Cellular Fibronectin in Failing Dental Implants

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*Purpose: Cellular fibronectin staining is decreased in adult periodontitis, which implies elastase-mediated degradation of periodontal tissues. The purpose of this study was to determine whether failing dental implants display similar changes. **Materials and Methods:** Cellular fibronectin and its integrin receptors were identified by immunohistochemistry and quantified by computerized image analysis. **Results:** Cellular fibronectin was found in blood vessel walls, epithelial basement membranes, and fibroblasts. Quantitative results of cellular fibronectin staining were as follows: failing dental implants, median 26.5% (Q3−Q1 = 23%); adult periodontitis, median 5.5% (Q3−Q1 = 5.6%); normal controls, median 12.2% (Q3−Q1 = 7.5%). Cellular fibronectin staining was increased around failing dental implants but decreased in adult periodontitis compared to healthy controls. **Discussion:** The distribution of integrin receptor subunits α4, α5, and β1 of cellular fibronectin was similar in failing dental implants. The pathomechanisms in adult periodontitis and failing dental implants seem to differ. **Conclusions:** Adult periodontitis is characterized by proteolysis/loss of cellular fibronectin, whereas failing dental implants are characterized by increased cellular fibronectin deposition, probably as a result of titanium-induced local synthesis and relatively modest degradation.* (Int J Oral Maxillofac Implants 2002;17:363–368)

Key words: *dental implants, fibronectin receptors, fibronectins, periodontitis*

The recent re-introduction of dental implants to clinical practice has been considered a milestone in modern dentistry. Dental implants are important in oral rehabilitation. The long-term failure rate is about 5% to 10%, with the main symptoms being increasing mobility and pain when chewing.[1–3] Late failure indicates loosening of an implant that was already osseointegrated, as a result of biomechanical overloading or peri-implantitis.[4] Fibronectin is an important component of the extracellular matrix of periodontium and might play a role in failing dental implants.[5] The periodontal pathogen *Actinobacillus actinomycetemcomitans* binds to fibronectin.[6] Changes in cellular fibronectin alter integrin-mediated matrix-cell signaling. Increased concentration of fibronectin fragments in gingival crevicular fluid reflects inflammation and tissue destruction in adult periodontitis.[7] Similar to adult periodontitis, failing dental implants are considered to be a result of progressive loss of peri-implant support. Therefore, it was hypothesized that the staining patterns of locally synthesized cellular fibronectin would be similar in adult periodontitis and in failing dental implants; thus these could indicate increased inflammation-induced degradation. Therefore, the distribution of cellular fibronectin and its α4β1 and α5β1 integrin receptors were examined in the present study.

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Table 1 Clinical Information

Group	Gender	Mean age (y) (range)	Procedure
Failing dental implants	7M, 3F	64.4 (59–77)	Failure of dental implants associated with pain, increased mobility, peri-implant bone loss
Adult periodontitis	6M, 4F	51.7 (27–71)	Flap-surgery treatment
Healthy	5M, 5F	51.1 (17–85)	Extraction of impacted third molar or end-stage caries

M = male; F = female.

MATERIALS AND METHODS

Gingival biopsies from 30 patients (Table 1) were frozen in liquid nitrogen, embedded in OCT (optimal cutting temperature) Tissue-Tek (Sakura, Zoeterwoude, Netherlands), and stored at –80°C. The protocol for collecting samples from human subjects was approved by the Ethical Committee of the Institute of Dentistry of the University of Helsinki, Finland. All subjects gave their informed consent.

Immunohistochemistry

Cryostat sections 6 µm thick were cut at a similar angle with respect to the epithelium. The primary mouse–anti-human antibodies used recognized cellular fibronectin (EDA-cellular fibronectin, 1:50 dilution)[8] and integrin α4 (A4-PUJI, 1:2000),[9] integrin α5 (SAM-1, 1:500),[10] or integrin β1 (102DF5, diluted 1:50) subunits.[11] The Vectastain Elite ABC Kit (Vector Laboratories, Burlingame, CA) was used for immunohistochemistry. Blocking was done using normal horse serum (1:50).

Quantitative Assessment

Using 400× magnification, the sections were inspected with a Leitz Diaplan microscope (Wetzlar, Germany). The connective tissue in each section was investigated from each sample. Images were stored in a computer supplied with a semiautomatic Analysis Pro 3.0 image analysis and processing system (Soft Analysis System, Münster, Germany) using a 12-bit PC digital image camera (SensiCam, Kelheim, Germany). The threshold for staining color was set and used for the rest of the analysis.

