Ultrastructural changes of collagen and elastin in human gingiva during orthodontic tooth movement.

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RÉSUMÉ

Après 15 jours de traitement orthodontique par mésialisation ou distalisation, 10 prémolaires permanentes ont été extraites chez de jeunes patients, ainsi que la gencive interdentaire adjacente. Le tissu conjonctif des papilles interdentaires comprimées ou étirées a été comparé avec celui d'échantillons de dents n'ayant pas subi de traitement. L'observation a été faite en microscopie classique et en microscopie électronique à transmission. Dans la gencive interdentaire comprimée, on observe de longs faisceaux de fibres de collagène faits de fibrilles qui présentent une périodicité de 64 nm et un diamètre inférieur à 75 nm. Ont été observées également de fibres élastiques d'une diamètre inférieur à 950 nm. Dans certaines zones centrales de gencive comprimée, on peut voir, à proximité dess fibres élastiques, des fibrilles de collagène réparties longitudinalement en microfibrilles très espacées.

Dans la gencive interdentaire étirée et dans celle non soumise au traitement orthodontique, les fibrilles de collagène présentent un diamètre de 66 nm et 57 nm respectivement. Dans ces deux groupes, on note un très petit nombre de fibres élastiques de 600 nm de diamètre. L'augmentation du nombre et de la taille des fibres élastiques dans la gencive comprimée indique que le système de fibres élastiques remplace la trame collagène qui a subi un collapsus.

SUMMARY

After 15 days of mesializing or distalizing orthodontic treatment, 10 permanent premolars of young patients were extracted with the interdental gingiva. The connective tissues of the compressed or stretched interdental papillae were compared to that of untreated samples by light and transmission electron microscope.

Large collagen fibres bundles represented by fibrils with a banding pattern of 64 nm and a mean diameter of 75 nm were observed in compressed interdental gingiva. Several elastic fibres with a mean diameter of 950 nm were also present. In some central areas of compressed gingiva collagen fibrils longitudinally split into widely spaced microfibrils were often observed in proximity to the elastic fibres.

In stretched and untreated interdental papillae the collagen fibrils presented a mean diameter of 66 nm and 57 nm respectively. In both groups, few elastic fibres ranging in diameter 600 nm were seen. The increased size of the gingival collagen fibrils undergoing pressure and tension is indicative of remodelling of the fibrous collagen system.

The fair increase in number and size of elastic fibres in compressed gingiva suggests that the elastic fibre system takes over the place whenever a collapse of the collagenous framework occurs.

INTRODUCTION

The extracellular connective tissue matrix of both free and attached gingiva is largely made up of collagen which contains 60% of total proteins (Page, 1972; Shluger et Coll., 1977). This is described as mainly Type I and Type III collagen (Ballard and Butler, 1974; Chavrier et Coll., 1981, Chavrier et Coll., 1984) in a ratio of 7: 1 (Nathan et Coll., 1979; Chavrier et Coll., 1984). Type IV collagen is also reported to be restricted to the basal laminae and around the blood vessels (Chavrier et Coll., 1984). In oral mucosa, type V collagen is considered an interstitial fibrillar collagen rather than a basement membrane collagen (Schuppan et Coll., 1986; Fonzi and Ruggeri, 1988).

A minor component of the gingival connective tissue matrix is elastin. Elastic fibres are found in the central portion of the dog free gingiva while oxytalan fibres are prevalently observed close to the epithelial basal lamina (Soames and Davies, 1975; Soames and Davies, 1978). Lopez and Co-workers (1976) reported also the presence of elastin-like fibres in human attached gingiva. Their histological indentification has been further demonstrated by processing the tissue with a preliminary treatment with elastase (Porter et Coll., 1977).

In various tissues undergoing stress the presence, arrangement, shape and dimension of collagen and elastin are closely related to environmental changes (Parry et Coll. 1978; Jonas and Riede, 1980; Craig and Baines, 1981; Pasquale-Ronchetti and Fornieri, 1984; Parry and Craig, 1984; Strocchi et Coll., 1988).

