Gingival Wound Healing in the Presence of Plaque-
Induced Inflammation*

Jose A. Yumet and Alan M. Polson

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An accelerated loss of connective tissue attachment has been reported following surgery in plaque-infected dentitions. It was the purpose of the present study to evaluate histologically the healing of incisal wounds in the gingival supracrestal region in the presence and absence of bacterially induced inflammation.

In the experimental group, marginal periodontitis was induced around the teeth by tying plaque-retentive ligatures at the gingival margins and 10 weeks later an incisal wound was made from within the gingival sulcus to the crest of the bone. In the control group, similar incisal wounds were made in normal gingiva. In each group, three specimens were available for histologic and histometric analysis 1, 3, 7 and 21 days after wounding. In the control group, the wound healing sequence in the supracrestal region was similar to that reported following incisal cutaneous wounds. In the experimental group, epithelial continuity across the wound was re-established earlier, and marked invagination of epithelium occurred into the incision. This invagination was significantly greater than in control specimens at all time points. In addition, within the experimental group a second significant epithelial migration into the wound occurred between 7 and 21 days. The epithelial invagination extended through the major portion of the supracrestal area and terminated near the cementum surface. It is conceivable that marked epithelial invagination into a supracrestal wound may predispose to an accelerated loss of connective tissue attachment.

One of the most adverse effects of chronic marginal periodontitis is progressive destruction of connective tissue attachment to the root surface. A major goal of therapy is to halt, or significantly inhibit, further loss of connective tissue attachment. An integral component of many therapeutic modalities involves raising surgical flaps to facilitate access to exposed root surfaces for cleaning, and to eliminate periodontal pockets. It has been shown in a number of long-term clinical studies that this therapeutic approach, when combined with effective removal of bacterial plaque from the tooth surface, is able to maintain existing levels of connective tissue attachment. However, surgical therapy undertaken in situations where control of bacterial plaque is not effective has been followed by loss of connective tissue attachment occurring at a rate significantly greater than that of untreated marginal periodontitis, i.e., an accelerated loss of attachment occurred following surgery in plaque-infected dentitions. Although it has been recognized that a wound subject to bacterial contamination does not heal at an optimal rate, investigations describing the detailed aspects of healing in the presence of pre-existing inflammation are lacking, especially in the supracrestal connective tissue region of the periodontium. Consequently, the aim of the present study was to evaluate histologically the healing of incisal wounds in the supracrestal region in the presence and absence of plaque-induced inflammation.

MATERIALS AND METHODS

The study used 4 young adult squirrel monkeys with all permanent teeth erupted, caries free and exhibiting minimal attrition. The animals were sedated for all procedures by intramuscular injection of Ketaset (ketamine hydrochloride, 100 mg/ml) 10 mg/kg body weight. Six weeks prior to the beginning of the study, an oral hygiene regimen was begun which consisted of mechanical plaque removal three times a week. The first four cleanings included instrumentation for removal of subgingival deposits.

The teeth and surrounding gingiva of the areas to be included in the study were divided equally into an

* From the Department of Periodontology, Eastman Dental Center, Rochester, NY. Study was supported by Pluta Periodontal Fund.

† Bristol Laboratories, Syracuse, NY 13201.
experimental and control group. The areas were distributed around maxillary bicuspids and molars, and mandibular molars. In the experimental group, marginal periodontitis was induced around the teeth by tying plaque-retentive silk ligatures circumferentially at the gingival margins. Oral hygiene was stopped after initiating periodontitis. Ten weeks after initiating periodontitis an incisional wound was made through the marginal gingiva. The wound was made using a #11 Bard-Parker* scalpel blade. The incision began within the gingival sulcus, and it extended obliquely toward the tooth surface to the level of the alveolar crest. Wounds were made in the tissues on the buccal and lingual aspects of the teeth, and did not extend through the interproximal area. Wound margins were apposed using digital pressure for 30 seconds, but not sutured. The regions were evaluated 1, 3, 7 and 21 days after incisinal wounding. Three specimens were available at each of the postwounding time points.

In the control group, incisional wounds were made into normal gingiva in areas which corresponded to those used in the experimental group. The oral hygiene regime which had been in effect prior to making the surgical incision was continued for the remainder of the study. The number of specimens available for analysis after wounding was the same as that present in the experimental group.

