Effect of Personal Oral Hygiene on Bleeding Interdental Gingiva*

Histologic Changes

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A previous study demonstrated that the combination of subgingival scaling and improved oral hygiene resulted in a reduction of clinical and histological signs of interdental gingival inflammation, changes that were associated with a cessation of interdental gingival bleeding. The present study compared, histologically, the interdental tissues of bleeding sites with sites that initially bled but had been converted to nonbleeding by an oral hygiene program alone. Morphometric analysis of interdental gingiva demonstrated that conversion from bleeding to nonbleeding was associated with a significant reduction in the inflamed connective tissue component. This study showed that an oral hygiene program consisting of toothbrushing and interdental cleaning could significantly reduce interdental inflammation, and that bleeding determinations monitored the effects of this therapy.

Diagnosis of periodontal disease and evaluation of the results of periodontal therapy are, to a large extent, based on detection of inflammatory lesions within the periodontium. Visual signs of inflammation have been used for this diagnosis, however, the interdental gingiva is generally inaccessible for direct visual examination. In the facial and lingual gingiva, sulcular bleeding, after stimulation with a periodontal probe, has also been shown to be associated with the presence of an inflammatory lesion. In addition, bleeding of the interdental tissues after stimulation with interdental cleaners has been considered a clinical sign indicating gingival inflammation and was found to be associated with inflammatory lesions within the connective tissues of the midinterproximal gingiva. This information has established the rationale for the use of bleeding in the diagnosis of periodontal status.

Clinical trials have demonstrated that gingival bleeding can be reduced or eliminated by therapy aimed at control of bacterial plaque. It has been shown, histologically, that interproximal bleeding sites subjected to thorough scaling and daily plaque control stopped bleeding and had a substantially reduced inflammatory lesion. It would be of interest to determine the contribution of personal plaque control in the reduction of bleeding and the decrease in the inflammatory infiltrate. Consequently, the purpose of this investigation was to compare, histologically, the interproximal tissues of bleeding sites with sites that initially bled but had been converted to nonbleeding by personal oral hygiene techniques alone.

MATERIALS AND METHODS

An interdental gingival biopsy was obtained from each of 30 patients, 13 male and 17 female, ages 26 to 74 years (mean age 44.2), who were in good health. The biopsies were obtained during periodontal surgery to treat periodontal disease in adjacent areas. One month prior to surgery, all patients were instructed in oral hygiene techniques. These instructions included patient education in the use of a conventional toothbrushing technique combined with interdental cleaners for the interproximal areas.

Interdental areas included in the study had initial pocket depths and attachment loss ≤4 mm, and radiographic bone loss ≤20%. These sites had both a normal tooth alignment and gingival embrasure space. To test for interdental bleeding, a wooden interdental cleaner was inserted between the teeth from the facial aspect in

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such a way as to depress the interdental papilla 1 to 2
mm.\textsuperscript{14,15} The path of insertion was horizontal, taking
care not to direct the point apically. In this manner,
the cleaner was inserted and removed four times. The
presence or absence of bleeding within 15 seconds was
recorded.\textsuperscript{15}

All sites included in the study responded with bleeding
at the initial examination. Immediately prior to
surgery, four weeks ± one week after oral hygiene
instructions, the interproximal sites were again assessed
for bleeding tendency. Fifteen patients had not mas-
tered good oral hygiene techniques, and their interprox-
imal sites still bled (bleeding group); whereas, in the 15
patients that had mastered these techniques (including
use of interdental cleaners), the sites were converted to
nonbleeding (stopped-bleeding group). The technique
for interproximal gingival biopsy has been described
previously.\textsuperscript{14}

Biopsy specimens were fixed in 10% neutral buffered
formalin for 24 hours, washed for six hours in tap
water, dehydrated in ascending alcohols and cleared for
paraffin embedding. The biopsies were embedded in
three pieces: facial, interproximal and lingual.\textsuperscript{14} The
interproximal specimen was sectioned in a mesiodistal
plane, while the facial and lingual pieces were retained
for future analysis. Sections from the interproximal
piece were cut 6 μ in thickness, and step-serial sections
at 144 μ intervals were mounted on glass slides and
stained with the Van Gieson picric acid-fuschin
method.\textsuperscript{23,24}

Histologic Analysis. The step serial sections (6 to 20
sections per biopsy) were analyzed with a calibrated
grid mounted in the ocular of a light microscope at
magnification × 125 using the technique introduced by
Weibel\textsuperscript{25} and modified by others.\textsuperscript{10,14,16,17,26,27} All tissue
in the coronal 1620 μ of the sections was analyzed and
recorded separately for the apical and coronal aspects
of the sections (Fig. 1).