In specific types of orthodontic movements, relapse is supposed to be largely determined by the supra-alveolar structures and in particular the gingival collagen fibre bundles (Reitan, 1959; Pinson and Straham, 1974; Rygh et Coll., 1982). The purpose of the present study was to examine the structural and quantitative changes occurring in the fibrous content (collagen and elastin) of gingival areas submitted to pressure and tensile stresses in the course of orthodontic treatment.

MATERIALS AND METHODS

10 permanent upper and lower premolars were to be extracted for orthodontic reasons from 5 young patients aged 11-12 years. 7 of these teeth were extracted after 15 days of orthodontic treatment, during which they were subjected to 200 g of mezializing or distalizing force controlled every 3 days. The remaining 3 premolars, which didn't received any orthodontic treatment, were used as controls.

With a written patient consent, both the treated and untreated teeth were extracted after 15 days by removing the adiacent interdental gingiva. The samples were then immediately fixed in 3% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) for 24 hours. After fixation every sample was immersed in EDTA water solution for 8 hours and then carefully divided into two parts for examination with both light and transmission electron microscopy respectively. Specimens for light microscopy were dehydrated in the series of alcohols and embedded in paraffin. 5-6 μm thick sections were stained with Haematoxilin-Eosin, Sirius Red for the identification of collagen fibres (Junqueira et al., 1979) and Orcein for the identification of elastic fibres. The samples for transmission electron microscopic study were washed in a 0.1 M phosphate buffer (pH 7.2), post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in the series of alcohols and embedded in araldite. Thin sections were stained with uranyl acetate and lead citrate. The diameters of the collagen fibrils were measured using an image analyser (Leitz ASM). The instrumental magnifications were determined by comparison with a suspension of latex particles (Balzers Union).

RESULTS

Histological analysis of compressed gingiva showed a thin interdental papilla. In the deep area of the papilla, the supra-alveolar collagen fibres bundles appeared denser than those of the control samples and of those undergoing tensile stress (Fig. 1). In all specimens, orcein staining failed to show a significant presence of elastin.

The ultrastructural observations of compressed gingiva demonstrated large collagen fibres bundles represented by fibrils with a banding pattern of 64 nm. The collagen fibril diameter measured on transverse sections averaged 75 nm, 18 nm more than that of untreated samples (Table I). Several elastic fibres were found adjacent to the collagen fibrils and oriented in the same

TABLE I.
Assessment of the average diameter of the collagen fibrils in gingiva undergoing either compression, tensile stress or in controls.
Diamètre moyen des fibres de collagène dans la gencive soumise à compression ou étirement et dans la gencive témoin.

	Pressure	Tension	Normal
Number/compon	120	120	166
Minimum	44.1607 nm	40.7141 nm	23.8423 nm
Mean	75.057 nm	65.6246 nm	57.1801 nm
Maximum	101.3052 nm	84.0795 nm	84.1234 nm
Stand. Dev.	0.964775	0.687622	0.912569
Var. Coeff.	16.0674	13.0976	19.4629

direction. These elastic fibres showed a mean diameter of 950 nm and appeared composed of a large central core of amorphous material surrounded by a few microfibrils. Occasionally, immature elastic fibres composed of numerous microfibrils intermingled with patches of amorphous material were seen (Fig. 2-3). In some central areas of compressed interdental papillae bundles of collagen fibrils whose characteristic banding was lost were often observed in proximity to the elastic fibres. These fibrils indeed appeared sometimes longitudinally split into widely spaced 5.0-7.5 nm thick microfilaments (Fig. 4). Some areas moreover comprised remnants of collagen fibrils and elastic fibres immersed in a network of very many microfilaments which presumibly corrisponded to a high level of collagen fibrils swelling (Fig. 5).

