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Wound Healing following Demineralization of Resected Root Ends in Periradicular Surgery

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The objective of this study was to determine the effect of demineralization of resected root ends on the temporal and qualitative healing of the dentoalveolar (apical attachment apparatus) and alveolar (osseous) tissues in the excisional wound site created during periradicular surgery. Root end resections to orthograde gutta-percha obturations were performed on the mandibular premolars of six mongrel dogs. Twenty-four experimental root ends were demineralized by citric acid burnishing of the resected surfaces. The remaining 24 standard treatment root ends were not demineralized.

Microscopic evaluations at postsurgical intervals from 4 to 45 days revealed that the demineralized root ends were associated with more rapid and complete healing than the nondemineralized root ends. It is proposed that demineralization enhances cementogenesis, the key to dentoalveolar healing, by removing the smear layer barrier and exposing the organic component (collagen fibrils) of resected cementum and dentin.

Root end resection is a commonly used surgical technique in endodontics. This procedure involves reflection of a mucoperiosteal flap, surgical penetration of radicular bone, exposure of the root, and resection of the apical root segment. The resection results in an exposed root surface comprised primarily of dentin with a periphery of cementum and a centrally located canal system which may or may not contain obturating materials. However, covering the resected dentin and cementum is a debris-like substance known as the smear layer.

A smear layer results from any mechanical instrumentation of the dentinal or cemental surfaces and has both inorganic and organic components (1, 2). The dentinal, cemental, bacterial, and tissue remnants which comprise the smear layer adhere to the manipulated surface, clogging the dentinal tubules. Its presence is a potential barrier to obtaining an effective seal with coronal restorations used in operative dentistry and with obturating materials used in endodontics (3, 4). Additionally, the smear layer has been shown to act as a barrier to connective tissue new attachment with supracrestal cementum or dentin following periodontal mucoperiosteal flap surgery (5).

Postsurgical excisional wound healing following periradicular surgery entails *dentoalveolar healing* (i.e. reestablishment of an apical attachment apparatus) and *alveolar healing* (i.e. osseous repair of trabecular and cortical bone). Cementum deposition on the resected root end is considered the critical step in dentoalveolar wound healing (6). Consequently, creating an environment which is conducive to cementogenesis should enhance the healing process following surgical endodontic treatment. The presence of a smear layer on the resected root surface may delay or prevent cementum deposition and, thus, delay or prevent excisional wound healing.

The smear layer can be removed by the application of certain acidic solutions. Complete smear layer removal with acidic solutions has been shown to result in dentinal (and cemental) surface demineralization, producing a mat of projecting collagen fibrils (7). In periodontal surgery, demineralization dentin leads to enhanced connective tissue new attachment through splicing of exposed dentinal collagen with new collagen fibers produced during wound healing and early deposition of cementum on the dentinal surfaces (8–10). Therefore, removal of the smear layer on resected root end surfaces may increase the rate of cementogenesis and result in enhanced periradicular wound healing.

The purpose of this study was to evaluate the effect of surface demineralization of resected root ends upon the temporal and qualitative wound healing of radicular and periradicular tissues following endodontic surgery. The objectives were to determine whether smear layer removal affects the rate and quality of cementogenesis, dentoalveolar healing, and alveolar healing.

MATERIALS AND METHODS

The mandibular third and fourth premolars of six healthy, male mongrel dogs were used in this investigation, providing a total of 24 teeth with 48 roots. The animals were housed at the Baylor College of Dentistry Animal Research Unit under approved and controlled conditions. Nonsurgical and surgical endodontic treatment was conducted under general anesthesia by intramuscular injection of Rompun (1.1 mg/kg) and ketamine (22 mg/kg). Intraoral anesthesia was established by

inferior alveolar nerve block injection of 1.8 ml of 2% lidocaine with 1:100,000 epinephrine. For the surgical sessions, an additional injection of 2% lidocaine with 1:50,000 epinephrine was administered by infiltration in the surgical site for hemostasis.

For each animal, three general anesthesia sessions were required. The first session was for prophylaxis followed by nonsurgical endodontic treatment of the mandibular premolars. Canals were accessed, chemomechanically debrided, and obturated with thermoplasticized gutta-percha and sealer. Access cavities were sealed with a composite restorative material and radiographs were taken before and immediately after treatment. Two surgical sessions were subsequently scheduled for each animal to provide four demineralized and four nondemineralized root ends at postsurgical intervals of 4-, 8-, 12-, 16-, 30-, and 45 days, for a total of 48 root specimens.

A triangular, full mucoperiosteal flap design was used with a vertical incision at the distal line angle of the second premolar joining an intrasulcular incision extending distally to the midbuccal surface of the first molar. The mucoperiosteal flaps were reflected with the undermining elevation technique (11), cortical and cancellous bone were removed with a #8 round bur in a high-speed handpiece with copious water spray, allowing exposure of the apical thirds of the premolar roots. The roots were resected near the junction of the middle and apical thirds with a surgical fissure bur.

The four premolar roots in each mandibular quadrant were assigned to two groups with two roots per group. In the experimental group, resected surfaces were burnished with 50% citric acid at a pH of 1 for 2 min. In the standard treatment group, resected surfaces received no treatment. All root surfaces were irrigated thoroughly with sterile saline following completion of the citric acid treatment.

Surgical flaps were reapproximated and compressed with saline-moistened gauze for 2 min prior to suturing with plain gut sutures. The flaps were further compressed an additional 2 min following suturing. Animals were placed on soft diets and administered ibuprofen (400 mg twice daily) for 2 days after each surgical procedure.

