The Microvasculature of Human Infant Oral Mucosa Using Vascular Corrosion Casts and India Ink Injection II. Palate and Lip

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THE MICROVASCULATURE OF HUMAN INFANT ORAL MUCOSA USING VASCULAR CORROSION CASTS AND INDIA INK INJECTION II. PALATE AND LIP

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Abstract

The microvasculature of human hard and soft palate and lip originating from four infant males and six females, aged 6 months to 2 years was studied by scanning electron microscopy of vascular corrosion casts and light microscopy of India ink injected specimens. The capillary loops of the hard palate mucosa and vermilion border of the lips were found to be tall, numerous and consisted of primary, secondary and tertiary loops. Those of the soft palatal and labial mucosa were short, few in number and demonstrated a simple hair-pin shape originating directly from the subpapillary vascular network. It was concluded that the configuration of capillary loops is not only determined by the shape of the connective tissue papillae in the lamina propria but also influenced by the functional demands characteristic of the different areas of the oral mucosa.

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Introduction

The basic structure of the oral mucosa varies in different areas of the oral cavity in accordance with the functional demands of each region. There are three main types of oral mucous membrane: masticatory, lining and specialized mucosa. Masticatory mucosa includes gingiva and hard palate and is subjected to the major forces of mastication. The dorsum of the tongue is characterized by a complex pattern of papillae and taste buds and is classified as specialized, although functionally it is a masticatory mucosa. The lining mucosa covers much of the surface of the lips and cheeks, the floor of the mouth, alveolar and vestibular mucosae, the ventral surface of the tongue and soft palate. The histological differences have been described in detail in several publications (Schroeder, 1981; Meyer et al., 1984; Ten Cate, 1985).

The vasculature of the mammalian oral mucosa has received considerable attention over the years (Prichard and Daniel, 1953, 1954; Hodde et al., 1977; Ichikawa et al., 1977; Kishi et al., 1986a, 1986b, 1990; Nobuto et al., 1987; Kajiwara, 1989; Kuramae, 1989; Aharinejad et al., 1990; Ike, 1990; Lametschwandtner et al., 1990; Inoue and Toda, 1991; Lee et al., 1991; Aharinejad and Lametschwandtner, 1992). However, there are relatively few reports concerning the microvasculature of human oral mucosa (Yuang et al., 1985; Ohta et al., 1992). A thorough knowledge of the microvasculature is likely to contribute to our understanding of the human oral mucosa in health and disease.

Using vascular corrosion casting and India ink injection, we have previously studied the microvasculature of human infant tongue papillae (Yu et al., 1992). In this paper, we present the results of observations on the microvasculature of the human infant lip and palatal mucosa using the same techniques.

Materials and Methods

A total of 10 human infant palate and lip specimens from 4 males and 6 females, ages 6 months to 2 years,

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were studied. All these children had died from infectious diseases of the respiratory and gastrointestinal tracts and their bodies were donated by their parents to the Faculty of Stomatology at the Sun Yat-Sen University of Medical Sciences. The oral mucosa including the lip and palate was clinically healthy. The following procedures were started within 16 hours after death. The internal jugular veins were dissected and kept open for drainage. An anticoagulant (500 ml saline solution containing 2 ml heparin comprising 125,000 IU) was injected into the common carotid arteries. The vascular system was subsequently irrigated with a solution consisting of 40 ml dextran, 500 ml glucose and 25 ml mannitol, until the colour of the fluid draining from the internal jugular veins appeared clear. Subsequently, a fixative solution of 2% glutaraldehyde in phosphate buffer solution (pH 7.4) was perfused through the vascular system in order to fix the vessel walls.

India ink injection

Five subjects (2 males and 3 females) were selected randomly from the above-mentioned individuals. The mixed India ink solution (10 ml India ink and 90 ml normal saline) was injected into the vessels through plastic tubes. The opened blood vessels were ligated when the colour of the skin of the head turned black. The heads were cut at the thyroid gland level and fixed in 10% formalin for one month. Specimens were then removed by dissection. Paraffin sections (100 μm thick) were mounted in Permount® (Fisher Scientific) and examined under light microscopy.