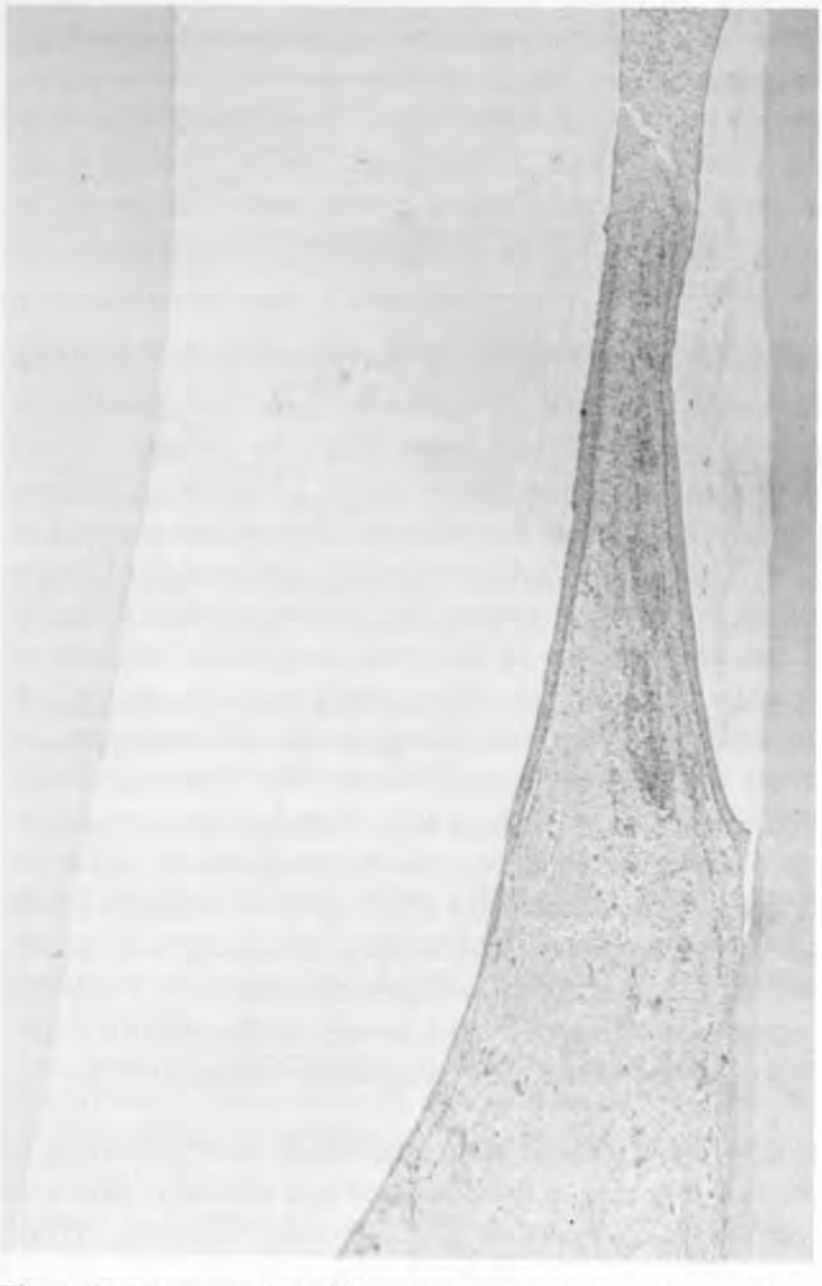

Fig. 1: In compressed papillae, the supra-alveolar collagen fibres bundles appear densly packed.
Fig. 1: Papille soumise à compression. Les trousseaux de fibres de collagène sont très denses.

Samples of gingiva under tension and of untreated patients showed 64 nm D-period collagen fibrils with a mean diameter of 66 nm and 57 nm respectively. In both samples groups, few elastic fibres with a dense central core surrounded by scant microfibrils ranging in diameter 600 nm were observed. They appeared aligned in the same direction of the collagen fibrils.