After sacrifice, block sections of the mandibular quadrants were retrieved and placed in 10% buffered formalin for fixation. Specimen blocks were later trimmed with an anatomical rotary saw and placed in 0.5 *M* EDTA for decalcification. When partial decalcification was attained, specimens were further trimmed into blocks containing individual premolar roots. Decalcification of specimens was monitored by radiographic means.

Following decalcification, specimens were sectioned with scalpel blades in an axiobuccolingual plane, bisecting the roots and the central areas of the osseous and apical defects (Fig. 1). The sections were dehydrated in alcohol, embedded in paraffin, and serially sectioned at 7-μm thicknesses. Beginning in the center of the canal and progressing at two-section intervals, one section was stained with hematoxylin and eosin and the second stained with Snook's reticulin stain. The sectioning sequence was continued until specimens no longer provided evaluative information.

Light microscopic evaluations of the coded spcimens were based upon observed sequential biological events related to *clotting and inflammation* and *connective tissue healing.* The temporal and qualitative events which encompass excisional wound healing were graded using the following criteria.

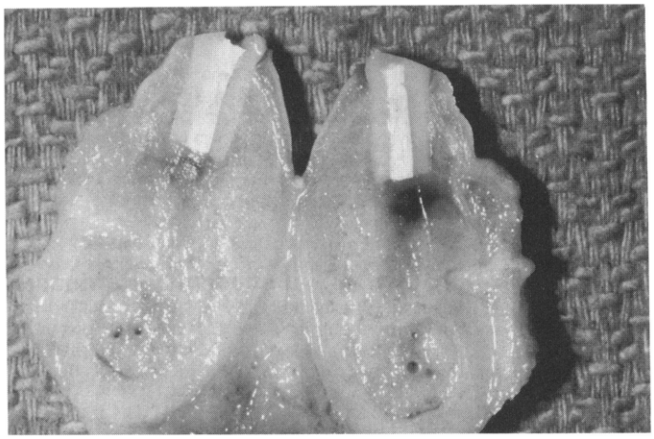

FIG 1. After decalcification, each specimen was sectioned in an axiobuccolingual plane with a scalpel blade, bisecting the root (and the root canal with its obturating materials) and the central areas of the surgically created osseous and apical defects. After removal of the obturating materials, the specimens were dehydrated in alcohol, embedded in parrafin, and serially sectioned at 7-μm thicknesses. Sectioning was continued until specimens no longer provided evaluative information.

CLOTTING AND INFLAMMATION

Clot Formation

#, Not evident = no fibrin clot; coagulum present.

+, Evident = fibrin clot formed: areas of coagulum.

++, Very evident = fibrin clot occupies entire wound site.

##, Event completed = reparative tissues occupy wound site.

Polymorphonuclear Leukocytes

#, Not evident = no polymorphonuclear leukocyte (PMN) infiltrate in wound site.

+, Evident = PMN infiltrate evident in wound site.

++, Very evident = PMN are predominant inflammatory cell type.

##, Event completed = PMN infiltrate no longer evident.

Macrophages

#, Not evident = no macrophage (MP) infiltrate in wound site.

+, Evident = MP infiltrate evident in wound site.

++, Very evident = MP are predominant inflammatory cell type.

##, Event completed = MP infiltrate no longer evident.

CONNECTIVE TISSUE HEALING

Bone Devitalization

#, Not evident = no bone devitalization at perimeter of excisional wound.

+, Evident = bone devitalization at <50% of perimeter.

++, Very evident = bone devitalization at >50% of perimeter.

##, Event completed = devitalized perimeter of bone resorbed or covered by direct apposition of new bone.

Bone Apposition

#, Not evident = no bone apposition on perimeter bone.

+, Evident = bone apposition on <50% of perimeter.

++, Very evident = bone apposition on >50% of perimeter.

##, Event completed = bone apposition on entire perimeter.

Endosteal Proliferation

#, Not evident = no proliferation of endosteal tissues into excisional wound site.

+, Evident = <50% of wound site occupied by endosteal tissues.

++, Very evident = >50% of wound site occupied by endosteal tissues.

##, Event completed = endosteal tissues and associated woven bone trabeculae occupy entire wound site.

Woven Bone Formation

#, Not evident = no woven bone formation in wound site.

+, Evident = woven bone occupies <50% of wound site.

++, Very evident = woven bone occupies >50% of wound site.

##, Event completed = woven bone trabeculae and associated endosteal tissues occupy entire wound site.

Periosteum Formation

#, Not evident = no forming periosteal tissues at interface of excisional wound site and base of flap.

+, Evident = forming periosteal tissues occupying <50% of interface.

++, Very evident = forming periosteal tissues occupying >50% of interface.

##, Event completed = functioning periosteum occupies entire interface.

Cementum Formation

#, Not evident = no cementum deposition on resected root surface.

+, Evident = cementum deposition on <50% of resected root surface.

++, Very evident = cementum deposition of >50% of resected root surface.

##, Event completed = cementum deposition on entire resected root surface.

Root End Encapsulation

#, Not evident = no granulation tissue proliferating from the periodontal ligament (PDL).

+, Evident = tissue proliferating from the PDL encapsulating <50% of the resected root end.

++, Very evident = tissue proliferating from the PDL encapsulating >50% of the resected root end.

