Connective tissue organization of healthy human gingiva

Ultrastructural localization of collagen types I-III-IV

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Healthy human gingival connective tissue appears to be arranged in two patterns of organization at the ultrastructural level: Pattern I (PI) and Pattern II (PII). PI is a dense pattern of organization mainly constituted of large, dense bundles of thick collagen fibers, whereas PII is a loose pattern of organization, mainly constituted of short, thin collagen fibers mixed with a fine reticular network, especially located under or around basement membranes. Ultrastructural immunoperoxidase labelling of types I, III, and IV collagen demonstrates that gingival connective tissue is made of an intricate pattern of type I and III collagen where type I collagen fiber are preferentially organized in large dense bundles in PI, whereas a fibrous and fibrillar type II collagen network is predominant in PII. Type IV collagen, which does not exist in fibrous or fibrillar form, appears to be the main collagenous component of the basement membrane.

(Accepted for publication November 22, 1983)

Introduction

The healthy human gingival connective tissue, attached to the external part of the alveolar bone and the cervical region of the teeth, protects and maintains the integrity of periodontium. Its main structural and fibrillar component is collagen, which accounts for about 60% of the total tissue protein (Page 1972, Schlueter et al. 1977). Numerous biochemical studies have demonstrated that types I and III collagen were the two main collagenous components of healthy human gingival connective tissue (Ballard & Butler 1974), with a type I:type III ratio of 7:1 (Nathan et al. 1979). Together these interstitial collagen types account for 99% of the total extractable collagen (Narayan & Page 1983), while type IV collagen accounts for less than 1%. Immunolabelling studies at the light microscopic level have shown that types I and III collagen were distributed throughout healthy human gingival connective tissue with a predominance of type III collagen in the gingival papillae underlying gingival basement membrane, and around blood vessel walls, while type IV collagen was the main collagenous component of basement membrane (Chavrier et al. 1981).

Although the ultrastructural feature of gingiva has been quite well studied in the
past (for review see: Schluger et al. 1977), little attention has been given to a possible correlation between some particular morphological pattern of organization of the gingival connective tissue and the nature of its collagenous components. The existence of such a correlation would be of great interest in the understanding of the remodelling ability of gingival connective tissue under physiological and pathological conditions. This study has been undertaken in order to establish such a correlation at the ultrastructural level by using the indirect immunoperoxidase labelling procedure.

Materials and Methods

Gingival biopsies

Seven, twenty-one-year-old students in dental surgery, showing healthy gingiva characterized by a gingival index (Löe & Silness 1965) ranging from 0.03 to 0.05 and the absence of gingival exudate (Löe & Holm-Pedersen 1965) were selected for the experiments after having given their informed consent. Under local anesthesia, 6 mm² of attached gingiva was removed from each patient in superior incisive area. Samples were then cut in small blocks of about 2 mm³ volume and divided in two groups: one was prepared for standard electron microscopy whereas the other one was prepared for indirect immunolabelling procedure.

Standard electron microscopy

The blocks were immediately placed in 2% glutaraldehyde-0.1 M sodium cacodylate buffer (pH: 7.4) for 2 h at 4°C. After washing they were finally fixed in 1% osmium tetroxide-0.1 M sodium cacodylate buffer (pH: 7.4) for 2 h at 4°C. Dehydration was performed with ethanol and samples were embedded in Epon 812. Ultrathin sections were contrasted with uranyl acetate and lead citrate and observed with a Philips 300 electron microscope.

Immunolabelling for electron microscopy

For indirect immunolabelling using the peroxidase procedure, the blocks were immediately fixed with 4% formaldehyde-0.1 M cacodylate (pH: 7.4) for 8 h at 4°C, washed, and frozen. Cryostat sections (10 μm) were cut and treated with 0.3% hyaluronidase (bovine testis type 1 Sigma) for 30 min at room temperature and by sodium azide 0.01 M in the same conditions. Sections were then washed and placed overnight in 0.07% bovine serum albumin at 4°C. After washing, the sections were incubated overnight at 4°C in highly purified antibodies (20 μLA) against type I, III, or IV collagen prepared from normal and fibrotic human livers according to Grimaud et al. (1980), washed, and then reacted with peroxidase conjugated antiserum. The bound peroxidase complexes were visualized by treatment with D.A.B. according to Graham and Karnovsky.

Fig. 1. Typical gingival connective tissue observed under the electron microscope following the same procedure. Pattern I (P1) is a dense pattern of organization. Collagen fibers are coarse (60-70 nm), arranged in large and dense bundles. On the other hand, pattern II (P2) is a loose pattern of organization underlying the basement membrane. Collagen fibers are short, thin (40-80 nm) and mixed with an amorphous non-pigmented material. × 10,000. Insert shows P2 to higher magnification, × 20,000. ED: Epithelial cell.

Fig. 2. Ultrastructural immunoperoxidase labelling of type IV collagen. Type IV collagen appears as the collagenous component of gingival basement membrane (arrow). × 12,000. ED: Epithelial cell.