Vascular corrosion casting

The remaining 5 subjects, including 2 males and 3 females were used for vascular corrosion casting. The casting medium (comprising 0.2 g of benzoyl peroxide, 6.0 ml hydroxy-propylmethacrylate monomer and 0.3 ml n,n-dimethyl-aniline in 14.0 ml pre-polymerized methyl-methacrylate) was injected into the vessels through plastic tubes at a constant pressure. The opened blood vessels were ligated when the colour of the skin of the head turned pink. After the initial setting of the plastic, the heads were removed and submerged into a 60°C water bath for 24 hours to complete the polymerization. The lip and palate mucosa were then removed by dissection, corroded with 20% potassium hydroxide and washed thoroughly in tap water until the plastic casts were clean. The casts were frozen in distilled water, sectioned with a single-side razor blade, air-dried and mounted on copper stubs with conductive colloidal carbon. The specimens were then sputter-coated with gold in a sputter ion coater operated at 1.2 kV and 5 mA for 8 minutes. The casts were examined and photographed using a scanning electron microscope (JXA-840, JEOL, Japan) operated at an accelerating voltage of 5 kV.

Legends for Figures on the facing page 135.

(SEM and LM: scanning electron and light micrograph).

Figure 1. Microvasculature of one of the transverse rugae of the hard palate. Top of the ruga (*) and the inter-rugal (**) area. SEM; bar = 1 mm.

Figure 2. Capillary loops of a palatal ruga. (a) Higher magnification of ruga shown is in Figure 3; SEM; bar = 100 μm; (b) India ink section. LM; bar = 100 μm.

Figure 3. Capillary loops of the hard palate showing primary (P), secondary (S) and tertiary (T) loops. SEM; bar = 100 μm.

**Figure 4**. The SPCN (*) of the hard palate: (a) SEM; bar = 100 μm; (b) India ink section. LM bar = 100 μm.

Figure 5. Vessels surrounding excretory duct openings (*). SEM; bar = 100 μm.

Figure 6. Capillary loops of the soft palate showing "hair-pin" pattern. SEM; bar = 10 μm.

Results

The anterior part of the hard palate is characterized by a series of transversely aligned rugae which consists of crests and troughs. The microvasculature within an individual ruga is shown in Figure 1. The capillary loops followed the morphology of the rugae as demonstrated in Figures 2a and 2b. These loops could be divided into primary, secondary and tertiary ones (Figure 3). The largest primary capillary loop comprised an ascending root following a course towards the connective tissue papilla and finally converging into a descending root. Tertiary loops in the individual papilla, consisting of one ascending and one descending root, demonstrated a hair-pin pattern. The location of the secondary capillary loops were intermediate between the primary and tertiary loops. These loops were more complex in structure and often anastomosed with each other. The above mentioned capillary loops were found not only at the crest of each ruga but also on the slopes, the inter-rugal troughs, and the posterior parts of the hard palate. The crest loops were tall and more dense than those in other areas. There was a subpapillary capillary network (SPCN) under the capillary loops in the hard palate. Two zones of SPCN could be identified (Figure 4). The superficial zone consisted of arterioles and venules which interlinked directly with the capillary loops. The deep zone was composed of larger arterioles and venules which connected directly with the deep vessels of the hard palate. Excretory ducts of the palatine salivary glands opened onto the posterior part of the hard palate

