

# Effects of Personal Oral Hygiene and Subgingival Scaling on Bleeding Interdental Gingiva\*

Jack Caton, Otis Bouwsma, Alan Polson, and Mark Espeland

THE AIM OF THIS STUDY was to determine the effects of personal oral hygiene and subgingival scaling on bleeding interdental gingiva. The Eastman Interdental Bleeding Index (EIBI) was used to clinically evaluate interdental gingival status. Forty-seven bleeding interdental sites in 47 patients were divided into three groups. Sites in Group I bled on stimulation with wooden interdental cleaners. Groups II and III initially bled but were converted to nonbleeding with oral hygiene alone or oral hygiene combined with subgingival scaling, respectively. Interdental gingival biopsies were obtained and subjected to morphometric analysis to compare the three groups. The findings from this study indicated that: (A) personal oral hygiene reduced the magnitude and extent of the interdental inflammatory lesion; (B) subgingival scaling plus oral hygiene decreased the interdental inflammatory lesion to a greater extent than oral hygiene alone; (C) significant repair of the interdental lesion occurred within four weeks; and (D) the EIBI was an effective method for monitoring the effects of therapy directed towards resolution of the interdental inflammatory lesion.

Several studies have examined the effect of scaling and personal oral hygiene in the treatment of periodontal disease. These effects included a reduction in plaque,<sup>1-13</sup> gingival inflammation,<sup>1-13</sup> bleeding,<sup>1,3-10,12,13</sup> pocket depth,<sup>1-10,12,13</sup> increased gingival recession,<sup>1-3,5-9,12</sup> and gain in probing attachment level.<sup>1-3,5-9,12</sup> Only a few clinical<sup>1,3,12</sup> and histological<sup>1,31</sup> studies, however, have evaluated the effects of scaling and personal oral hygiene, independently. The histological studies examined facial and lingual gingival biopsies and no reports could be found related to the interdental area.

Clinical evaluation of interdental gingival status has been based upon bleeding tendency after stimulation with wooden interdental cleaners.<sup>14-22</sup> Bleeding after stimulation has been considered a clinical sign indicating gingival inflammation and associated histologically with inflammatory lesions within the connective tissues of the interdental gingival region.<sup>16,18-22</sup> These studies have provided a scientific rationale for the diagnosis of gingival inflammatory lesions within the interdental gingiva.

The purpose of the present study was to evaluate the effect on interdental gingival status of personal oral hygiene alone and in combination with subgingival scaling. A further aim was to establish if bleeding determinations were effective in monitoring the effect of these therapies.

## MATERIALS AND METHODS

An interdental gingival biopsy was obtained from each of 47 patients, 24 male and 23 female, ages 26 to 74 years (mean age 43.2 years), who were in good health. Gingival biopsies were obtained during the course of periodontal surgery to treat periodontal disease in adjacent areas. One month prior to surgery, all patients were instructed in oral hygiene techniques, which included 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 probing attachment loss  $\leq 4$  mm, and radiographic bone loss  $\leq 20\%$ . These sites had a normal tooth alignment and gingival embrasure space. To test for interdental bleeding, the method described for the Eastman Interdental Bleeding Index (EIBI) was used.<sup>16-18</sup> A wooden interdental cleaner was inserted between the teeth from the facial aspect in such a way as to depress the interdental papilla 1 to 2 mm. 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.

All sites included in the study had a positive bleeding response at the initial examination. Immediately prior to surgery, four weeks  $\pm$  one week after oral hygiene

\* Department of Periodontology, Eastman Dental Center, 625 Elmwood Avenue, Rochester, NY.