Gingival inflammation was assessed during the period of the study. Immediately after the killing of an animal, the jaws were dissected out, placed in fixative and processed for histologic evaluation as described previously. Specimens were embedded in paraffin and sectioned in a buccal-lingual plane with a microtome set at 6 µm. Step-serial sections representing intervals of 96 µm were stained with hematoxylin and eosin. The sections cut immediately before the hematoxylin and eosin sections were stained using a silver impregnation technique, to delineate connective tissue fibers.

Methods of Analysis. The supracrestal connective tissue region was examined histologically on five step-serial sections from each specimen (the midsection and two step-serial sections to the mesial and distal of the midsection). The central three sections were used for the histometric analysis which measured the length of epithelial projection into the incisinal wound. Sections were projected at a magnification × 200, and the region of epithelium extending into the coronal aspect of the wound was traced on a sheet of white paper. The length of the epithelial projection was measured as the distance between its termination in the connective tissue and its junction with the basal cell layer of the surface epithelium. This distance was measured along the midaxis of the epithelial projection using a map-measurer. The

RESULTS

Clinical Observations

At the start of the study (which was after the initial 6-week period of oral hygiene) the gingival margins were without signs of clinical inflammation. Ten weeks after periodontitis had been induced (experimental group), the gingival tissues were enlarged, deep red, and bled upon provocation. No clinical changes were noted in the degree of inflammation at any of the time points after incisinal wounding. The gingiva of control areas (no induced periodontitis) bled less than experimental areas when the incisinal wound was made. These areas exhibited mild inflammation 1 and 3 days after wound-

Figure 1. Control specimen. One day after wounding. Supracrestal region (hematoxylin and eosin stain, original magnification × 51).

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* Becton, Dickinson and Co, Rutherford, NJ 07070.
ing, but were without signs of clinical inflammation at the later time points.

**Histological Observations**

**Control Specimens.** One day after wounding: The supracrestal region from control specimens one day after wounding exhibited severe disruption (Figs. 1 and 2) compared with corresponding regions from normal specimens (Figs. 3 and 4). The coronal end of the incision in control specimens was located in the apical region of the gingival sulcus. The incision extended obliquely through the supracrestal connective tissue and terminated on, or near, the cementum surface, coronal to the crest of the alveolar bone (Fig. 1). The cementum surface at the apical termination of the incision was often notched, and small cementum particles were present in the adjacent supracrestal connective tissue. The incision area was occupied by a fibrin network into which were enmeshed inflammatory cells and erythrocytes. At either side of the incision, there appeared to be reduced cellularity. The epithelium that lined the gingival sulcus had slight invagination into the coronal aspect of the incision, and was often absent over the connective tissue fibers remaining on the root surface. Evaluation of sections stained to delineate fiber continuity showed a distinct corono-apical break in the region of the incision (Fig. 2). Fibers remained attached to the root surface.
Three days after wounding: The coronal end of the incision within the epithelium was difficult to delineate precisely due to the fact that, in the majority of cases, epithelial continuity had been re-established within the gingival sulcus, and with minimal invagination into the incisional wound (Fig. 5). In the supracrestal connective tissue area, the wound margins appeared to be closer together than they had been at the 1-day time point. Associated with the closer approximation of the wound margins, the fibrin network had become more condensed and was more basophilic when stained with hematoxylin and eosin. Some cell repopulation had occurred in areas adjacent to the wound margins. The break in supracrestal fiber continuity was still apparent.

Seven days after wounding: Epithelial continuity was present within the gingival sulcus, and slight invagination of epithelium into the area of the wound indicated the original location of the incision (Fig. 6). In the supracrestal connective tissue region, the repair process had advanced as evidenced by a regaining of cellularity in the connective tissue adjacent to the incision. The incision area itself had become repopulated with small, oval cells which often had an orientation parallel to the direction of the wound. Fibrin was no longer present, and it had become replaced by fine and delicate fibers which restored continuity between the severed ends of the connective tissue fibers (Fig. 7). These new fibers

Figure 5. Control specimen. Three days after wounding. Supracrestal region (hematoxylin and eosin stain, original magnification \( \times 64 \)).