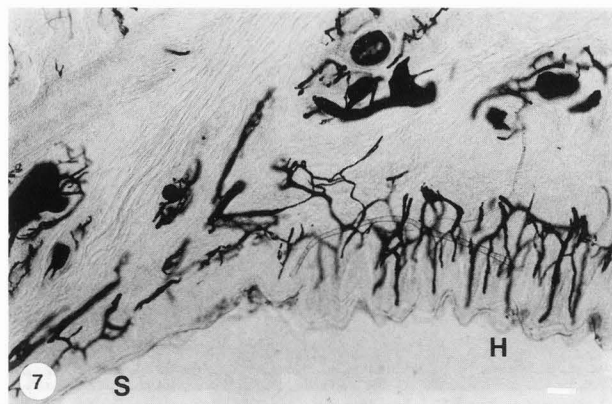Q.X. Yu, K.M. Pang, W. Ran, H.P. Philipsen, X.H. Chen
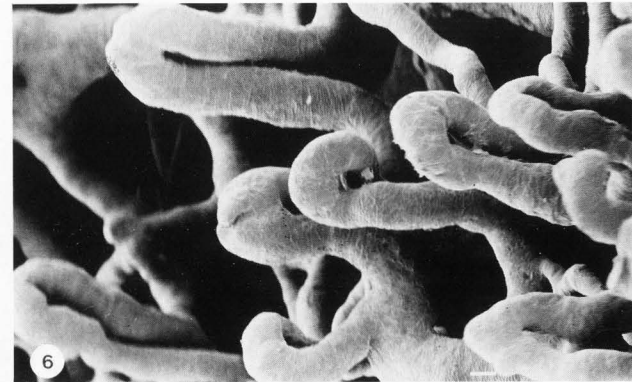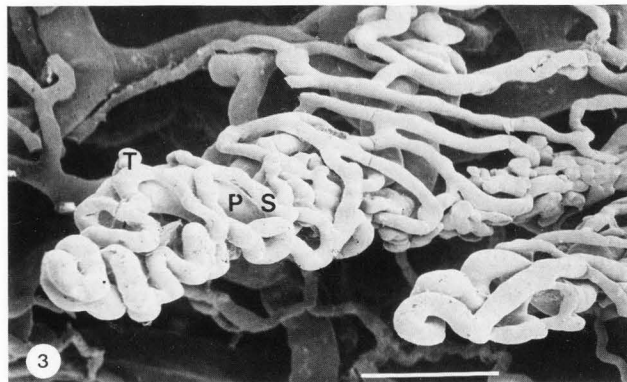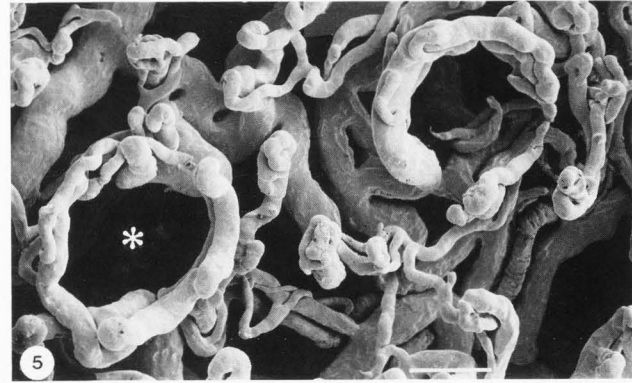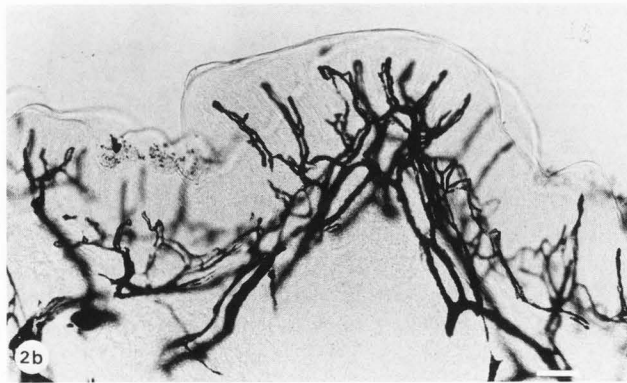