Collagenase activity and protein content of sulcular fluid after scaling and occlusal adjustment of teeth with deep periodontal pockets


Three teeth from each of 14 adult patients with advanced periodontitis were included in this study. The Test Tooth was an incisor or canine with increased mobility associated with an occlusal interference and a $\geq 5$ mm deep pathologic pocket. The Infected Control Tooth was a non-mobile incisor or canine with a $\geq 5$ mm pocket. A non-mobile incisor or canine with pockets $\leq 3$ mm served as the Healthy Control Tooth. At least 7 d prior to Day 0 the patients were taught an effective oral hygiene regimen and received a supragingival prophylaxis. At Day 0, sulcular fluid for assay of protein content and collagenase activity was collected from each chosen pocket. Immediately thereafter the Test Teeth of 7 subjects were scaled and root planed and the Test Teeth of 7 subjects occlusally adjusted. At Day 14, the treatments were reversed for the two groups. Sulcular fluid for the assays was again collected at Days 14 and 28. The protein content and collagenase activity in deep pockets was elevated during periodontitis in both mobile and immobile teeth. After establishment of a supragingivally clean oral environment, a rapid decrease of the collagenase activity took place following scaling and root planing of the root surfaces within the periodontal pockets ($p<0.05$). Also, occlusal adjustment of the hypermobile teeth with deep pathological pockets reduced the protein content and collagenase activity in sulcular fluid ($p<0.02$). There was a further reduction of collagenase activity when occlusally adjusted teeth were scaled and root planed ($p<0.02$). No change in the protein content or collagenase activity was observed in the deep pockets of the untreated Control Teeth in the same patients.

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Introduction

Collagenase activity has been detected in sulcular fluid by several groups. The enzymatic cleavage has been shown to result in 3/4 and 1/4 fragments of the original native collagen molecule characteristic of vertebrate collagenase (1, 2). A high correlation has been found between the collagenolytic activity of sulcular fluid and the gingival pocket probing depth (3). The collagenolytic activity in sulcular fluid increases with the degree of clinically measured periodontal inflammation (4, 5), and a higher collagenolytic activity has been demonstrated in clearly inflamed gingivae than in clinically noninflamed gingivae (6, 7). In experimental periodontitis in dogs collagenolytic activity in sulcular fluid increased, although not at an even rate (8). This fluctuation in collagenase activity was suggested to reflect the alternation between destructive and quiescent phases in the progression of the periodontal disease. In humans monitored during treatment of juvenile periodontitis, collagenase activity was higher around diseased teeth than in healthy control teeth in the same mouth and decreased following clinical treatment (9).

Despite intensive investigation, the role of occlusal adjustment in the treatment of marginal periodontitis is still not clear. Although the importance of bacteria as a causal factor of inflammation is evident (10-13), the impression still remains among clinicians that elimination of occlusal interferences speeds up the healing of the periodontium. To clarify this question we earlier measured the quantity of sulcular fluid flow around occlusally adjusted teeth and around scaled and root planed teeth both before and after each treatment (14). The results of that study suggested that occlusal interferences have no effect on the quantity of sulcular fluid flow, and it was suggested that the presence of occlusal trauma would
The mean age of the patients was 49 yr (range 35–65 yr), and all had localized, advanced periodontitis. Some of the patients were treated at the Department of Periodontology, University of Helsinki, the remainder in the principal investigator's (KH) private practice. The general health of the patients was normal for their age. None of the patients was diabetic or pregnant. None had received antibiotics during the previous 5 months.

After the initial periodontal examination, 3 teeth per patient were assigned for the experiment, according to the following criteria:

1) The mobile and infected Test Tooth: an incisor or canine tooth with increased mobility associated with an occlusal interference either in centric occlusion, protrusion, or during lateral excursions, and with and infected, ≥5 mm deep periodontal pocket.

2) The Infected Control Tooth: a non-mobile incisor or canine with an infected, ≥5 mm deep periodontal pocket.

3) The Healthy Control Tooth: a non-mobile incisor or canine without pathologic periodontal pockets >3 mm.

After the initial examination, each patient received a supragingival prophylaxis and was taught an effective oral hygiene regimen. The thorough oral hygiene education was given at least 7 d prior to Day 0.

