

# Journal of Dental Research

<http://jdr.sagepub.com>

---

## **Collagenases in Different Categories of Peri-implant Vertical Bone Loss**

J. Ma, U. Kitti, O. Teronen, T. Sorsa, V. Husa, P. Laine, H. Rönkä, T. Salo, C. Lindqvist and Y.T. Kontinen

*J DENT RES* 2000; 79; 1870

DOI: 10.1177/00220345000790110901

The online version of this article can be found at:  
<http://jdr.sagepub.com/cgi/content/abstract/79/11/1870>

---

Published by:



<http://www.sagepublications.com>

On behalf of:

International and American Associations for Dental Research

**Additional services and information for *Journal of Dental Research* can be found at:**

**Email Alerts:** <http://jdr.sagepub.com/cgi/alerts>

**Subscriptions:** <http://jdr.sagepub.com/subscriptions>

**Reprints:** <http://www.sagepub.com/journalsReprints.nav>

**Permissions:** <http://www.sagepub.com/journalsPermissions.nav>

**Citations** <http://jdr.sagepub.com/cgi/content/refs/79/11/1870>

J. Ma<sup>1,3</sup>, U. Kitti<sup>1,8</sup>, O. Teronen<sup>5</sup>,  
T. Sorsa<sup>3</sup>, V. Husa<sup>4</sup>, P. Laine<sup>5</sup>, H. Rönkä<sup>7</sup>, T.  
Salo<sup>8</sup>, C. Lindqvist<sup>5</sup>, and Y.T. Konttinen<sup>1,2,6\*</sup>

<sup>1</sup>Department of Anatomy, Institute of Biomedicine, PO Box 9, FIN-00014, University of Helsinki, Finland; <sup>2</sup>Department of Oral Medicine, Helsinki University Hospital;

<sup>3</sup>Departments of Periodontology, <sup>4</sup>Prosthetics, <sup>5</sup>Surgery, and <sup>6</sup>Oral Medicine, Institute of Dentistry, University of Helsinki;

<sup>7</sup>Medix Biochemica Oy Ab, Kauniainen, Finland;

and <sup>8</sup>Department of Pathology, University of Oulu, Finland;

\*corresponding author, yrjo.konttinen@helsinki.fi

J Dent Res 79(11):1870-1873, 2000

## ABSTRACT

The loosening of dental implants is associated with peri-implant vertical bone loss. The mechanisms and mediators of this bone destruction are not known. To test the hypothesis that collagenase-2 and collagenase-3 might be markers or maybe even mediators in this process, we measured collagenase-2 (time-resolved immunofluorometric assay) and collagenase-3 (quantitative immunoblot) in peri-implant sulcus fluid in 49 implant sites in 13 patients. Vertical bone loss was graded as being < 1 mm, from 1 to 3 mm, or > 3 mm. The severity of inflammation, as rated according to Gingival Index, did not correlate with the category of bone loss ( $p > 0.05$ ). Collagenase-2 and collagenase-3 were higher ( $p < 0.05$ ) in the group which had lost > 3 mm of bone than in the two other groups. Gingival Index is not a clinically important marker for bone loss, but collagenase-2 and collagenase-3 in peri-implant sulcus fluid are. They might participate in peri-implant osteolysis.

**KEY WORDS:** collagenase, gingival index, peri-implant vertical bone loss.

# Collagenases in Different Categories of Peri-implant Vertical Bone Loss

## INTRODUCTION

Dental implants have been accepted as a method of restoring both function and esthetics of teeth. Long-term survival rates of dental implants have been satisfactory: 88% for maxillary and 93% for mandibular teeth (O'Roark, 1997). However, some implants undergo loosening and eventually are completely lost. Especially in elderly patients, general and local contraindications may restrict the possibilities for re-implantation. It would be beneficial if dental implants could last for a lifetime.

Loosening may be defined as a result of an adverse biological host response combined with cyclic occlusal loading and oral bacterial pathogens (Gross, 1988; Swanberg and Henry, 1995; Leonhardt *et al.*, 1999). Clinically, we defined loosening as increasing mobility of the dental implant associated with pain. The Gingival Index makes it possible to assess the quality (the severity of the lesion) and the location (quantity) of inflammation as related to the four (buccal, mesial, distal, and lingual) areas surrounding teeth or implants (Löe, 1967) and might be useful in the assessment of the degree of peri-implant vertical bone loss. Furthermore, collagenase-1, collagenase-2, and collagenase-3 degrade the triple helical structures of native types I, II, and III collagens, which are the main components of alveolar bone matrix (Batge *et al.*, 1992; Delaisse and Veas, 1992). Collagenase-2 activity has been found to be high in peri-implant sulcus fluid around clinically loosening implants (Teronen *et al.*, 1997). Golub and his co-workers have suggested that collagenase-3 may even reflect bone-type collagen degradation in adult periodontitis (Golub *et al.*, 1997). Therefore, the aim of this study was to investigate if the presence of the two collagenases, as measured in peri-implant sulcus fluid, correlates with the amount of bone lost around the implant. At the same time, the usefulness of the Gingival Index for this purpose was also assessed.