Morphometric (grid intersection) point counting was
performed to determine the number of grid intersects
falling over the entire tissue area, to include normal
connective tissue, inflamed connective tissue and epi-
thelium.\textsuperscript{14,16} Intersects that fell over empty tissue spaces
or artifacts were not counted and intersects on the
connective tissue-epithelial interface were counted as
connective tissue.

The connective tissue exhibiting inflammation was
defined as that portion that had a high cell density (cell
rich) and was stained poorly (collagen-poor) by the Van
Gieson method.\textsuperscript{24} The Van Gieson picric acid-fuschin
method stains normal collagen red. In areas infiltrated
with inflammatory cells, the collagen is lost or altered,
losing its affinity for the acid fuschin, and is colored
yellow by the picric acid component.\textsuperscript{23}

Statistical Analysis. The morphometric measure-
ments tended to show systematic changes with relative
facial-lingual depth into the biopsy. These changes were
examined by calculating for each section, its percentile
depth through the biopsy by using the method described
previously\textsuperscript{16} and regressing the morphometric measure-
ments versus the calculated percentiles. Cubic equa-
tions were found to yield adequate fits to the patterns
for all measurements. Plots of these fitted equations
were prepared (Figs. 2–6). Upper and lower 95% con-
fidence bands about these plots were included to indi-
cate the precision of the fitted regression lines.

Hypotheses tests comparing the two groups (bleeding
and stopped-bleeding) were performed using random
effects model analyses of variance. First, overall cubic
regressions were performed on the combined data from
both groups to model changes with percentile depth.\textsuperscript{16}
Residuals were obtained by calculating the assigned
distance each data point was from the fitted cubic
regression curve. These residuals were then analyzed
using the analysis of variance based on a model in
which specimens were viewed as random representa-
tives of the two groups and the residuals from each
section were repeated measurements from specimens.
This analysis yielded overall P values associated with
the average difference between groups throughout the
relative depths. These comparisons were supplemented
with a rerandomization procedure\textsuperscript{25} that assessed dif-
frences between group patterns across relative depth.
Since the rerandomization tests resulted in P values
that were nearly identical with those from the analyses
of variance, these supplemental analyses will not be
discussed.

Group differences of the coronal and apical aspects
of the sections were examined using paired t tests based
on average differences across each specimen. Within
group comparisons of coronal versus apical aspects were
performed in an identical way. Patterns for each aspect
with relative percentile depth were plotted.
Errors of the Method. Duplicate measurements taken one-week apart were performed on ten randomly selected sections. The errors of the method were less than 2% for epithelium and total tissue area, and less than 4% for normal and inflamed connective tissue. All measurements were made by the same investigator (OB).

RESULTS

The results were based on 30 biopsy specimens taken from 30 individuals and were equally divided between bleeding and stopped-bleeding specimens. The biopsy technique for obtaining the interdental tissues consistently yielded intact specimens suitable for histologic evaluation.

The data obtained from the morphometric evaluation were grouped to: (1) compare the various measured parameters between bleeding and stopped-bleeding specimens, (2) compare the coronal and apical aspects between the bleeding and stopped-bleeding specimens and (3) compare the coronal and apical aspects of the specimens within bleeding and stopped-bleeding groups.

Comparison of Parameters from Bleeding Versus Stopped-Bleeding Specimens. The results of overall comparisons of the tissue components in bleeding versus stopped-bleeding biopsies are shown in Table 1. No significant differences between the groups were observed for the tissue components of epithelium or connective tissue. However, when the connective tissue aspect was considered alone, the bleeding specimens had a significantly greater mean percentage of inflamed connective tissue than the stopped-bleeding specimens (68.1 ± 3.6% vs. 43.3 ± 3.3%, P < 0.002). Thus, it appeared that the major difference between the groups was in the relative proportion of connective tissue that was inflamed.

There was strong evidence (P < 0.0001) for curvature in the pattern of per cent-volume-density inflamed connective tissue across the relative facial-lingual width (Fig. 2) when both coronal and apical aspects were combined. The bleeding group had a significantly higher (P < 0.002) mean percentage of inflamed connective tissue across the entire facial-lingual width of the interproximal area. The fitted regression curves, portraying the patterns in percentage of inflamed connective tissue across percentile depth, varied between stopped-bleeding and bleeding specimens (Fig. 2). There appeared to be very little change in percentage of inflamed connective tissue with percentile depth for the stopped-bleeding group; in the bleeding group, there appeared to be a marked parabolic relationship between mean percentage of inflamed connective tissue and percentile depth, with the maximum percentage of inflamed connective tissue approximating the midinterproximal section.

