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SCIENTIFIC ARTICLES

Wound Healing in the Tissues of the Periodontium following Periradicular Surgery. I. The Incisional Wound

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Periradicular surgical procedures were performed on rhesus monkeys and the wound healing responses of the tissues of the periodontium were evaluated by light microscopy. This article, Part I of the investigation, reports the mucoperiosteal tissue wound healing responses to incisional wounds of the triangular and submarginal rectangular flap designs. Little difference was found in the temporal and qualitative healing responses to incisional wounds of the two flap designs. However, the submarginal rectangular design showed less predictable results, with a greater intersample variation of wound healing responses in the earlier postsurgical evaluation periods.

Vital connective tissue and epithelium, although not visible clinically, remain attached to the root surfaces following reflection of flaps which include an intrasulcular incision. Preservation of these root-attached tissues prevented apical epithelial downgrowth along the root surfaces and loss of soft tissue attachment levels. Vitality of root-attached tissues was preserved by preventing dehydration, avoiding curettement of root surfaces, and using a flap reflection technique which eliminates reflective forces in the intrasulcular incisional wound site.

The most common type of endodontic surgery used in clinical practice is periradicular surgery which involves reflection of mucoperiosteal tissues for the purposes of curettage, root end resection and/or root end filling. Periradicular surgery is initiated to eliminate etiological factors of endodontic origin which cause periradicular pathosis. Thus, pathological injury is corrected by intentional surgical injury, initiated for the purpose of improving the overall health of the patient and based on the predictability of the surgical wound healing that will follow.

Wound healing is the response of living tissues to any injury which causes disruption of the continuity and/or function of those tissues and involves a complex series of biological events, some occurring simultaneously and others dependent upon the completion of prior events. Wound healing is basically dependent upon the type of tissue wounded and the type of wound that the tissue receives (1–3).

The tissues wounded in periradicular surgery are the mucoperiosteal tissues (gingiva, alveolar mucosa, palatal mucosa, and underlying periosteum), periradicular tissues (bone, gingival ligament, and periodontal ligament), and radicular tissues (cementum and dentin). These tissues, with the exception of dentin, are collectively termed the periodontium and form the supporting structures of the teeth. The tissues of the periodontium receive three types of surgical wounding during periradicular surgery: incisional wounding, blunt dissectional wounding, and excisional wounding. Incisional wounds are made with a scalpel, outline the perimeter of the flap, and involve the mucoperiosteal tissues. Blunt dissectional wounds are made with a periosteal elevator, separating mucoperiosteal tissues from cortical bone during the flap reflection procedure. Excisional wounds are made with a rotary instrument in removing bone and resecting the root end (3).

Thus, with multiple types of oral tissues receiving various types of surgical wounding, the entire wound healing response to periradicular surgery is understandably diverse and complicated. Applied surgical concepts and techniques should be based on the knowledge of factors which promote rapid and complete wound healing. This, in turn, is based on the response of the wounded tissue to the type of surgical wound inflicted. Therefore, knowledge of the wound healing responses is essential for evaluating and developing the most effective surgical regimen. Very little information exists in the scientific literature regarding wound healing following periradicular surgery. This is in direct contrast to the plethora of studies evaluating wound healing responses to periodontic surgery.

This investigation, one of a series of wound-healing studies recently completed at Baylor College of Dentistry, was designed to investigate early wound-healing responses of the

tissues of the periodontium to the incisional, dissectional, and excisional wounding of periradicular surgery.

The purpose of Part I of this report is to describe the events of the wound healing response of mucoperiosteal tissues to incisional wounding at postsurgical periods of 1 to 4 days and 14 and 28 days. Parts II and III will describe the wound healing response to dissectional and excisional wounding.

MATERIALS AND METHODS

Four mature rhesus monkeys (*Macaca mulatta*), ranging in age from 3 to 5 yr and weighing 23 to 35 kg, served as the research models for this investigation. Nonhuman primates were chosen because of their close phylogenetic relationship to man; evidence that their pathophysiological wound-healing responses are remarkably similar to man, and the morphology and size of their oral cavity which allows ease of access for intraoral surgical procedures (4). The animals were housed at the Baylor College of Dentistry Animal Research Unit facilities under approved and controlled conditions.

On the day of surgery, each animal was anesthetized by intramuscular injection of ketamine (10 mg/kg) and Rompun (1.5 mg/kg). An infiltration injection of 2% lidocaine with 1:50,000 epinephrine into the alveolar mucosal tissues in each surgical site was given for local anesthesia and hemostasis.

Surgical procedures were scheduled to provide six flaps in each animal for a total of 24 experimental flaps. Two flap designs were used, a triangular flap with an intrasulcular incision and a single vertical releasing incision; and a submarginal rectangular (Ochsenbein-Leubke) flap with a horizontal incision in attached gingiva and two vertical releasing incisions. The underlying periosteum was reflected with each flap, exposing cortical bone.

In each animal, two general anesthesia sessions were required, one session for two flaps which would provide either 14- or 28-day postsurgical evaluation periods, and a second session for four flaps which would provide a 1-, 2-, 3-, or 4-day postsurgical evaluation periods. Thus, two flaps of each design at each postsurgical evaluation period were made. The maxillary left and mandibular right posterior sextants and the right half of the maxillary and mandibular anterior sextants were utilized as experimental surgical sites. A maxillary and mandibular surgical site was used for each flap design at each postsurgical period. Unoperated areas served as controls, allowing comparison of the surgically injured tissues with normal tissues.

Following incision and reflection of the flap with a periosteal elevator, an excisional wound was made in interdental bone by penetrating through the cortical plate and into cancellous bone with a #10 round bur in a high-speed handpiece with water coolant. Penetration was to the depth of the cutting head of the bur. This was accomplished to evaluate the wound healing response of the osseous tissues to excisional wounds similar to that accomplished in periradicular surgery.

Each flap was retracted for 15 min with frequent irrigation of the tissues with sterile, physiological saline. The flaps were reapproximated, and wound closure and stabilization was effected by interrupted plain gut sutures. The tissues were then compressed for 3 min by applying firm finger pressure to saline-soaked gauze pads placed over the surgical site. This was done to effect hemostasis, prevent hematoma formation, and promote formation of a thin fibrin clot in the incisional and dissectional wounds by enhancing good tissue reapproximation (5).

The animals were placed on a soft diet supplemented with vitamin C and fresh fruit postoperatively for 4 days or until sacrifice. One crushed tablet of Vicodin was added to the food daily for 2 days (or until sacrifice) after surgery to minimize discomfort from the surgical wounding. The gut sutures were allowed to absorb, obviating the need for additional general anesthesia sessions for suture removal. Photographs were taken of the surgical sites prior to surgery, during surgery, immediately after surgery, and at the time of sacrifice. The photographs were compared and evaluated for clinical evidence of delayed healing (e.g. swelling, flap dislodgement, sinus tract formation, scarring, etc.), which might provide additional evidence to support or explain microscopic findings.