## MATERIALS & METHODS

Forty-nine randomly selected dental implant sites in 13 patients were studied (Tables 1, 2). All patients had regular maintenance history. No systemic diseases were found. Only one implant was found to have failed. No other implants showed increasing mobility because of fixed prostheses and overdenture treatments. Implants were grouped into three categories according to the amount of bone loss in the vertical dimension: < 1 mm, from 1 to 3 mm, or > 3 mm (Jeffcoat *et al.*, 1995). We determined the distance of the fixture and alveolar crest using the threads of the fixture of the inserted dental implant as an internal dimensional reference (Strid, 1985).

The four gingival areas of each implant were scored from 0 to 3 as follows: 0 = Normal gingiva; 1 = Mild inflammation, slight change in color and edema, no bleeding on probing; 2 = Moderate inflammation, redness, edema and glazing, bleeding on probing; and 3 = Severe inflammation, marked redness, edema and

ulceration, tendency to spontaneous bleeding. These scores were used to calculate the Gingival Index (Löe, 1967).

Peri-implant sulcus fluid samples, one sample *per* implant, were collected with a filter strip placed into the sulcus for 4 min. The absorbed fluid was diluted in 50 µL Tris-based buffer and centrifuged. The supernatants were stored at -20°C (Ingman *et al.*, 1994). The protocol for the collection of human samples was approved by the ethical committee of the Institute of Dentistry, University of Helsinki, Finland. All subjects gave their informed consent.

**Collagenase-2 Immunofluorometric Assay**

A catching monoclonal antibody MoAb 8708 (Medix Biochemica, Kauniainen, Finland), which recognizes only the activated collagenase-2, was used to coat microtitration wells. The peri-implant sulcus fluid samples were incubated in the coated wells for 1 hr with assay buffer (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 5 mM CaCl<sub>2</sub>, 50 µM ZnCl<sub>2</sub>, 0.5% bovine serum albumin) followed by a wash and incubation with a monoclonal tracer antibody MoAb 8706 (Medix Biochemica) for 1 hr. A 100-µL quantity of enhancement solution was added (Wallac, Turku, Finland), and we determined collagenase-2 activity levels (ng/mL) by measuring fluorescence after 5 min in a 123 Delfia Research Fluorometer (Wallac, Turku, Finland) (Hanemaaijer *et al.*, 1997).

**Quantitative Collagenase-3 Immunoblot**

Two-µg-protein peri-implant sulcus fluid samples were heated for 5 min at 100°C with Laemmli's buffer, separated on 7.5% SDS-PAGE, and electrophoretically transferred to a nitrocellulose membrane (Bio-Rad). Non-specific binding was blocked by 3% gelatin in Tris-HCl, pH 8.0, 22 mM NaCl, 0.05% Tween (TST) for 1 hr at 37°C. The membrane was incubated with polyclonal rabbit anti-human collagenase-3 antibody (0.5 mg/mL) for 6 hrs. After repeated washes in TST, the membrane was incubated with biotinylated anti-rabbit immunoglobulins (1:100) for 1 hr. After washes, the color was developed by nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Sigma Chemical Co., St. Louis, MO, USA). The secondary antibody alone did not recognize the band detected by collagenase-3 (Teronen *et al.*, 1997). Known concentrations of collagenase-3 antigen were used as positive controls and standards for quantification. The collagenase-3 antibody was kindly prepared and provided by Prof. Carlos López-Otín, Department of Biochemistry,