Figure 6. Control specimen. Seven days after wounding. Supracrestal region (hematoxylin and eosin stain, original magnification \( \times 51 \)).

Figure 7. Control specimen. Seven days after wounding. Supracrestal region (silver impregnation stain, original magnification \( \times 64 \)).
had an orientation which was often perpendicular to the existing supracrestal connective tissue fibers.

**Twenty-one days after wounding:** Although the epithelium lining the gingival sulcus was continuous and had a morphology characteristic of normal sulcular epithelium, there was slight invagination of the epithelium into the former area of the incision in some cases. No apical migration of the sulcular epithelium had occurred along the root surface. The cell and fiber components in the supracrestal connective tissue resembled a normal situation (Figs. 8 and 9). The junction between previously transected collagen fibers and the newly formed intracisal collagen was difficult to delineate. The fibers which had formed in the former region of the incision had a directional alignment similar to existing supracrestal fibers. The cells had become aligned in the direction of the fibers and were perpendicular to the original direction of the incision.

**Experimental Specimens. One day after wounding:** Examination of the dento-gingival area of experimental specimens 1 day after incisinal wounding showed that the gingival sulcus was occupied by bacterial plaque surrounding the silk ligature. An established marginal periodontitis was present characterized by loss of connective tissue attachment to the root surface with the apical end of the sulcular epithelial located on the cementum surface. The coronal end of the incisional wound was located within the gingival sulcus. Epithelial continuity was present across the incision, and the epithelium had invaginated markedly into the coronal aspect of the incision (Fig. 10). The wound extended in an oblique direction through the supracrestal connective tissue to terminate close to the cementum surface and crest of the alveolar bone.

The supracrestal connective tissue subjacent to the epithelium was densely infiltrated with inflammatory cells. Areas of acellularity were present in the deeper connective tissue adjacent to the margins of the incisinal wound. The area of the incision itself which was not occupied by epithelium contained fibrin. Concentrations of inflammatory cells were present within the fibrin network and seemed to be more densely aggregated around the termination of the epithelial invagination into the incision area. The supracrestal fibers had a distinct break in continuity in the area of the incision, and fiber remnants were distinguishable and attached to the cementum surface.

**Three days after wounding:** The coronal end of the incision in the supracrestal connective tissue was not identifiable through either severed collagen fibers or presence of fibrin, but by the distinct invagination of epithelium into the connective tissue (Fig. 11). The invaginated epithelium occupied a major portion of the incisinal wound. At the apical extent of the invaginated epithelium, fibrin was present between the wound
margins. The fibrin appeared to be more condensed and amorphous than at the 1-day time point. An accumulation of inflammatory cells was present within the fibrin and adjacent to the terminal end of the invaginated epithelium.

Seven days after wounding: The original coronal location of the incision in the supracrestal connective tissue could only be identified by the distinct epithelial projection extending into the region (Fig. 12). The apical termination of the epithelial projection appeared to coincide with the apical border of the inflammatory cell infiltrate in the supracrestal region. Examination of the epithelial projection indicated that the basal cells had different morphological characteristics on the different surfaces (Fig. 13). The basal cell layer adjacent to the more densely infiltrated connective tissue had large, rounded cells in contrast to the flattened, low-columnar basal cell shape on the other side of the projection. These different morphologies were also apparent at the site where the projection originated from the epithelium lining the gingival sulcus (Fig. 14). Apical to the termination of the epithelial projection, fibrin was no longer present, and the region consisted of a cellular connective tissue. The fiber composition of this region consisted of fine and delicate fibrils which had bridged the space between the severed wound margins.

Twenty-one days after wounding: Distinct invagination of the epithelium into the supracrestal connective tissue was a pronounced feature of the specimens 21 days after incisinal wounding (Fig. 15). In some specimens there appeared to have been a confluence between epithelia from different locations, i.e., between epithelial projections originating from sulcular epithelium and oral epithelium (Fig. 15). The epithelial projection into the supracrestal connective tissue extended virtually to the cementum surface, and terminated at a
level considerably apical to the coronal level of connective tissue attachment. Although the basal cell layers on either side of the epithelial projection had different morphologic characteristics, the differences were not as marked as they had been at the 7-day time point. Apical to the termination of the epithelium, the connective tissue exhibited normal cellularity and a fiber system was present, although the latter lacked distinct orientation.

