Collagen fibril arrangement and size distribution in monkey oral mucosa

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ABSTRACT

Collagen fibre organisation and fibril size were studied in the buccal gingival and hard palate mucosa of *Macacus rhesus* monkey. Light and electron microscopy analysis showed connective papillae exhibiting a similar inner structure in the different areas examined, but varying in distribution, shape and size. Moving from the deep to surface layers of the buccal gingival mucosa (free and attached portions), large collagen fibril bundles became smaller and progressively more wavy with decreasing collagen fibril diameter. This gradual diameter decrease did not occur in the hard palate mucosa (free portion, rugae and interrugal regions) where the fibril diameter remained constant. A link between collagen fibril diameter and mechanical function is discussed.

Key words: Palatal and gingival mucosa; connective tissue; collagen; elastin.

INTRODUCTION

Several studies on healthy or pathological human and animal gingival connective tissue have been reported, but most were limited to single selected areas (Svoboda et al. 1983; Chavier et al. 1984; Garnick & Walton, 1984). Other studies did not make a comparative analysis between different topographic regions or did not specify human or animal ages (Page et al. 1974; Chavier et al. 1981). Because of the different morphological appearances of the epithelium and collagen fibre arrangement in between different regions of the human oral mucosa together with variations with age in the collagen fibre distribution pattern and fibril diameter (Craig et al. 1989; Xu et al. 1993) and mechanical requirements (Craig et al. 1987), it is necessary to examine gingival collagen fibres in different topographical gingival areas in humans or animals of defined age. Differences in the appearances of the connective tissues and also different distributions and orientation of elastic fibres between the buccal and lingual gingiva have been described (Fleish, 1974). Moreover, a number of workers agree on the wide heterogeneity of collagen distribution in healthy and pathological human gingival connective tissue (Chavier et al. 1984; Romanos et al. 1993).

The immunohistochemical distribution of collagen has been studied in human gingiva (Chavier et al. 1981), in healthy gingival connective tissue of beagle dogs (Cho et al. 1987), and in rats, marmosets and humans (Romanos & Bernimoulin, 1990; Romanos et al. 1991*a*, *b*). According to Chavier et al. (1984) little attention has been paid to a possible correlation between particular morphological patterns of gingival connective tissue arrangement and the nature of its collagenous components. Some authors suggested that the different morphology exhibited by many fibres or fibrils may be due to specific functions in which the gingiva is involved (Garnick & Walton, 1984; Redlich et al. 1994). For this reason, we investigated the shape of the collagen fibres and size of related fibrils in different regions of the oral mucosa.

MATERIALS AND METHODS

Biopsies from mandibular buccal gingival (free and attached portions) and hard palate mucosa (free gingival portion, palatal rugae and interrugal regions) were taken from 4 female *Macacus rhesus* monkeys aged 3 to 4 y; no pathological lesions were present in their gingival tissues. The animals were killed while under intramuscular Ketalar anaesthesia by vascular perfusion of half-strength Karnovsky's fixative.

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Samples that included the epithelium and all the underlying connective tissue around premolars were removed from the bone by preparing a mucoperiosteal flap. Specimens processed for light microscopy were fixed in 10% neutral formalin, and paraffin-embedded sections stained by Picro-Sirius red and Weigert's resorcin-fuchsin, the latter either without any previous treatment or after oxidation with oxone (Fullmer et al. 1974).

Specimens for electron microscopy were fixed in Karnovsky's solution (4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4) at 4 °C; for elastic fibres, specimens were fixed in Karnovsky's solution containing 0.1% tannic acid. All samples were postfixed in 1% OsO_4, dehydrated in graded concentrations of ethanol, oriented to allow sections to be cut in the frontal plane and embedded in epoxy resin. Thin sections were obtained with a diamond knife on a Reichert OM-U3 ultramicrotome, collected on Cu–Rh grids, stained with uranyl acetate and lead citrate and observed using a Siemens Elmiskop 101 transmission electron microscope operated at 100 kV.