† Stim-U-Dent, Johnson & Johnson Products, Inc. New Brunswick, NJ.

instructions, the interdental sites were again assessed for bleeding tendency. Fifteen patients had not mastered good oral hygiene techniques and their interproximal sites still bled (Group I, bleeding group); whereas, in the 15 patients that had mastered these techniques, including use of interdental cleaners, the sites were converted to non-bleeding (Group II, stopped-bleeding by oral hygiene alone). Seventeen patients received a single episode of subgingival scaling (one month  $\pm$  one week prior to biopsy) and also mastered good oral hygiene techniques. The interproximal sites in these patients were converted to nonbleeding (Group III, stopped-bleeding by scaling and oral hygiene). The technique for interproximal gingival biopsy was described previously.<sup>16</sup>

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. Sections from the midinterproximal piece were cut 6  $\mu$  in thickness in a mesiodistal plane and step-serial sections at 144  $\mu$  intervals were mounted on glass slides and stained with the van Gieson picric acid-fuschin method.<sup>23,24</sup>

#### Histologic Analysis

Step-serial sections (6 to 22 sections per biopsy) were analyzed with a calibrated grid mounted in the ocular of a light microscope at magnification  $\times$  125 using the technique introduced by Weibel<sup>25</sup> and modified by others.<sup>16,19-22,26-30</sup> Morphometric point counting (grid intersection) was performed to determine the number of grid intersects falling over the entire tissue, and the tissue identified as normal connective tissue, inflamed connective tissue, and epithelium.<sup>16,19,22</sup> Intersects that fell over empty tissue spaces or artifacts were not counted and intersects on the connective tissue-epithelium interface were counted as connective tissue. In order to determine locations within the gingiva of inflammatory changes, the coronal 1620  $\mu$  of sections were analyzed in coronal and apical 810  $\mu$  portions.<sup>22</sup>

Inflamed connective tissue was defined as that portion of connective tissue which had a high cell density (cell-rich) and stained poorly (collagen-poor) with the van Gieson method.<sup>24</sup> 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.<sup>23</sup>

#### Statistical Analysis

The group means for the tissue components were calculated from the means of the individual biopsy specimens. Differences between the means of Groups I, II, and III were assessed using repeated measures analysis of covariance. The morphometric measurements 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.<sup>19,22,31</sup> Then, a cubic polynomial was fitted to describe changes in the percentage of tissue components through the biopsy specimens, so that these changes could be related to the percentile facial-lingual depth through the interdental area. Plots of these fitted equations were prepared (Figs. 1-6) and approximate upper and lower 95% confidence bands about these plots were included to indicate the precision of the fitted regression lines. The residuals from these regressions were then analyzed using nested, random effects analysis of variance. Separate analyses were performed for each pair of treatment groups and for coronal, apical, and combined sites. The *p*-values for the group differences were adjusted by the Bonferroni method to control for the propagation of Type I errors associated with multiple comparisons.<sup>32</sup> Differences between the coronal and apical portions were assessed using student paired student *t*-tests based on average differences across each student *t*-test.

#### Errors of the Method

Duplicate measurements taken one week apart were performed on randomly selected sections. The errors of the method<sup>33</sup> 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 47 interproximal gingival biopsies taken from 47 individuals. At the time of biopsy, the sites from Group I (15 patients) demonstrated bleeding. The sites of Group II (15 patients) were converted to nonbleeding as a result of personal oral hygiene alone. The sites of Group III (17 patients) had also converted to nonbleeding, but this group had a single episode of subgingival scaling in addition to personal oral hygiene. 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 tissue components between the three groups; (2) compare the coronal and apical aspects of the interdental gingiva between the three groups; and (3) compare the coronal and apical aspects within the three groups.

#### Comparison of Tissue Components from the Three Groups

The results of the overall comparisons of the tissue components in the groups are shown in Table 1. No significant differences between groups were observed for epithelium or total connective tissue. However, when the inflamed connective tissue portion was ex-

Table 1  
*Epithelium and Connective Tissue in Bleeding and Stopped-Bleeding Biopsies\**

	Bleeding	Stopped-Bleeding		<i>p</i> -Value
		Oral Hygiene	Scaling, Oral Hygiene	
Epithelium	42.2 ± 3.3	45.3 ± 1.9	48.3 ± 1.7	NS
Total connective tissue	56.7 ± 3.3	54.7 ± 1.9	51.3 ± 1.7	NS
Inflamed	68.1 ± 3.6	43.3 ± 3.3	25.6 ± 2.7	<0.001†
Noninflamed	31.9 ± 3.6	56.7 ± 3.3	74.4 ± 2.7	<0.001†

NS = Not significant.