Day 0 (baseline)

The following parameters were recorded for the 3 Test or Control teeth of each subject, always by the same examiner (KH).

1) Probing depth. The distance from the gingival margin to the depth of the pocket was measured to the nearest millimeter using a periodontal probe.

2) Gingival Index according to Löe & Silness (15).

3) Mobility score. The mobility score was recorded according to the method described by Lindhe (16), degree 1 denoting 0.2–1 mm horizontal mobility of the crown of the tooth and degree 2 denoting a horizontal mobility exceeding 1 mm.

4) Sulcular fluid was collected from the Test Tooth and the 2 Control Teeth. The teeth were isolated with cotton rolls and gently dried with a stream of air. A Munktell No. 3 filter paper strip, 1 mm wide and with rounded edges (17) was inserted into the pocket until resistance was felt, and was left in place for 5 min for fluid collection. Immediately after collection of the fluid the filter paper strips were frozen and stored at −20°C for no more than 2 months.

After baseline recordings, patients were alternately assigned to Group A and Group B, 7 patients in each group. In Group A, the tooth with both an infected periodontal pocket and increased mobility (Test Tooth) was meticulously scaled and root planed under local anesthesia at Day 0. In Group B, the corresponding Test Tooth was adjusted occlusally by grinding. The adjustment included elimination of all excessive occlusal contacts in centric occlusion, protrusion, and during the lateral excursions.

The Infected Control Teeth and the Healthy Control Teeth were not instrumented in any way. Meticulous personal oral hygiene measures were maintained throughout the study.

Day 14

Collection of sulcular fluid from the 3 Test and Control Teeth. Occlusal adjustment of the Test Teeth in Group A and scaling and root planing of the Test Teeth in Group B.

Day 28

Collection of sulcular fluid and recording of the Gingival Index and mobility score from the 3 Test and Control Teeth of each subject, as at Day 0. Probing depths were not recorded.

Collagenase assay

The filter paper strips were eluted with 300 µl of 0.05 M Tris-HCl-buffer, pH 7.8, containing 0.2 M NaCl, 0.005 M CaCl₂, 0.01% Brij 35 and 0.001 M sodium azide: 100 µl of the solution was then incubated with 1H-proline-labeled soluble type I chick embryo tendon collagen (20 000 dpm), for 6 h at 28°C (2). The incubation was performed in the presence of 1 mM aminophenyl mercuric acetate, which is known to activate the latent forms of human collagenase. The method therefore measures the total collagenase content (active plus latent) in the sulcular fluid. After incubation, the reaction was stopped by heating with 2% sodium dodecyl sulphate. The samples were then subjected to polyacrylamide gel electrophoresis under denaturing and reducing con-
The clinical data for the 3 groups of Test and Control Teeth in the study at Days 0 and 28. After the measurements at Day 0, the teeth of Group A were scaled and root planed and, after 14 d, occlusally adjusted. In Group B the sequence of treatment was the reverse. The Control Teeth were not treated. Probing depths were recorded only at Day 0.

Table 1. The clinical data for the 3 groups of Test and Control Teeth in the study at Days 0 and 28. After the measurements at Day 0, the teeth of Group A were scaled and root planed and, after 14 d, occlusally adjusted. In Group B the sequence of treatment was the reverse. The Control Teeth were not treated. Probing depths were recorded only at Day 0.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Probing depth (mm)</td>
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<tr>
<td></td>
<td>Day 0</td>
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<tr>
<td>Test Teeth</td>
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<tr>
<td>Group A N = 7</td>
<td>8.0 ± 0.6</td>
</tr>
<tr>
<td>Group B N = 7</td>
<td>8.1 ± 0.6</td>
</tr>
<tr>
<td>Infected Control Teeth</td>
<td></td>
</tr>
<tr>
<td>Group A N = 7</td>
<td>6.3 ± 0.4</td>
</tr>
<tr>
<td>Group B N = 7</td>
<td>7.0 ± 0.5</td>
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<tr>
<td>Healthy Control Teeth</td>
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<td>Group A N = 7</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Group B N = 7</td>
<td>1.8 ± 0.1</td>
</tr>
</tbody>
</table>