Instrumental magnification was assessed using a suspension of 0.091 μm latex beads (Balzer Union). Collagen fibril diameter, from the subepithelial connective tissue and the dense connective deep layer, was obtained by measuring 400 cross-sectioned fibrils of regular round profile for each different area of the oral mucosa from randomly selected micrographs with a Leitz ASM image analysis computer system. Statistical analysis of collagen fibril diameter was performed by Student's *t* test assuming statistical significance at $P < 0.05$.

All procedures involving monkeys were performed according to the ethical guidelines for animal experimentation issued by the University of Bologna.

RESULTS

Histological findings

The histological analysis of the subepithelial layer in buccal gingival (both free and attached portions) and hard palate mucosa (free gingival portion, rugae and

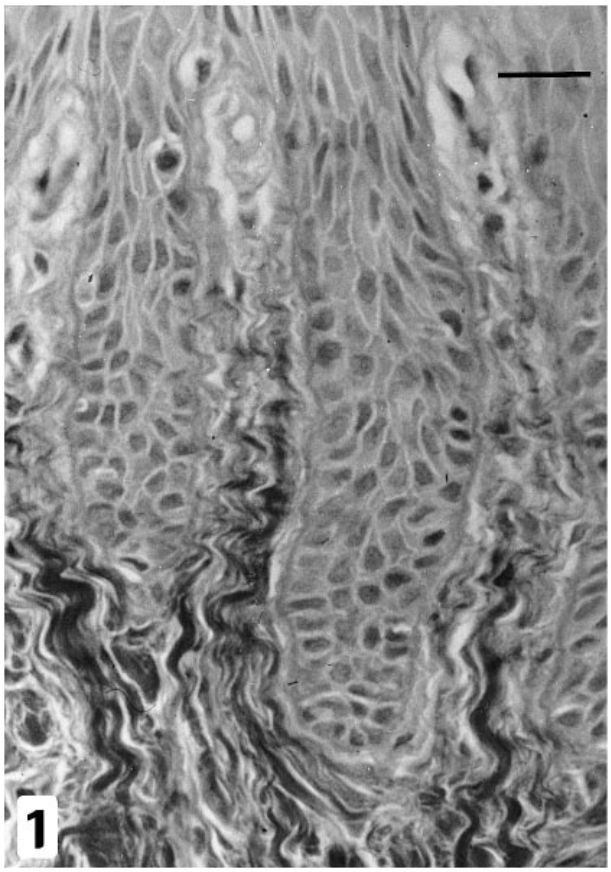

Fig. 1. Structure of the connective tissue of the papillary body. Small winding bundles of collagen fibres with a capillary loop running through them are visible. At the top of the papilla there is a dilatation of the capillary loop. Picro-Sirius red staining. Bar, 20 μm.

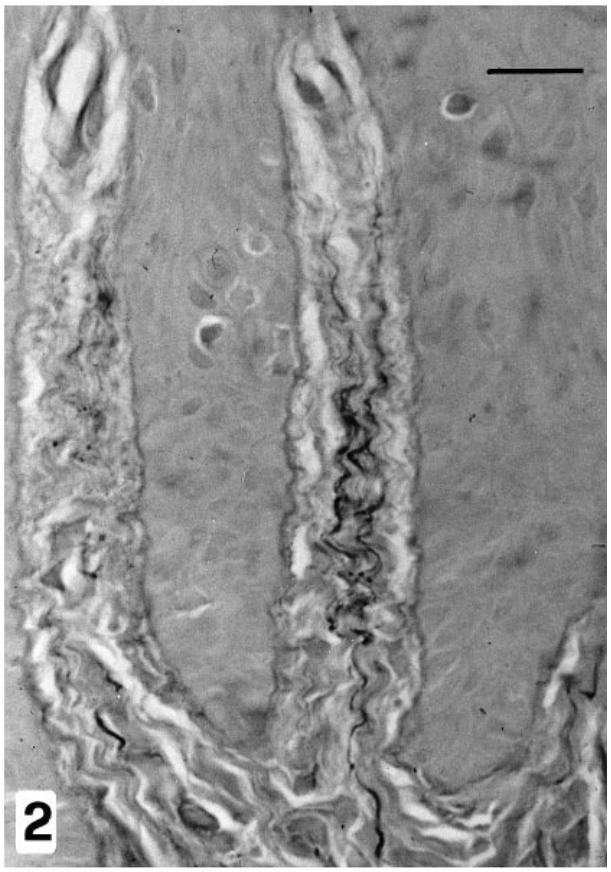

Fig. 2. Detail of the papillary body. In the connective tissue of the papillae, oxytalan fibres follow the winding course of the collagen fibres. Resorcin-fuchsin staining after oxidation with oxone. Bar, 20 μm.

