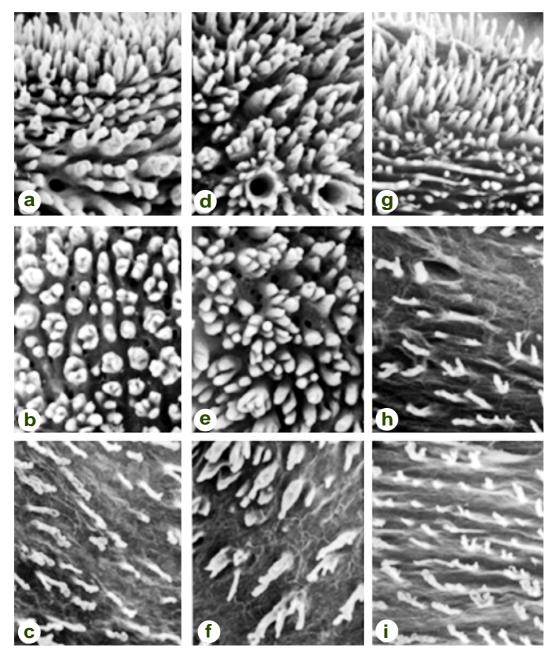
In the free gingiva, connective tissue papillae were slender and pointed (type I) (Figs. 4a, d and g). They were 20 to 60  $\mu$ m wide and 100 to 200  $\mu$ m high. They were distributed at a density of 200 to 300/mm<sup>2</sup>. Their height was reduced the farther they were from the free gin-

gival 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**:  $\times$  60.

ance (type II) except for those in the molar region (Figs. 4b, e and h). They measured 30 to 100  $\mu$ m wide and 50 to 120  $\mu$ m high and were distributed at the density of 80 to 120/mm<sup>2</sup>. 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 (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  $10 \times 120 \,\mu\text{m}$ . The height of the papillae ranged from 50 to 250  $\mu\text{m}$ . The density of the papillae was almost the same among the incisor, canine and molar regions (40–60/mm<sup>2</sup>).

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  $\mu$ m in diameter. They were most frequently observed in the attached gingiva of the canine region (60 to 70/mm<sup>2</sup>). In the other regions, the density of the opening was 30 to 40/ mm<sup>2</sup>. Larger openings, surrounded by the elevation of the papillae, were observed in the free gingiva of the canine region, showing flowerlike configurations (Fig. 4d). They measured 110 to 130  $\mu$ m 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  $\mu$ m). 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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