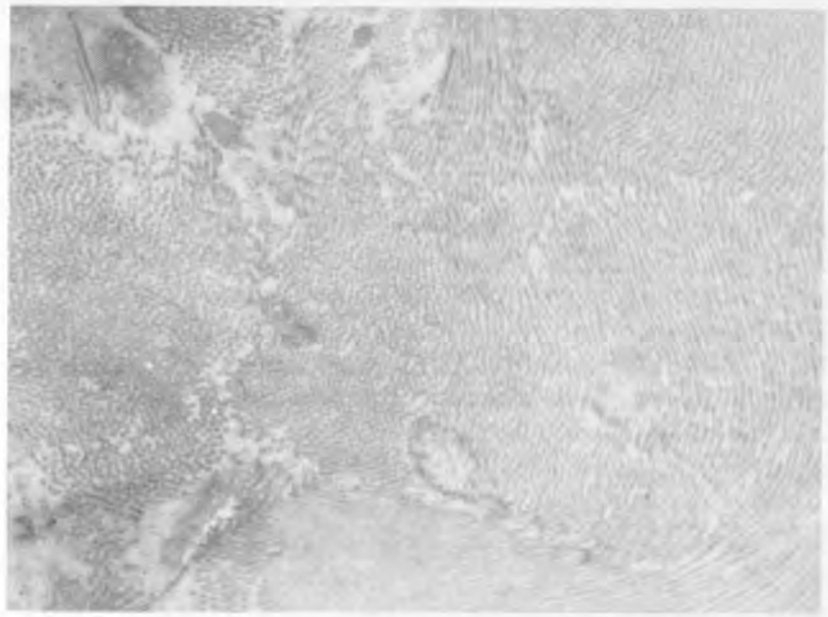

Fig. 2: Gingival connective tissue of the interdental papillae undergoing compressing stress. A fair number of elastic fibres (mean diameter 950 nm) are immersed in densly packed collagen fibrils.
Fig. 2: Tissu conjonctif gingival des papilles interdentaires soumises à compression. Un certain nombre de fibres élastiques (diamètre moyen, 950 nm) sont mêlées aux fibres de collagène très denses.

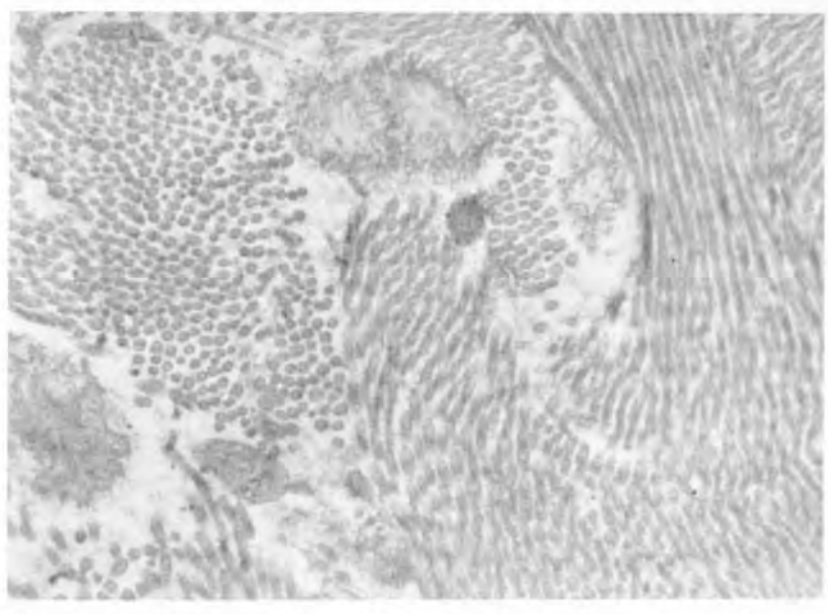

Fig. 3: Detail of figure above. Note the elastic fibres among the collagen fibrils.
Fig. 3: Détail de la figure 2. Noter la présence de fibres élastiques parmi les fibres de collagène.