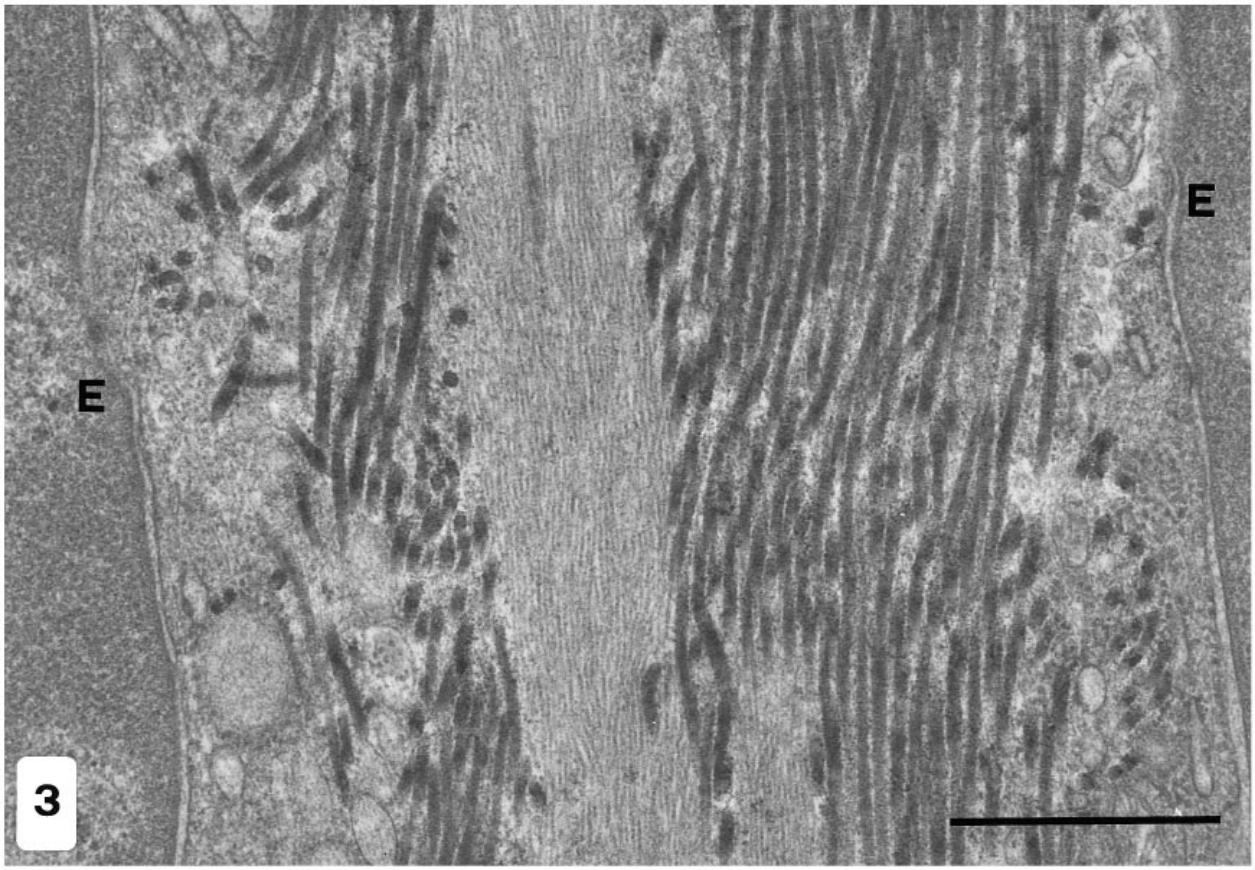

Fig. 3. Ultrastructural detail of Fig. 2. Free buccal gingiva stained with tannic acid. In the core of the connective tissue of the papilla, between the epithelial cells (E), an oxytalan fibre runs parallel to the collagen fibres. Bar, 1 μm.

