and pointed (type I) (Figs. 4a, d and g). They were 20 to 60 μm wide and 100 to 200 μm high. They were distributed at a density of 200 to 300/mm^2. Their height was reduced the farther they were from the free gingival margin, and the tips tended to exhibit bifurcation and twisting. In the molar region, the papillae were arranged in an parallel array along the gingival margin (Fig. 4g).

In the attached gingiva, the papillae were short and branched, showing a bud-like appear-

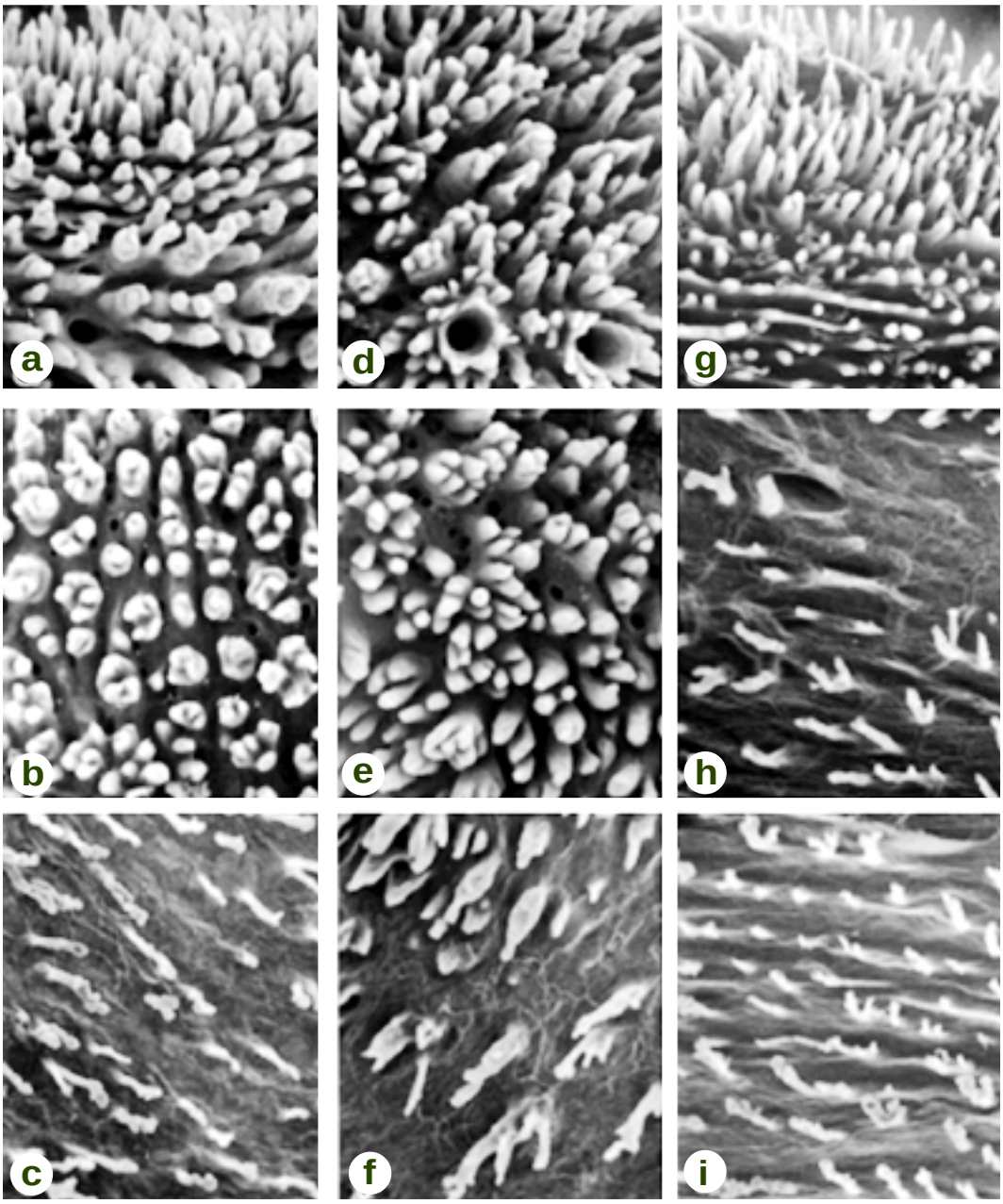

Fig. 4. Connective tissue papillae of the gingiva of the lower jaw prepared by the NaOH maceration method. **a**–**c**: incisor region, **d**–**f**: canine region, **g**–**i**: molar region. **c**, **f**, **i**: alveolar mucosa, **b**, **e**, **h**: attached gingiva, **a**, **d**, **g**: free gingiva. **a**–**i**: × 60.

ance (type II) except for those in the molar region (Figs. 4b, e and h). They measured 30 to 100 μm wide and 50 to 120 μm high and were distributed at the density of 80 to 120/mm^2. In the incisor region, type II papillae tended to be arranged in rows perpendicular to the gingival margin (Fig. 4b). In the canine region, type I papillae were mixed with type II papillae (Fig. 4e). In the molar region, neither type I nor type II papillae were visible, but fork-shaped type III papillae were observed (Fig. 4h).

