Epithelial-connective tissue boundary in the oral part of the human soft palate

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ABSTRACT

The papillary layer of the oral part of the human soft palate was studied in 31 subjects of different age by means of histological, immunohistochemical and scanning electron microscopical methods. For scanning electron microscopy a new maceration method was introduced. Results determine epithelial thickness, height and density of connective tissue papillae and their 3-dimensional architecture inside the lining epithelium as well as the collagenous arrangement of the openings of the glandular ducts. The individual connective tissue papillae of the soft palate are compared with the connective tissue boundary on the other side of the oral cavity. The connective tissue plateaux carrying a variable number of connective tissue papillae were found to be the basic structural units of the papillary body. The function of the epithelial-connective tissue interface and the extracellular matrix arrangement in the lamina propria are discussed in order to promote the comparability of normal with pathologically altered human soft palates.

Key words: Oral mucosa; connective tissue papillae; extracellular matrix.

INTRODUCTION

The human soft palate can be involved in pathological phenomena of the velopharyngeal region such as excessive snoring and sleep apnoea (Woodson et al. 1991) as well as in dermatological conditions (de las Heras et al. 1996). To understand these mechanisms, a basic understanding of the normal tissue morphology is necessary.

The epithelial-connective tissue boundary of the human oral mucosa is a highly variable interface which, owing to the uneven distribution of the projecting connective tissue papillae, can be extremely irregular in some sites and rather smooth in others (Horstmann, 1954; Karring & Loe, 1970; Klein-Szanto & Schroeder, 1977). In addition, Wentz et al. (1952), Horstmann (1954), Shklar (1966), Loe & Karring (1971) and Karring (1973) described age and sex variations in connective tissue papillary density. The connective tissue papillae are regarded as adaptive structures which enlarge the epithelial-connective tissue interface in order to achieve a broader anchorage for the epithelium and to provide a larger exchange surface for nutritional purposes (Horstmann, 1954; Karring, 1973; Klein-Szanto & Schroeder, 1977; Kobayashi et al. 1987; Nakano, 1991). In addition, transplantation experiments have suggested that the features of the epithelium overlying the connective tissue papillae result from the action of unknown connective tissue inducers (Plagman et al. 1974; Karring et al. 1975). Klein-Szanto & Schroeder (1977) divided the oral mucosa into 3 regions with different characteristics of the epithelial-connective tissue interface: (1) floor of the mouth, (2) lip and cheek, (3) gingiva and hard palate. So far there is no quantitative and detailed information in the literature about the epithelium-connective tissue interface in the human soft palate of the oral cavity. In view of the possible value of such information in the field of oral pathology the matter is investigated in some detail and compared with other regions of the oral cavity.

In this study a proved cell maceration method (Othani et al. 1987) and a new cell maceration technique for scanning electron microscopy were applied to the soft palate in order to demonstrate the 3-dimensional architecture of the connective tissue papillae and their anchorage inside the epithelium. Also, the extracellular matrix composition of the

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connective tissue papillae and the collagen fibrillar framework constituting the openings of the glandular ducts will be described.

MATERIALS AND METHODS

Unfixed normal soft palates of adults were obtained from 31 subjects of different ages (range 51–89 y) during autopsy within 48 h of death (Table). The specimens were taken from individuals free from recent trauma or infections of the oral cavity or of diseases which might involve or affect pharyngeal function.

Light microscopy

For light microscopy 6 soft palates (3 men, 3 women, aged 59–89 y) were fixed in 4% formaldehyde for 1 wk, dehydrated in graded concentrations of ethanol, and embedded in paraffin. Sagittal and transverse sections 7 µm in thickness were stained with toluidine blue (pH 8.5), resorcin-fuchsin-thiacine-picric acid, orcein and by the Gomori method (Romeis, 1989). In addition, 2 soft palates (1 male, 1 female, aged 61 and 79 y) were fixed in 3.5% glutaraldehyde (in 0.1 M Sørensen phosphate buffer solution at pH 7.4) at 4 °C for 1 wk. After dehydration in graded concentrations of ethanol they were embedded in Araldite. Semithin sections ranging (2.5–3 µm) were prepared and stained with toluidine-blue (pH 8.5) according to Romeis (1989). The slides were examined with a Zeiss Axiophot microscope.