\* Mean percent volume density ± standard error.

† Bonferoni comparisons:

Bleeding vs. oral hygiene *p* = 0.005

Bleeding vs. scaling, oral hygiene *p* < 0.001

Oral hygiene vs. scaling, oral hygiene *p* = 0.016

amed, the bleeding specimens had a significantly greater percentage volume density of inflamed connective tissue than the stopped-bleeding specimens (Group I: 68.1 ± 3.6% vs. Group II: 43.3 ± 3.3%, and Group III: 25.6 ± 2.7%). Within the stopped-bleeding groups, oral hygiene alone had significantly more inflamed connective tissue than the scaling plus oral hygiene (43.3 ± 3.3% vs. 25.6 ± 2.7%). The percentage inflamed connective tissue from the three groups was plotted against the relative percentage distance through the specimen in a facial-lingual direction (Fig. 1), and demonstrated that differences between the groups in percentage volume density of inflamed connective tissue was maintained throughout the entire facial-lingual width of the interdental area. The greatest percentage inflamed connective tissue in all three groups was located in the approximate middle of the interdental region.

#### Between Group Comparison of Coronal and Apical Locations

Descriptive data comparing coronal and apical aspects between the groups are presented in Table 2. There were no significant differences observed in coronal and apical measurements of epithelium and connective tissue between the bleeding and stopped-bleeding biopsies.

With respect to the percentage volume density of inflamed connective tissue, significant differences for both the coronal (Group 1: 46.1 ± 5.7% vs. Group II: 28.8 ± 5.2% vs. Group III: 11.1 ± 1.7%) and apical (Group 1: 75.1 ± 3.8% vs. Group II: 50.4 ± 5.0% vs. Group III: 32.6% ± 3.4%) locations of the interdental gingiva existed. Figures 2 and 3 demonstrated the results from coronal and apical locations, respectively, when percentage volume density of inflamed connective tissue was plotted against relative facial-lingual distance through the specimens. Coronal sites had less inflamed connective tissue than apical sites, and differences between groups were maintained throughout the entire facial-lingual width of the interproximal region. In both coronal and apical locations, the curvature of

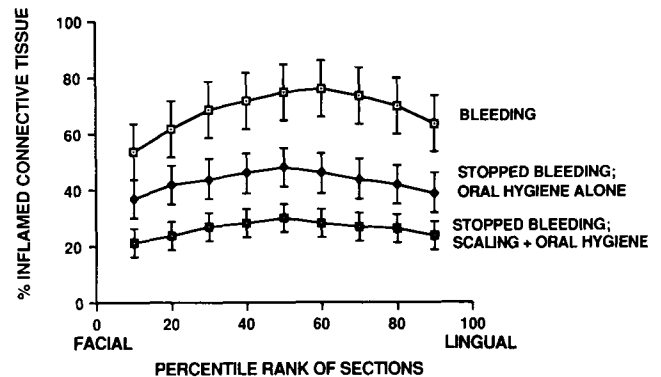


Figure 1. Plot of mean percent volume density of inflamed connective tissue, with approximate upper and lower 95% confidence bands, versus percentile rank of sections across the facial-lingual width of biopsies.

the bleeding group was more parabolic than stopped bleeding groups and this tendency was more pronounced in the coronal location.

#### Within Group Comparisons of Coronal and Apical Locations

Comparisons were also made between the coronal and apical locations within bleeding and stopped-bleeding groups (Table 2). The coronal location had significantly more epithelium and less total connective tissue in both the bleeding and stopped-bleeding groups. With regard to percentage volume density of inflamed connective tissue, the coronal location had significantly less inflamed connective tissue than the apical location in all three groups. Plots of percentage inflamed connective tissue against relative distance through the specimens for coronal and apical aspects of Groups I, II, and III are presented in Figures 4, 5, and 6, respectively. In each of these plots, there was a greater percentage of inflamed connective tissue in the apical location across the entire facial-lingual width of the interdental gingiva.