**Table 1. Implant Features**

Patients	Location	Length of Implant (mm)	Treatments	Peri-implant Vertical Bone Loss <sup>a</sup>
1	43 42 32 33	13 13 13 13	Removable overdenture	II III III III
2	15 13 12	13 13 8	Fixed prosthesis	I II III
3	43 42 32 33	13 13 13 13	Removable overdenture	II II I I
4	41 31 33	15 15 13	Removable overdenture	I I I
5	45 44 42 31 35	13 13 11 11 13	Fixed prosthesis	II II I I I
6	42 41 31 36 37	13 13 13 6 6	Fixed prosthesis	I I I II I
7	35 36 37	15 15 15	Fixed prosthesis	I I I
8	46 45 44 42 41 33	9 11 11 12 12 11	Fixed prosthesis	III I I I I I
9	45 44 41 31 32	18 15 10 14 14	Fixed prosthesis	I I II I I
10	14 12 22 24	12 12 12 14	Removable overdenture	III II I I
11	42	13	Removable overdenture	I
12	13 11 22	15 11 11	Removable overdenture	II II I
13	12 11 21	13 18 18	Fixed prosthesis	I I I

<sup>a</sup> I = < 1 mm, II = 1-3 mm, III = > 3 mm.

University of Oviedo, Spain (Freije *et al.*, 1994). Immunoblots were quantitated by image densitometer (Bio-Rad Model G5-700, Richmond, CA, USA) and the Molecular Analyst Program (Image analysis system version 1.4) for the determination of collagenase-3 levels (ng/mL).

**Statistics**

Statistical calculations were made by means of SPSS 7.5.2 for Windows software (SPSS Inc, Chicago, IL, USA). We used Pearson's correlation test to assess relationships and the Kruskal-Wallis test to evaluate differences between groups.

**RESULTS**

The Gingival Index was slightly higher in the group with from 1 to 3 mm of peri-implant vertical bone loss than in the group with < 1 mm of bone loss, and it was highest in the group with > 3 mm of bone loss. None of these differences was significant (*p* > 0.05) (Fig., panel A).

Collagenase-2 and collagenase-3 levels were significantly higher in the group with > 3 mm of bone loss (collagenase-2, *p* = 0.049; collagenase-3, *p* = 0.041) than in those groups that had lost less bone (Fig., panels B, C). The high standard deviations are due to both high dispersion of collagenase-2 and collagenase-3 values and the low number of implants in the category with > 3 mm of peri-implant vertical bone loss. There

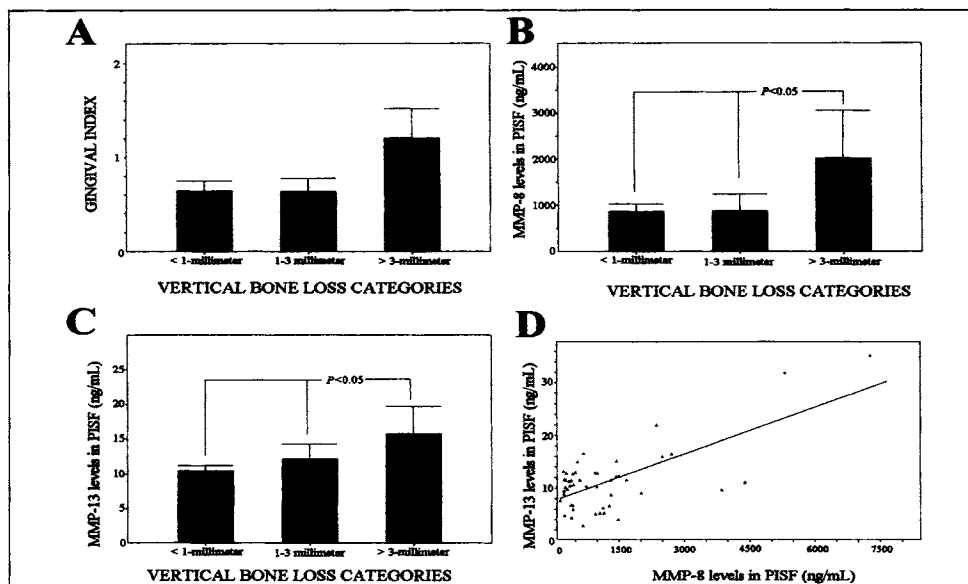
**1870Table 2. Patient Characteristics**

Categories	N <sup>a</sup>	Gender (M/F)	Age Mean (range)	Dentulous/Edentulous	Unloading Time (mos)	Implant Type <sup>b</sup>
< 1 mm PVBL <sup>c</sup>	32	7/4	48 (23-89)	26/6	4.9 (0-7)	13A; 15B; 4S
1-3 mm PVBL	11	4/3	57 (39-77)	8/3	6.7 (5.5-11)	8A; 2B; 1S
> 3 mm PVBL	6	1/3	67 (52-77)	6/0	8.7 (0-11)	5A; 1S

<sup>a</sup> N = Number of implants.