Table. Details of specimens investigated

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IM, investigation method; m, male; f, female; lm, light microscopy; sem, scanning electron microscopy; ih, immunohistochemistry; O, method of Ohtani; N, new maceration method.

Scanning electron microscopy (SEM)

For scanning electron microscopy the oral part of 18 human soft palates (12 men, 6 women, aged 51–77 y) were used. All soft palates were fixed in 4% formaldehyde for 1 wk. In 9 human soft palates the method of Ohtani et al. (1987) was used for the detailed demonstration of collagen fibrils. The tissue was macerated in 10% NaOH at room temperature for 3–7 d and then rinsed in distilled water for 1–2 d until the material was transparent. Another 9 human soft palates were macerated in 10% hypochloric acid (HClO) at 67 °C for 5, 10, 15 or 20 min and then briefly rinsed in distilled water.

All macerated tissue blocks were impregnated in 2.5% tannic acid for 2 d. Postfixation in 2% OsO₄ for 4 h was followed by dehydration in ethanol and drying in a critical point dryer. Preparations were coated with gold and analysed in a Philips scanning electron microscope.

Collagen, glycosaminoglycan and laminin immunostaining

Immunohistochemical investigation of extracellular matrix components was performed on unfixed material (4 men, 3 women, aged 54–78 y) which was snap frozen in liquid nitrogen. Sagittal and transverse frozen sections (7 µm) were cut with a cryostat at −21 °C and mounted on gelatine-coated slides. For immunohistochemistry, frozen sections were pre-treated with testicular hyaluronidase (Boehringer, Mannheim, Germany) in Tris-buffered saline (TBS) in a moist chamber at 37 °C for 30 min. The sections were washed 3 times with TBS and incubated with goat serum (Dako, Glostrup, Denmark) for 45 min at room temperature. Incubation with the primary antibody was carried out for 60 min at room temperature. The following antibodies were used: mouse
anticollagen (MAC) type I polyclonal antibody (Bio-Science Products, Emmenbrücke, Switzerland); MAC type II polyclonal antibody (Biodesign, Kennebunk, ME, USA); MAC type III polyclonal antibody (Bio-Science Products, Emmenbrücke, Switzerland); MAC type IX polyclonal antibody (gift from Prof. P. K. Müller, Lübeck, Germany); antichondroitin-4-sulphate monoclonal antibody (Chemicon International, Temecula, CA, USA), Clone BE-123; the antibody is directed against unsaturated uronic-acid residues bound to N-acetyl-galactosamine-4-sulphate; antichondroitin-6-sulphate monoclonal antibody (Chemicon International, Temecula, CA, USA), Clone MK-302; the antibody is directed against unsaturated uronic-acid residues bound to N-acetyl-galactosamine-6-sulphate; antikeratan sulphate monoclonal antibody (Bio-Science Products, Emmenbrücke, Switzerland), Clone 5-D-4; the antibody is directed against hexasaccharides of keratan sulphate; antidermatan sulphate proteoglycan monoclonal antibody (Bio-Science Products, Emmenbrücke, Switzerland), Clone 6-B-6; the antibody is directed against the core protein. The presence of basement membrane components was investigated using an antibody against laminin (Medac, Hamburg, Germany). Sections were labelled with the respective secondary antibody, fluoresceine isothiocyanate (FITC)-conjugated goat antirabbit or goat antimouse IgG for 45 min. In each case 2 negative control sections were used. One was incubated only with the FITC-conjugated antibody, the other only with the primary antibody. Tissues with defined antigen sites (human cartilage, skin, kidney, liver, spleen) were used as positive controls. The slides were examined by a Zeiss Axiophot microscope equipped for epifluorescence.