##, Event completed = tissue proliferating from the PDL encapsulating the entire root end.

Apical Periodontal Ligament Formation

#, Not evident = no functionally oriented apical PDL fibers.

+, Evident = functionally oriented fibers associated with <50% of resected root end.

++, Very evident = functionally oriented fibers associated with >50% of resected root end.

##, Event completed = functional PDL associated with entire resected root end.

RESULTS

Results of the microscopic evaluations of the dentoalveolar and alveolar healing responses to excisional wounding in periradicular surgery are shown as cumulative data in Tables 1 and 2. The wound healing response to periradicular surgery is diagramatically summarized in Fig. 2.

The 48 block specimens represented eight samples at each postsurgical interval, four experimental samples and four standard samples. The assigned cumulative grades were based on the majority of grades assigned for the samples in each group. The following is a descriptive summary of the wound healing responses observed at each postsurgical interval. Observations are recorded for the experimental (demineralized) group at each interval and are applicable to the corresponding standard (nondemineralized) group except as noted.

TABLE 1. Microscopic evaluation of healing events associated with demineralized root ends (experimental group)*

	Postsurgical Interval					
	Day 4	Day 8	Day 12	Day 16	Day 30	Day 45
Clot formation						
Alveolar	#	##	##	##	##	##
Dentoalveolar	#	##	##	##	##	##
Polymorphonuclear leukocytes						
Alveolar	+	+	##	##	##	##
Dentoalveolar	+	+	##	##	##	##
Macrophages						
Alveolar	+	++	##	##	##	##
Dentoalveolar	+	++	##	##	##	##
Bone devitalization	+	+	++	++	##	##
Bone apposition	+	+	++	++	++	##
Endosteal proliferation	++	##	##	##	##	##
Woven bone formation						
Alveolar	#	+	++	++	##	##
Dentoalveolar	#	#	+	++	++	##
Periosteum formation	#	#	#	+	##	##
Cementum formation	#	#	+	++	++	++
Root end encapsulation	+	##	##	##	##	##
PDL formation	#	#	#	#	+	++

* #, not evident; +, evident; ++, very evident; ##, event completed. Alveolar = area of osseous excisional wound, minus the dentoalveolar area. Dentoalveolar = area immediately subjacent to the resected root end.

TABLE 2. Microscopic evaluation of healing events associated with nondemineralized root ends (standard group)*

	Postsurgical Interval					
	Day 4	Day 8	Day 12	Day 16	Day 30	Day 45
Clot formation						
Alveolar	#	##	##	##	##	##
Dentoalveolar	#	##	##	##	##	##
Polymorphonuclear leukocytes						
Alveolar	+	+	##	##	##	##
Dentoalveolar	+	+	##	##	##	##
Macrophages						
Alveolar	+	+	##	##	##	##
Dentoalveolar	+	+	##	##	##	##
Bone devitalization	+	+	+	+	+	+
Bone apposition	#	+	++	++	+	++
Endosteal proliferation	++	##	##	##	##	##
Woven bone formation						
Alveolar	#	+	++	++	++	++
Dentoalveolar	#	#	+	+	+	++
Periosteum formation	#	#	#	+	+	+
Cementum formation	#	#	#	+	+	+
Root end encapsulation	+	##	##	##	##	##
PDL formation	#	#	#	#	#	#

* #, not evident; +, evident; ++, very evident; ##, event completed. Alveolar = area of osseous excisional wound, minus the dentoalveolar area. Dentoalveolar = area immediately subjacent to the resected root end.

Day 4 with Demineralization

Granulation tissue emanating from the severed PDL was proliferating into the coagulum at the periphery of the resected root surfaces. Cellular and fibrin adherence to the resected surfaces was seen in all specimens. Within the depths of the excisional wound, granulation tissue emanating from the endosteal tissues had replaced approximately 50% of the excisional wound coagulum (Fig. 2). This highly vascular tissue contained cells which were morphologically similar to osteoprogenitor cells, fibroblasts, fibroblast-like cells, undifferentiated ectomesenchymal cells, macrophages, and some polymorphonuclear leukocytes.

Cortical and trabecular osseous tissue at the periphery of the excisional wound showed evidence of devitalization (necrosis) in some areas with lacunae containing no osteocytes. However, this was noted only in areas of apparent contact by a rotary instrument during the resection procedures. The remainder of the trabecular bone bordering the excisional wound showed vital osteocytes in lacunae, indicating vital osseous tissue. Isolated areas within the deep excisional wound showed osteoblastic activity with early osseous apposition to vital bone.

Day 4 without Demineralization

The resected root surfaces had noticeably fewer areas of cellular and fibrin strand adherence, with less than 50% of the resected surfaces demonstrating any form of attachment. No evidence of osteoblastic activity was present in any of the specimens.