<sup>b</sup> A = Astra implant, B = Bränemark implant, S = Straumann Bonelit implant.

<sup>c</sup> Peri-implant vertical bone loss.



**Figure.** (A) The Gingival Index results did not show differences among different categories of peri-implant vertical bone loss ( $0.6 \pm 0.1$  in patients with < 1 mm,  $0.6 \pm 0.5$  in patients with 1-3 mm, and  $1.2 \pm 0.3$  in patients with > 3 mm bone loss,  $p > 0.05$ , Kruskal-Wallis test). (B) Collagenase-2 ( $2021 \pm 1038$ ,  $p < 0.05$ ) and (C) collagenase-3 ( $16 \pm 4$ ,  $p < 0.05$ ) in peri-implant sulcus fluid were higher in the group which had lost > 3 mm bone ( $n = 6$ ) compared with the two other groups, which had lost either < 1 mm ( $n = 32$ ,  $861 \pm 164$  and  $10 \pm 1$ , respectively) or from 1 to 3 mm ( $n = 11$ ,  $1265 \pm 508$  and  $12 \pm 2$ , respectively) bone (Kruskal-Wallis test). Panel D shows a linear relationship between collagenase-2 and collagenase-3 in peri-implant sulcus fluid ( $n = 49$ ,  $r = 0.69$ ,  $p < 0.005$ , Pearson's correlation test).

was also a significant linear correlation between collagenase-2 and collagenase-3 site-specific levels ( $r = 0.692$ ,  $p < 0.005$ ) (Fig., panel D).

## DISCUSSION

In trying to develop a means of predicting the extent of the peri-implant vertical bone loss, we first focused on peri-implantitis, which has traditionally been regarded as an etiologic factor. The Gingival Index is used to describe the clinical severity of inflammation in soft tissues surrounding the implant (Ciancio, 1986). It has been taken for granted that the higher the Gingival Index, the more severe is the extent of peri-implant vertical bone loss. However, our results show that the Gingival Index findings do not correlate with the extent of irreversible bone loss. Peri-implant vertical bone loss does not seem to be reflected by the severity of peri-implant mucosal inflammation. Even if the patient has a very low Gingival Index, dentists should pay attention to the possibility of underlying bone loss around the implants.

Activated collagenase-2 has been shown to be present in gingival crevicular fluid in adult periodontitis (Ingman *et al.*, 1996). Collagenase-2 seems to originate mainly from extravasated and degranulated neutrophils. Non-neutrophil-lineage mesenchymal cells, such as human gingival and periodontal ligament fibroblasts and chondrocytes, may also produce collagenase-2 (Chubinskaya *et al.*, 1996; Hanemaaijer *et al.*, 1997). Similarly, higher collagenase-2 levels in peri-implant sulcus fluid have been detected in loosening dental implants than in well-fixed implants (Teronen *et al.*, 1997). In our study, collagenase-2 levels were high in the high-bone-loss group. Collagenase-2 may play a

pathological role in peri-implant bone loss, which has been found to be the case in adult periodontitis (Golub *et al.*, 1997). Peri-implantitis seems to share this feature with adult periodontitis.

Collagenase-3 is most active against type II collagen substrate (Billinghurst *et al.*, 1997), whereas collagenase-2 prefers type I collagen (Hasty *et al.*, 1987). Increased expression of collagenase-3 has been considered to contribute to loosening of hip implants (Imai *et al.*, 1998). Elevated collagenase-3 activity in gingival crevicular fluid seems to increase bone-type collagen degradation in adult periodontitis (Golub *et al.*, 1997). In our present study, collagenase-3 levels were high in those implants which had lost > 3 mm of peri-implant bone in the vertical dimension. Collagenase-3 may also play a role in peri-implant bone loss. Furthermore, scatter plots of collagenase-3 vs. collagenase-2 showed a linear relationship. It seems that both may serve as markers of the category of peri-implant vertical bone loss.

We conclude that collagenase-3 and collagenase-2, produced by adjacent bone osteoclast cells, neutrophils, and mesenchymal cells, reflect irreversible peri-implant vertical bone loss around loosening dental implants. Measurements of collagenase-3 and/or collagenase-2 could be used as markers to indicate the degree of peri-implant vertical bone loss. These findings also offer new insights into the pathomechanisms that contribute to the loosening of dental implants.

## ACKNOWLEDGMENTS

This study was supported by grants from the Finnish Dental Society and the Academy of Finland and by an EVO Clinical Research Grant (TKIO 601).