RESULTS

Light microscopy

In the oral surface of the soft palate, the lamina propria consisted of 3 connective tissue layers (Fig. 1a): (1) a broad deep layer of dense connective tissue composed of reticular, collagen and elastic fibres running in direction parallel vertical to the oral surface; (2) a broad intermediate layer of loose connective tissue bordering on (3) a narrow superficial layer of dense connective tissue. The superficial layer consisted mostly of reticular fibres forming long and finger-like connective tissue papillae (CTP) which penetrated the basal half of the lining epithelium and showed signs of bifurcation (Fig. 1b). Between reticular fibres some elastic fibres were also evident (Fig. 1c). The oral side of the soft palate was covered consistently and uniformly in all investigated subjects by nonkeratinised stratified squamous epithelium about 10 to 20 cell layers thick (Fig. 1a, b, d).

Taste buds were found in the lining epithelium especially near the transition into the hard palate (Fig. 1d). The lamina propria formed finger-like CTP in such areas projecting around the taste buds. Excretory ducts of the palatal glands opened into the oral surface of the soft palate especially in the anterior half and were surrounded by dense connective tissue of the lamina propria of the soft palate.

Scanning electron microscopy (SEM)

NaOH treatment removed the cellular elements as well as the basement membrane and permitted a view on the architecture of the papillary body, the individual connective tissue papillae, and the basal surface of the lamina propria between the CTP (Fig. 2a-f).

The subepithelial connective tissue surface of the human soft palate exhibited a papillary body which was characterised by a high density of CTP that extended between 70–100 mm² (Fig. 2a, b). The distribution was symmetric but showed no preferential alignment or organisation in completely parallel rows. The papillae appeared to arise from a flat connective tissue surface (Fig. 2a, b), although connective tissue ridges supporting the papillae were seen in some places (Fig. 2d). The CTP were about 200 μm long (Fig. 2b, d), usually with a conical or cylindrical shape and a narrow base about 20–25 μm in width (Fig. 2c). Most of the CTP showed bifurcations, trifurcations or more extensive branching (Fig. 2b). They terminated in spread tips or dome-shaped endings which often displayed angulations at their tips (Fig. 2b). Seen at high magnification, the conical CTP consisted of a delicate meshwork of thin collagen fibrils which appeared to be twisted along the whole axis of the papilla (Fig. 2e). These characteristic papillary features not only varied slightly from case to case but were also more evident in the mucosa of the paramedian zone where branches, tip angulations and dome-shaped endings were less numerous (Fig. 2d). The openings of the glandular ducts were circular or elliptical in form (Fig. 2e–f). CTP of the lamina propria surrounding the opening reached the margin of the opening but were not seen inside the glandular duct (Fig. 2f). Higher magnification revealed the opening to be rimmed by thick collagen fibrils running concentrically on the wall of the duct into its depth.
Fig. 1. Histological and immunohistochemical findings. (a) Sagittal section of the soft palate of a 51-y-old male, showing the oral side covered with nonkeratinised stratified squamous epithelium (e) and lamina propria consisting of a superficial layer (arrows), an intermediate layer (il) and a deep layer (dl). Gomori, × 57. (b) Region from a at higher magnification showing the superficial layer of lamina propria which consists of reticular fibres forming long and finger-like connective tissue papillae (arrowheads). The CTP penetrate the lining epithelium and show signs of bifurcation. Gomori, × 114. (c) Semithin section of a connective tissue papillae of a 61-y-old female showing elastic fibres (arrows) inside the papilla. Toluidine blue, × 359. (d) Sagittal section of the soft palate of a 63-y-old female showing a taste bud (arrowheads) inside the lining epithelium. Resorcin-fuchsin-thiacine picric acid, × 228. (e) Immunohistochemical proof of laminin (male, 63 y). Reactivity is visible along the basement membrane (arrows). Additionally, subepithelial blood vessels are marked by the antibody, in particular inside the connective tissue papillae (open arrows). e, epithelium, × 208.

(Fig. 2f). Thin collagen fibrils arose from such concentrical fibrils and reached the larger collagen fibrils of the surrounding lamina propria.

Treatment with 10% hypochloric acid at 67 °C allowed a fast and uniform maceration into the depth of the tissue, preserving the cytoskeleton of cells and connective tissue (Fig. 3a–h).