Comparison of Coronal and Apical Aspects between Bleeding and Stopped-Bleeding Specimens. Descriptive data comparing coronal and apical aspects between the groups are presented in Table 2. There were no significant differences observed in the coronal and apical aspects of the epithelium and connective tissue of the bleeding and stopped-bleeding specimens. When the connective tissue aspect was factored into its inflammatory or noninflammatory components, significant differences were present for both the coronal and apical aspects of the inflamed connective tissue (46.1 ± 5.7% vs. 28.8 ± 5.2%, P < 0.02; 75.1 ± 3.8% vs. 50.4 ± 5.0%, P < 0.001, respectively). Corresponding, significant differences were also present for the noninflamed connective tissue components.

When the percentage of coronal inflamed connective tissue for bleeding and stopped-bleeding specimens was regressed against the facial-lingual rank of sections, Figure 3 was generated. The mean percent inflamed

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Connective Tissue and Epithelium in Bleeding and Stopped-Bleeding Biopsies*</th>
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<tbody>
<tr>
<td></td>
<td>Bleeding</td>
</tr>
<tr>
<td>Tissue Components</td>
<td></td>
</tr>
<tr>
<td>Epithelium</td>
<td>43.2 ± 3.3</td>
</tr>
<tr>
<td>Connective Tissue</td>
<td>56.7 ± 3.3</td>
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<tr>
<td>Connective Tissue Aspect</td>
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<tr>
<td>Inflamed</td>
<td>68.1 ± 3.6</td>
</tr>
<tr>
<td>Noninflamed</td>
<td>31.9 ± 3.6</td>
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* Mean per cent volume density ± SE.
† NS = not significant.
Table 2

| Comparison of Coronal and Apical Aspects for Bleeding and Stopped-Bleeding Groups* |
|-----------------------------------|-----------------------------------|
|                                    | Bleeding                         | Stopped bleeding | \(P\)     |
| Tissue Components                  |                                  |                  |
| Epithelium:                        |                                  |                  |
| Coronal                            | 60.4 ± 3.8                      | 66.4 ± 2.4       | NS†       |
| Apical                             | 25.9 ± 3.3                      | 24.3 ± 1.9       | NS        |
| \(P\)                              | <0.0001                         | <0.0001          |
| Connective Tissue                  |                                  |                  |
| Inflamed                           | 39.6 ± 3.8                      | 33.6 ± 2.4       | NS        |
| Apical                             | 74.1 ± 3.3                      | 75.7 ± 1.9       | NS        |
| \(P\)                              | <0.0001                         | <0.0001          |
| Noninflamed                        |                                  |                  |
| Coronal                            | 53.9 ± 5.7                      | 71.2 ± 5.2       | NS        |
| Apical                             | 24.9 ± 3.8                      | 49.6 ± 5.0       | <0.001    |
| \(P\)                              | <0.0006                         | <0.0001          |

* Mean per cent volume density ± SE.
† NS = not significant.

More towards the lingual aspect of the interproximal tissue than the facial.

Figure 4 is similar to Figure 3 except that the apical inflamed connective tissue was compared between the two groups. This figure showed the two groups presented similar curves except that the bleeding group averaged approximately 30% more inflamed connective tissue. Both curves appeared slightly skewed towards the facial aspect of the facial-lingual width of the biopsy.

Comparison between the Coronal and Apical Aspects of the Specimens within Bleeding and Stopped-Bleeding Groups. Comparisons were also made between the coronal and apical aspects within bleeding and stopped-bleeding groups (Table 2). The coronal component had significantly more epithelium in both the bleeding and stopped-bleeding groups (60.4 ± 3.8% vs. 25.9 ± 3.3%, \(P < 0.0001\) and 66.4 ± 2.4% vs. 24.3 ± 1.9%, \(P < 0.0001\)) and significantly less inflamed connective tissue (46.1 ± 5.7% vs. 75.1 ± 3.8%, \(P < 0.0001\) and 28.8 ± 5.2% vs. 50.4 ± 5.0%, \(P < 0.0001\)).

Figure 5 showed a regression curve involving the percentage inflamed connective tissue from the apical and coronal aspects of the stopped-bleeding group. The coronal mean percentage of inflamed connective tissue appeared to be fairly stable (between 20% and 30%) for most of the facial-lingual width of the biopsy. The apical mean percentage of inflamed connective tissue appeared slightly more curved and reached a maximum at approximately 50% inflamed connective tissue. Figure 5 illustrates that there is significantly more inflamed connective tissue in apical portions when compared with coronal portions.
Figure 6 shows the apical and coronal sites for the bleeding group. The apical curve was skewed to the facial aspect of the interproximal biopsy and reached a maximum inflamed connective tissue of 80%. The coronal portion contained inflamed connective tissue ranging from less than 20% to 65%. The maximum inflamed connective tissue appeared to be located toward the lingual aspect of the interproximal biopsy. Figure 6 illustrates that there is significantly more inflamed connective tissue in apical portions when compared with coronal portions.