Animals were sacrificed while under deep general anesthesia produced by Nembutal. The thoracic cavity was entered, the left ventricle opened, and a cannula passed into the ascending aorta. The descending aorta was clamped with a hemostat to concentrate perfusion to the head and neck. Transcardiac perfusion, under 120 mm Hg pressure, was initiated through the aortic cannula with 800 to 1000 ml of physiological saline to clear the vascular channels of blood. Perfusion was then completed with 800- to 1000-ml solution of 2% glutaraldhyde and 2% paraformaldehyde to provide immediate internal fixation of tissues. The maxilla and mandible were removed, hemisected, and placed in 10% formalin for 1 wk of further fixation. Additional sectioning was then accomplished with a thin rotary saw, separating each surgical site and control site into small blocks which were coded and placed in 5% formic acid for decalcification. The progress of the decalcification process was monitored by radiographic means.

Following decalcification, the block sections were further trimmed and sectioned with scalpel blades to provide specimens that would allow cross-sectional viewing of the approximated edges of both the vertical and horizontal incisional wounds of each flap design. From each block section containing 1 of the 24 experimental surgical sites, two block specimens were cut, one containing the vertical incision and one the horizontal incision. The 48 block specimens were embedded in paraffin and serial sectioned at a thickness of 7 μm. At 20 section intervals, 4 sections were stained with hematoxylin and eosin, 4 sections were stained with Masson's trichrome, and 4 sections were stained with Snook's reticulin stain. This sequencing was continued until it was determined that additional sectioning would not provide further information. The coded, stained specimens were examined by light microscopy.

Evaluation of the soft tissue responses to incisional wounding was based on anticipated events as extrapolated from wound-healing studies of both oral and nonoral tissues (6–9). These events were *clotting and inflammation, epithelial healing,* and *connective tissue healing,* representing three major phases of wound healing (1–3). Within each of these wound healing phases, specific biological events were graded, using the following criteria.

CLOTTING AND INFLAMMATION

Clot Formation

(#) Absent = fibrin clot has not formed; coagulum present.
(+) Evident = fibrin clot has formed; areas of coagulum.
(++) Very evident = thin fibrin clot occupies the wound site.
(##) Event completed = fibrinolysis very evident; clot partially or totally replaced by reparative tissues.

Polymorphonuclear Leukocytes (PMN's)

(#) Absent = no evidence of PMN infiltrate in the wound site.
(+) Evident = PMN infiltrate evident in the wound site.
(++) Very evident = heavy PMN infiltrate; PMN clearly the predominant inflammatory cell type.
(##) Event completed = PMN infiltrate no longer present; scattered PMN's in wound site.

Macrophages (MP's)

(#) Absent = no evidence of MP infiltrate in the wound site.
(+) Evident = MP infiltrate evident in the wound site.
(++) Very evident = heavy MP infiltrate; MP are clearly the predominant inflammatory cell type.
(##) Event completed = MP's greatly reduced in number; MP's no longer the predominant cell type in the wound site.

EPITHELIAL HEALING

Epithelial Cell Migration

(#) Absent = no evidence of epithelial migration.
(+) Evident = epithelial streaming apparent from at least one wound edge.
(++) Very evident = epithelial migration and streaming are apparent from both wound edges.
(##) Event completed = epithelial migration completed; contact of epithelial cells from opposing wound edges.

Epithelial Seal Formation

(#) Absent = contact of migrating epithelium from opposing wound edges has not been established.
(+) Evident = epithelial seal established by contact of epithelium from opposing wound edges.
(++) Very evident = epithelial seal established by multiple layers of epithelial cells.
(##) Event completed = maturation of epithelium of the seal into identifiable strata of stratified squamous epithelium.

Epithelial Barrier Formation

(#) Absent = no evidence of identifiable strata.
(+) Evident = evidence of two or more identifiable strata.
(++) Very evident = evidence of all strata, including superficial strata.
(##) Event completed = maturation of epithelium complete.

CONNECTIVE TISSUE HEALING

Fibroblast Migration

(#) Absent = no evidence of fibroblast migration into the wound site.
(+) Evident = fibroblasts are evident in the wound site.
(++) Very evident = fibroblasts and fibroblast-like cells are the most predominant reparative cell type in the wound site.
(##) Event completed = number of fibroblasts has decreased in healing wound site.

Collagen Synthesis

(#) Absent = no evidence of collagen fibers in the wound site.
(+) Evident = type III collagen fibers in the wound site.
(++) Very evident = type I and III collagen fibers present in the wound site; fibers are thin and lack fiber density.
(##) Event completed = remodeling and maturation of collagen in healing wound site.

Granulation Tissue Formation

(#) Absent = no evidence of granulation tissue formation in the wound site.
(+) Evident = granulation tissue apparent in the wound site.
(++) Very evident = granulation tissue present throughout the wound site.
(##) Event completed = granulation tissue maturing or replaced by fibrous connective tissue.

Fibrous Connective Tissue Formation

(#) Absent = no fibrous connective tissue in the wound site.
(+) Evident = evidence of early maturation of granulation tissue into fibrous connective tissue in areas of the wound site.
(++) Very evident = maturation of granulation tissue into fibrous connective tissue in all but isolated areas of the wound site.
(##) Event completed = fibrous connective tissue in the wound site undergoing maturation and remodeling.

RESULTS

The 48 block specimens represented two samples of each vertical and each horizontal incisional wound in the two flap designs at each postsurgical evaluation period. Eight of the block specimens, each from different incisional wounds, could not be microscopically evaluated because of processing errors. Of the remaining incisional wounds with two samples, inter-

sample variations at each postsurgical period were noted but, primarily involved differences between (+) and (++) grading. Where these differences existed, the more advanced healing grade was assigned. If the samples differed by more than one grade, the intermediate grade was assigned. Results of the microscopic evaluation of the healing responses of mucoperiosteal tissues to vertical and horizontal incisional wounding are presented in Tables 1 and 2. The healing responses to the vertical incisions in both types of flap design were essentially the same. Only minor differences were observable in some of the evaluated biological events, and these variations were no greater than the intersample variations noted in the same flap designs at the same postsurgical period. Healing responses to the horizontal incisional wounds in the two flap designs showed a greater variation in wound-healing responses in the earlier (1- to 4-day) postsurgical evaluation periods.