## REFERENCES

- Batge B, Diebold J, Stein H, Bodo M, Muller PK (1992). Compositional analysis of the collagenous bone matrix. A study on adult normal and osteopenic bone tissue. *Eur J Clin Invest* 22:805-812.
- Billinghurst RC, Dahlberg L, Ionescu M, Reiner A, Bourne R, Rorabeck C, *et al.* (1997). Enhanced cleavage of type II collagen by collagenases in osteoarthritis articular cartilage. *J Clin Invest* 99:1534-1545.
- Chubinskaya S, Huch K, Mikecz K, Cs-Szabo G, Hasty KA, Kuettner KE, *et al.* (1996). Chondrocyte matrix metalloproteinase-8: up-regulation of neutrophil collagenase by interleukin-1 beta in human cartilage from knee and ankle

- joints. *Lab Invest* 74:232-240.
- Ciancio SG (1986). Current status of indices of gingivitis. *J Clin Periodontol* 13:375-378, 381-382.
- Delaisse JM, Veas G (1992). Mechanism of mineral solubilisation and matrix degradation in osteoclastic bone resorption. In: *Biology and physiology of the osteoclast*. Rifkin BR, Gay C, editors. Boca Raton, FL: CRC Press, pp. 290-314.
- Freije JM, Diez-Itza I, Balbin M, Sanchez LM, Blasco R, Tolivia J, *et al.* (1994). Molecular cloning and expression of collagenase-3, a novel human matrix metalloproteinase produced by breast carcinomas. *J Biol Chem* 269:16766-16773.
- Golub LM, Lee HM, Greenwald RA, Ryan ME, Sorsa T, Salo T, *et al.* (1997). A matrix metalloproteinase inhibitor reduces bone-type collagen degradation fragments and specific collagenases in gingival crevicular fluid during adult periodontitis. *Inflamm Res* 46:310-319.
- Gross UM (1988). Biocompatibility-the interaction of biomaterials and host response. *J Dent Educ* 52:798-803.
- Hanemaaijer R, Sorsa T, Kontinen YT, Ding Y, Sutinen M, Visser H, *et al.* (1997). Matrix metalloproteinase-8 is expressed in rheumatoid synovial fibroblasts and endothelial cell. *J Biol Chem* 272:31504-31509.
- Hasty KA, Jeffrey JJ, Hibbs MS, Welgus HG (1987). The collagen substrate specificity of human neutrophil collagenase. *J Biol Chem* 262:10048-10052.
- Imai S, Kontinen YT, Jumppanen M, Lindy O, Ceponis A, Kemppinen P, *et al.* (1998). High levels of expression of collagenase-3 (MMP-13) in pathological conditions associated with a foreign-body reaction. *J Bone Joint Surg Br* 80:701-710.
- Ingman T, Könönen M, Kontinen YT, Siirilä HS, Suomalainen K, Sorsa T (1994). Collagenase, gelatinase and elastase activities in sulcular fluid of osseointegrated implants and natural teeth. *J Clin Periodontol* 21:301-307.
- Ingman T, Tervahartiala T, Ding Y, Tschesche H, Haerian A, Kinane DF, *et al.* (1996). Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *J Clin Periodontol* 23:1127-1132.
- Jeffcoat MK, Wang IC, Reddy MS (1995). Radiographic diagnosis in periodontics. *Periodontol* 2000 7:54-68.
- Leonhardt A, Renvert S, Dahlén G (1999). Microbial findings at failing implants. *Clin Oral Implants Res* 10:339-345.
- Löe H (1967). The gingival index, the plaque index and retention index systems. *J Periodontol* 38:610-612.
- O'Roark WL (1997). Survival rate of dental implants: an individual practitioner's anecdotal review of 25 years of experience. *J Oral Implantol* 23:90-103.
- Strid K (1985). Radiographic results. In: *Tissue-integrated prostheses, osseointegration in clinical dentistry*. Brånemark P-I, Zarb GA, Albrektsson T, editors. Chicago: Quintessence Publishing Company, pp. 187-198.
- Swanberg DF, Henry MD (1995). Avoiding implant overload. *Implant Soc* 6:12-14.
- Teronen O, Kontinen YT, Lindqvist C, Salo T, Ingman T, Lauhio A, *et al.* (1997). Human neutrophil collagenase MMP-8 in peri-implant sulcus fluid and its inhibition by clodronate. *J Dent Res* 76:1529-1537.