In the alveolar mucosa, type III connective tissue papillae were scattered on a relatively smooth epithelium-connective tissue interface (Figs. 4c, f and i). The papillae showed a plate-like appearance whose tips were forked into several tips, showing a fork-appearance as a whole. The type III papillae tended to be arranged in rows. The basal portion of the papillae measured 10 × 120 μm. The height of the papillae ranged from 50 to 250 μm. The density of the papillae was almost the same among the incisor, canine and molar regions (40–60/mm^2).

Small openings were often visible among the connective tissue papillae in the free gingiva (Figs. 4a, d and g) and attached gingiva in the incisor (Fig. 4b) and canine regions. They measured 10 to 30 μm in diameter. They were most frequently observed in the attached gingiva of the canine region (60 to 70/mm^2). In the other regions, the density of the opening was 30 to 40/mm^2. Larger openings, surrounded by the elevation of the papillae, were observed in the free gingiva of the canine region, showing flower-like configurations (Fig. 4d). They measured 110 to 130 μm in diameter.

Discussion

The interface between the epithelium and lamina propria, lined by a sheet of the basal lamina, is an important place from the viewpoint of material exchange and invasion of cancer cells (Barsky et al., 1983; Ishikura, 1995). Since the basal lamina firmly connects the basal epithelial surface with the superficial surface of the lamina propria, it is generally difficult to observe the epithelium-connective tissue interface. Several specimen preparation methods have been developed, by which an epithelial layer is mechanically peeled off (Klein-Szanto and Schroeder, 1977; Ooya and Tooya, 1981). Other preparation methods using chemical digestion have offered good results in cleaving the interface (Scaletta and Maccallum, 1974; Takahashi-Iwanaga and Fujita, 1986; Kobayashi, 1990; Ushiki and Murakumo, 1991; Inoué and Gabella, 1992). At first, we applied the 6 N NaOH digestion method (Inoué and Gabella, 1992) to the gingiva, but the ultrastructural preservation of the connective tissue papillae was unsatisfactory as shown in Fig. 1. In contrast, the 2N NaOH maceration technique (Ohtani et al., 1988) offered good results in the preservation of connective tissue papillae (Figs. 2 to 4).

The three-dimensional architecture of the connective tissue papillae of the human gingiva has also been examined by the reconstruction of paraffin sections using light microscopy (Karring and Löe, 1970; Löe and Karring, 1971). Karring and Löe (1970) have classified the connective tissue papillae into two types: “papillae” and “ridges”. They are identical to types I and III in this study, respectively. But since the wax remodeling technique was insufficient in reconstructing ultrastructures, the short type II papillae would not have been recognizable.

In the human epidermis, essential changes occur at an advanced age: a thinning of the epidermis and a reduction in the height and number of epidermal ridges (Hill and Montgomery, 1940). However, in the gingiva, the height of epithelial ridges increases with age (Wentz et al., 1952). According to Löe and Karring (1971), distinct differences exist in the morphology of the epithelium-connective tissue interface of the gingiva between young and old individuals. They described that the essential age change of the epithelium-connective tissue interface is the conversion of the connective tissue “ridges” to “papillae”. According to the present SEM study, the connective tissue papillae were slender and pointed at the tip in the free gingiva (type I). They were gradually reduced in height towards the attached gingiva and the tip became rounded (type II). In the alveolar mucosa, the connective papillae became flattened (type III).

Considering the findings of Löe and Karring (1971), it is reasonable to assume that all parts of the connective tissue papillae in the free gingiva show type I, because the materials used in this study were obtained from old individuals.

Klein-Szanto and Schroeder (1977) examined the connective tissue papillae of the alveolar mucosa and attached gingiva in the molar region by SEM. Their findings including their morphometric data are almost identical to those obtained in this study. They also studied various part of the oral mucosa, and found that the number of papillae in the oral floor was the lowest in the oral cavity. They considered that the development of the papillae was closely related to the contact with food. The interaction between the gingiva and food is more intimate in the free gingiva than the attached gingiva and alveolar mucosa. The high density of the pointed papillae (type I) in the free gingiva may be concerned with such interaction. Another factor influencing the number and height of the papillae is dental plaque and dental calculus. When this plaque and calculus exist around the teeth, inflammation may occur in the free gingiva. Since papillae contain lots of capillaries, it is reasonable to assume that the elongated papillae of type I are intensively distributed in the free gingiva.

The present SEM study clearly demonstrated the presence of small openings in the upper and lower incisor and canine regions. Such openings have not been reported in previous SEM studies to the best of our knowledge. Although they appeared as openings in the glandular ducts (Moss-Salentijn and Applebaunm; 1972), the diameters of the ducts must have been much larger (over 50 μm) than those observed in this study (10–30 μm). Another possibility for the pores is that they are structures associated with small depressions referred to as stipplings. The stipplings occurred in young adults and were restricted to the attached gingiva (Orban, 1948; Rosenberg and Massler, 1967), ranging from 100 to 400 μm in width and 30 to 500 μm in depth (Rosenberg and Massler, 1967), and are limited to only the epithelial layer (Orban, 1948). Judging from the size and location, it is obvious that the openings observed in this study are not directly related to the stipplings. Although the functional significance of the openings remains unknown at present, it would be interesting in a further study to see whether the openings occur in young individuals or not. If not, they may be concerned with the retrogressive change of the gingiva. Further studies including transmission electron microscopic studies are expected.

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