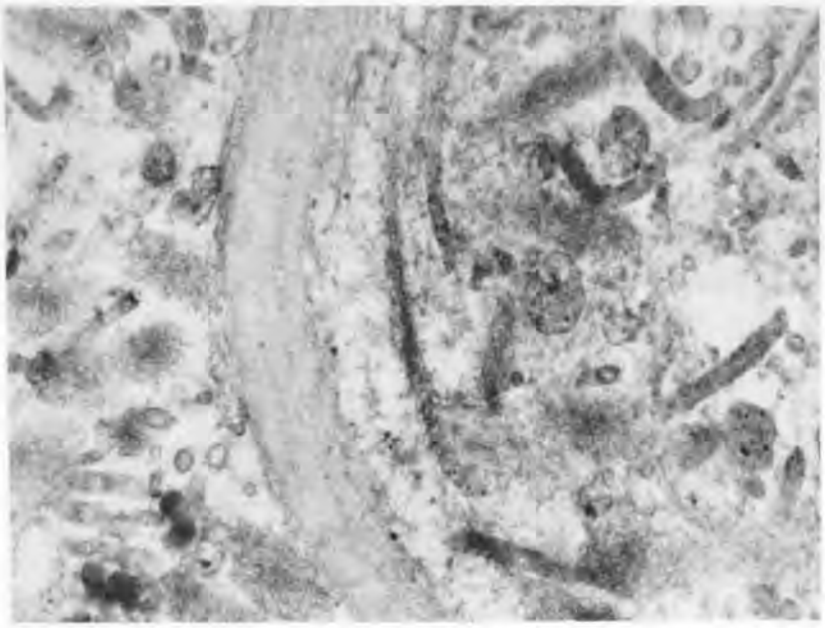

Fig. 4: Central zone of the compressed interdental papilla. An elastic fibre and collagen fibrils without banding can be seen. In some areas they appear split into numerous microfilaments of 5.0–7.5 nm diameter.
Fig. 4: Zone centrale de la papille interdentaire soumise à compression. On voit une fibre élastique ainsi que des fibres de collagène sans striation périodique. En certains endroits, elles ont éclaté en nombreux microfilaments de 5,0-7,5 nm de diamètre.

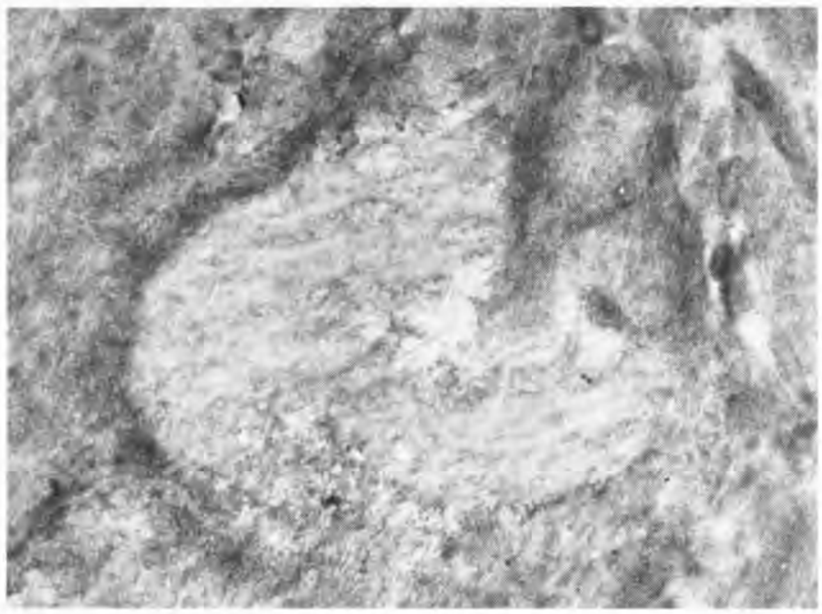

Fig. 5: In compressed interdental papillae the deep connective tissue comprises numerous elastic fibres intermingled with the collagen fibrils longitudinally split into numerous microfilaments.
Fig. 5: Papille interdentaire soumise à compression. Le tissu conjonctif profond comprend de nombreuses fibres élastiques mêlées à des fibres de collagène éclatées longitudinalement en de nombreux microfilaments.