interrugal regions) showed that connective papillae varied in distribution, shape and size in the different areas. Their internal structure, however, was similar in all areas. In Picro-Sirius red stained sections of all samples, the connective tissue core of the papillae was represented by wavy bundles of collagen fibres running from the inner deep layer of the lamina propria apically to the papilla and branching into thinner bundles. Among these bundles, a capillary loop was detectable (Fig. 1). In all sections stained with resorcin-fuchsin, after oxidation with oxone, very thin sinuous fibres belonging to the elastic component were seen to follow the axial course of the collagen fibres with the main orientation parallel or orthogonal to the gingival surface. In this areas, few resorcin-fuchsin stained fibres were present (Fig. 2).

In the deep layer of the lamina propria, beneath the subepithelial layer, straight and parallel large fibre bundles were detectable in Picro-Sirius red stained sections.

Electron microscopy

Transmission electron microscope (TEM) analysis of all gingival areas showed that the connective tissue of the papillae include wavy bundles of collagen fibrils running along the space between the central capillary vessel and the surrounding epithelium. In sections of specimens treated with tannic acid these collagen fibril bundles included thin bundles of beaded 10 μm microfibrils recognisable as oxytalan fibres associated with amorphous electrondense material (Fig. 3). These thin bundles were considered to correspond to the elastic component shown in paraffin sections after oxone-fuchsin treatment and recognizable as oxytalan fibres.

Both in buccal gingival and hard palate mucosa, in proximity to the epithelial basement membrane, thin collagen fibrils, randomly loose or collected into small bundles, and oxytalan fibres were detectable (Fig. 4*a*, *b*). Other thin fibrils, loose or assembled in bundles around the capillary vessel walls, were observed. The deep layer of the lamina propria, beneath the subepithelial layer, was occupied by thick straight bundles of collagen fibrils, mostly parallel to the surface of the gingiva (Fig. 5*a*). In these bundles we observed rare thin elastic fibres stainable with tannic acid (Fig. 5*b*).

TEM analysis of collagen fibril cross sections showed different size distributions between the gin-