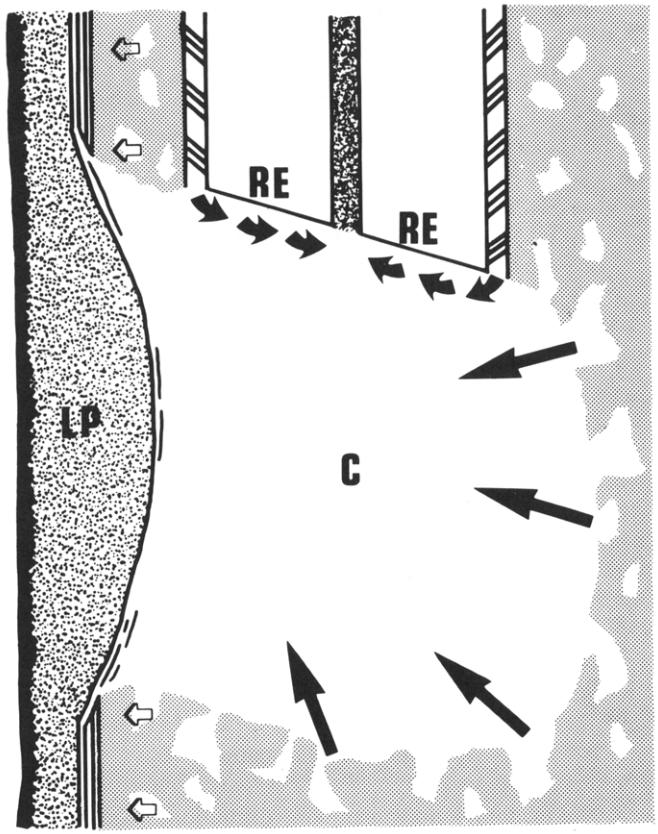

FIG 2. Following periradicular surgery, the excisional wound is bounded by cortical bone (*open arrows*), severed PDL tissues, the resected root end (*RE*) with its obturating materials, trabecular (cancellous) bone and associated endosteal tissues, and the lamina propria (*LP*) of the mucoperiosteal tissues. Granulation tissues emanating from the severed PDL (*small arrows*) and endosteal tissues (*large arrows*) proliferate into the coagulum (*C*) occupying the bone cavity and are responsible for repair of the excisional wound. The PDL-derived tissue subsequently encapsulates the resected root end and plays the primary role in dentoalveolar healing. The endosteal-derived tissue plays the primary role in alveolar healing.

Day 8 with Demineralization

The coagulum had been completely replaced by granulation tissue proliferating from the severed PDL and endosteal tissues. The tissue migrating from the PDL had encapsulated the root end and could be distinguished from the endosteal-derived tissue occupying the remainder of the excisional wound. The density and orientation of the encapsulating collagen fibers, with more mature fibers oriented parallel to the resected root surface, was in sharp contrast to the endosteal-derived granulation tissue which was more vascular, contained more cells per unit area, and possessed a haphazard orientation of delicate, thin collagen fibers.

Cells and fibers of the PDL-derived encapsulating tissue were closely adherent to the demineralized root surfaces in all specimens. The appearance was one of complete unity between the granulation tissue and the resected root surfaces, suggesting that the fibers of the former had become spliced to the organic matrices of the resected surfaces exposed by the demineralization process. The encapsulating tissue, including the tissue directly subjacent to the root canal, was not in-

flamed, indicating that neither the canal system, the obturating materials, nor the resected root surfaces were sources of irritation.

Osteoblastic activity was evident in numerous areas of the endosteal-derived granulation tissue occupying the remainder of the excisional wound site. Woven bone formation was observed as appositional growth on peripheral vital and devitalized trabecular and cortical bone, and as developing woven bone trabeculae within the granulation tissue.

At the interface of the excisional wound site and the overlying lamina propria, a delimiting membrane (12) had developed. This consisted of a distinct zone of fibrous connective tissue with collagen fibers oriented parallel to the plane of the cortical plate.

Day 8 without Demineralization

The PDL-derived encapsulating tissue showed no evidence of cellular or fibrous adherence to the resected root surfaces.

Day 12 with Demineralization

The PDL-derived granulation tissue encapsulating the resected root ends showed increasing maturity as evidenced by increased collagen fiber density and thickness, and decreased cellular content as compared with day 8 specimens. PMN and MP were rarely observed. Fiber orientation remained primarily parallel to the resected root surfaces, with the encapsulating tissue in direct contact with these surfaces.

New cementum, with its external surface lined with cementoblasts, had been deposited on the resected root surfaces (Fig. 3). This deposition occurred equally across the resected surfaces, not preferentially at the periphery with progression toward the central canal zone. Separation of new cementum from the resected surfaces was not observed in any specimen.

Osteoblastic activity was very evident throughout the excisional wound. Woven bone formation was observed as appositional growth on vital and devitalized peripheral bone and as osteoblast-lined trabeculae within the granulation tissue. Near the level of the resected root, isolated trabeculae were in direct contact with the encapsulating tissue (Fig. 3). The excisional wound site was separated from overlying lamina propria by a well-defined delimiting membrane.

Day 12 without Demineralization

Similar to the 8-day specimens, the PDL encapsulating tissue showed no evidence of cellular or fibrous adherence. Cementum deposition was not observed (Fig. 4).

Day 16 with Demineralization

A band of acellular cementum had been deposited on the resected surfaces and was adherent to the root ends, without any separations from the resected surfaces. The pattern of deposition was similar to that seen at 12 days, but thicker (Fig. 5). Reticulin stain revealed a narrow (5 to 7 μm) clear zone between the resected dentin and new cementum. This unstained zone was continuous with a similar zone separating dentin and cementum on the lateral root surfaces.