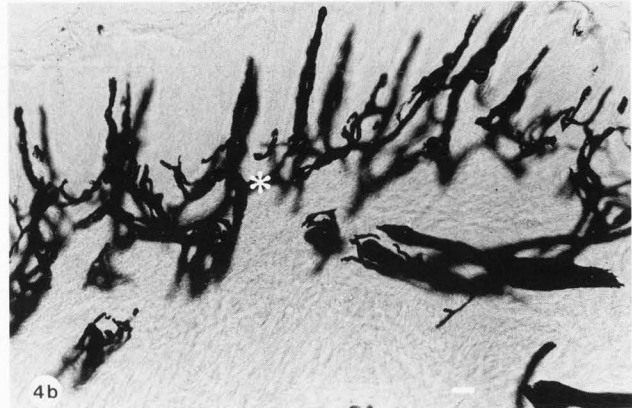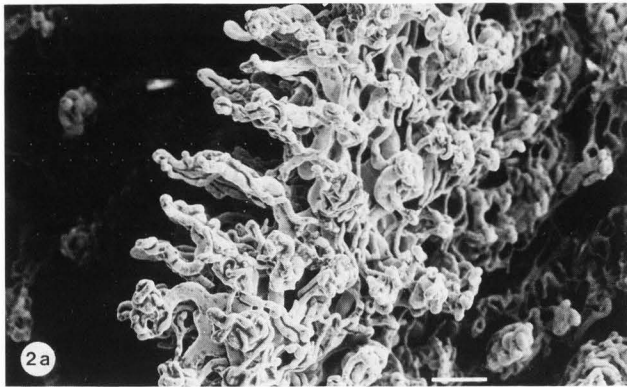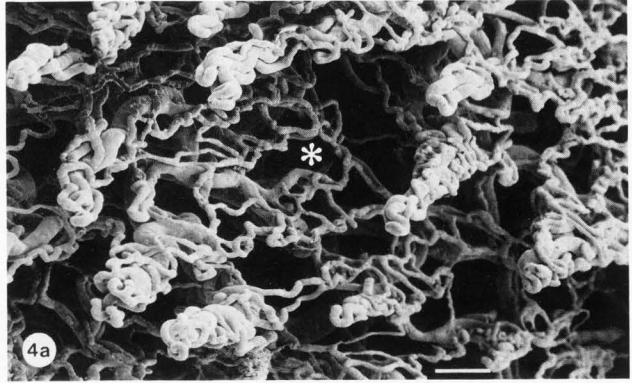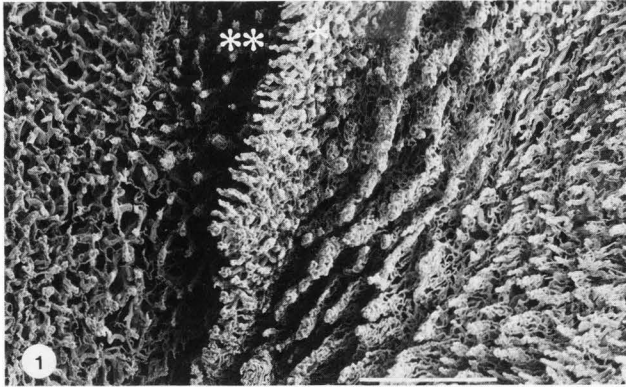
Figure 7. Difference in the capillary loops between the hard (H) and soft (S) palate. LM; bar = 100 μm.