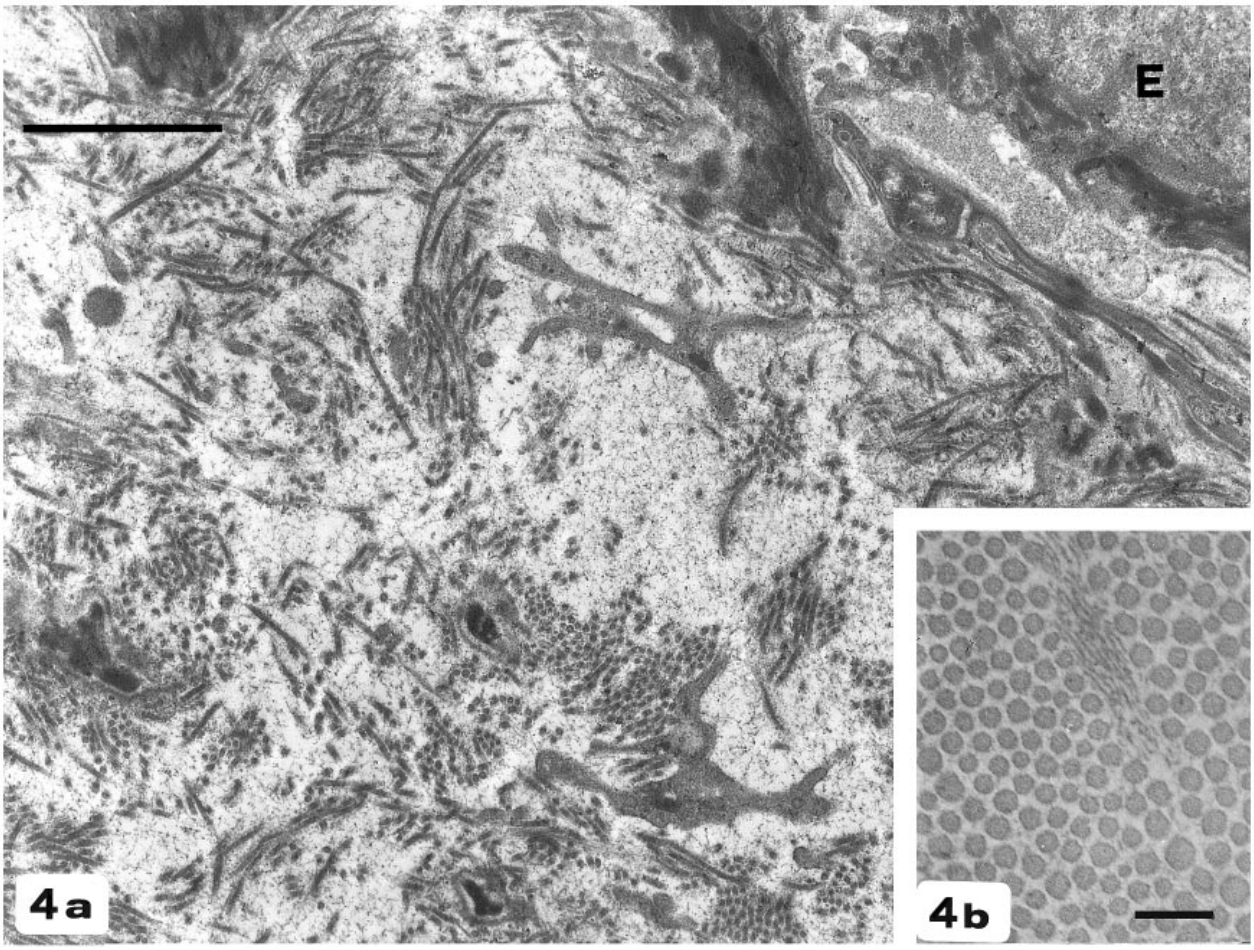

Fig. 4. Micrograph of free buccal gingiva. (*a*) In the subepithelial connective tissue layer, beneath the epithelium (E), thin loose collagen fibrils are visible. Bar, 2 μm. (*b*) Cross-sectioned collagen fibrils and an oxytalan fibre. Bar, 250 nm.

gival and hard palate mucosa. Subepithelial collagen fibrils in buccal gingival (free and attached portions) and hard palate mucosa (free portion, rugae and interrugal regions) presented relatively small diameters ranging from 38.5 ± 7.1 nm to 50.8 ± 9.1 nm (Fig. 6). Collagen fibrils in the deep layer of the connective tissue were of relatively large diameter in buccal gingiva (70.1 ± 10.5 nm in the free portion, 71.8 ± 9.2 nm in the attached portion), while a smaller diameter was evident in the hard palate mucosa (53.5 ± 5.2 nm in the free portion, 42.1 ± 8.6 nm in the rugae, 43.5 ± 8.8 nm in the interrugal regions) (Fig. 6).

DISCUSSION

In line with previous studies (Klein-Szanto & Schroeder, 1977; Ooya & Tooya, 1981), the morphology and fibre distribution of the connective tissue of the papillae varied between the different areas of the oral mucosa that we examined. The internal structure of the papilla, however, was similar in all areas, presenting a connective tissue stroma with a capillary loop running along its axis and sinuous bundles of collagen fibres running downwards in a loose environment and emerging at the apex of the papilla. Fine sinuous fibres that stained for elastin ran through these bundles. Their ultrastructural features were consistent with those of immature elastic fibres (oxytalan fibres). The sinuous course of the axial bundles of the papilla stroma and the presence of an elastic component can be explained by the chewing stress to which each papilla is subjected. Ultrastructural analysis disclosed that the sinuous collagen fibrils composing these bundles and running through the subepithelial connective tissue layer were usually small in diameter (38.5 ± 7.1–50.8 ± 9.1 nm). In particular, the smallest diameter of these fibrils was observed in the hard palate mucosa 38.5 ± 7.1 nm and 39.0 ± 8.3 nm. Here we also found fibres with an elastic component. Our findings are in line with previous observations (Raspanti et al. 1989) sug-

