Ultrastructural Immunohistochemical Study of Interstitial Collagenous Components of the Healthy Human Keratinized Mucosa Surrounding Implants

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The aim of this study was to investigate the ultrastructural and immunohistochemical organization of the main collagenous components of healthy human keratinized mucosa surrounding endosseous implants. Eight patients with completely edentulous mandibles were selected. Four endosseous implants were placed in the mandible of each patient, connected with a bar to support a complete overdenture, and loaded 4 months later. Two years after placement, biopsies of surrounding soft tissue, including the sulcular and junctional epithelium with the underlying and supracrestal connective tissue, were routinely prepared for standard electron microscopy and for ultrastructural immunolabeling of Types I, III, and IV collagen. The connective tissue located under the junctional epithelium comprised Types I and III collagen, whereas the supracrestal connective tissue was composed mainly of Type I collagen. Type IV collagen was located exclusively in the basement membrane of the junctional epithelium.
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The soft tissues surrounding successful endosseous implants are in many ways similar to those that surround the natural dentition.[1] As in the normal dentition, each implant is surrounded by a gingival sulcus, the apical part of which is lined by the cells of the junctional epithelium (JE) attached to the abutment via hemidesmosomes.[2]

Underlying the sulcular epithelium (SE) and the JE, the gingival connective tissue is in direct contact with the implant surface in the supracrestal area. This connective tissue is made up mainly of 2 interstitial fibrous collagen types (I and III), which account for 99% of the total extractable collagen, and of an amorphous collagen (Type IV collagen), which accounts for less than 1%.[3] Previous studies have shown that the distribution of these collagen types is very similar to that in periodontal and peri-implant keratinized mucosa.[4,5] The ultrastructural organization of these collagenous components in healthy human gingiva and in diseased gingiva surrounding the natural teeth has been previously described,[6–8] but it has never been studied in the soft tissue surrounding implants. The aim of this study was to observe the ultrastructural organization of these interstitial collagenous components in the connective tissue of the healthy keratinized mucosa surrounding endosseous implants.

Materials and Methods

Biopsies. Eight patients with completely edentulous mandibles (4 males and 4 females) aged 55 to 81 years were selected for this study. All patients were in good health, and each signed a consent form following explanation of the study and were given the option to withdraw at any time. Four Calcitek Omniloc implants (Sulzer Calcitek,

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Mountain View, CA) were placed in the anterior mandible and located 4 months later. The implants were connected with a gold alloy bar, and the complete mandibular overdenture was retained by 2 or 3 gold alloy clips. Following prosthetic restoration, all patients were recalled every 6 months. At each follow-up visit, the osseointegration of each implant was checked according to the criteria described by Albrektsson et al.[9] Marginal soft tissue reactions were evaluated by measuring the Plaque Index,[10] the Sulcus Bleeding Index,[11] and the presence or absence of soreness around the abutments. After 2 years, examination of 4 sites at each implant revealed the presence of plaque on 10 to 15% of the sites and the absence of bleeding on probing in 85% of the sites. The mean probing depth was 3 mm ± 1 mm. Biopsies from the attached keratinized mucosa surrounding the implants were obtained under local anesthesia. The biopsies, including oral, sulcular, and junctional epithelium with the underlying and supracrestal connective tissue, were cut into 2 blocks.

Standard Electron Microscopy. The first block of each biopsy was placed in a 2% glutaraldehyde, 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 2 hours at 4°C. After being washed, they were post fixed in 1% osmium tetroxide 0.1 mol/L sodium cacodylate buffer (pH 7.4) for 2 hours at 4°C. Dehydration was performed with ethanol, and samples were embedded in Epon 812. Ultrathin sections were contrasted with lead citrate and uranyl acetate and observed with a JEOL 1200 electron microscope (JEOL, Tokyo, Japan).