**Histometric Analysis**

Statistical comparisons between the length of the epithelial projections in control and experimental specimens supported the histologic impression of more rapid and extensive migration into the supracrestal connective region of the experimental group. The comparisons showed that, at each of the time points, the length of the epithelial projection was significantly greater in experimental specimens (Table 1). Comparisons within each group across time using the analyses of variance showed that, in the control group, there were no significant differences in the length of the epithelial projection. In contrast, in the experimental group, the length at 21 days was significantly greater than that at 1, 3, and 7 days. The latter finding indicated that a secondary phase of epithelial migration into the incisional wound had occurred between 7 and 21 days.
Figure 15. Experimental specimen. Twenty-one days after wounding. Supracrestal region (hematoxylin and eosin stain, original magnification × 40).

Table 1
Length of Epithelial Projection (Mean ± SE, microns)

<table>
<thead>
<tr>
<th></th>
<th>Control*</th>
<th>Experimental†</th>
<th>t</th>
<th>P</th>
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<tbody>
<tr>
<td>1 Day</td>
<td>50.5 ± 12.1</td>
<td>290.5 ± 28.7</td>
<td>7.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>3 Days</td>
<td>70.4 ± 14.8</td>
<td>343.3 ± 8.8</td>
<td>15.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7 Days</td>
<td>68.7 ± 19.0</td>
<td>299.4 ± 34.2</td>
<td>5.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>21 Days</td>
<td>59.5 ± 23.1</td>
<td>569.1 ± 29.2</td>
<td>13.6</td>
<td>&lt;0.01</td>
</tr>
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* Incisional wound in normal supracrestal connective tissue.
† Incisional wound in presence of plaque induced inflammation.

DISCUSSION

The histologic observations indicated that the wound healing sequence in the supracrestal fiber area of the control group was similar to that reported following incisinal cutaneous wounds and tooth replantation. Epithelial continuity was re-established within 3 days after incisinal wounding, and had acquired the characteristic morphology of normal sulcular epithelium at 7 days. Some limited invagination of the surface epithelium into the coronal aspect of the wound occurred. Early time-points were characterized by connective tissue fiber discontinuity and a fibrin clot which occupied the incision area. As wound-healing progressed, the fibrin appeared to alter its morphology, and the wound margins became more closely apposed. At 7 days fibrin was absent and had been replaced by cells and fine reticulin fibers which were oriented perpendicular to the severed ends of the adjacent connective tissue fibers. The healing at 21 days had restored normal morphology. The cells and fibers had changed their orientation within the region of the wound and were now aligned parallel to the adjacent connective tissue fibers.

In the experimental group, where healing after incisional wounding occurred in the presence of plaque-induced inflammation, several marked differences were present compared to the healing in the control group. Initially, there was early establishment of epithelial continuity across the wound surface, and into the incision itself, observations which suggested the presence of a more “active” epithelium compared to control specimens. Regarding the more rapid establishment of epithelial continuity, it has been shown that epithelium has a dependence upon the underlying connective tissue during development, subsequent morphology and function. Proliﬁerative changes in epithelium have been associated with the presence of chronic inﬂammation in the underlying connective tissue. Inflammation may increase the mitotic activity of the basal cell layer. Mediators released from inﬂammatory cells in the connective tissue may further contribute to increased epithelial proliferation. Epithelial proliferation may also result from bacterial activity. Oral bacteria are known to produce various toxins and enzymes capable of causing tissue destruction. Although most of these have the effect, primarily, of increasing epithelial permeability by widening intercellular spaces, the enzyme hyaluronidase has been shown to have the additional capability of increasing the mitotic rate.