The submarginal rectangular flaps evidenced more unpredictability in wound healing responses between the two samples in the earlier postoperative periods. Intersample variations of more than one grade occurred in 13 instances, as compared with only 3 instances for the triangular flap. Macroscopic evidence obtained from photographs suggested that

TABLE 1. Microscopic evaluation of wound healing events occurring in vertical incisional wounds of two flap designs at varying postoperative periods

	Flap Design*											
	T	SR	T	SR	T	SR	T	SR	T	SR	T	SR
	Postoperative Day 1	Postoperative Day 1	Postoperative Day 2	Postoperative Day 2	Postoperative Day 3	Postoperative Day 3	Postoperative Day 4	Postoperative Day 4	Postoperative Day 14	Postoperative Day 14	Postoperative Day 28	Postoperative Day 28
Clotting and inflammation												
Clot formation	+†	+	+	++	+	++	##	##	##	##	##	##
PMN's	++	++	+	+	+	+	##	##	##	##	##	##
MP's	+	+	++	++	++	++	++	++	##	##	##	##
Epithelial healing												
Epithelial migration	+	+	++	++	##	##	##	##	##	##	##	##
Epithelial seal	#	#	++	++	##	##	##	##	##	##	##	##
Epithelial barrier	#	#	#	+	++	+	##	##	##	##	##	##
Connective tissue healing												
Fibroblast migration	#	#	+	+	++	++	++	++	##	##	##	##
Collagen synthesis	#	#	#	#	+	+	++	++	##	##	##	##
Granulation tissue	#	#	#	#	#	+	+	+	##	##	##	##
Fibrous connective tissue	#	#	#	#	#	#	#	#	++	##	##	##

* T, triangular flap design; SR, submarginal rectangular flap design.
† # = absent; + = evident; ++ = very evident; ## = event completed.

TABLE 2. Microscopic evaluation of wound healing events occurring in horizontal incisional wounds of two flap designs at varying postoperative periods

	Flap Design*											
	T	SR	T	SR	T	SR	T	SR	T	SR	T	SR
	Postoperative Day 1	Postoperative Day 1	Postoperative Day 2	Postoperative Day 2	Postoperative Day 3	Postoperative Day 3	Postoperative Day 4	Postoperative Day 4	Postoperative Day 14	Postoperative Day 14	Postoperative Day 28	Postoperative Day 28
Clotting and inflammation												
Clot formation	++†	++	+	+	+	++	##	+	##	##	##	##
PMN's	++	++	++	+	+	+	##	+	##	##	##	##
MP's	+	+	+	+	++	++	++	++	##	##	##	##
Epithelial healing												
Epithelial migration	++	++	##	++	##	++	##	##	##	##	##	##
Epithelial seal	+	+	+	+	++	++	##	##	##	##	##	##
Epithelial barrier	#	#	#	#	#	#	+	+	##	##	##	##
Connective tissue healing												
Fibroblast migration	#	#	+	+	+	+	++	+	##	##	##	##
Collagen synthesis	#	#	+	+	+	+	++	+	##	##	##	##
Granulation tissue	#	#	#	#	#	#	+	+	##	##	##	##
Fibrous connective tissue	#	#	#	#	#	#	#	#	++	++	##	##

* T, triangular flap design; SR = submarginal rectangular flap design.
† # = absent; † = evident; ++ = very evident; ## = event completed.

FIG 1. A multilayered epithelial seal (*arrows*) in the vertical incisional wound of a triangular flap at 2 days after surgery. Epithelial cells have invaginated into the wound site, but identifiable strata have not yet formed. Remnants of the surface coagulum (*SC*) are seen above the seal. *FC*, fibrin clot (hematoxylin and eosin; original magnification ×66).

these intersample variations might have resulted from the greater difficulty encountered in obtaining good flap reapproximation and stabilization with the submarginal rectangular flaps.

VERTICAL INCISION: TRIANGULAR FLAP

At 1 day clot formation was evident with fibrin strands oriented parallel to the plane of the wound and PMN's and some MP's present on the strands. Epithelial cell migration and streaming were evident from the unflapped wound edge. The flapped wound edge did not show streaming but basal and suprabasal cells were apparently dedifferentiating and undergoing morphological changes in preparation for migration.

At 2 days MP's were the predominant inflammatory cell in the wound site and the number of PMN's were reduced. Migration of epithelial cells from both wound edges had resulted in the formation of a multilayered epithelial seal (Fig. 1). Undifferentiated ectomesenchymal cells and fibroblasts had migrated into the wound site and were evident along the fibrin strands. Collagen formation was not observed.

At 3 days MP's continued to be the predominant inflammatory cell type in the wound site. Maturation of the ells of the epithelial seal had resulted in epithelial barrier formation with identifiable basal, prickle, and superficial cell layers (Fig. 2). Fibroblasts and undifferentiated ectomesenchymal cells had become the predominant reparative cells and reticulin staining revealed evidence of type III (reticular) collagen formation in the wound site.

At 4 days MP's were still very evident, but fibroblasts and undifferentiated ectomesenchymal cells were the predominant cells in the wound site. Epithelial barrier formation had progressed with maturation into epithelial strata similar to adjacent tissues. Collagen formation was very evident with both type I and III fibers evident throughout the wound site. Granulation tissue had replaced most of the fibrin clot (Fig. 3).

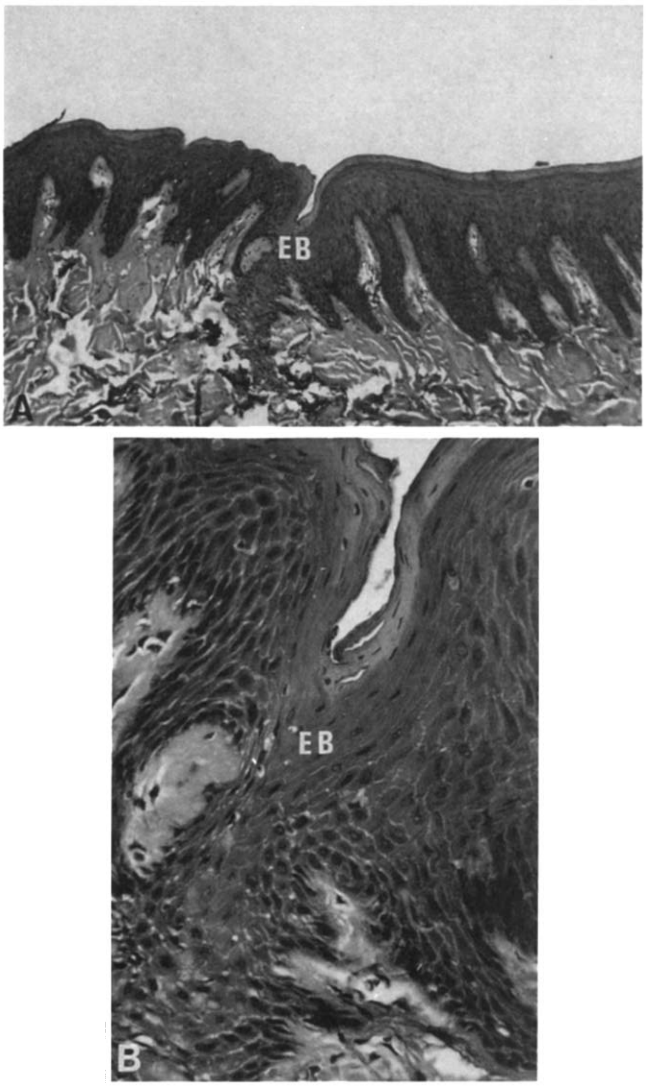


FIG 2. *A* and *B*, Epithelial barrier (*EB*) formation in vertical incisional wound of triangular flap at 3 days after surgery. Identifiable strata of stratified squamous epithelium are evident (hematoxylin and eosin; original magnification *A*, ×33; *B*, ×132).