Immunolabeling for Electron Microscopy. For indirect immunolabeling using peroxidase, the second block of each biopsy was immediately fixed with a 4% paraformaldehyde, 0.1 mol/L phosphate buffer (pH 7.4) for 8 hours at 4°C; the blocks were then washed and frozen. Cryostat sections 10 µm thick were cut and treated with 0.3% hyaluronidase (bovine testis type I sigma) for 30 minutes at room temperature and with 0.1 mol/L sodium azide under the same conditions. Sections were washed and placed overnight in 0.07% bovine serum albumin at 4°C, then incubated in specific antisera to human gamma G immunoglobin for 5 hours at 4°C. After washing, the sections were incubated in the peroxidase-conjugated antisera. The bound peroxidase complexes were visualized by treatment with dimethylaminoazobenzene.[12] Sections were then fixed with 1% osmium tetroxide, dehydrated, and flat embedded in Epon (Cipek, Paris, France). Ultrathin sections were prepared and observed, with no further staining, using a JEOL 1200 electron microscope. Control sections were incubated in 0.1 mol/L phosphate buffer without immune serum and in peroxidase-conjugated antisera alone.

Results

Standard Electron Microscopy. The connective tissue surrounding implants and underlying the JE is made up of fibroblastic cells surrounded by dense collagenous extracellular matrix. Most of the fibroblasts are characterized by an enlarged rough endoplasmic reticulum associated with a large number of secretory granules. The surrounding extracellular matrix consists mainly of bundles of thick collagen fibers sectioned in both cross and longitudinal orientation (Fig 1). In some areas, underlying the basement membrane of the JE, the collagenous fibers are short, curved, and oriented in all directions (Fig 2). Sometimes, rare degranulated mast cells are observed in the extracellular matrix (Fig 3).

The supracrestal connective tissue contains few fibroblastic cells. No inflammatory cells are observed. The extracellular matrix is composed of large and dense bundles of thick collagen fibers oriented transversely and longitudinally.

Immunoperoxidase Electron Labeling. Immunolabeling of the collagenous components located under the JE clearly shows the presence of Types I and III collagen (Figs 4 and 5). The Type IV collagen is exclusively located in the lamina densa of the basement membrane separating the epithelium from the connective tissue (Fig 6). In the supracrestal connective tissue the dense bundles of thick collagen fibers are made up mainly of Type I collagen (Figs 7a and 7b).

Discussion

According to this study, the connective tissue surrounding implants can be divided into 2 parts: an upper part underlying the JE, and a lower part closely bound to the implant and composing the supracrestal connective tissue.

The upper part underlying the JE is relatively rich in fibroblastic cells characterized by a great number of secretory elements. These cells have the ability to synthesize most of the components of the extracellular matrix. This may reflect an important turnover of the connective tissue in this area. Furthermore, the particular feature of the collagen fibers in this area (short and curved collagen fibers oriented in all directions) associated with the presence of Type III collagen, which is an early type of collagen found

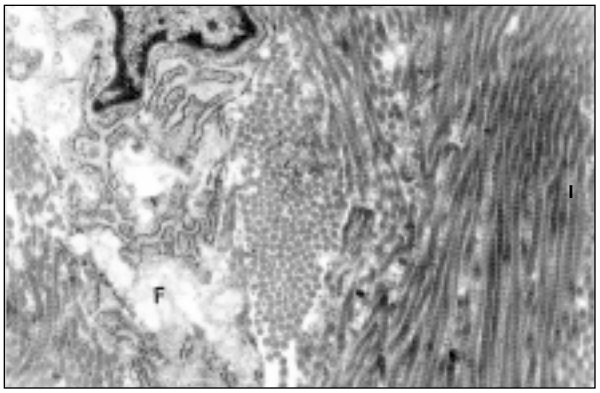

Fig 1 Fibroblast underlying the junctional epithelium, characterized by an enlarged rough endoplasmic reticulum associated with secretory granules. The extracellular matrix is composed of large, dense bundles of thick collagen fibers oriented transversely and longitudinally (uranyl acetate and lead citrate; magnification ×10,000). F = fibroblast; I = implant location.