Statistical comparison:
- **Probing depth at Day 0:** no difference between Groups A of the Test and the Infected Control Teeth, nor between Groups B of the Test and the Infected Control Teeth (paired t-test; p > 0.05). No difference between Groups A and B of the Test, of the Infected Control or the Healthy Control Teeth (Student’s t-test; p > 0.05).
- **Gingival Index at Days 0 and 28:** no differences between Groups A of the Test and the Infected Control Teeth, nor between Groups B of the Test and the Infected Control Teeth (paired t-test; p > 0.05). No differences between Groups A and B in the Test, Infected Control or Healthy Control Teeth (Student’s t-test; p > 0.05).
- **Mobility score at Days 0 and 28:** no difference between Groups A and B of the Test Teeth (Student’s t-test; p > 0.05).

**From Day 0 to Day 28:** no change in Gingival Index of the Test, Infected Control or Healthy Control Teeth. No change in the mobility score of the Test Teeth (p > 0.05).

The Test Teeth were compared to the Control Teeth of the same individual and thus the teeth could not be considered to be independent. Also, because of the small sample size in which a normal distribution could not be presumed, non-parametric tests were used to test the significances of the differences within Groups A and B. Thus, the effect of treatment on the protein content and the collagenase activity of the sulcular fluid at Day 28 was assessed using Friedman’s two-way analysis of variance. The location of the significant difference during Day 0, 14 and 28 was tested using Wilcoxon’s signed-rank test. The same procedure was applied when testing the difference of protein content or collagenase activity at Day 0 within Groups A and B. For the clinical parameters, the paired t-test was used to test the difference between means of dependent groups and Student’s t-test was used for independent groups.

The study conforms to the guidelines of the Helsinki Declaration 1975.

**Results**

**Clinical parameters (Table 1)**

- **Probing depth.** At Day 0 no difference was found between Groups A of the Test and the Infected Control Teeth, nor between Groups B of the Test and the Infected Control Teeth. There was no difference between Groups A and B within the groups of the Test Teeth and the Infected Control Teeth or the Healthy Control Teeth (p > 0.05). **Gingival Index.** At Days 0 and 28 there was no difference between Groups A of the Test and the Infected Control Teeth, nor between Groups B of the Test and the Infected Control Teeth. Neither at Day 0 nor at Day 28 was there a difference between Groups A and B of the Test, Infected Control or Healthy Control Teeth (p > 0.05). **Mobility score.** At Days 0 and 28 the scores did not differ between Groups A and B of the Test Teeth (p > 0.05).

From Day 0 to Day 28 no change in the mean Gingival Index scores of the Test Teeth, Infected Control Teeth or Healthy Control Teeth was observed (p > 0.05). The same was found for the mobility scores of the Test Teeth.
Table 2. Protein content and collagenase activity in sulcular fluid collected for 5 min from deep periodontal pockets of hypermobile teeth. The Test Teeth in Group A were scaled and root planed at Day 0 and occlusally adjusted at Day 14. In Group B the sequence of treatment was the reverse.

<table>
<thead>
<tr>
<th></th>
<th>Test Teeth</th>
<th>Infected Control Teeth</th>
<th>Healthy Control Teeth</th>
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<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 14</td>
<td>Day 14</td>
</tr>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td></td>
<td>Protein content (µg)</td>
<td>Collagenase activity (cpm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Test Teeth</td>
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<tr>
<td>Group A N = 7</td>
<td>0</td>
<td>41 ± 9</td>
<td>1293 ± 67</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>29 ± 7</td>
<td>203 ± 299</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>22 ± 5</td>
<td>1680 ± 308</td>
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<tr>
<td>Group B N = 7</td>
<td>0</td>
<td>60 ± 13</td>
<td>3543 ± 240</td>
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<td></td>
<td>14</td>
<td>37 ± 7</td>
<td>2815 ± 186</td>
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<tr>
<td></td>
<td>28</td>
<td>24 ± 6</td>
<td>1409 ± 262</td>
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<tr>
<td>Infected Control Teeth</td>
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<td>39 ± 7</td>
<td>2674 ± 133</td>
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<td>44 ± 6</td>
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<td>45 ± 6</td>
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<tr>
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<td>14</td>
<td>48 ± 9</td>
<td>3436 ± 310</td>
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<tr>
<td></td>
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<td>49 ± 7</td>
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<td>Healthy Control Teeth</td>
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<td>19 ± 3</td>
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<td></td>
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<td>1855 ± 313</td>
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<td>28</td>
<td>15 ± 2</td>
<td>2123 ± 199</td>
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<tr>
<td>Group B N = 7</td>
<td>0</td>
<td>13 ± 5</td>
<td>1915 ± 239</td>
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<td>14 ± 3</td>
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<td>14 ± 4</td>
<td>1691 ± 338</td>
</tr>
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</table>