The histologic observations reinforced the morphometric data. In bleeding gingiva, the connective tissue was densely infiltrated with inflammatory cells which often largely replaced red staining collagen. In contrast,

**Table 2**  
**Comparisons of Coronal and Apical Aspects for Bleeding and Stopped-Bleeding Biopsies\***

	Bleeding	Stopped-Bleeding		p-Value
		Oral Hygiene	Scaling, Oral Hygiene	
Epithelium				
Coronal	60.4 ± 3.8	66.4 ± 2.4	65.5 ± 1.7	NS
Apical	25.9 ± 3.3	24.3 ± 1.9	31.1 ± 1.7	NS
p-Value	<0.001	<0.001	<0.001	
Total Connective Tissue				
Coronal	39.6 ± 3.8	33.6 ± 2.4	34.5 ± 1.7	NS
Apical	74.1 ± 3.3	75.7 ± 1.9	68.9 ± 2.2	NS
p-value	<0.001	<0.001	<0.001	
Inflamed connective tissue				
Coronal	46.1 ± 5.7	28.8 ± 5.2	11.1 ± 1.7	0.001†
Apical	75.1 ± 3.8	50.4 ± 5.0	32.6 ± 3.4	0.001‡
p-value	<0.001	<0.001	<0.001	

NS = Not significant.

\* Mean percent volume density ± standard error

† Bonferoni comparisons:

Bleeding vs. oral hygiene  $p = 0.063$

Bleeding vs. scaling, oral hygiene  $p < 0.001$

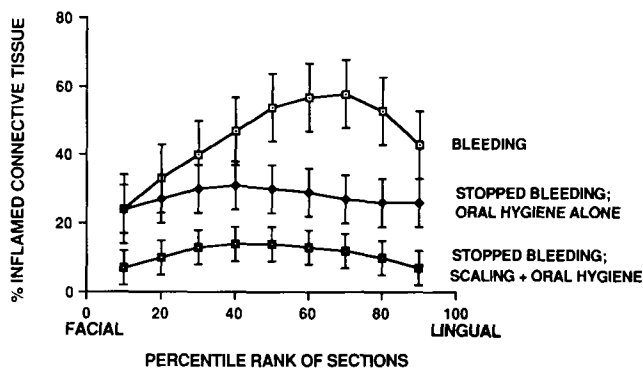
Oral hygiene vs. scaling, oral hygiene  $p = 0.022$

‡ Bonferoni Comparisons:

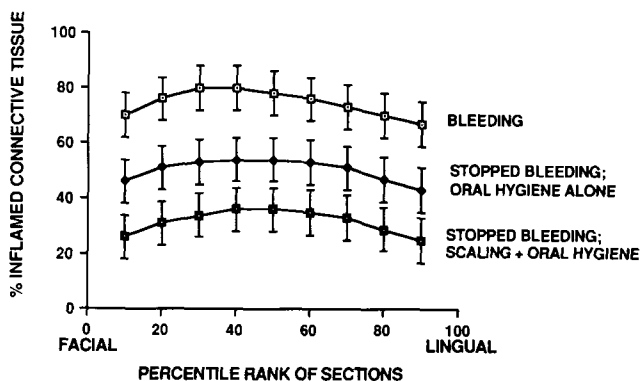
Bleeding vs. oral hygiene  $p = 0.003$

Bleeding vs. scaling, oral hygiene  $p < 0.001$

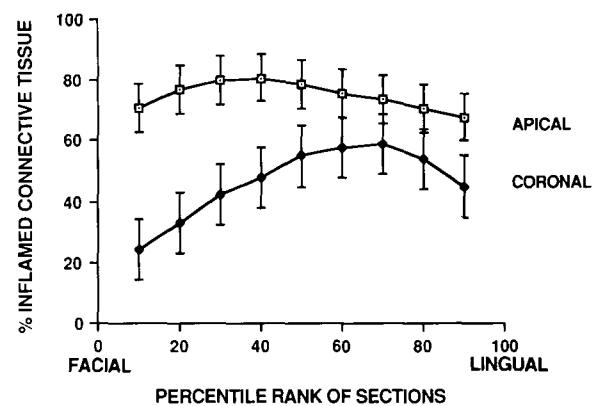
Oral Hygiene vs. scaling, oral hygiene  $p = 0.025$



**Figure 2.** Plot of mean percent volume density of coronal inflamed connective tissue, with approximate upper and lower 95% confidence bands, versus percentile rank of sections across the facial-lingual width of biopsies.