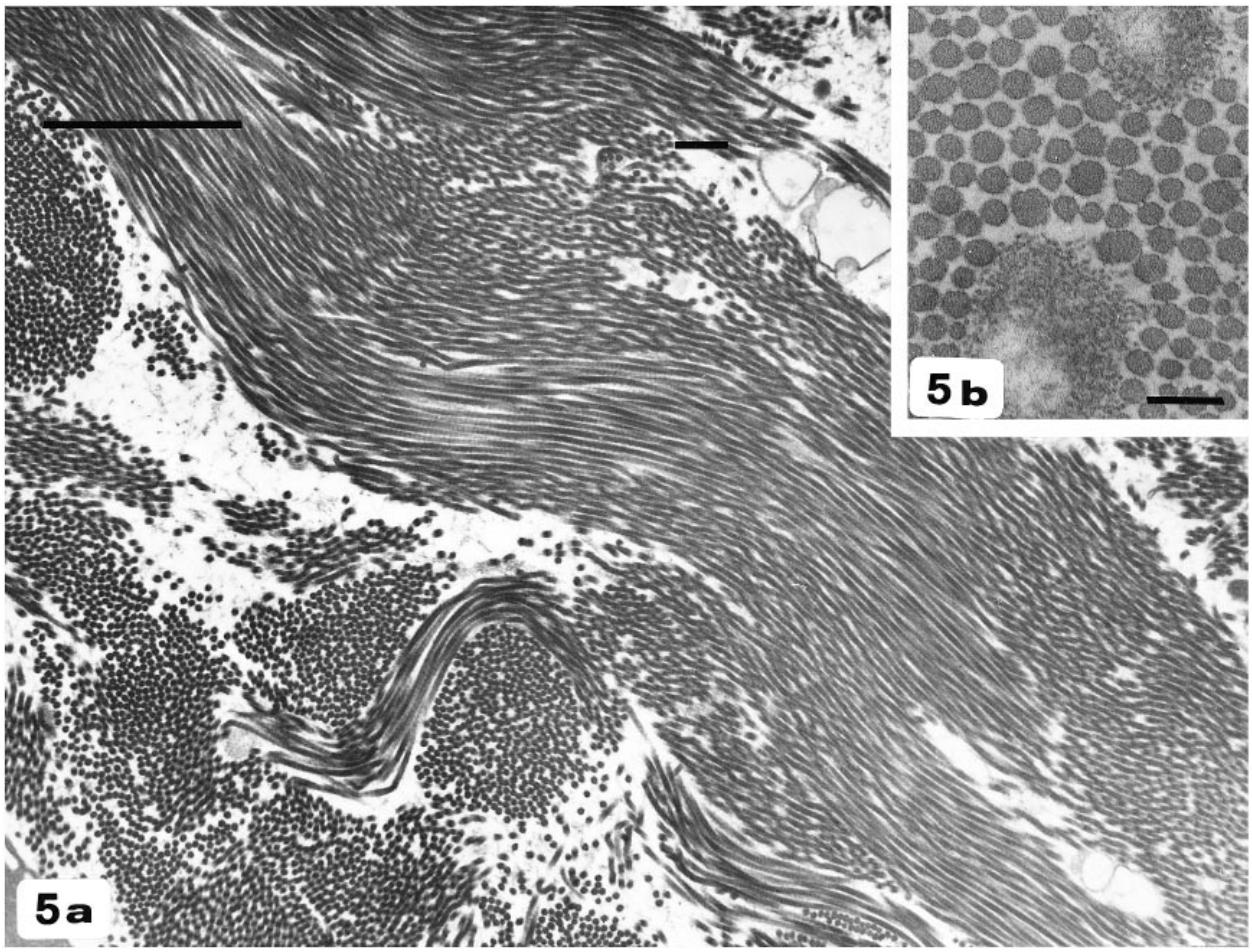

Fig. 5. Micrograph of free buccal gingiva. (*a*) The deep connective tissue layer of the lamina propria includes thick collagen fibril bundles. Bar, 2 μm. (*b*) Cross-sectioned collagen fibrils and elastic fibres. Bar, 250 nm.

gesting a broad correspondence between pressure stress (i.e. pluridirectional mechanical stress) and fibril diameter.