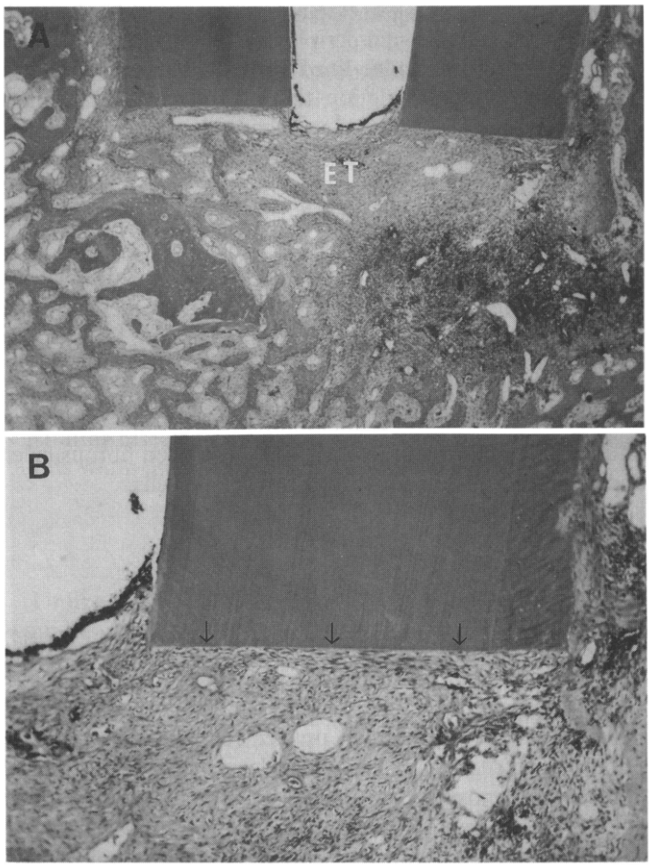

FIG 3. Excisional wound healing associated with an experimental (demineralized) root end at 12 days after periradicular surgery. *A*, The PDL-derived encapsulating tissue (*ET*) has surrounded the resected root end and shows no evidence of an inflammatory infiltrate. Woven bone trabeculae are evident in the wound site. *B*, Cementum deposition (*arrows*) is apparent on the resected root surfaces (hematoxylin and eosin; original magnification *A*, ×13; *B*, ×33).

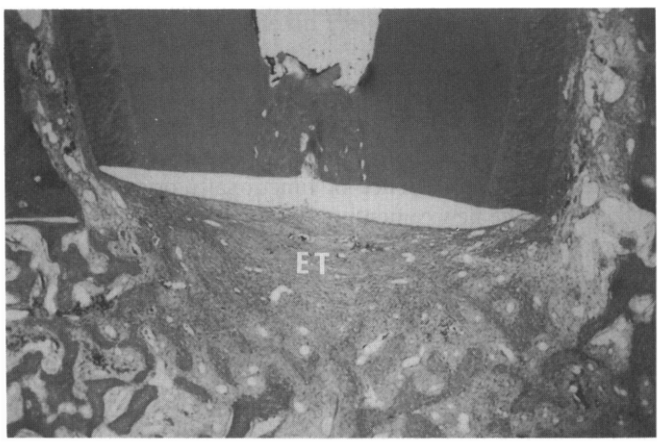

FIG 4. Excisional wound healing associated with a standard (nondemineralized) root end at 12 days after periradicular surgery. A lack of fibrin, cellular, or fibrous adhesion of the PDL-derived encapsulating tissue (*ET*) to the resected root surface is apparent. Woven bone trabeculae are evident in the wound site. No cementum has been deposited on the resected root surfaces (hematoxylin and eosin; original magnification ×13).

Osteoblastic activity was extensive with appositional growth on both vital and nonvital peripheral bone, and formation of interconnecting trabeculae lined with osteoblasts within the endosteal-derived granulation tissue. Trabecular formation subjacent to the resected root ends had occurred, with trabeculae in direct contact with the encapsulating tissue. A developing attachment apparatus was evident with collagen fibers becoming functionally oriented, connecting newly formed cementum and subjacent trabeculae (Fig. 5).

In several specimens, new trabeculae had formed subjacent to the delimiting membrane at the interface between the excisional wound and overlying lamina propria. Cells of the membrane lined the superficial surfaces of these trabeculae and were morphologically similar to active osteoblasts. In these areas, the delimiting membrane had the appearance of a functioning periosteum with a well-developed fibrous layer and a cambium layer containing osteogenic cells.

Day 16 without Demineralization

PDL-derived encapsulating tissue was in contact with the resected surfaces in the majority of specimens. Dentinoclastic and cementoclastic activity was also evident, and the resected surfaces showed an irregular, uneven topography unlike the smooth resected surfaces seen in the demineralized specimens. Cementum deposition was also seen, but the quantity was noticeably less, and the pattern was inconsistent as compared with the demineralized surfaces (Fig. 6). Separation of new cementum from dentin was seen in some areas.

Day 30 with Demineralization

The excisional wound repair was remarkably advanced. In the dentoalveolar component, maturing trabeculae were very prominent at the periphery of the PDL-derived encapsulating tissue. Lined with osteoblasts on their PDL surface, these trabeculae occupied greater than 80% of the dentoalveolar area subjacent to the root ends. The trabeculae also had coalesced and had a more mature appearance as compared with 12 and 16 days.

Cementum deposition was very evident and the development of a physiological attachment apparatus was essentially complete with the presence of functionally oriented apical PDL fibers. The new cementum was adherent to dentin with no areas of separation. The canal space, with its obturating material, was covered by a minimally inflamed fibrous connective tissue occupying the central space within the newly formed apical attachment apparatus. Reticulin staining revealed a narrow, unstained clear zone separating resected dentin and the new cementum.