At 14 days, some isolated areas of granulation tissue had not been replaced by fibrous connective tissue, but all other evaluated healing events were completed.

At 28 days all evaluated healing events were completed.

VERTICAL INCISION: SUBMARGINAL RECTANGULAR FLAP

The chronology of healing events in the vertical incision is essentially the same as in the triangular flap, with the following exceptions. Epithelial barrier formation was evident at 2 days and granulation tissue had replaced portions of the fibrin clot at 3 days. Maturation of fibrous connective tissue was more advanced at 14 days.

HORIZONTAL (INTRASULCULAR) INCISION: TRIANGULAR FLAP

At 1 day clot formation was similar to the vertical incision and PMN's were clearly the predominant inflammatory cell

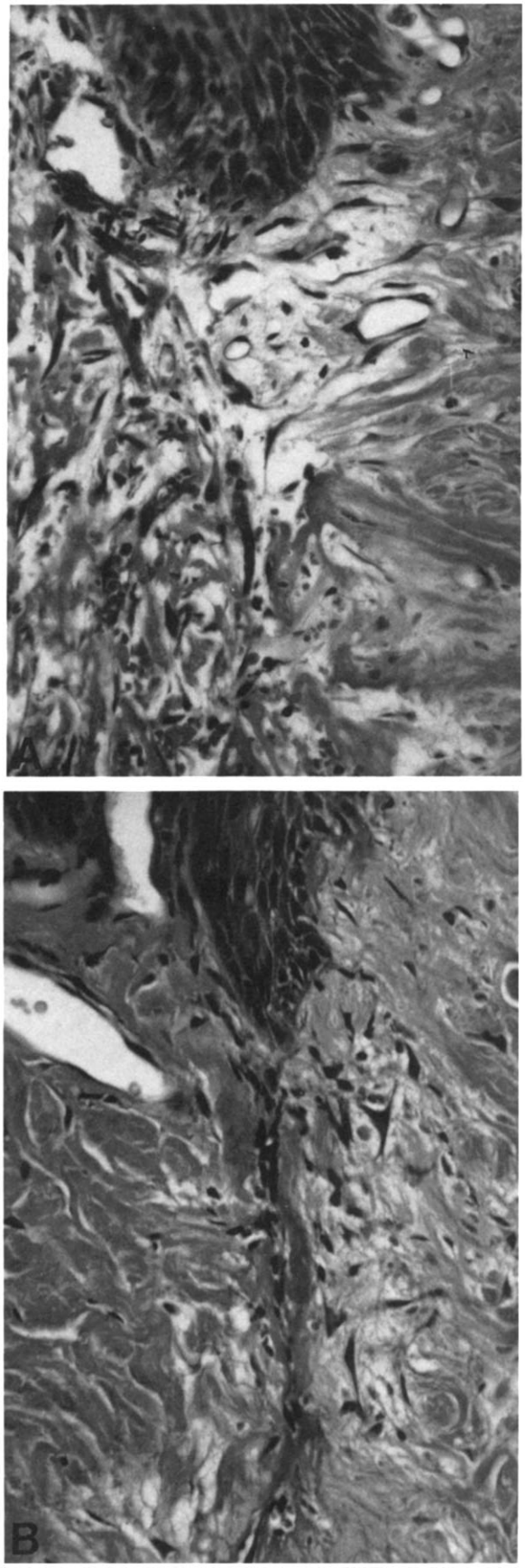


FIG 3. *A* and *B*, Replacement (organization) of fibrin clots by granulation tissue in vertical incisional wounds of triangular flaps at 4 days after surgery. Note the predominance of reparative cells, evidence of collagenation and angiogenesis, and the paucity of inflammatory cells. Basal and suprabasal cells of the epithelial barrier are seen at the *top center* of each specimen (hematoxylin and eosin; original magnifications ×132).

type. The incision penetrated sulcular epithelium, marginal and attached gingiva, and associated gingival ligament fibers to crestal bone. Vital sulcular epithelium, junctional epithelium, and fibrous connective tissue remained attached to the cementum surface. The thin fibrin clot, with its fibrin strands oriented parallel to the plane of the incisional wound, formed between these root-attached tissues and the flapped tissues.

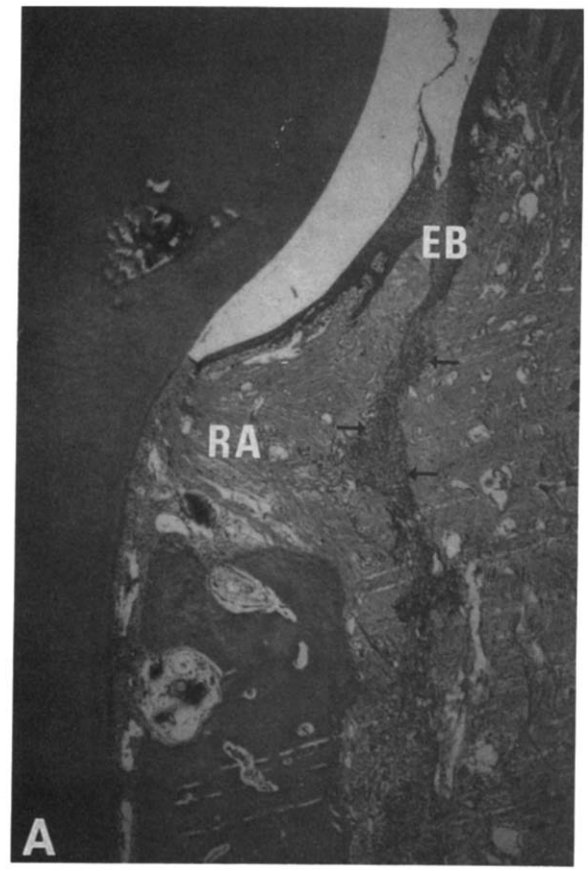
Except for the most superficial cell layers, the root-attached sulcular epithelium had intact cell membranes with normal staining cytoplasm and nuclei, and showed evidence of dedifferentiation and early migration with widening of intercellular spaces and elongation of cells at the wound edge. The root-attached junctional epithelium appeared to be normal in all respects, with no evidence of apical epithelial downgrowth along the root surface. Epithelial cell migration and streaming were evident from both wound edges and a thin epithelial seal had been established across the incisional wound site.