The histologic observations reinforced the morphometric data. In the bleeding specimens, the connective tissue was densely infiltrated with inflammatory cells which often, totally, replaced red-staining collagen. In contrast, the stopped-bleeding specimens had substantially greater amounts of red-staining collagen, and the inflammatory infiltrate was generally found subjacent to the epithelium (Fig. 7).

**DISCUSSION**

The purpose of the investigation was to compare histologically the interdental tissues of bleeding sites with sites that initially bled and had been converted to nonbleeding by personal oral hygiene techniques alone. The results demonstrated that conversion from a bleeding to a nonbleeding interdental site was associated with a significant reduction in the inflamed connective tissue component of the midinterproximal gingiva (68.1% vs. 43.3%, \( P < 0.002 \), Table 1). This appears to be the first study to relate histologically a “stopped bleeding” state in a patient with periodontitis to a decrease in the inflamed connective tissue component of the midinterproximal region, brought about solely by patient compliance with a personal oral hygiene program. No additional professional therapy such as scaling, polishing and root planing was performed on any of the patients. These results are in agreement with earlier work, which showed that effective oral hygiene can result in a decrease of gingival inflammation. The findings are also consistent with results from previous investigations in which bleeding and nonbleeding sites on the radicular and interproximal aspects were compared, and which showed that bleeding was a reliable clinical sign for the presence of a significantly greater inflammatory lesion within the gingival connective tissue.

A comparison was made between the results of the present study and an earlier study from this laboratory, which included a therapeutic episode of subgingival scaling and root planing in addition to a similar oral hygiene program. The only significant difference in the data between the two studies is in the inflamed connective tissue component of the stopped-bleeding groups (previous study 27.9 ± 0.8% vs. current study 43.3 ± 3.3%, \( P < 0.05 \)). These differences agreed with other studies that reported that scaling and root planing, in addition to an oral hygiene program, led to a decrease in gingival inflammation that was more pronounced than seen with the oral hygiene program alone.

Evidently, scaling and root planing contributed to a reduction in the inflamed portion of the connective tissue.

These differences in therapeutic effect at the histological level may be due to the inability of oral hygiene measures alone to remove bacterial plaque from the more apical regions of periodontal pockets.
Waerhaug\textsuperscript{12,33} reported that oral hygiene techniques that combined sulcular brushing and interdental cleaning effectively removed supragingival plaque but only removed subgingival plaque to depth of 0.5 to 2.5 mm. Our segmented morphometric analysis that examined separately the coronal and apical aspects of lesions facilitated comparisons of inflamed connective tissue within these regions. In the stopped-bleeding group, the apical region was significantly more inflamed than the coronal region (50.4 vs. 28.8\%, \( P < 0.0001 \)). This suggests that the plaque removal with wooden interdental cleaners was effective in reducing inflammation more in the coronal than the apical regions of the interdental gingival pocket. Scaling, if it had been performed, may have, more effectively, reduced the inflammation in the apical region of the pocket. A study to clarify this aspect of therapeutic response is in progress.

Figure 7. Photomicrographs of midinterproximal areas from bleeding (A and B) and stopped-bleeding (C and D) sites. A. Overview from bleeding site. B. Higher magnification from A of the epithelial-connective tissue border in the sulcular region. The connective tissue is characterized by a lack of collagen and the presence of a dense inflammatory cell infiltrate. C. Overview from a stopped-bleeding site. D. Higher magnification from C of the epithelial-connective tissue border in the sulcular region. The connective tissue consists primarily of dense collagen, with inflammatory cells interspersed between fiber bundles.
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The ability to convert an interdental area from bleeding to nonbleeding may depend on pocket depth, and it should be emphasized that pockets in the present study were ≤4 mm. Previous investigators have indicated that personal oral hygiene has a limited effect on inflammation in deep pockets, but biopsies from these studies did not include the critically important interdental area. After a month of personal oral hygiene, reduction in bleeding has been reported in deeper pockets by some investigators but not by others. Therefore, additional histologic studies should be performed on interdental areas with deep pockets to determine the relative significance of personal oral hygiene, and professional instrumentation, on the inflammatory lesion.

REFERENCES