In the deep layers of the buccal mucosa the connective tissue stroma is arranged in large compact parallel bundles. In these regions fibril diameter is large and varies from 70.1 ± 10.5 m to 71.8 ± 9.2 nm. These fibrils are clustered into large collagen bundles which correspond to terminals of numerous gingival ligaments. Therefore, in addition to pressure stress, these bundles are subjected to mainly unidirectional tensor mechanical stresses which accounts for the relatively large fibril diameter as demonstrated in other connective tissues (Parry et al. 1978; Buck, 1987; Mattew & Moore, 1991).

In the deep layers of the hard palate mucosa the connective stroma includes collagen fibrils with a smaller diameter (42.1 ± 8.6–53.5 ± 5.2 nm) than that of the buccal regions. The largest average diameter was 53.5 ± 5.2 nm in the fibrils of the gingival free portion of the hard palate mucosa, where the largest clusters of gingival ligaments are found. In general, there were no major differences in fibril diameter between the subepithelial and deep layers of the hard palate mucosa. The small and uniform diameter exhibited by all the collagen fibrils of the hard palate mucosa (free gingival portion, rugae and interrugal regions) may be consistent with the stronger and more frequent compressional stress to which this tissue is subjected during the movements of the tongue.

The mechanical properties of a connective tissue are related to collagen content, fibril size and fibril orientation (Parry & Craig, 1984; Craig et al. 1987; Raspanti et al. 1989; Mattew & Moore, 1991). A tissue is continually remodelled between birth and maturity in order to adjust to its changing mechanical requirements (Flint et al. 1984; Raspanti et al. 1990). The primary factors determining the ultimate morphological and biochemical characteristics of the tissue are likely to be the various environmental stimuli to which the tissues and cells are subjected (Parry et al. 1978). Literature reports on dental tissues