The remainder of the excisional wound, the alveolar component, was filled with maturing trabecular bone and associated endosteal tissues. New cortical bone was being formed, under apparent control of a functioning periosteum which had developed from the tissues of the delimiting membrane. The cortical level was depressed as compared with the adjacent cortical bone, indicating that cortical repair and remodeling was not complete.

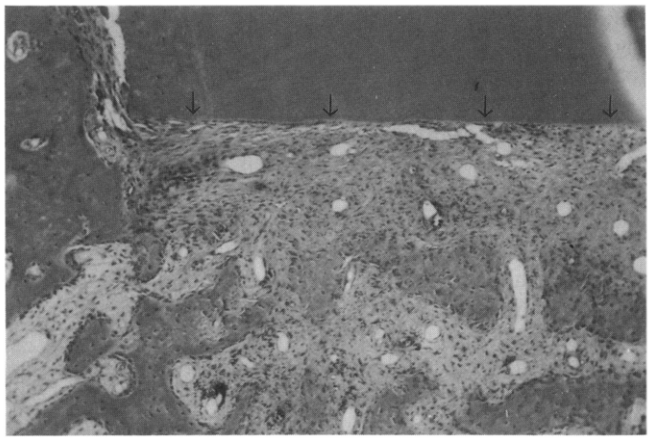

FIG 5. Dentoalveolar wound healing associated with an experimental (demineralized) root end at 16 days after periradicular surgery. Woven bone trabeculae have formed subjacent to the resected root surface and in direct contact with the PDL-derived encapsulating tissue. A thin layer of cementum (*arrows*) is apparent along the entire resected root surface and a developing attachment apparatus (collagen fibers) connects the cementum and subjacent trabeculae. The root canal is seen in *upper right corner* (hematoxylin and eosin; original magnification ×33).

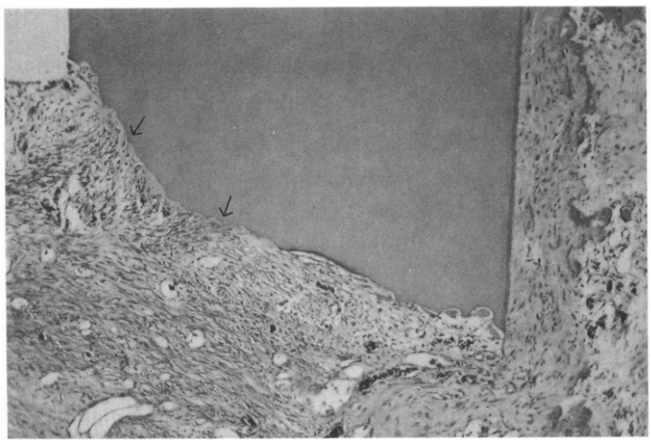

FIG 6. Dentoalveolar wound healing associated with a standard (nondemineralized) root end at 16 days after periradicular surgery. Woven bone trabeculae have not formed subjacent to this resected root surface. A small amount of cementum has been deposited on the root surface but is limited to the area bounded by *arrows*. Dentinoclastic activity is evident on resected root surface at *right*. There is no apparent developing attachment apparatus. The root canal is seen in *upper left corner* (hematoxylin and eosin; original magnification ×33).

Day 30 without Demineralization

Evidence of dentinoclastic and cementoclastic activity was apparent, with cementum deposition far less pronounced and more irregular in pattern than in the experimental group. Functionally oriented PDL fibers were also absent. Separation of new cementum from dentin was observed.

Peripheral bone devitalization was evident, with a pattern similar to that described at 4 and 8 days. Appositional bone growth was less advanced, as was trabecular maturity and overall osseous repair. Periosteum formation was evident, but

not as extensive as that associated with the experimental root ends.

Day 45 with Demineralization

Dentoalveolar healing was more advanced than at 30 days. Resected root ends were repaired with cementum, subjacent bone had re-formed the apical tooth socket, and these two hard tissues were connected by functionally oriented apical PDL fibers (Fig. 7). The root canals, with their obturating materials, were covered by a fibrous connective tissue lacking any inflammatory cell infiltrate. The collagen fibers of this connective tissue were oriented parallel to the resected root surfaces and were attached to the surrounding apical periodontal ligament fibers.

Cementum deposition was greater than at 30 days and was consistent in width across the resected surfaces. Separation of cementum from the root ends was not seen in any of the specimens. Hematoxylin and eosin staining demonstrates the presence of Sharpey's fiber insertions into both new cementum and subjacent bone. Reticulin staining also demonstrated Sharpey's fiber insertions and the presence of an unstained, clear zone separating resected dentin and new cementum.