Significant difference of recordings at Day 0 (Friedman’s two-way analysis of variance and Wilcoxon’s signed rank-test: p < 0.05): * Test Teeth versus Healthy Control Teeth, † Test Teeth versus Infected Control Teeth, ‡ Infected Control Teeth versus Healthy Control Teeth. Brackets indicate significant differences between recordings following treatment of the Test Teeth (Friedman’s two-way analysis of variance: Group A, p < 0.05; Group B, p < 0.001. Wilcoxon’s signed-rank test: Group A, *p < 0.05; Group B, **p < 0.02). The corresponding significance in Group B of the Healthy Control Teeth was p < 0.05 (Friedman) and p < 0.02 (Wilcoxon).

1 Collagenase activity is expressed as radioactivity degraded from soluble type I 1H-collagen α-chains isolated by polyacrylamide gel electrophoresis.

Protein content of sulcular fluid

Group A (Scaling followed by occlusal adjustment). At Day 0 the protein content of the fluid from the pockets of the Test Teeth was significantly (p < 0.05) higher than that of the fluid from the pockets of the Healthy Control Teeth. The same was found for the fluid from the pockets of the Healthy Control Teeth when compared to the fluid from the pockets of the Healthy Control Teeth. Between Days 0, 14 and 28 the mean protein content in the fluid collected from the Test Teeth did not change significantly (Table 2). Of the content at Day 0 the protein content was 79% at Day 14 and 70% at Day 28 (Fig. 2).

Group B (Occlusal adjustment followed by scaling). At Day 0 the protein content of the fluid from the pockets of the Test Teeth was significantly higher (p < 0.05) than of the fluid from the pockets of the Healthy Control Teeth (Table 2). The same was found for the fluid from the pockets of the Infected Control Teeth when compared to the fluid from the pockets of the Healthy Control Teeth (Table 2). In Group B there was, however, a significant (p < 0.02) decrease of the content from Day 0 to Day 14 and to Day 28, as well as from Day 14 to Day 28 (Table 2, Fig. 2). Of the content at Day 0 the protein content had decreased to 65% at Day 14 and to 40% at Day 28 (Fig. 2).

In both Groups A and B the protein content of the fluid from the pockets of the Infected Control Teeth and the Healthy Control Teeth did not change from Day 0 to Day 14 or to Day 28 (Table 2, Fig. 2).

Collagenase activity in sulcular fluid

An example of the polyacrylamide gel electrophoresis of the fluid from a Test Tooth (Group A) and the corresponding Infected Control Tooth in the same mouth, incubated with type I collagen, is shown in Fig. 1.

Group A (Scaling followed by occlusal adjustment). At Day 0 there was a significantly (p < 0.05) higher collagenase activity in the fluid collected from pockets of the Test Teeth than the Healthy Control Teeth and from the Infected than the Healthy Control Teeth (Table 2). Also, there was more collagenase activity in the fluid from the Test Teeth than in the fluid from the Infected Control Teeth (p < 0.05). The collagenase activity of the Test Teeth diminished significantly (p < 0.05) from Day 0 to Days 14 and 28 (Table 2, Fig. 3). At Day 14, the collagenase activity had decreased to 70% of the activity at Day 0 and, at Day 28, to 59% (Fig. 3).

Group B (Occlusal adjustment followed by scaling). At Day 0 there was a significantly (p < 0.05) higher collagenase activity in the fluid collected from pockets of the Test Teeth than the Healthy Control Teeth and from the Infected Control Teeth than the Healthy Control Teeth (Table 2). A decrease in collagenase activity was observed for the Test Teeth from Day 0 to Days 14 and 28 (p < 0.02). Also the decrease from Day 14 to Day 28 was significant (p < 0.02) (Table 2, Fig. 3). At Day 14, the collagenase activity had decreased to 81% of the activity at Day 0 and, at Day 28, to 41% (Fig. 3).