After 10 min of maceration with 10% hypochloric acid at 67 °C the upper half of the lining epithelium was removed and the tips of the CTP inside the epithelium were visible (Fig. 3a–c, e). The dome-shaped endings of the CTP were surrounded by a dense trimming with small epithelial cells forming a ring around each CTP (Fig. 3a, c). Epithelial cells in areas between the CTP were larger and were lying less close together than the cells covering the CTP (Fig. 3a, b). Bifurcations or trifurcations of the CTP inside the epithelium extended like the branches of dowels in a wall (Fig. 3e). At higher magnification a basement membrane between CTP and epithelial cells was not visible and seemed to be removed by the maceration procedure. Each tip of a CTP consisted of thin bundles of numerous interwoven collagen fibrils which came off the inner surface of the CTP and were arranged spirally (Fig. 3d). The delicate fibrillar meshwork was frequently perforated at the top by one large fenestration 4–6 µm in diameter and many smaller fenestrations of various sizes (0.4–2.1 µm in
diameter) (Fig. 3d). The collagen fibrils ran in a circular manner along the rim of the fenestrations at their periphery (Fig. 3d).

Epithelial cells at the openings of the glandular ducts showed a circular arrangement (Fig. 3f). After 20 min of maceration with 10% hypochloric acid at 67 °C the arrangement of collagen fibrils inside the wall of a glandular duct opening was visible (Fig. 3g, h). The opening was rimmed by thick collagen fibrils running concentrically (Fig. 3g). Many thin fibrils arose from the concentrical fibres and turned into periphery. At the inner edge of the openings, the concentric fibres continued to the fibres descending on the wall of the duct into its depth (Fig. 3h).
Fig. 3. Scanning electron microscope appearances after maceration with HClO. (a) Tips of connective tissue papillae (arrows) inside the epithelium of the soft palate (female, 77 y) are visible after 10 min of maceration. Bar, 100 µm. (b) Tips of connective tissue papillae (arrows)
**Immunohistochemistry**

Immunohistochemistry revealed that the lamina propria and especially the CTP consists mainly of type III and type I collagen. Use of antibodies against type II and type IX collagen showed negative results. The presence of chondroitin-4-sulphate, chondroitin-6-sulphate, keratan sulphate and dermatan sulphate was demonstrated. Immunoreactivity reached its maximal fluorescence for chondroitin-4-sulphate.

The presence of basement membrane components was investigated using an antibody against laminin. The antibody indicated a clear border between the epithelium and the connective tissue papilla within the epithelium of the soft palate (male, 53 y) extend like branches of a dowel in a wall. Bar, 20 µm. (d) Dome-shaped ending of a connective tissue papilla as shown in c at higher magnification. Many thin collagen fibrils are derived from the connective tissue boundary in human soft palate mucosa shown by SEM. (d) Direct observation of the CTP of the tongue by SEM, after removal of the epithelium, has been undertaken (Kunze, 1969; Schenk & Wersäll, 1975; Toyoshima & Shimamura, 1982; Hull & Warfel, 1986; Suzuki & Takeda, 1987; Kobayashi et al. 1987, 1988, 1989a–c, 1994; Nagato et al. 1989; Kobayashi, 1990; Ohshima et al. 1990). For separating the epithelial-connective tissue interface, some investigators treated the unfixed material with trypsin (Schenk & Wersäll, 1975), EDTA (Klein-Szanto & Schroeder, 1977) or sodium bromide (Hull & Warfel, 1986). Ohtani and coworkers introduced a cell-maceration method with NaOH. They reported that with this method it is possible to remove cellular elements much more effectively and consistently than with any other maceration method (Ohtani, 1987; Ohtani et al. 1988). To study the integration of CTP inside the overlying epithelium of the oral surface of the human soft palate a new maceration method was developed, treating the material briefly with 10% hypochloric acid at 67 °C. With this method it is possible to study the arrangement of epithelial cells around CTP as well as around the openings of the glandular ducts of the human soft palate by SEM.