Statistics

The rank sum test in the statistical software package BMDP-PC 7.01 (BDMP Statistical Software, Los Angeles, CA) was applied to analyze the differences between group means.

RESULTS

Cellular Fibronectin

Cellular fibronectin staining patterns were similar at failing dental implants, adult periodontitis, and healthy samples, but there were clear-cut quantitative differences between the groups (Fig 1, Table 2). Moderate to strong cellular fibronectin staining was found around blood vessels and epithelial basement membrane, but there was no staining in the epithelium. Staining of the fibroblasts in the lamina propria was weak to moderate. Metal particles embedded in the extracellular matrix were often surrounded by a capsule containing cellular fibronectin. Percentages of positive cellular fibronectin staining were as follows: failing dental implants, median 26.5% (Q3–Q1 = 23%); adult periodontitis, median 5.5% (Q3–Q1 = 5.6%); and normal controls, median 12.2% (Q3–Q1 = 7.5%). Compared to controls (n = 10), cellular fibronectin was increased around failing dental implants ($P < .01$, n = 10), but decreased in adult periodontitis ($P < .05$, n = 10; rank sum test).

Distribution of α4, α5, and β1 Integrin Subunits

α4 Subunit. Keratinocytes were α4 negative, but some fibroblasts in the connective tissue stroma were moderately positive. Almost all postcapillary venules and capillary endothelial cells were negative or very weakly stained (Figs 2a and 2b).

Figs 1a to 1f Histologic sections showing the similarity of the distribution of cellular fibronectin in failing dental implants, adult periodontitis, and healthy controls.
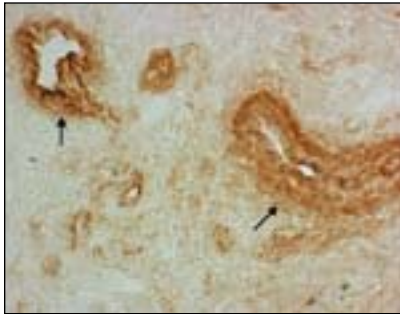

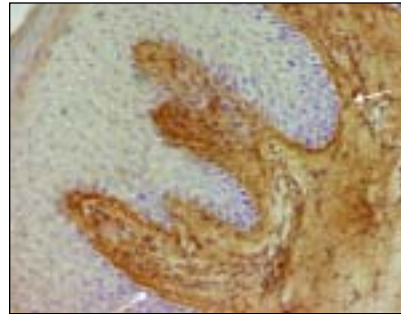**Fig 1a** Cellular fibronectin staining was moderate to strong in the blood vessel walls *(arrows)*.

Fig 1b The epithelial basement membrane *(arrows)* and connective tissue papillae were usually moderately to strongly positive, but the epithelium was negative.
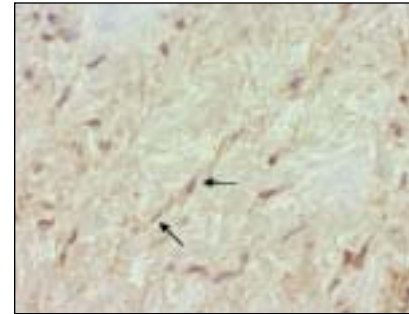

Fig 1c Fibroblasts in the lamina propria *(arrows)* were weakly to moderately positive.

Fig 1d A 15-μm metal particle in the connective tissue is surrounded by an approximately 6-μm-thick cellular fibronectin capsule *(black arrow)*. The other particle in this field, approximately 8 μm in diameter, is located in the epithelium and is not surrounded by cellular fibronectin *(white arrow)*.
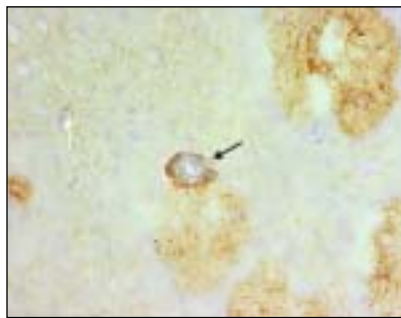
Fig 1e Staining controls with a monoclonal antibody and an irrelevant specificity, used at the same concentration as the primary antibody, were negative.
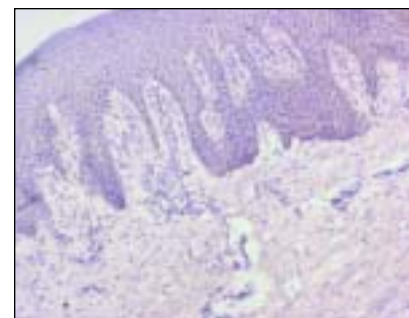

