

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

Tetsuya Yura, Hitoshi Osatake\* and Takao Inoué\*

*Department of Oral and Maxillofacial Surgery and \*Second Department of Anatomy, Faculty of Medicine, Tottori University, Yonago 683-0826, Japan*

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

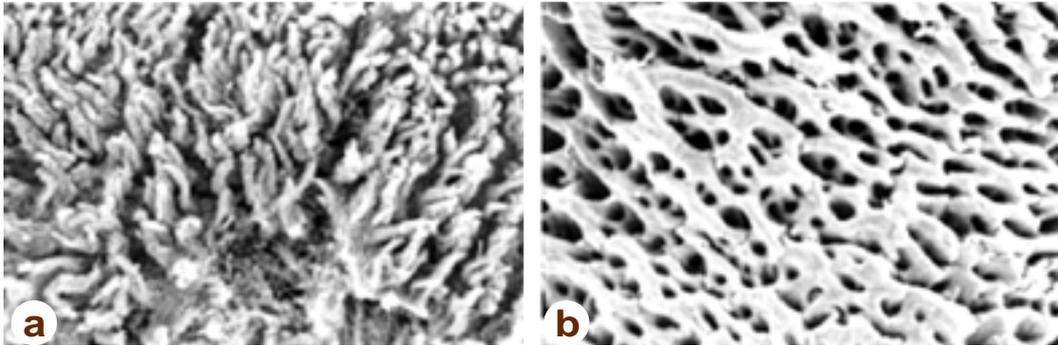
The gingiva, which covers the neck of the tooth and a part of the alveolar bone, is a soft tissue preventing the invasion of foreign materials and bacteria into underlying tissues. It is grossly divided into “free gingiva” and “attached gingiva”, which are separated from each other by a fine line running 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.

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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:  $\times 40$ , b:  $\times 40$ .

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

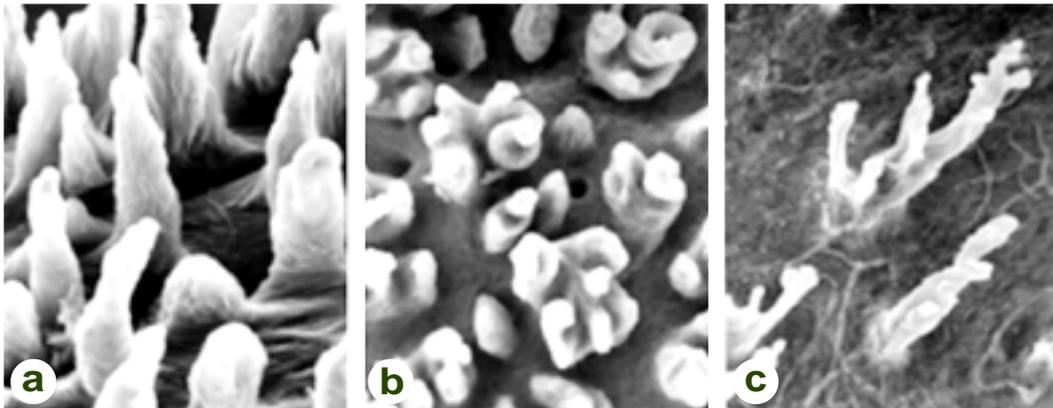
Specimens were obtained from human cadavers (two males: 63 and 80 years old, one female: 50 years old). These cadavers were initially fixed with a 10% formalin solution through the right femoral artery. The gingiva was excised from the incisor, canine and molar regions together with the alveolar mucosa 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  $5 \times 10$  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  $60^\circ\text{C}$  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  $20^\circ\text{C}$  (Ohtani et al., 1988). After rinsing in the same buffer, they were postfixated in 1% osmium tetrox-

ide 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:**  $\times 160$ , **b:**  $\times 95$ , **c:**  $\times 90$ .

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  $\mu\text{m}$  and 200 to 250  $\mu\text{m}$ , 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/ $\text{mm}^2$  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  $\mu\text{m}$  and 50 to 100  $\mu\text{m}$ , respectively. They were distributed at the density of 90 to 150/ $\text{mm}^2$ . 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