**Figure 3.** Plot of mean percent volume density of apical inflamed connective tissue, with approximate upper and lower 95% confidence bands versus percentile rank of sections across the facial-lingual width of biopsies.

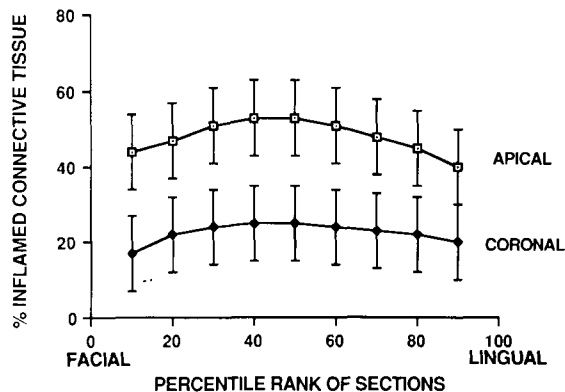


**Figure 4.** Plot of mean percent volume density of coronal and apical inflamed connective tissue for Group I (bleeding group) with approximate upper and lower 95% confidence bands, versus percentile rank of sections across the facial-lingual width of biopsies.

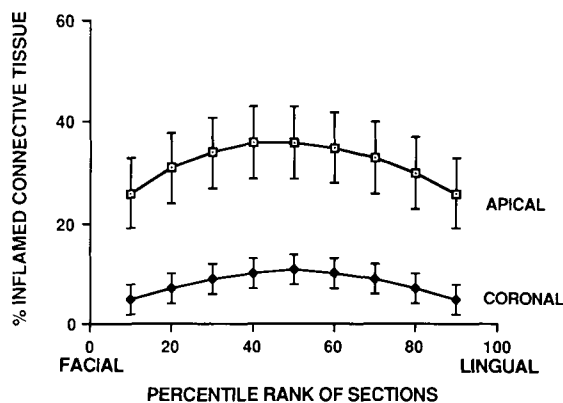
the stopped-bleeding gingiva had substantially greater amounts of red-staining collagen, and the inflammatory infiltrate was generally more pronounced subjacent to the epithelium and in the apical 810 micron location.

### DISCUSSION

The purpose of the present investigation was to evaluate the effect on interdental gingival status of personal oral hygiene alone, and in combination with subgingival scaling, using clinical bleeding determinations and histological methods. The results demonstrated that conversion from bleeding to nonbleeding was associated with a significant reduction in the inflamed connective tissue component of the midinterdental gingiva.



**Figure 5.** Plot of mean percent volume density of coronal and apical inflamed connective tissue for Group II (stopped-bleeding-oral hygiene alone group) with approximate upper and lower 95% confidence bands, versus percentile rank of sections across the facial-lingual width of biopsies.



**Figure 6.** Plot of mean percent volume density of coronal and apical inflamed connective tissue for the Group III (stopped-bleeding oral hygiene plus subgingival scaling), with approximate upper and lower 95% confidence bands, versus percentile rank of sections across the facial-lingual width of biopsies.

This appeared to be the first study that related histologically a stopped-bleeding state in patients with periodontitis to a decrease in the inflamed connective tissue component of the midinterdental region, brought about by either patient compliance with a personal oral hygiene program alone or in combination with a single episode of subgingival scaling. These results agreed with earlier work<sup>1,2,13</sup> in which oral hygiene alone or in conjunction with scaling resulted in a decrease of gingival inflammation clinically<sup>1-13,19-22</sup> and histologically.<sup>1,3,10,19-22</sup>

The histologic data obtained in this study agreed with results from previous investigations<sup>1,3,10</sup> in which there was a decrease in the inflammatory infiltrate and concurrent increase in the collagen content of the gingival connective tissues. The present findings highlighted the rapid repair (four weeks) of gingival tissue which took place following removal of plaque and calculus. This repair was apparently responsible for the clinical changes which took place following a single episode of subgingival scaling and controlled oral hygiene.<sup>7,8</sup> These

clinical changes took place within three weeks and included pocket reduction via gingival recession and gain in probing attachment level, and cessation of bleeding after probing for pocket depth.<sup>7,8</sup>