The root-attached connective tissue had a normal collagen fiber architecture with intact microvessels and a normal component of vital fibroblasts. Scattered inflammatory cells (PMN's and MP's) were observed in this tissue which retained its Sharpey's fiber attachments to cementum.

At 2 days PMN's continued to be the predominant inflammatory cell in the wound site but MP's were seen in increasing numbers. Vital root-attached epithelium and connective tissue were apparent and differed little from the observation at 1 day. There was no evidence of apical epithelial downgrowth along the root surface. An epithelial seal was present but barrier formation had not occurred. Undifferentiated ectomesenchymal cells were the predominant reparative cell in the wound site and reticulin staining revealed the presence of type III reticular collagen throughout the site.

At 3 days MP's had become the predominant inflammatory cell in the wound site and the number of PMN's were greatly reduced. The epithelial seal had matured into multiple layers but without identifiable strata and the epithelial cells had extended into the underlying connective tissue along the incisional wound. Vital root-attached epithelium and connective tissue were evident with the junctional epithelium appearing to be normal and no evidence of apical epithelial downgrowth along the root surface. Fibroblasts and undifferentiated ectomesenchymal cells were evident in large numbers along the fibrin strands, which showed evidence of fibronolytic activity, and a network of fine, branching reticulin fibers was present throughout the wound site.

At 4 days fibrinolytic activity was very evident, with few fibrin strands observable. PMN's were further reduced in numbers and appeared only as occasional, scattered cells. Root-attached epithelium and connective tissue remained vital and were apparently actively participating in the wound-healing process. At the incisional wound site, an epithelial barrier had formed, joining the root-attached epithelium with the sulcular epithelium of the flapped tissue (Fig. 4). Basal and suprabasal layers of the barrier had migrated into the underlying connective tissue along the incisional wound. Fi-



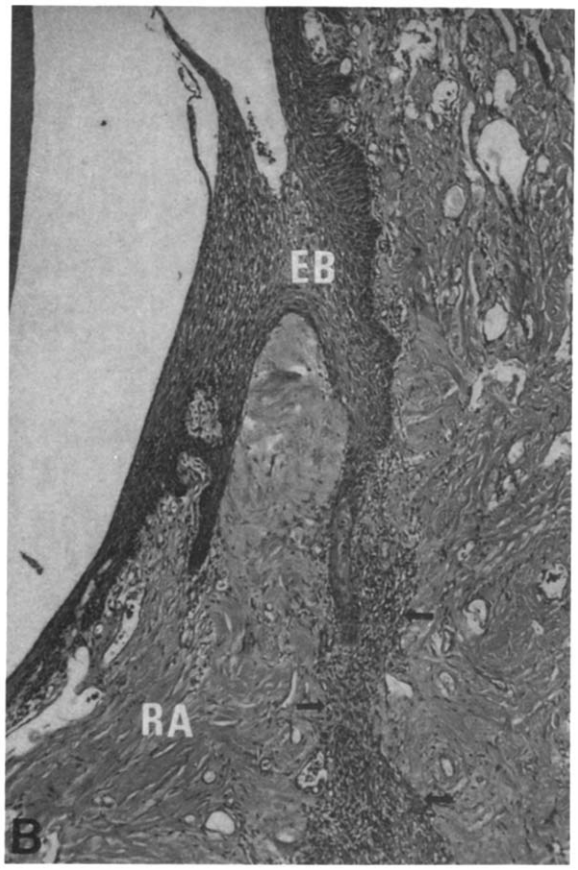


FIG 4. *A* and *B*, Intrasulcular incisional wound at 4 days after surgery. An epithelial barrier (*EB*) has formed. Root-attached tissues (*RA*) (epithelium and connective tissue) have remained vital and demonstrate essentially normal architectural patterns. Granulation tissue (*small arrows*) occupies the wound site in lamina propria. There is no evidence of apical epithelial downgrowth along the root surface (hematoxylin and eosin; original magnification *A*, ×13; *B*, ×33).

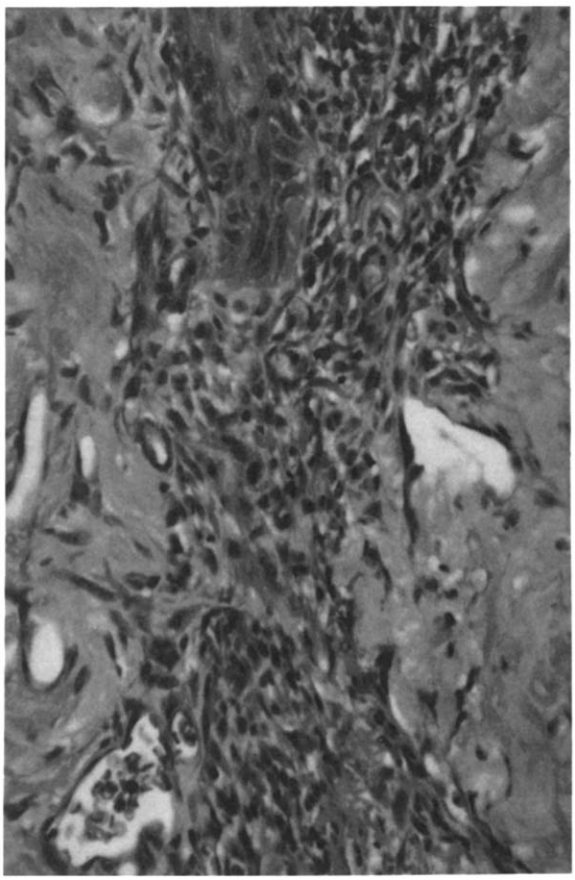


FIG 5. Photomicrograph of same specimen as in Figure 4. Maturing granulation tissue in the intrasulcular incisional wound. Note the predominance of reparative cells (fibroblasts and undifferentiated ectomesenchymal cells), evidence of collagenation and angiogenesis, and the paucity of inflammatory cells. Cells of the epithelial barrier are seen at *top center*. On the *left* is the vital root-attached connective tissue, and on the *right* is the vital lamina propria of the flapped tissues (hematoxylin and eosin; original magnification ×132).

broblasts were now the predominant reparative cell and type I and III collagen fibers were present throughout the wound site. Most of the fibrin clot has been replaced by granulation tissue (Fig. 5). The new collagen fibers appeared to connect and join the severed collagen fibers of the flapped lamina propria and the root-attached connective tissue. The junctional epithelium remained normal in appearance, with no evidence of apical epithelial downgrowth along the root surface (Fig. 6).