In a normal wound healing sequence, fibrin formation occurs almost immediately, and provides a provisional matrix over which epithelial cells may migrate. The chemotactic agent or agents responsible for the directional movement are apparently derived from fibrin or its breakdown products. The early migration of epithelium into the wound of the experimental group was in marked contrast to the healing observed in the control group. There are several potential mechanisms which may be responsible for the rapid migration of epithelium into the incision area, and they relate to the process of fibrinolysis. Fibrinolysis is the process which degrades either fibrin, or fibrinogen substrates, by enzyme action. Fibrinolytic enzymes can originate from several sources. Plasma normally con-
tains the protein plasminogen, which under the influence of various activators is created into its active form plasmin. Bacterial products (e.g., streptokinase), leukocytes, endothelial cells, activated Hageman Factor and other proteases (kallikrein, thrombin, trypsin) activate plasminogen into plasmin. In addition, granular leukocytes contain one or more fibrinolysins (proteases) which can mediate fibrinolysis either through the release of proteases that digest fibrin directly or through the release of a plasminogen activator. 

Additionally, bacteria produce powerful fibrinolysins. In the experimental group, it seems, therefore, that the elements for a more rapid degradation of fibrin were present. Specifically, plasmin from blood plasma, the presence of leukocytes at the site of injury, and the presence of bacterial plaque may have been interacting with, and degrading, fibrin. In this respect, gingival fluid from inflamed crevices has been shown to possess greater plasmin activity than that from healthy crevices.

Epithelial migration into the wound may also be related to the ability of epithelial cells to degrade fibrin. Epithelium in wound healing can penetrate the fibrin clot, suggesting that the epithelial cells are capable of secreting fibrinolytic enzymes. In a study estimating fibrinolytic activity of gingival epithelial cells, it was reported that lysis of fibrin occurred with epithelium associated with a dense inflammatory cell infiltration, but not with epithelium overlying a normal connective tissue. Fibrinolytic activity of the epithelium was also related to the rate of cell turnover, and higher rates of turnover were associated with more activity. Thus, the increased mitotic activity of the epithelium overlying inflamed connective tissue may increase the ability of the epithelial cells to degrade fibrin, and thereby contribute further toward a more rapid and deeper epithelial migration into the tissues.

In addition to the greater epithelial migration into the wound of the experimental specimens, it was also observed that, within the experimental group, a secondary phase of epithelial invagination occurred between 7 and 21 days. During this latter time period in incisional wound healing, fibrin is no longer present, and has been replaced by collagen. Consequently, mechanisms facilitating the secondary phase of epithelial migration into the wound probably related to collagenolysis, rather than to fibrinolysis. The collagenolytic system comprises, primarily, cells of epithelial, connective tissue, and vascular origin that have an ability to synthesize the enzyme collagenase, and other proteases, that mediate degradation of collagen.

Collagenase activity has been demonstrated during the wound healing process, and distinct collagenases have been demonstrated from epithelial cells, and from the connective tissues at the epithelial-mesenchyme interface. In the experimental situation under investigation, namely, where healing occurred in the presence of a plaque-induced inflammation, there were several possible sources of collagenase. Although bacterial collagenases have been identified, it seems more likely that collagenase activity in the presence of inflammation originates primarily from the tissues rather than the bacteria. Within connective tissues, fibroblasts, macrophages, and polymorphonuclear leukocytes (PMNs) have been implicated in the production of collagenase. It has been established that PMNs and macrophages are prevalent in the supracrestal infiltrate of the experimental periodontitis lesion in the squirrel monkey. In addition to their ability to synthesize collagenase, macrophages and PMNs are able to synthesize proteolytic enzymes which can complete collagen degradation, and destroy an inhibitor of collagenase. Macrophages can not only produce collagenase, but are a primary source of prostaglandin synthesis. The presence of prostaglandins may contribute to the collagenolytic process by increasing collagenase production by macrophages and fibroblasts.

The specific and nonspecific substances which are capable of activating inflammatory cells to produce, or release, collagenase are present in bacterial plaque, with an ability to penetrate into the gingival tissues. They are also present in the connective tissue as products of the inflammatory response.