The EIBI was based on interdental gingival bleeding after stimulation with wooden interdental cleaners. Previous reports have shown that such bleeding was associated with a substantial inflammatory lesion in the midinterdental gingiva, and the absence of bleeding with a relatively normal gingival connective tissue. This information provided the scientific rationale for the use of bleeding determinations for periodontal diagnosis of the interdental region. The present study indicated that this type of bleeding determination could also be used for monitoring the effects of periodontal therapy aimed at resolution of the interdental inflammatory lesion, since the conversion from bleeding to nonbleeding was associated with resolution of the lesion. The interdental area apical to the contact point is usually inaccessible for visual inspection and a common site of periodontal pathosis. The EIBI could prove to be a valuable tool for evaluation of this region. Furthermore, the EIBI was a more sensitive indicator for detection of interdental gingival pathosis than the Papilla Bleeding Index in a clinical trial which compared the two indices.<sup>34</sup>

The results of the present study verified earlier studies, which showed that the middle area of the interdental gingiva contained the greatest amount of inflamed connective tissue.<sup>16,19</sup> The result of both the therapeutic regimes was a uniform and significant reduction in inflamed connective tissue across the entire facial-lingual aspect of the biopsies (Fig. 1). This seemed to indicate that both regimes were effective in decreasing the inflammatory lesion not only on facial and lingual aspects, but also in the less accessible regions that were under contact points.

Inspection of Figures 1 to 3 indicated that for each of the groups the most facial and most lingual sections had a similar percent inflamed connective tissue. The exception was the coronal aspect from the bleeding group (Fig. 2). The interdental tissue from this group was more inflamed on the lingual aspect. This suggested that these patients were more effective with plaque removal on the facial aspect. Generally the data from the three groups resulted in similar parabolic curves with the most inflamed tissue in the middle of the interdental area, which suggested that the middle area was the most difficult to accomplish thorough elimination of plaque. This middle area corresponded to the more inaccessible region under the contact point. Since the inflammatory lesion was greatest in the midinterdental area and the oral hygiene regime specifically focused on that area, the results of this study suggested that these regions should be carefully monitored for signs of possible periodontal destruction.

In order to obtain data concerning the distributional nature of the inflammatory lesion in an apico-coronal

plane, the biopsies were analyzed in apical and coronal halves. The data showed coronal areas had less inflamed connective tissue than apical areas. Furthermore, oral hygiene apparently contributed to approximately 50% of the total decrease in inflamed connective tissue of both coronal and apical locations (Table 2).

The differences in coronal versus apical inflammation may be due to the inability of oral hygiene measures to remove bacterial plaque from the more apical regions of periodontal pockets. Plaque removal techniques incorporating wooden interdental cleaners were apparently more effective in reducing inflammation in the coronal regions of the interdental gingival pocket than in the apical regions. Waerhaug<sup>35,36</sup> reported oral hygiene techniques that combined sulcular brushing and interdental cleaning effectively removed supragingival plaque, but removed subgingival plaque to a depth of only 0.5 to 2.5 mm. Other observations have shown that even after meticulous scaling and root planing, deeper pockets had more residual calculus and plaque<sup>37,38</sup> and this is consistent with our findings that the inflammatory lesion was greater in the apical half of the gingiva for all groups (Figs. 4-6).

#### Clinical Significance

The findings from this study indicated that: (A) personal oral hygiene reduced the magnitude and extent of the interdental inflammatory lesion; (B) subgingival scaling plus oral hygiene decreased the interdental inflammatory lesion to a greater extent than oral hygiene alone; (C) significant repair of the interdental lesion occurred within four weeks; and (D) the Eastman Interdental Bleeding Index was an effective method for monitoring the effects of therapy directed towards resolution of the interdental inflammatory lesion.

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Send reprint requests to: Dr. Jack G. Caton, Eastman Dental Center, 625 Elmwood Avenue, Rochester, NY 14620.

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