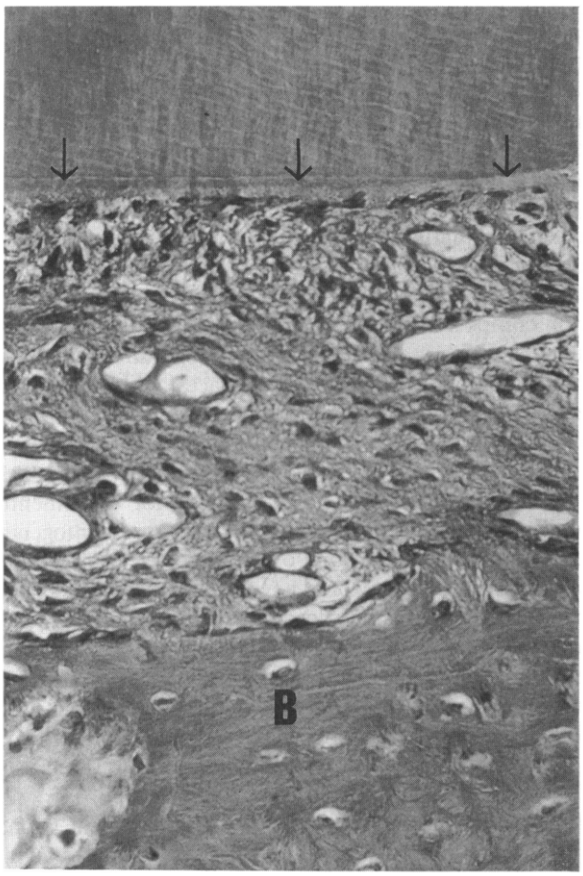

FIG 7. Dentoalveolar wound healing associated with an experimental (demineralized) root end at 45 days after periradicular surgery. Functionally oriented PDL fibers have formed between the new cementum (*arrows*) on the resected root surface and the subjacent bone (*B*) forming the apical tooth socket (hematoxylin and eosin; original magnification ×132).

Wound healing in the alveolar component of the excisional wound was similar to that seen at 30 days. The area of cortical bone penetration contained a depression in the surface, indicating incomplete cortical remodeling. A functioning periosteum was present and was participating in cortical repair.

Day 45 without Demineralization

Osseous formation and maturity subjacent to the resected roots was less advanced and was similar in appearance to that seen at 30 days. Cementum deposition was less pronounced, appearing primarily in areas demonstrating previous dentinoclastic activity. Functionally oriented apical PDL fibers were not observed. Reticulin staining demonstrated a clear zone separating resected dentin and new cementum in some areas, but its width and presence was inconsistent.

DISCUSSION

Unlike incisional and dissectional wounding, excisional wounding requires the intentional removal of tissues from the surgical site, entailing a more complex and prolonged series of wound healing events. All of the excised tissues possess reparative powers with the exception of dentin which depends upon cementum for repair of its surface.

Initially, the excisional wound site fills with a coagulum that does not promote wound healing, but acts as a barrier that must be removed prior to the progression of wound healing (12). Granulation tissues emanating from the severed PDL and endosteum proliferate into the coagulum and are responsible for repair of the excisional wound of periradicular surgery. The PDL-derived tissue is primarily responsible for dentoalveolar healing and endosteal-derived tissue is responsible for alveolar (osseous) healing (Fig. 2).

Proliferation of granulation tissue from the severed PDL was observed at 4 days, and the tissue had completely encapsulated the root ends at 8 days. In the experimental groups, cementum deposition was observed at 12 days and, by 16 days, cementum covered more than 50% of the surface areas of the resected root ends. In the standard treatment groups, cementum deposition was not observed until 16 days and, even at 45 days, cementum covered less than 50% of the surface areas of the resected root ends. Dentoalveolar healing with re-formation of an apical attachment apparatus was evident at 45 days in the experimental group, but not in the standard treatment group.

Thus, demineralization of the resected root ends enhanced the temporal and qualitative healing of the dentoalveolar tissues. Two potential explanations for this observation may be conjectured. First, demineralization removes the smear layer which may act as a partial barrier to induction mechanisms for cementum deposition emanating from resected dentin or cementum (5, 7). It is possible that the smear layer, created by the resection of the root end with a rotary instrument, partially inhibits these inductive mechanisms. Second, demineralization exposes the organic (collagen) matrix of the resected dentin and cementum, promoting early adherence of fibrin, fibronectin, and reparative cells to the resected root surfaces (7, 10). Adherence of cells with cementogenic potential to these inducting surfaces enhances early cementum deposition (9). This is supported by the observation that

adherence was not apparent with nondemineralized root surfaces and cementum deposition was greatly delayed.

The significance of clastic activity in attaining connective tissue new attachment on nondemineralized root surfaces has been described by Egelberg (13). Dentinoclasis and cementoclasis, like the demineralization procedure, remove the smear layer and inorganic matrices, resulting in exposure of the organic matrices (collagen fibrils) of dentin and cementum. In the absence of clastic activity or demineralization, Egelberg (13) suggests that cementum deposition can occur but is attached to the (resected) root surface only through interlocking mineral (inorganic) crystals connecting the new cementum, through the smear layer, to the root surface. There is no organic interlocking component to support this tenuous inorganic connection. This concept is supported by the frequent observation of artifactural separations between new cementum and root surfaces in histological and ultrastructural preparations. It is further supported by observations that these separations rarely occur on root surfaces that have been demineralized or have undergone prior clastic activity (14, 15).

In this investigation, nondemineralized root ends were associated with delayed cementogenesis, an irregular pattern of cementogenesis (inconsistently distributed along the root surfaces), and separations between new cementum and root surfaces except in areas where clastic activity had previously occurred. Conversely, demineralized root ends were associated with early cementogenesis (evenly distributed along the root surfaces), adherence of new cementum to root surfaces (without separations), and no clastic activity. This contrasted with Andreasen's (6) findings of preferential deposition at the periphery of the resected root, with progression toward the central canal creating a wedge-shaped layer of reparative cementum (thicker at the periphery and thinner toward the canal). However, root ends were not demineralized in the latter study.