At 14 days the sulcular epithelium appeared to be essentially normal throughout, with the only identifiable microscopic evidence of the site of incisional wounding being an epithelial extension into the underlying connective tissue. Fibrous connective tissue occupied the incisional wound site in the lamina propria and differed from surrounding connective tissue only by its thinner fibers and greater numbers of fibroblasts per unit area. The junctional epithelium appeared

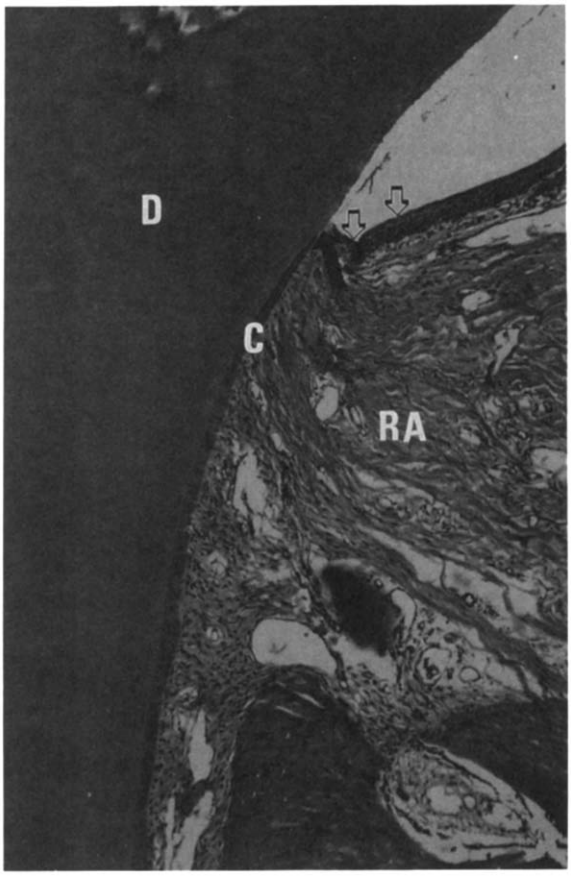


FIG 6. Photomicrograph of same specimen as seen in Figure 4. The junctional epithelium (*open arrows*) remains attached at the level of the cementoenamel junction (*large arrow*) at 4 days after surgery. Preservation of the vitality of root-attached connective tissue (*RA*) has prevented apical migration of epithelium along the root surface. *C*, cementum; *D*, dentin (hematoxylin and eosin; original magnification ×33).

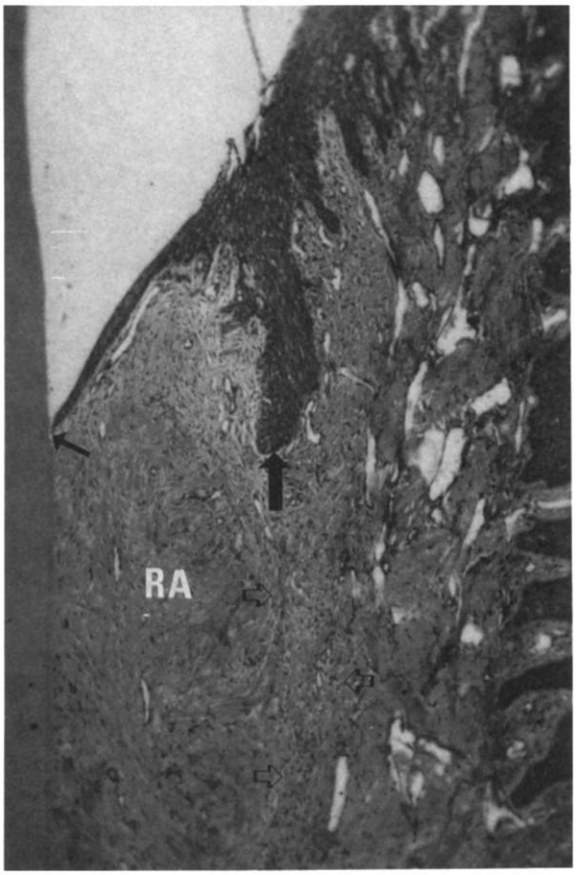


FIG 7. Photomicrograph of area of intrasulcular incisional wound at 14 days after surgery. Note the invagination of sulcular epithelium (*large arrow*) and the maturing fibrous connective tissue (*arrowheads*) now occupying the incisional wound site. Junctional epithelium remains attached at the level of the cementoenamal junction (*small arrow*) with no apical migration along the root surface. Root-attached connective tissue (*RA*) appears to be normal and uninflamed (hematoxylin and eosin; original magnification ×33).

to be normal with no evidence of apical epithelial downgrowth along the root surface (Fig. 7).

At 28 days all evaluated and observed biological events in the wound-healing process were completed (Fig. 8).

HORIZONTAL INCISION: SUBMARGINAL RECTANGULAR FLAP

Healing of the horizontal incisional wound of the submarginal rectangular flap was very similar to the healing patterns reported for the intrasulcular wound of the triangular flap. The following differences were noted.

At 2 days the number of PMN's were reduced from the 24-h period and MP's and PMN's were equally evident. In one sample, epithelial migration from both wound edges was observable but no epithelial seal had formed. In the second sample, epithelial migration was complete and epithelial barrier formation had occurred. In the latter sample, fibroblasts were the predominant reparative cell in the wound site.

At 3 days similar discrepancies between the two samples were evident, with one sample showing much more advanced epithelial and connective tissue healing than the other.

At 4 days fibroblast migration and collagen synthesis were less evident than in the intrasulcular wound of the triangular flap.

DISCUSSION

Results of this study indicate that the healing responses of the soft tissues of the periodontium to incisional wounding in periradicular surgery are remarkably rapid. The two types of flap designs showed similar wound healing potential but not similar wound healing predictability. The submarginal rectangular flap was less predictable with much greater intersample variations between rapid and delayed healing at the earlier postsurgical intervals. However, at 14 and 28 days there were essentially no differences in the temporal and qualitative degrees of healing.

Great care was exercised in reapproximating, stabilizing, and compressing the flapped tissues following surgery. In most specimens this resulted in a thin hiatus between wound edges and a well-formed, thin blood clot with fibrin strands oriented parallel to the plane of the incisional wound. The parallelism of the strands indicates that they have undergone contraction