It is of interest to note that collagenase released from PMNs is effective against newly synthesized collagen formed in a wound healing situation, but inactive against mature collagen. The susceptibility of newly formed collagen in the healing wound is probably due, in part, to its greater solubility. In addition, the degree of cross linking in the collagen molecule affects the rate of degradation, and noncross linked reconstituted fibrils are degraded faster than mature fibrils, and older tissue collagens. It seems likely, therefore, that in the situation where wound healing was occurring in the presence of a plaque-induced inflammation there was a combination of bacterial, cellular and tissue factors which would facilitate a rapid degradation of newly synthesized collagen. Since this degradation would be occurring in the immediate vicinity of the terminal end of the migrating epithelium, it could facilitate further invagination of epithelium into the healing wound and thereby account for the secondary phase of migration observed between 7 and 21 days. While the latter considerations have related to mechanisms of collagenolysis, it is also conceivable that the plaque-induced inflammation could have had an effect upon the synthesis of collagen during the wound healing process.

The methodology in our present study did not permit evaluation of that aspect of healing, but it warrants further evaluation since the evidence is conflicting regarding collagen synthesis in the presence of inflammation.

It was apparent that the basal cell layers on the
different surfaces of the epithelial projections within the connective tissue of the experimental group had markedly different morphologies. One basal cell layer had flat to low columnar shaped cells and had an organized architectural arrangement, whereas, on the other side of the projection, the oval cells had large, plump nuclei which occupied most of the intracellular area. The latter arrangement of the epithelial projections appeared to correspond with the morphologic characteristics of migrating and proliferating cells. Movement of migrating epithelial cells during wound healing may take place by a "leap frog" process or by movement of a coherent, intact sheet. It appears that there is evidence to support both theories of cell migration, and the dominant mechanism remains unclear. The basal cell layers on either side of the epithelial projections in our experimental group had morphologic characteristics associated with both types of migration. It was not possible to determine which of these mechanisms was operative, or dominant, at the various time points.

It was interesting to observe, however, that the basal cells with the large plump nuclei were adjacent to the more densely infiltrated connective tissue, and it is possible to speculate regarding this association. Inflammatory cell infiltrates may have a modifying influence on the epithelial cells through release of chemical mediators. These mediators are able to detach epithelial cells through destruction of intercellular attachments and facilitate detachment from the underlying connective tissues thereby increasing permeability and cell proliferation. It could also be of significance that these mediators can increase epithelial cell proliferation by elevating the mitotic rate of the basal cell layer. Although the nature of the proliferating signal remains unknown, cyclic nucleotides, prostaglandins, and a number of protein factors, e.g., fibroblast growth factor, epidermal growth factor, tumor angiogenesis factor, may be involved in the initiating signal.

In addition to the direct effects which the infiltrated connective tissue may have upon epithelial cell morphology and function, there may be indirect effects through actions on the basal lamina. The basal lamina is necessary for maintenance of morphology acts as a scaffold in tissue formation and repair and its integrity may be one of the factors limiting cell proliferation. Breaks in the continuity of the basal lamina consequent to an adjacent infiltrated connective tissue have been reported and the enzymes hyaluronidase and collagenase may degrade the basal lamina. In the experimental specimens in the present study, it was not possible to ascertain the status of the basal lamina; however, it too appears to warrant further investigation.

The major goal of the present investigation was to evaluate the healing of incisal wounds in the supra-crestal region in the presence and absence of plaque induced inflammation to determine if there were any differences which might contribute toward the accelerated loss of connective tissue attachment reported after surgery in plaque-infected dentitions and the principal theories involve interactions between proliferation of the sulcular epithelium adjacent to the root surface and degeneration of the underlying gingival and supracrestal fibers. In one of these theories, epithelial cells, stimulated by inflammation, migrate between connective tissue fibers and attach themselves further apically on the cementum. The latter relationship results in secondary fiber degeneration and concomitant loss of connective tissue attachment. With regard to the results of our current study, it is conceivable that if a marked epithelial downgrowth occurred into an incisal wound after periodontal surgery in the presence of a plaque-induced inflammation, and the terminal region of the epithelium were to approximate the root surface, the situation could predispose toward an increased loss of connective tissue attachment. Although the latter extrapolation is speculative, the marked contrast with the situation when healing occurred in the absence of an infiltrated connective tissue, i.e., minimal epithelial invagination, seems to provide clinical emphasis for performing periodontal surgery in tissues where inflammation has been resolved.

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Gingival Healing in Presence of Plaque

Send reprint requests to: Dr. Alan M. Polson, Department of Periodontology, Eastman Dental Center, 625 Elmwood Ave, Rochester, NY 14620.