(Figure 5). Capillary loops encircled the orifices of these ducts and formed anastomoses in a corolla-like network.

The distribution of the capillary loops in the soft palate was the same as that in the non-rugal areas of the hard palate. However, the profile of the individual capillary loops in the soft palate appeared much more simple. They consisted of only one ascending and one descending root, forming a hair-pin like loop (Figure 6). These loops were connected with the SPCN directly and thus lacked primary and secondary loops. Compared with the hard palate, the capillary loops of the soft palate were shorter in height and fewer in number (Figures 7 and 8). The SPCN in the soft palate (Figure 8) was identical to that of the hard palate. Excretory ducts of the palatine salivary glands opened onto the entire surface of the soft palate. The microvasculature of these duct openings was also similar to that of the hard palate.

The microvasculature of the lip showed topographical variation. In the cutaneous part, regularly distributed capillary loops were seen encircling the hair follicles (Figure 9). The capillary loops in the skin were quite dense and were composed of hair-pin loops originating from the SPCN.

The vermilion border was characterized by many long, slender capillary loops (Figure 10a). The loops were similar to those of the hard palate in terms of structure, height and density. In India ink sections (Figure 10b), the tertiary loops were found to be covered by only a thin layer of mucosal epithelium. The loops were closely packed so that the SPCN was difficult to detect in cast specimens (Figure 10a). However, the SPCN could be clearly demonstrated in India ink sections (Figure 10b). The loops were taller and more numerous in the commissural areas than in the central part of the lip.

Figure 8. Capillary loops arising directly from the SPCN in the soft palate: (a) SEM; bar = 100 μm; (b) India ink section; LM; bar = 100 μm.

Figure 9. Microvasculature of the cutaneous part of the lip. The hair follicles (*) are evident. (a) SEM; bar = 100 μm; (b) India ink section; LM; bar = 100 μm.

Figure 10. Microvasculature of the vermilion border of the lip showing long, slender loops (*), compare with Figure 3. (a) SEM; bar = 100 μm; (b) India ink section; LM; bar = 100 μm.

Figure 11. Microvasculature of the lip mucosa showing hair-pin loops: (a) SEM; bar = 100 μm; (b) India ink section; LM; bar = 100 μm.

In the lip mucosa, the majority of the capillary loops were of the hair-pin type, resembling those of the soft palate although they were often more tortuous at the end of the loops (Figure 11). The loop arrangement around secretory duct openings possessed the same morphology as those of the palate. The SPCN was demonstrated more clearly in cast specimens of the mucosa because the capillary loops were fewer in number and of simple morphology. The microvasculature showed no obvious differences between upper and lower lips.

Discussion

The present study has shown that each of the studied mucosal regions has a unique microvasculature. Masticatory mucosa (hard palate) is thus characterized by units of primary, secondary and tertiary capillary loops originating from the SPCN. Some Japanese authors found the capillary loops in the connective tissue papillae are positioned at right angles to the transverse plica, forming regular lines parallel to the antero-posterior axis of the palate (Toda, 1986; Kajiwara, 1989; Ike, 1990; Inoue and Toda, 1991; Ohta et al., 1992). These findings were identical to those demonstrated in the present study. The above investigators also pointed out that the capillary loops of the palate appeared as hair-pins not only in cat, dog, Japanese monkey (*Macaca fuscata*), rabbit and squirrel monkey (*Saimiri sciureus*) but also in human. We found hair-pin loops only appeared in the soft palate, while in the hard palate they became more complicated and three levels of capillary could be identified. Similar units are found in the papillae of the lingual mucosa (Yu et al., 1992). Although the dorsum of the tongue is classified as specialized mucosa, functionally it actually belongs to the masticatory mucosa and its filiform papillae in particular are heavily keratinized.