Klein-Szanto & Schroeder (1977) divided the mucous membrane of the oral surface in 3 regions with different characteristics of the epithelium-connective tissue interface: (1) floor of the mouth; (2) lip and cheek; (3) gingiva and hard palate. The floor of the mouth showed the lowest CTP density, the smallest papillae, and connective tissue plateaux separated by narrow grooves. All papillae were small slender structures, about 10–50 µm long, implanted on a base ~8–13 µm wide. Generally, they were straight and finger-like. Branching was rarely seen. These papillae had a wrinkled surface with protruding villus-like projections, which appeared to be twisted spirally along the axis of the papillae. Lip and cheek mucosae showed an intermediate density, the papillae frequently being bifurcated and angulated. They were aligned in rows and either implanted in isolation or supported by a narrow connective tissue ridge. There
were 76 ± 10 papillae per mm² connective tissue surface. The papillae were tubular, finger-shaped, and often without a broader base. The basal epithelial surface had similar characteristics to that of the buccal mucosa. The proportion of its surface occupied by CTP orifices was 15 ± 3%. Gingiva and hard palate were characterised by the highest papillary density and by papillae which were cylindrical, slender and erect. The papillae reached 114 ± 16 mm² in the hard palate. The frequency of the various papillary features varied here to some extent from case to case, but no differences could be established between the mucosa of the midline and that of the paramedian zone of the hard palate. The vestibular gingiva showed the highest density of CTP (119 ± 27 mm²). The size of these structures ranged between 80–200 μm in length with a diameter of ~ 60 μm. The individual papillae of the human soft palate investigated in the present study by light microscopy, SEM and immunohistochemistry revealed some structural details which correspond at the earliest to the connective tissue boundary of the gingiva and hard palate. The subepithelial connective tissue surface exhibited a papillary body which was characterised by a high density of CTP that reached 70–100 mm². The distribution was symmetric but were not aligned into strictly parallel rows. The papillae were ~ 200 μm in length and usually had a conical or cylindrical shape and a narrow base, ~ 20–25 μm in width. Most of the CTP showed bifurcations, trifurcations or more numerous branches. They terminated in expanded tips or dome-shaped endings which were often angulated at their tips. The processes are of special interest at the papillary tips, which have also been described for the filiform papillae of the human tongue (Kunze, 1969; Schenk & Wersäll, 1975). These surface structures, as well as the branching and peripheral angulations with dome-shaped endings of the CTP which are integrated in the epithelium like dowels in a wall, in addition to the tip fenestrations and vascularisation of the CTP, are interpretable as being still another way of increasing the epithelial-connective tissue interface and providing an optimally large surface for anchoring and nourishing the epithelium.

Data on the hard palate and gingiva obtained by Klein-Szanto & Schroeder (1977) as well as the present data of the soft palate indicate 'keratinising' epithelia to be associated with a high connective tissue papillary density, whereas the density for 'non-keratinising' epithelia elsewhere in the mouth is much lower. Horstmann (1952) argued that this relationship might be thought to favour a functional adaptation to external mechanical stimuli. However, Hale (1952) and Horstmann (1954) found that the configuration of these structures is already established before birth. Transplantation experiments, which showed that the features of the connective tissue do not change when the entire tissue is transferred to areas with different mechanical conditions, confirm these findings (Smith, 1970; Karring et al. 1971). In addition, the typical papillary architecture, and the type of epithelium covering the papillary body, have been demonstrated to result from the action of unknown connective tissue inducers (Plagman et al. 1974; Karring et al. 1975).