Between Days 0, 14 and 28 the collagenase activity in the fluid did not change for the Infected Control Teeth in either Group A or B (Table 2, Fig. 3). The activity in the fluid from pockets of the Healthy Control Teeth did not change in Group A. In Group B, however, the activity decreased (p < 0.02) from Day 14 to Day 28 (Table 2).

No clear changes were noted in the collagenase activity in relation to the protein content of the fluid.

Discussion

Hydrolitic enzymes are a group of substances in sulcular fluid that have been thought to reflect the condition of the periodontium (23, 24). Collagenase appears to be the most important of these enzymes, since it initiates the enzymatic degradation of collagen fibers in tissue. A synthetic aminoacid substrate was used in a study on sulcular fluid collagenase in gingivitis (25). Even though the substrate is convenient to use, it is
The collagenase activity in sulcular fluid decreased significantly as a result of both treatment modes. Treatment only by scaling and root planing (Group A) led to a significant decrease in collagenase activity within 14 d. This is in accordance with the results of Larivée et al. (9). The finding that occlusal adjustment alone (Group B) also resulted in decreased collagenase activity was somewhat unexpected. In a previous investigation the flow rate of sulcular fluid was not decreased by occlusal adjustment (14). In the present study, scaling and root planing was, however, the more effective of the two treatment modes, since a further significant decrease of sulcular fluid collagenase activity was noted when the teeth were scaled and root planed 14 d after occlusal adjustment (Table 2, Fig. 3). The reason for the decrease of collagenase activity in sulcular fluid after occlusal adjustment is not clear. The rather crude method of measuring the mobility of the Test Teeth in this study did not reveal changes in the short period of 28 d. But the jiggling in conjunction with movements of the jaw was reduced or eliminated from the Test Tooth by occlusal adjustment. It has been suggested that the jiggling of the traumatized tooth enhances the inflammatory reaction in the periodontium by a “pumping action” (27).

The prostaglandin levels of inflamed gingiva have been found to be significantly increased in comparison to the levels found in healthy gingiva (29, 30, 31). Prostaglandins are released from
mammalian tissues in response to physiological and pathological, including mechanical, stimuli (32, 33). It has also been demonstrated that prostaglandin E is involved in the activation of macrophages with resultant production of collagenase (34). The mechanical stimulation caused by the jiggling of the hypermobile tooth could enhance prostaglandin synthesis and, through that, also collagenase activity.

Collagenase activity in the fluid from the pockets of the Healthy Control Teeth of Group B decreased significantly from Day 14 to Day 28 (Table 2). The reason for this fluctuation in collagenase activity of untreated shallow pockets is not clear. In a study on dogs, the fluctuation was suggested to reflect the alternation between destructive and quiescent phases of the periodontal disease (8).

The protein content of the deep pockets showed the same trends as the collagenase activity (Table 2, Fig. 2). It has also been shown earlier that the protein flow increases with periodontal disease (23).

The origin of sulcular fluid collagenase has not yet been conclusively established. As observed in our preliminary study, sulcular fluid collagenase was found to originate mainly from human cells, even though traces of bacterial collagenase were present in some samples. It is possible that most of the collagenase is of polymorphonuclear leukocyte origin (28). A significant proportion of this enzyme may thus be released from the cells not in the connective tissue but in the gingival pocket. Therefore, the enzyme activity in sulcular fluid may not necessarily reflect the degradation of the periodontal connective tissue.

In conclusion, the protein content and collagenase activity in deep pockets is increased during periodontitis in both mobile and immobile teeth. A relatively rapid decrease of the collagenase activity takes place after establishment of a supragingivally clean oral environment and following the removal of subgingival bacterial plaque and calculus and infected cementum from the root surface within the periodontal pocket.

Also occlusal adjustment of hypermobile teeth with deep pathological pockets reduces the protein content and collagenase activity in sulcular fluid after the establishment of a supragingivally clean oral environment. No changes in the protein content or in collagenase activity were observed in the deep pockets of the untreated Control Teeth in the same patients. Further studies are needed to clarify the significance of these results.

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