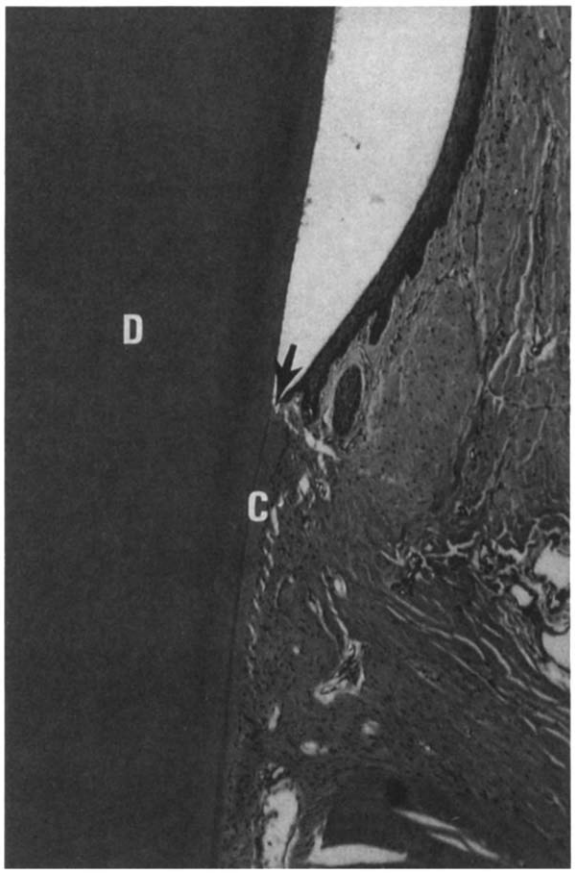


FIG 8. Photomicrograph of area of intrasulcular incisional wound at 28 days after surgery. Junctional epithelium remains attached at the level of the cementoenamel junction (*arrow*), with no evidence of apical migration along the root surface. *C*, cementum; *D*, dentin (hematoxylin and eosin; original magnification ×33).

after initial polymerization (1, 9–11), and now provide a tenuous attachment between wound edges (9, 11) and pathways for migration of inflammatory and reparative cells (8).

PMN's were the dominant inflammatory cell at 24 h but were reduced in numbers by 48 h as the macrophage became the predominant cell in the wound site during the 48- to 72-h period. The presence of increasing numbers of MP's and decreasing numbers of PMN's is highly significant in that it signals the transition from the clotting/inflammatory phase to the connective tissue healing phase (8, 12). MP's are the supervisors of the reparative process, creating by phagocytosis an environment conducive to connective tissue healing and directing the migration of cells that will effect regeneration or repair into the wound site (1, 8, 13). The MP is more than an inflammatory cell, it is also the most important reparative cell in the very early wound healing process, directly influencing the temporal and qualitative course of repair (3, 12).

The early reduction in numbers of PMN's is also important as it indicates a rapid destruction of microorganisms within the wound site and wound edges, reducing the need for continued migration of these cells. The primary function of PMN's in wound healing is the destruction of microorganisms and, when this is satisfactorily accomplished, the inflammatory mediators attracting these cells to the site are no longer released and the short-lived PMN's in the area soon degenerate (9). The early reduction in PMN's also attests to the effectiveness of the epithelial seal, observed as early as 24 h, in inhibiting the ingress of microorganisms from the oral environment into the underlying tissues.

Epithelial barrier formation, resulting from maturation of the cells forming the epithelial seal into identifiable layers of stratified squamous epithelium, occurred in the vertical incisions during the 48- to 72-h period. However, in the intrasulcular incision, barrier formation was not observed until 96 h, although an epithelial seal was evident at 24 h. This delay in barrier formation may be due to the hostile environment of the gingival sulcus and associated plaque accumulation. However, it is interesting to note that the intrasulcular epithelial seal was sufficiently protective to allow collagen fiber formation in the wound site between 24 and 48 h. Collagen fiber formation was not observed in the vertical incisions until 72 h. This difference may result from earlier epithelial seal formation or greater collagen synthesis capabilities in the tissues receiving intrasulcular incisional wounding.

Epithelial migration and streaming occurred more rapidly from the wound edge of unflapped tissue than flapped tissue in all incisions, with the exception of the intrasulcular incision where the rate was about the same from both wound edges. Epithelium also migrated to varying depths along the wound edges into underlying connective tissue in all incisional wounds. Some of these epithelial projections showed post-wounding acanthosis as described by Ordman and Gillman (10).

Connective tissue healing appeared to be directly related to the speed with which epithelial healing occurred. Collagen synthesis in the wound site was evident by light microscopy within 24 h after the formation of an epithelial seal and increased rapidly with epithelial barrier formation. Granulation tissue was observed in the wound site within 24 h after the formation of an epithelial barrier in vertical incisions. Barrier formation occurred as early as 48 h in some specimens and by 72 h in all specimens. Granulation tissue was evident in the horizontal incisions at 96 h.

By light microscopic examination, evidence of collagen synthesis is delayed until aggregation by extracellular collagen molecules has progressed to fiber formation, even with special stains (14). At the highest level of magnification possible with the electron microscope, collagen can be seen only after aggregation of collagen molecules to fibrils which have a diameter of about 0.3 nm. It is necessary for fibrils to further aggregate to fibers, with a diameter of about 0.2 μm, before detection by light microscopy is possible (14). Therefore, the observation that new collagen fibers are present in vertical incisions at 72 h and in horizontal incisions at 48 h indicates that actual collagen synthesis has preceded this observation by several hours, probably as many as 8 to 16 h. This demonstrates the remarkable rapidity with which the oral mucoperiosteal tissues can respond to incisional surgical wounding.

By far the most interesting finding, with significant clinical implications, is the important role played by the thin layer of epithelial and connective tissues that remains attached to the root surfaces. In previous pilot studies on monkeys and dogs it was observed that apical epithelial downgrowth along the root surface, resulting in deep sulcular "pocket" formation, occurred with alarming frequency following reflection of full mucoperiosteal flaps with an intrasulcular incision. These cases were associated with delayed wound healing in the

intrasulcular incisional wound site. It was further noted that some flaps of the same design healed very rapidly and showed no evidence of apical epithelial downgrowth along the root surface. In an attempt to determine a plausible explanation for such disparate surgical results, representative microscopic sections from each group at varying postsurgical time intervals were compared. This resulted in the observation that in some early postsurgical specimens, a thin layer of apparently vital connective tissue and epithelium survived the surgical procedures and remained attached to the root surfaces. The fibrin clot had formed between this tissue and the flapped tissues. In other specimens, this root-attached tissue was not present and the clot had formed along the root surface. A review of the literature relating to wound healing following mucoperiosteal flap procedures in periodontal surgery provided evidence suggesting that maintaining the vitality of root-attached tissues would greatly enhance rapid wound healing and impede apical epithelial downgrowth along the root surface (15–17).

In the present investigation, meticulous care was taken to protect the root-attached tissues, which are not clinically visible. First, reflection of the flap was not initiated in the intrasulcular incision, as the reflective forces would likely damage or destroy these tissues. Instead, reflection was initiated in the vertical incision against attached gingiva and an undermining elevation technique (5) was used to elevate the flap. Second, curettement or planing of the root surfaces, a standard periodontal surgical procedure sometimes used in endodontic surgery, was avoided. Third, the root-attached tissues were prevented from dehydration by frequent irrigation with sterile physiological saline during the surgical procedure. These clinical protective measures were successful, as all triangular flap specimens showed evidence of vital root-attached tissues.