Fig 1f The results of the quantitative morphometric assessment are expressed as the percentage of cellular fibronectin (CFn) –positive area of connective tissue measured as follows: failing dental implants (FDIs), median 26.5% (Q3–Q1 = 23%); adult periodontitis (AP), median 5.5% (Q3–Q1 = 5.6%); and normal controls (Normal), median 12.2% (Q3–Q1 = 7.5%).
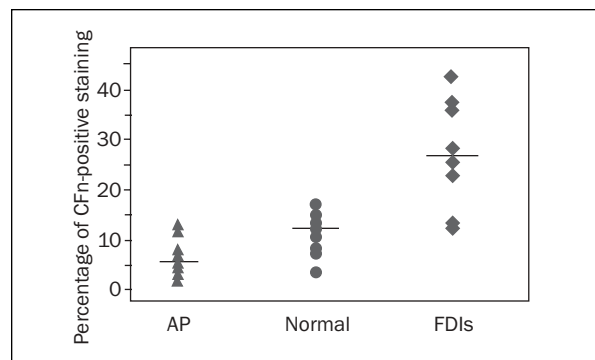

Table 2   Staining of Cellular Fibronectin and Its Integrin Receptors in Gingival Tissue

	Cellular fibronectin	α4 receptor	α5 receptor	β1 receptor
Oral epithelium				
Keratinized layer	Negative	Negative	Negative	Negative
Granular layer	Negative	Negative	Negative	Negative
Prickle cell layer	Negative	Negative	Negative	Moderate
Basal cell layer	Negative	Negative	Negative	Strong
Basement membrane	Moderate to strong	Negative	Negative	Negative
Connective tissue				
Fibroblasts	Weak to moderate	Moderate	Weak to moderate	Weak to moderate
Vascular edothelial cells	Moderate to strong	Negative to very weak	Moderate to strong	Strong

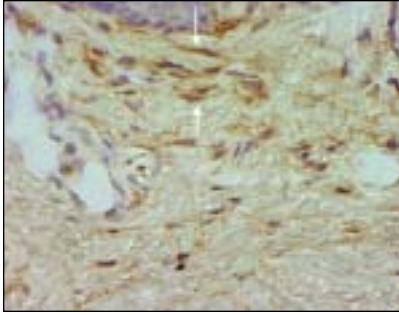**Figs 2a to 2g**   Distribution of integrin receptors for fibronectin.

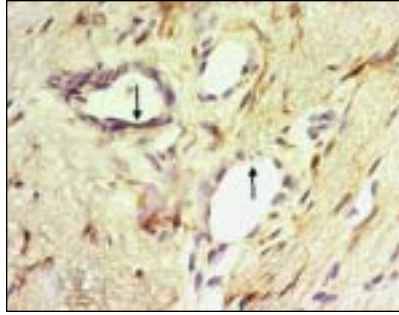**Fig 2a**   Some fibroblasts in the connective tissue stroma were stained moderately α4 positive (arrows).

Fig 2b   Almost all postcapillary venules and capillary endothelial cells were weakly positive or negative (arrows).

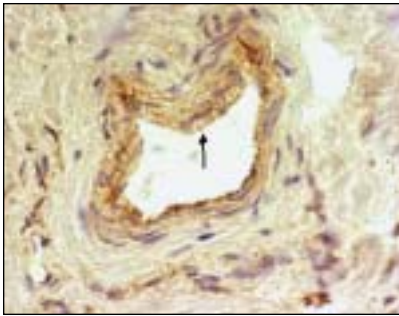**Fig 2c**   Moderate α5 integrin subunit immunoreactivity was found in the endothelium of capillary blood vessels and postcapillary venules (arrow) in connective tissue.
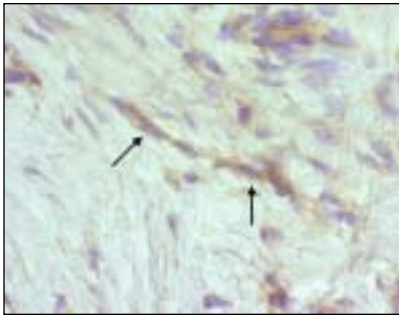

Fig 2d   Fibroblasts were weakly to moderately α5 positive (arrows).