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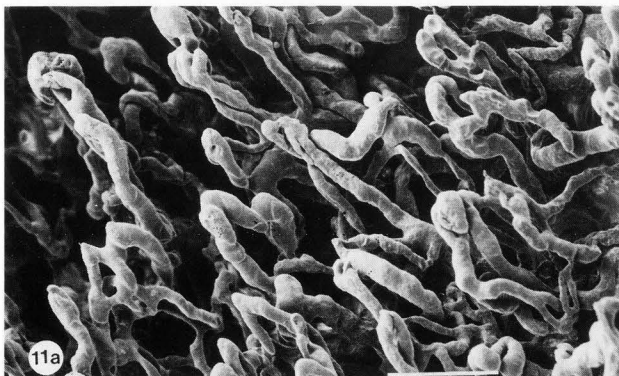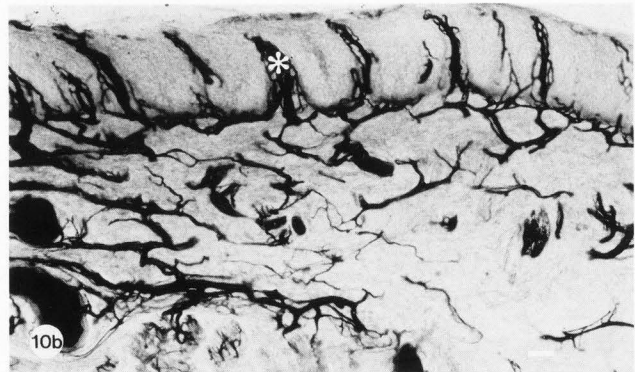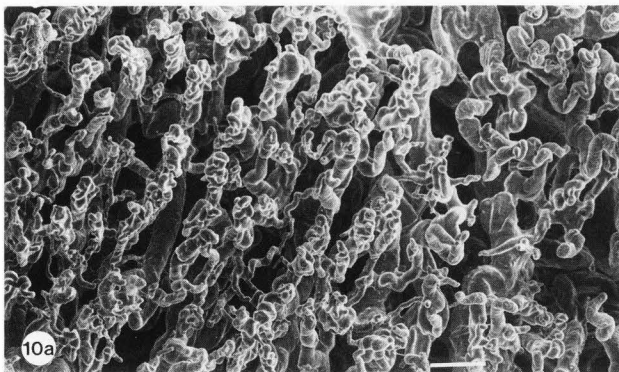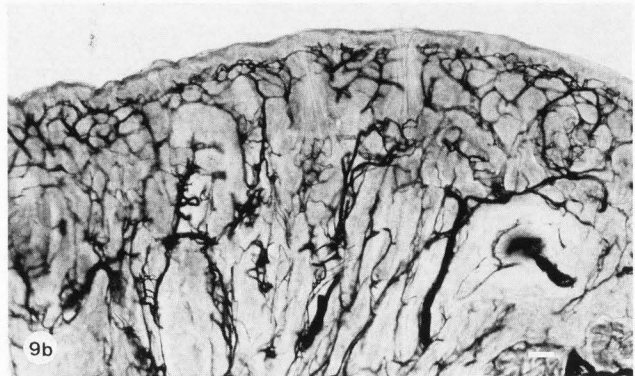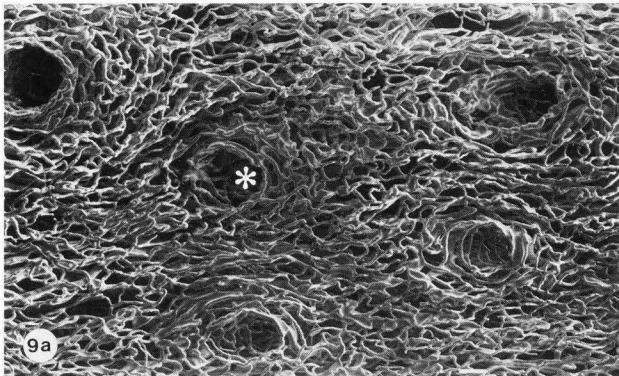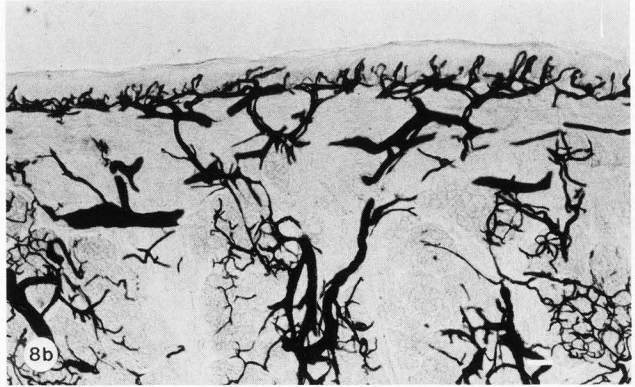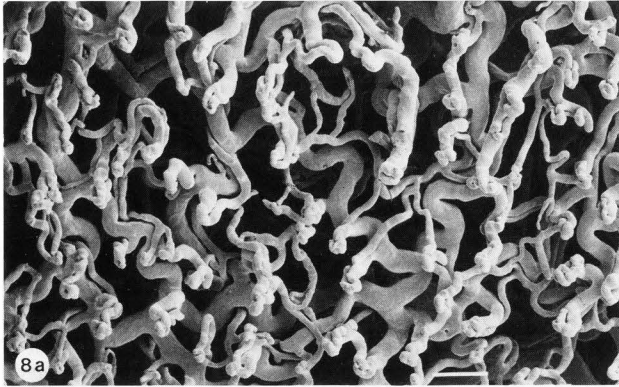
Lining mucosa, including soft palate and lip mucosa, are both characterized by simple vascular units: hair-pin shaped capillary loops raising directly from the SPCN. Ohta *et al.* (1992) considered that the high connective tissue papillae in both the anterior slope and plical top may be of a resistant form for mollifying exhaustion, affected by the periodicity and mastication function. Stablein *et al.* (1982) stated that the geometry of the microvasculature in rat oral mucosa suggests functional adaptation of the capillary loops to masticatory forces. Our study in humans demonstrates differences in microvasculature between masticatory and lining mucosa which may be regarded as functional adaptation.