Löe & Karring (1971) have demonstrated that distinct differences exist in the morphology of the epithelium-connective tissue interface of the gingiva between young and old individuals. The essential feature is that in older individuals connective tissue papillae predominate whereas younger individuals show mainly connective tissue ridges. A similar development of the epithelium-connective tissue interface of tongue, labial and soft palate has been reported by Horstmann (1954) as well as by Coslet & Cohen (1968). The change from connective tissue ridges to papillae involves the formation of epithelial cross-ridges (Karring, 1973). It is surprising that in spite of these alterations, no change occurs in the volume of the epithelium with age. Probably this may be explained by a concurrent decrease in the distance between the tip of the CTP and the epithelial surface as demonstrated by Wentz et al. (1952). Age-related changes of the soft palates analysed in the present study which only include the older age group are in conformity with the findings of Horstmann (1954) and Löe & Karring (1971) that in older age groups CTP predominate and that a change from connective tissue ridges to papillae must already have occurred in the younger groups. No sex differences in the morphology of the soft palate were detected.

A special role in the epithelial-connective tissue boundary is exerted by the basement membrane (BM) (see Junqueira & Carneiro, 1996). Three major functions have been established for BM: (1) physical support, (2) cell attachment, and (3) filtration (Martinez-Hernandez & Amenta, 1983). The combination of these 3 functions permits individual cells, or groups of cells, to generate and maintain their optimal environment. The presence of a BM can be revealed using an antibody against the BM component laminin. Just as in other epithelial areas of the oral mucosa, it could be demonstrated that BM is a ubiquitous component of the extracellular matrix at the boundary between the epithelial cells and the connective tissue stroma. The antibody outlined the whole epithelial-connective tissue boundary in the
oral part of the human soft palate including each CTP.

The underlying superficial zone of the lamina propria consisted of elastic, collagenous and reticular fibres. Immunohistochemically, reticular fibres resemble type III collagen. They have been described as being tension-elastic or flexion-elastic (Bargmann, 1977). The second predominant collagen type was shown immunohistochemically to be type I collagen. In addition, the distribution of glycosaminoglycans within the lamina propria was established. Because of the combination of collagen fibrils, elastic fibres and glycosaminoglycans the superficial zone of the lamina propria of the human soft palate including the CTP is endowed with the mechanical properties both of tensile strength and simultaneous flexibility.

Taste buds are present (Fig. 1d) beneath the overlying epithelium of the soft palate. They are well known to occur in the oral surface of the soft palate of primates (Hoffmann, 1875; Yamamoto et al. 1959; Klein & Schroeder, 1979). Their distribution and their relationship to the CTP in the human soft palate have been well described by Imfeld & Schroeder (1992) using light microscopy and scanning and transmission electron microscopy.

The integrity of the lining epithelium of the human soft palate is only interrupted by the openings of the glandular ducts. The configuration of the collagen fibrils constituting the openings of the glandular ducts was originally studied by Nakano (1991). He observed the openings of the glandular ducts in the mouse soft palate by SEM at high magnification. Openings of the glandular ducts in the human soft palate are almost indistinguishable from those of the mouse soft palate with regard to the arrangement of collagen fibrils. Thick collagen fibrils run concentrically around the rim of the openings. Thin collagen fibrils derived from such concentric fibrils and reached thick collagen fibrils of the surrounding lamina propria. The concentric arrangement of the collagen fibrils inside the wall of the glandular ducts is suggested to play an important role in resisting the extending forces of the ducts during secretion (Nakano, 1991).

The use of the techniques used to examine the human soft palate in the present study, along with the possibility of comparing normal with pathologically altered epithelial-connective tissue boundaries, should promote a better understanding of lesions of the soft palate. The occurrence of bullae in dermatological diseases (de las Heras et al. 1996) or extracellular matrix changes in the CTP in snorers and patients with sleep apnoea are possibly caused by alterations at the epithelial-connective tissue boundary. Formation of bullae in several dermatological conditions results in a detachment of the overlying epithelium from the underlying connective tissue. Changes in the connective tissue interface especially in the CTP could play a major role in these lesions. Woodson et al. (1991) showed that histological differences occur in snorers and in patients with sleep apnoea compared with nonsnorers, visible as mucous gland hypertropy, focal atrophy of muscle fibres, and extensive oedema of the lamina propria. No investigation has been carried out in such clinical syndromes on the connective tissue interface and the CTP. Further investigation of the connective tissue boundary in such pathological states will lead to a better understanding of these diseases and may open new perspectives for currently existing therapeutic concepts.

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