Fig. 3. Ultrastructural immunoperoxidase labelling of type I collagen in pattern II, underlying gingival basement membrane (black arrow). Note the alternation of positive and negative reactions. × 11,000. ED: Epithelial cell/Fibroblast.

Fig. 4. Ultrastructural immunoperoxidase labelling of type III collagen in pattern II, underlying gingival basement membrane (black arrow). Note the alternation of positive and negative reactions. × 11,000. ED: Epithelial cell/Fibroblast.
sky (1966). Sections were then fixed with 1% OsO₄, dehydrated, and flat embedded in Epon. Ultrathin sections were prepared and observed with no further staining in a Philips 300 electron microscope.

Controls section were incubated in 0.1 M phosphate buffer without immune serum and in peroxidase conjugated antiserum alone.

Results

Standard electron microscopy
At the ultrastructural level, gingival connective tissue seems to consist of two patterns of organization each having characteristic features and a particular location. To clarify comments on the observations the following two patterns were designated: pattern I and pattern II.

Pattern I (Figs. 1-5). Pattern I consists of a particularly dense tissue and appears to be the predominant type of organization of the gingival connective tissue. It is generally composed of large, dense bundles of long thick striated collagen fibers with a mean diameter ranging from 60-70 nm. These fibers are often in close contact with mature fibroblasts.

Pattern II (Figs. 1-5). Pattern II on the other hand, is a loose connective tissue which appears to be the main type of organization in areas underlying gingival basement membrane or surrounding basement membranes of blood vessel walls. It is generally composed of short, thin (40-60 nm) striated collagen fibers, scattered or grouped in small bundles, mixed with an abundant non-striated fibrillar material (10-20 nm). In addition to fibroblasts, a significant number of resident mast cells and plasma cells can be observed.

Immunoperoxidase electron labelling
Pattern I. In sections both parallel and perpendicular to the fibers, type I collagen appears to be the main fibrillar component of this pattern of organization. In longitudinal section peroxidase deposits are closely bound to collagen fibers and periodically arranged (spacing around 64 nm). In cross sections, immunolabelling is emphasized by dark fibroblastic peroxidase deposits and punctate intracellular deposits (Figs. 7-12). The type I collagen fibers are generally thick (60-70 nm) and arranged in large and dense collagen bundles.

In PI tissue, fibers reacting with anti-type III antiserum (Fig. 13) are rare, thinner (60 nm), and generally isolated throughout dense bundles of predominantly type I collagen fibers. Immunolabelling yields the same selective contrast of typical periodicity as for type I collagen.

Pattern II is essentially a mixed pattern of type I and III collagen (Figs. 3, 4, 7, 8, 9, 10), where type III collagen seems to be present.

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Fig. 5. Typical gingival connective tissue around a blood vessel, observed under the electron microscope following the routine procedure. Pattern II (P2) is a loose pattern of organization surrounding basement membrane (arrow) of blood vessel wall. Collagen fibers are short, thin (40-60 nm) and mixed with an abundant non-striated fibrillar material. On the contrary, pattern I (P1) is mainly composed of coarse fibers (60-70 nm) and arranged in large dense bundles. × 15,000. BMG: Smooth muscle cell. P: Fibroblast.

Fig. 6. Ultrastuctural immunoperoxidase labelling of type IV collagen which seems to be the main collagen component of blood vessel basement membrane (arrows). × 15,000. BV: Blood vessel.

Fig. 7. Ultrastuctural immunoperoxidase labelling of type I collagen around a blood vessel in the two patterns of organization PI and P2. × 12,000. BV: Blood vessel. Arrows: Basement membrane. I: Type I collagen.

Fig. 8. Ultrastuctural immunoperoxidase labelling of type III collagen around a blood vessel in PI. Collagen III appears most often as a widespread thread-like material. × 12,000. BV: Blood vessel. Arrow: Basement membrane.
the or surrounding bone. The blood vessel walls, if it is of short, thin, and coarse fibers, scattered or bundles, mixed with an associated fibrillar material. The contribution to fibroblasts, a large population of resident mast cells and fibroblasts.

Electron labelling shows both parallel and crossed fibers, type I collagen a main fibrillar component organization. In longitu- dinal deposits are larger fibers and periodicity around 64 nm. In unlabelled is enhanced fibrillar periodicity. Type I collagen fibers are 70 nm and arranged in a helical pattern.

Immunolabelling with anti-type I (13) are rare, thinner (40 nm) isolated throughout the basement membrane. Dominantly type I collagen. Immunolabelling yielded the highest level of typical periodicity.

6. Presence of a mixed pattern of collagen (Figs. 3, 4, 7, 8, 9, 10). The basement membrane seems to be perforated by the electron microscope and mixed with an important coarse fibers (80-70 nm). Type I collagen is the main collagenous fiber.
dominant and organized in two forms: the fibrous form and the fibrillar one.