DISCUSSION

These components are continually subjected to stress during physiological movements or orthodontic treatment and may be largely responsable for relapse after othodontic treatment (Reitan, 1959; Pinson and Straham, 1974; Rygh et Coll., 1982).

The fibrous component of the gingival matrix is mainly composed of Type I and III. Type IV and V collagen represent minor components (Schuppan et Coll., 1986; Fonzi and Ruggeri, 1988; Ballard and Butler, 1974; Nathan et Coll., 1979; Chavrier et Coll., 1981). Elastin is also present either as oxytalan or elastic fibres (Fonzi and Ruggeri, 1988).

Our histological investigation of human interdental gingiva undergoing orthodontic pressure shows a thinning of the papillae associated with the presence of densly packed collagen fibres bundles. At the ultrastructural examination the same samples show collagen fibrils with 64 nm banding, and a certain number of mature elastic fibres. These ones have the same arrangement as the collagen fibrils, are more numerous and may be considered more mature if compared to those both in the samples undergoing tension and controls. In central areas of compressed interdental papillae, around the elastic fibres, swollen and scattered collagen fibrils without any banding pattern are observed. Similar collagen fibrils are also described in the periodontal hyalinazed zone of teeth undergoing orthodontic pressure (Rygh, 1973a; Rygh, 1973b; Franchi et al., in press) or in pathological conditions of the periodontium (Selvig, 1966) as an expression of accentuated tissue distress.

Morphometric analysis of the microfibrils shows the apparently normal collagen fibrils in compressed gingiva to be statistically larger than the collagen fibrils in tissue undergoing tensile stress. These latter, moreover, are larger than the collagen fibrils of untreated gingiva. These variations presumably indicate the fibrous connective system adaptation to new stresses. Actually it has been shown in different anatomical regions of animals that both the arrangement and diameter of collagen fibrils differ according to the different type of stress applied (Craig and Baines, 1981; Parry et Coll., 1978; Strocchi et Coll., 1988). Further immunohistochemical investigations are in progress in order to verify if the increased size of the gingival collagen fibrils undergoing pressure and tension is indicative of remodelling of the fibrous collagen system with a relative increase of collagen Type I, as seen in the papillary dermis of striae albae (De Pasquale et Coll., 1987).

A new observation, which needs an interpretation, is the fair increase in number and size of elastic fibres in compressed gingiva. It is noteworthy that our orthodontic treatments cause dramatic remodelling of collagen matrix associated with numerous elastic fibres that may represent a substituting fibrous system. Although the functional importance of the morphometric observations is speculative, it is noteworthy that several biological examples suggest that the elastic system fibres takes over when any collapse of the collagenous framework occurs. Thus, unpublished results (G.S. Montes, 1988 - personal communication) show that there is a remarkable increase in elastic fibres in the uterine cervix at term, which provide for a framework that maintains the structure of this organ during the collagenolysis that is undergoes to permit the softening of its fibrous tissue at parturition. As well as in striae albae, the weakening of dermal resistance due to an alteration of the collagenous arrangement is overcome by an increase of the elastic fibre system (De Pasquale et Coll., 1987). Patients with osteogenesis imperfecta develop the «floppy valve syndrome» in which collagen is seriously affected. The integrity of the heart valves, however, is provided by an increase in the elastic fibre system (L.C.V. Junqueira, 1988, personnal communication).

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