The presence of root-attached tissues provides an intrasulcular incisional wound site with wound edges of like tissues (i.e. epithelium to epithelium and fibrous connective tissue to fibrous connective tissue), which is a tremendous advantage because both wound edges can contribute to the healing process. If, however, flapped tissues are replaced onto a denuded root surface, only one wound edge can contribute and fibrous attachment of flapped tissue to root is greatly delayed.

In the presence of vital root-attached epithelium, an epithelial seal was rapidly established and observable at 1 day postsurgically, with both epithelial wound edges contributing to the seal. Connective tissue healing followed quickly, with collagen fiber formation evident in the wound site 24 h later.

If the root-attached tissues are destroyed or intentionally removed, sulcular epithelium from the flapped wound edge migrates apically along the root surface until it encounters connective tissue that is attached to the cementum surface (7). At this juncture, the epithelium will form a new attachment to cementum. It is the level of this new attachment that is of particular concern because this determines the postsurgical sulcular depth. As the level of apical epithelial migration is dependent upon the level of connective tissue attachment to cementum, the importance of rapid connective tissue new attachment to denuded cementum becomes apparent. The longer the delay in connective tissue new attachment, the greater the apical migration of epithelium (19). In periodontal surgery, root surfaces are routinely planed or curetted to remove diseased tissues and it is this postsurgical race between apically migrating epithelium and connective tissue new attachment to cementum that ultimately determines the success or failure of the procedure.

However, in endodontic surgery there is usually no necessity to denude the root surfaces and this provides a tremendous advantage in wound healing by allowing "reattachment" rather than "new attachment." Connective tissue reattachment is rapid and occurs when connective tissue of the flap is reunited with vital connective tissue fibers retained on the root surface. Connective tissue new attachment is slower and requires formation of new cementum with incorporation of collagen fibers or, root surface demineralization with citric acid to expose collagen of the organic matrix of cementum or dentin (19).

If connective tissue reattachment or new attachment does not occur at the presurgical level, the result is a loss of soft tissue attachment level caused by epithelial downgrowth along the root surface (15), creating increased sulcular depth and predisposing the patient to periodontal diseases (16, 18, 19). Thus, the endodontic problem may have been resolved but a periodontic problem may have been created. This problem is compounded by its lack of recognition and identification as a potential untoward postsurgical sequela of endodontic surgery. The focus has been almost exclusively on esthetics as the primary concern regarding loss of soft tissue attachment level. If there is little or no change in the clinical appearance of the marginal gingiva in relation to the teeth, particularly anterior teeth, the assumption is that there is no loss of soft tissue attachment level. However, postsurgical examination of the surgical site after several days or weeks may reveal esthetically pleasing results, despite the presence of increased postsurgical sulcular depths. It is suggested that a comparison of presurgical and postsurgical probing depths wound be the most effective means of detecting this problem, and that this should be incorporated into the routine presurgical and postsurgical regimen in clinical practice.

The results of this study show that apical epithelial downgrowth (i.e. loss of soft tissue attachment level) can be prevented following periradicular surgery by maintaining the vitality of root-attached tissues. These findings are in agreement with Levine (17) and others (16) who found that epithelial migration along the root surface was absent or minimal if connective tissue attached to the root surface was not destroyed during periodontal mucoperiosteal flap surgery.

The present findings differ considerably from those of Kramper et al., (20) who microscopically evaluated postsurgical healing following periradicular surgery in dogs. Comparing the intrasulcular incisional wound with incisional wounds in attached gingiva and alveolar mucosa, these investigators found delayed wound healing in the intrasulcular wound. In contrast to the other wounds, intrasulcular wounds showed no epithelial closure (seal) at 2 days, no collagen formation in the wound site at 7 days, persistence of inflammation at 156 days, and measurable loss of soft tissue attachment levels. These authors concluded that the flap design of choice, when not otherwise contraindicated, would include a submarginal incision in attached gingiva rather than an intrasulcular incision. Results of our study showed little difference in the healing of intrasulcular and submarginal incisional wounds. Both wounds showed an epithelial seal at 1 day, collagen formation at 2 days, no persistence of inflammation at 14 days, and no evidence of loss of soft tissue attachment level

resulting from apical epithelial downgrowth. The differences in findings between these two studies may have resulted, at least in part, from the preservation of root-attached tissues in the present study.

SUMMARY AND CONCLUSIONS

A light microscopic evaluation of the wound healing responses of the soft tissues of the periodontium to incisional wounding in periradicular surgery was conducted, using the rhesus monkey as an experimental model. The chronology of specific, key biological events in wound healing was recorded and observations made regarding the similarities and differences in incisional wounds of triangular and submarginal rectangular flap designs. Within the parameters of this investigation, the following conclusions were drawn:

1. Wound healing responses of the mucoperiosteal tissues to incisional wounding in periradicular surgery are remarkably rapid.

2. Few differences in the temporal and qualitative degrees of healing of incisional wounds were noted between the two types of flap designs, although the submarginal rectangular incisions showed a less predictable healing pattern with greater intersample variations in the first 4 postoperative days.

3. The intrasulcular incision leaves a thin layer of vital tissues attached to supracrestal root surfaces. This root-attached connective tissue and epithelium are not clinically visible.

4. With close flap reapproximation and the formation of a thin fibrin clot in the wound site, apical epithelial downgrowth along the root surface does not occur if the vitality of the root-attached tissues is maintained during and after periradicular surgery. Thus, loss of soft tissue attachment level following periradicular surgery with an intrasulcular incision is not inevitable but is preventable.

5. In the presence of vital root-attached tissues, the temporal and qualitative wound healing in the intrasulcular incisional wound site is essentially the same as that of other incisional wounds evaluated in this study.

6. Vitality of root-attached tissues can be predictably maintained by (a) initiating reflection and elevation of the flap in the vertical incision and using undermining elevation to reflect the flap; (b) avoiding curettement or planing of the supracrestal root surfaces; and (c) preventing the dehydration of these tissues with frequent irrigation.

7. Preservation of root-attached epithelium promotes rapid epithelial seal formation, and preservation of root-attached connective tissue enhances connective tissue reattachment rather than new attachment.

8. At 14 and 28 days postsurgery, there is essentially no difference in the incisional wound healing progress of the two flap designs in any of the evaluated or observed biological events of wound healing.

9. In vertical incisional wounds of both flap designs, epithelial closure occurs rapidly, with a multilayered epithelial seal established between 24 and 48 h and epithelial barrier formation occurring between 48 and 72 h. Collagen synthesis in the wound site also occurs early, with aggregation of collagen macromolecules to form fibers between 48 and 72 h.

10. In horizontal wounds of both flaps designs, epithelial closure is extremely rapid; with a thin epithelial seal established at 24 h, a multilayered seal between 48 and 72 h, and epithelial barrier formation occurring between 72 and 96 h. Collagen fibers are formed in the wound site between 24 and 48 h.

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