The vermilion border, usually not covered by the above-mentioned classification of the oral mucosa because this region represents a transitional zone between the cutaneous and mucosal part of the lips, interestingly shows vascular units identical to those found in masticatory mucosa (hard palate and lingual papillae). In spite of closely similar vascular structures in the vermilion border and the palatal mucosa, there is a marked difference in colour between the two regions, the vermilion border being distinctly red. The vermilion border is lined by a rather thin epithelium which allows the blood vessels of the connective tissue papillae to shine through.

Furthermore, our results show that in human oral mucosa, the configuration of the capillary loops follows the shape of the connective tissue papillae of the lamina propria. This is in agreement with the results of Forsslund (1959) on the capillary system in gingiva. It should be stressed that our findings are based on the study of specimens from young infants. It is likely, although at present unknown, that age may influence the microvasculature of the human oral mucosa.

The present study has shown that a combination of scanning electron microscopy of corrosion casts and light microscopy of India ink preparations can reliably identify structural variations in the microvasculature of human oral mucosa.

Acknowledgements

We would like to thank Professors W.I.R. Davies and S.H.Y. Wei, Faculty of Dentistry, University of Hong Kong, for their assistance in making arrangements for this collaborative study and for financial support. We also sincerely thank Dr. F. White, Department of Anatomy, University of Hong Kong, for his kindness in reading through this manuscript with several valuable suggestions.

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Discussion with Reviewers

S. Aharinejad: Why did you use a fixative prior to India ink and/or methylmethacrylate injection?
Authors: We used a fixative prior to India ink and/or methylmethacrylate injection in order to prevent post-mortem decomposition and to preserve and set as closely as possible the structure they had in life, so as to maintain the original shape and size of the vessels.

S. Aharinejad: Why are the lips red? Are the different colours of skin-covered and transitional zone of the lips due to a different histological pattern (skin versus mucosa), or the underlying vascular pattern (including the density of vessels), or both of them?
Authors: The structure and arrangement of the vascular loops varies from area to area within the human oral mucosa, the vermilion border and adjacent skin. So does the thickness and degree of keratinization of the covering epithelium. All these factors, in combination, determine the clinical appearance (and more specifically the colour) of a particular area.

S. Aharinejad: Have you ever seen arterio-venous (AV)-anastomoses or other peculiar structures (sphincters) in the oral cavity microvasculature?
Authors: We have not come across AV-anastomoses nor have we encountered structures like sphincters in the oral cavity microvasculature. If this is related to the very young age of the human material examined (6 months to 2 years), we cannot tell.

Y. Ohta: Schematic illustrations of the vascular architecture in the lip and palatal mucosa were not made. Are any differences on the capillary loop construction found essentially between the two regions or among all regions of the oral mucosa?
Authors: Schematic illustrations of the vascular architecture of the lip and palatal mucosa were not produced as the light microscopic (India ink) and scanning electron microscopy illustrations quite clearly demonstrate the characteristic similarities and differences between the mucosal areas examined. Thus, the soft palate and lip mucosa were very much alike with respect to capillary loop construction. The capillary loops of the vermilion border and the hard palate were of the same basic configuration which clearly deviated from that of the soft palate and the vermilion border.