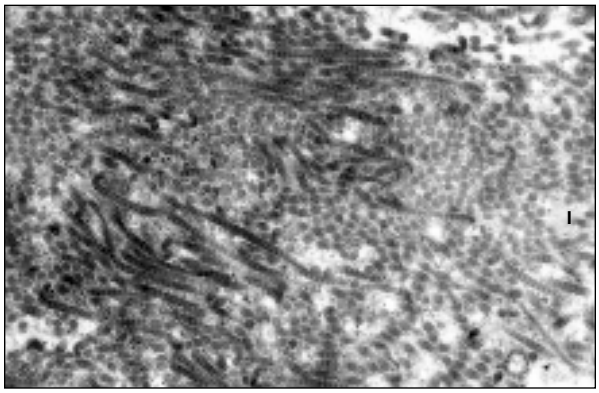

Fig 2 Extracellular collagenous matrix in the area underlying the junctional epithelium (magnification ×12,000). I = implant location.

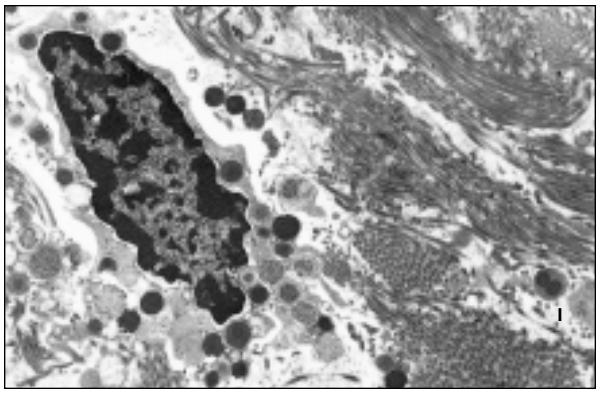

Fig 3 Degranulated mast cell in the area underlying the junctional epithelium (magnification ×7,500). I = implant location.

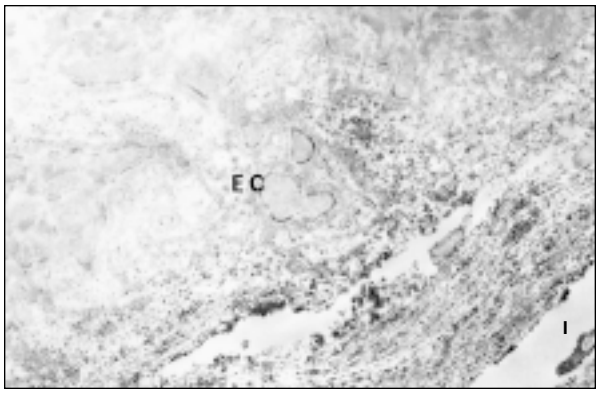

Fig 4 Immunolabeling of Type I collagen of the connective tissue underlying the junctional epithelium (magnification ×10,000). EC = epithelial cell; I = implant location.

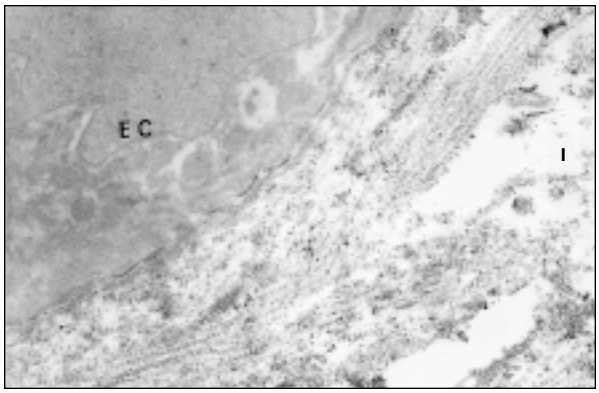

Fig 5 Immunolabeling of Type III collagen of the connective tissue underlying the junctional epithelium (magnification ×10,000). EC = epithelial cell; I = implant location.