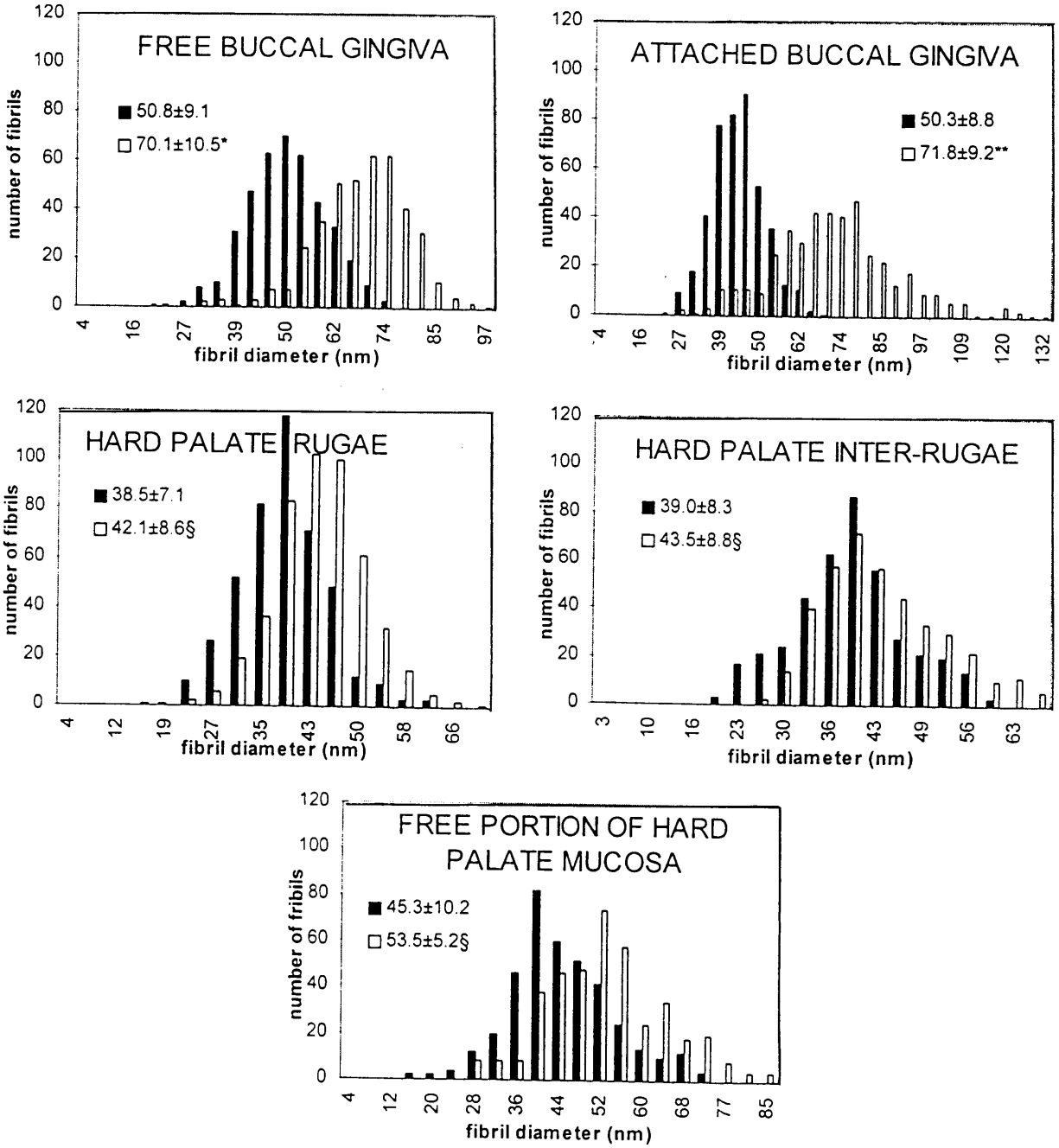

Fig. 6. Collagen fibril diameters in different areas of oral mucosa. Figures represent the mean of 4 animals ± S.D. Statistical analysis of collagen fibril diameter was performed by Student's t test comparing fibrils within the same area. Statistical significance was assumed for $P < 0.05$; * $P = 0.022$; ** $P = 0.005$; § = non significant. ■ = subepithelial layer; □ = deep layer.

and oral mucosa (Fleish, 1974; Luke, 1992) suggest that collagen fibrils are affected by their different mechanical role. In particular, Fleish (1974) demonstrated in the vervet monkey that collagen in the lamina propria and in the submucosa of the alveolar mucosa was much thicker and more densely arranged on the buccal than on the lingual side. The author suggested that collagen in the lamina propria and submucosa was subjected to different stresses on the buccal and lingual sides and, in particular, that the buccal side might be subjected to far greater demands of flexibility. Fullmer et al. (1974) suggested that the intensity of stress, together with its frequency and direction, may influence the diameter of the collagen fibrils as well as their inner structure. Therefore different areas of buccal gingival or hard palate mucosa may include collagen fibrils with different diameters predicted to resist stresses of different intensity, frequency or direction. In our study, in buccal gingival and hard palate mucosa, collagen

fibrils of the subepithelial and deep layers showed different diameters. These findings can be interpreted as being related to different mechanical stresses. These stresses may influence the metabolism of fibroblasts by significant elevation of prostaglandin E (Ngan et al. 1990) and intracellular calcium ions (Ten Cate et al. 1976; Svoboda, 1983; Arora & McCulloch, 1994) that result in the production of different collagen types. The difference in diameter size of collagen fibrils may also be related to a functional turnover of collagen in the extracellular matrix (Svoboda et al. 1983; Lekic & McCulloch 1996). Svoboda et al. (1983) indicated that larger collagen fibrils impair the ability of cells to phagocytose and degrade them; that is, fibril diameter may be a regulating factor in collagen turnover.

Finally, in this study large collagen fibrils appeared to be associated with mature elastic fibres, while small collagen fibrils were related to immature oxytalan fibres, confirming previous literature reports (Fleish, 1974) on the distribution and diameter of elastic fibres in the buccal and lingual sides of alveolar mucosa subjected to different stresses.

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