The fibrous form consists of short and thin (40 nm) striated fibers with the same selective labeling contrast as for type I collagen (Fig. 10). However, the most characteristic feature of type III collagen immunolabeling is the visualization of a widespread thread-like material (Figs. 4-8) which comprises the fibrillar form of type III collagen. In addition, the peroxidase deposit resulting from anti-type IV collagen labeling is exclusively limited to the lamina densa of the basement membranes underlying these structures (Figs. 2-6), and the pattern is always associated with the gingival epithelium or blood vessels.

Discussion

The present data confirm previous biochemical and immunohistological results, showing the heterogeneity of collagen distribution in healthy human gingival connective tissue: Ballard and Butler (1974), Chavrier et al. (1981). The particular focus of the present work is to demonstrate that healthy gingival connective tissue can be morphologically divided into two types of organization and, at the same time, that the nature of its collagenous components is different.

Indeed, although types I and III collagen always seem to be intermixed at the ultrastructural level, it appears that one type predominates in each of the two patterns of gingival connective tissue organization. Pattern I is matrix composed of dense type I collagen bundles. On the other hand, pattern II is composed of both types I and III collagen but shows a predominance of type III collagen.

These collagen types are probably both synthesized by gingival fibroblasts (Engel et al. 1980) in variable ratios (Narayanan & Page 1976). According to our data, gingival fibroblasts of pattern I tissue should be able to synthesize both types I and III collagen with a high type I/III ratio whereas gingival fibroblasts of pattern II could produce both type I and type III collagen with a lower type I/II collagen ratio. Factors affecting which collagen type is preferentially produced have been reviewed (Burnstein & Sage 1980) but are still unclear.

Since it is known that type III collagen is an early type of collagen (Epstein 1974, Prockop et al. 1979), it can be concluded that pattern II is a type of organization of gingival connective tissue that the ability for remodelling is greater and more important than in pattern I. Furthermore, the association of pattern II with basement membranes of gingiva and blood vessel walls correlates the remodelling ability of such a structure of organization under physiological conditions. On the other hand, predominance of type I collagen in pattern I tissue may testify to the

Fig. 9. Ultrastructural immunoperoxidase labeling of type I collagen in pattern II underlying gingival basement membrane. Periodically disposed peroxidase-labeled deposits along fibers can be noted (thin arrow).

EC: Epithelial cell.

Fig. 10. Ultrastructural immunoperoxidase labeling of the fibrous form of type III collagen in pattern II underlying gingival basement membrane. Note the periodically disposed peroxidase-labeled deposit along type II collagen fiber (arrow). In contrast no positive reaction could be detected on coarse fibers (thin arrow).

EC: Epithelial cell.

Fig. 11. Ultrastructural immunoperoxidase labeling of type I collagen in a longitudinal section of pattern I. The periodically disposed peroxidase-labeled deposits along type I collagen fibers (arrow). x 20,000.

EC: Epithelial cell.

Fig. 12. Ultrastructural immunoperoxidase labeling of type I collagen in a cross section of pattern I. Observe perifibrillar granular deposit around collagen fibers. x 20,000.

EC: Epithelial cell.

Fig. 13. Ultrastructural immunoperoxidase labeling of type II collagen in pattern I. Observe the selective labeling of two type II collagen fibers (arrow) with peroxidase deposits periodically arranged. x 40,000.
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[Image of a page with diagrams and text]

The document appears to discuss the organization of healthy human gingiva, although the specific content is not entirely clear due to the nature of the images and text. It seems to involve the study of collagen types I and III in the context of gingival tissue.

Collagen types I and III are mentioned, which are important components of connective tissue. The text hints at the use of these collagens in the context of gingival health and possibly the role of factors like basement membrane proteins.

The text also references other studies, indicating a comparative or integrative approach to understanding the tissue organization.

Overall, the document seems to be a scientific study or review focusing on the structural and functional aspects of healthy human gingiva, with a particular emphasis on collagen types I and III.
stability of such a dense connective tissue organization. In addition, it is noted that the ultrastructural fibrous feature of type I collagen is in agreement with the notion of stability ( Peyrol & Grimaud, 1981) while the fibrillar nature of type III collagen is in agreement with the notion of remodeling ability. Finally, appearance of types I and III collagen found in gingiva in this study by means of ultrastructural immunoperoxidase labelling correlates previous studies on other connective tissue: Lapierre, Nussens and Pierard (1977), Wick, Bjorn and Timpi (1978), Gay and Miller (1978), Harnisch et al. (1978), Grimaud et al. (1977, 1980), Fleischmajer et al. (1981), Magloire et al. (1982).

Collagen immunotyping represents a considerable advance in the attempt to distinguish different patterns of organization in healthy gingival connective tissue. Such methods have the potential to establish a correlation between morphological and biochemical remodeling abilities in normal and pathologic conditions of gingival corium.

Acknowledgements
The authors gratefully acknowledge Simone Peyrol and P. Ledger for critical comments and are indebted to D. Herbage for the generous gift of collagen antigens. This work was supported by CNRS (RCP 553).

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