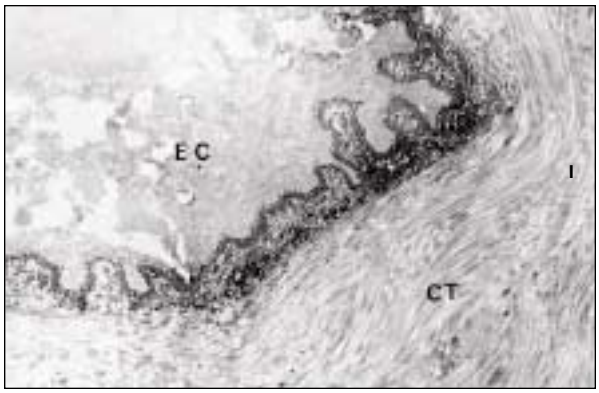

Fig 6 Immunolabeling of Type IV collagen in the lamina densa of the basement membrane of the junctional epithelium (magnification ×7,500). EC = epithelial cell; CT = connective tissue; I = implant location.

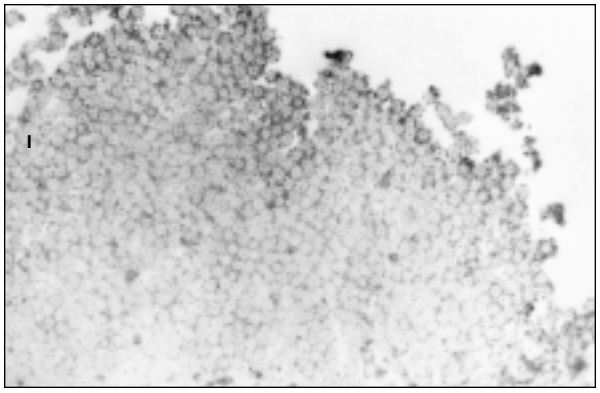

Fig 7a Immunolabeling of Type I collagen in a cross section of dense bundles of collagen fibers in supracrestal peri-implant connective tissue (magnification ×15,000). I = implant location.

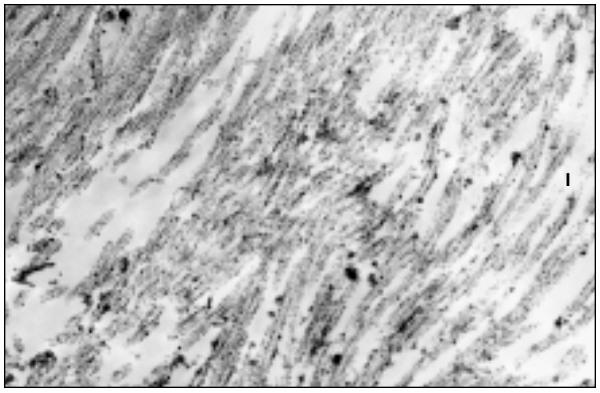

Fig 7b Immunolabeling of Type I collagen in a longitudinal section of bundles of collagen fibers in supracrestal peri-implant connective tissue (magnification ×12,000). I = implant location.

mainly in the initial stages of soft connective tissue wound healing,[13,14] underscores the important remodeling ability of peri-implant connective tissue underlying the JE. The presence in some sections of degranulated mast cells reflects an inflammatory response to bacterial aggression in this part of the peri-implant connective tissue. However, the well-defined lamina densa of the gingival basement membrane underlying the JE, characterized by Type IV collagen, reflects the good health of this tissue in spite of the weak inflammation.

The supracrestal connective tissue is poor in cells, and the extracellular matrix is organized mainly into large and dense bundles of thick Type I collagen fibers. This type of organization, similar to that of a scar,[15] increases the mechanical resistance of the tissue and is responsible for its stability.[16] It could be related to the role played by the peri-implant mucosa, which functions as a barrier at the transmucosal passage of the abutment to ensure implant success.[17–19]

Conclusion

The connective tissue surrounding successful endosseous implants can be divided into 2 parts:

1. The upper part, which is located under the JE, is rich in Types I and III collagen. It is an area of exchange where the transformation of collagen seems to be important.
2. The lower part, which is closely bound to the implant, represents the supracrestal connective tissue. It is rich in Type I collagen and adds mechanical resistance and stability to the peri-implant soft tissues.

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