The PDL-derived tissues encapsulating the root ends were remarkably free of inflammatory cell infiltrates. In a few specimens, a mild infiltrate of inflammatory cells (PMN and round cells) occupied a thin zone (about 50 μm) subjacent to the root canals and their obturating materials. In most specimens, the latter zone showed only occasional, scattered inflammatory cells, with reparative cells and neovasculature clearly predominating in the zone. This finding indicated that the resected root surfaces, the root canal systems, and the obturating materials were well tolerated and were not sources of irritants which were impeding the healing process.

Alveolar healing was a product of endosteal proliferation into the excisional wound site. At later postsurgical intervals, alveolar healing (osseous repair) was progressing in both the experimental and standard treatment groups, indicating that alveolar healing was not totally dependent upon cementogenesis. The primary differences noted in the 30- and 45-day specimens were that maturation of the woven bone trabeculae and repair of the cortical plate were not as advanced in the standard treatment group.

The peripheral trabecular and cortical bone forming the wound edges of the excisional defect showed evidence of devitalization with a variably wide zone of lacunae without osteocytes. Apposition of new woven bone directly to devitalized trabecular and cortical surfaces was observed in all specimens from 8 to 45 days postsurgically. Osteoclastic activity was not seen on the devitalized boney surfaces. The formation of new bone within the excisional wound site began in the internal areas and progressed externally toward the level of the former cortical plate. As the more external woven bone trabeculae were formed in contact with the overlying delimiting membrane, the latter tissue becomes a functioning periosteum (12, 16). In the 30- and 45-day specimens, the new cortical plate was being formed and remodeled with osteoblastic activity on the external surfaces under apparent control of the newly functional periosteum, and osteoblastic activity on the internal surfaces under control of the endosteal tissues. All of these observations are in agreement with findings of previous wound healing studies (16, 17).

Woven bone formation near the resected root ends and in contact with the PDL-derived encapsulating tissue was noted in the 12- and 16-day specimens. As more woven bone trabeculae were deposited in the later intervals (30 and 45 days) subjacent to the encapsulating tissue, a tissue response similar to that described for the delimiting membrane in becoming a functional periosteum became apparent. Osteoid was being deposited on the PDL surface of these trabeculae by large, plump osteoblasts in direct contact with the PDL-derived encapsulating tissues. Some osteoblastic activity was also noted on the opposite (endosteal) surface by osteoblasts in direct contact with endosteal tissues. This suggests that the initial formation of woven bone subjacent to the encapsulating tissue may be a product of the endosteal tissues and that the encapsulating tissues may be induced by this endosteal-derived new bone to begin osteogenic activity. Thus, endosteal-derived new bone may be necessary to induce the delimiting membrane to become a functioning periosteum and to induce the encapsulating tissue to initiate the osteogenic activity of a functioning periodontal ligament.

The presence of a clear (nonstaining) zone separating the resected dentinal surfaces from the new cementum was revealed by reticulin staining. The absence of staining indicates that the zone has an extremely low organic content and, thus, is not composed of dentin or cementum. This zone was continuous with a similar clear zone separating dentin and cementum along the lateral root surfaces, where its location coincides with that of intermediate cementum. Lindskog et al. (18) reported that intermediate cementum is actually composed of enameloid produced by cells of Hertwig's epithelial root sheath and that this layer forms prior to cementum deposition, and may be a prerequisite for cementogenesis. The presence of a clear zone on the resected root ends was noted only on surfaces where new cementum was deposited. Further investigations are needed to determine whether the clear zone represents an enameloid layer and, if so, what cells (presumably epithelial) are responsible for its synthesis. It is tempting to conjecture that this may be the long-sought function for the cell rests of Mallesez which are known to proliferate in the presence of an inflammatory response such as that occurring following surgical wounding.

Bone slivers, apparently dislodged from cortical surfaces during elevation of mucoperiosteal flaps, were frequently observed in the deep laminae propria of the previously flapped tissues. This finding agrees with the observation by Melcher (19) that mechanical forces applied with a periosteal elevator during flap reflection can dislodge bony slivers from the cortical surface. This observation also confirms the observation by Harrison and Jurosky (16) that these bone slivers

become surrounded by new connective tissue with a normal fiber architecture and cellular content, showing no evidence of inflammation or fibrous encapsulation.

Lingual root tags were evident in many specimens. These root tags, many of them sharply pointed, were well tolerated and were not associated with an inflammatory infiltrate. Reparative tissues predominated at every postsurgical interval in close approximation with the root tags. This finding strongly suggests that the presence of root tags, previously considered detrimental to dentoalveolar healing, does not interfere with the healing process (20).

CONCLUSIONS

Within the parameters of this investigation, the following conclusions were drawn:

1. Demineralization of resected root ends with citric acid enhances cementogenesis and dentoalveolar healing.
2. Cementum deposition on resected root ends is essential for optimal dentoalveolar repair which includes re-formation of a functional periodontal ligament between new cementum and subjacent new bone.
3. Granulation tissue emanating from the severed periodontal ligament encapsulates the resected root ends and is primarily responsible for dentoalveolar healing and the formation of an apical attachment apparatus.
4. Granulation tissue emanating from severed endosteal tissues is primarily responsible for alveolar healing, which begins in the internal areas of the excisional wound and progresses toward the external (cortical) surface.
5. Alveolar healing associated with demineralized root ends was slightly more advanced at 30 and 45 days than that associated with nondemineralized root ends.
6. Demineralization of resected root ends should be incorporated into the procedures of periradicular surgery.

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