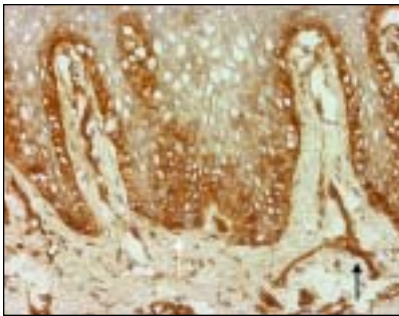**Fig 2e**   A strong β1 subunit staining was detected in vascular endothelium (black arrow). In the epithelium, the basal cell layer was strongly positive and prickle cell layer was moderately positive (white arrow).
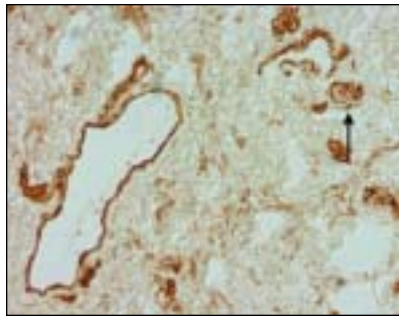

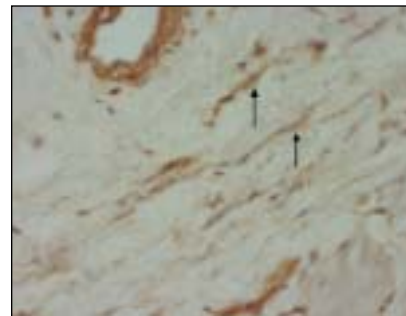**Fig 2f**   Strong β1 subunit staining was found in postcapillary venules in endothelial cells (arrow).

Fig 2g   Integrin β1 subunit staining of fibroblasts was weak to moderate (arrows).

α5 Subunit. Keratinocytes were α5 negative, but moderate to strong immunoreactivity was found in the endothelium of postcapillary venules and capillary blood vessels in the connective tissue. Fibroblasts were weakly to moderately positive (Figs 2c and 2d).

β1 Subunit. Cells in the basal and prickle cell layer were strongly or moderately positive, respectively, whereas the other epithelial layers were negative. Vascular endothelial cells were strongly positive. Fibroblasts in the lamina propria stained weakly or moderately (Figs 2e to 2g).

DISCUSSION

Human neutrophil elastase activity has been found to be increased in gingival crevicular fluid and saliva in adult periodontitis and to decrease after periodontal treatments.[12] Elastase is considered as a useful biochemical marker to monitor the course and treatment of adult periodontitis,[13] and it degrades fibronectin.[14] Elastase has also been considered to be the main proteolytic enzyme responsible for the degradation of fibronectin in skin wounds.[15] In this study, the extent of staining of cellular fibronectin in adult periodontitis was found to be low in areas where elastase was localized in adult periodontitis.[12] Decreased cellular fibronectin staining in adult periodontitis might be the result of increased elastase activity, which leads to the cleavage of fibronectin. This interpretation is supported by high levels of fibronectin fragments, which have been found in gingival crevicular fluid in untreated adult periodontitis patients.[7] Cellular fibronectin fragments, together with increased elastase activities in gingival crevicular fluid, might be useful markers of progressive connective tissue degradation in adult periodontitis.

In contrast to adult periodontitis, elastase activities are not up-regulated in peri-implant sulcus fluid or saliva in peri-implantitis.[16] Accordingly, in the present study cellular fibronectin staining was found to be intense in peri-implant samples around failing dental implants. Titanium induces cellular fibronectin expression in fibroblast cell culture.[17] According to the present study, this was also seen in peri-implant tissues around titanium implants and debris particles found embedded in the extracellular matrix. Strong cellular fibronectin staining around failing dental implants may be a result of increased local synthesis and relatively modest degradation. It is possible that the differences in the local disease activity could perhaps explain the differences observed between the study groups. However, this is unlikely, because the differences were opposite in peri-implantitis and in adult periodontitis.

Changes in extracellular matrix may reflect cellular history and initiate various metabolic cascades.[18] Integrin α5 and β1 subunits were detected in endothelial cells, apparently able to interact with extracellular fibronectin ligand. The α5β1 integrin receptor increases human neutrophil migration to inflammatory sites if cellular fibronectin is overexpressed in the blood vessel wall.[19] Integrin α4, α5, and β1 subunits were found in peri-implant fibroblasts. The α5β1 and α4β1 integrins regulate matrix metalloproteinase expression via adherence to fibronectin.[20]

CONCLUSIONS

The difference in cellular fibronectin staining between failing dental implants and adult periodontitis reflects differences in the pathomechanisms operative in failing dental implants and natural teeth. Although adult periodontitis and failing dental implants may superficially demonstrate very similar tissue-destructive processes, they seem to differ in this and probably in many other aspects as well.

ACKNOWLEDGMENTS

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