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Wound Healing in the Tissues of the Periodontium following Periradicular Surgery. III. The Osseous Excisional Wound

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Excisional wounds were made in the maxillas and mandibles of rhesus monkeys, and the osseous wound-healing responses at postsurgical intervals ranging from 1 to 28 days were evaluated by light microscopy.

The excisional defects were initially filled with a coagulum which was subsequently replaced by granulation tissue emanating from the endosteal tissues. Cortical and trabecular bone forming the wound edges was devitalized, as evidenced by an absence of osteocytes in the peripheral lacunae. At 14 days postsurgery, woven bone trabeculae occupied most of the defect, with the more superficial trabeculae in direct contact with a thick band of dense fibrous connective tissue separating the osseous defect from overlying mucosal tissues. Within the defect, new bone was deposited on devitalized bone without evidence of preceding osteoclastic activity. At 28 days, the woven bone trabeculae were more mature and a functioning periosteum was now active in repair of the cortical plate.

The hard and soft tissues of the periodontium are subjected to three basic types of surgical wounding during periradicular surgery. Incisional wounding affects the mucoperiosteal tissues; dissectional wounding affects the mucoperiosteal and osseous tissues; and excisional wounding involves the removal of periradicular (osseous, endosteal, and periodontal ligament tissues) and radicular (cementum and dentin) tissues. The wound-healing responses of the soft and hard tissues of the periodontium to incisional and dissectional wounding have been detailed in previous reports (1–3). The purpose of this report is to describe the osseous response to excisional wounding at postsurgical intervals ranging from 1 to 28 days.

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 research models for this investigation. The animals were housed at the Baylor College of Dentistry Animal Research Unit facilities under approved and controlled conditions.

Six mucoperiosteal flaps were reflected in the anterior and posterior sextants of the maxilla and mandible, providing a total of 24 flaps for evaluation of excisional wounding. Unoperated areas served as controls, allowing comparison of the surgically injured tissues with normal tissues at each postsurgical interval.

Before 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 was given in each surgical site for local anesthesia and hemostasis. In the mandible, an inferior alveolar nerve block injection was also administered. Surgical sessions were scheduled to provide four flaps at each of the postsurgical intervals of 1 to 4 and 14 and 28 days.

Following incision and reflection of the mucoperiosteal flap, an excisional wound was made in interdental bone (i.e. bone between the roots of adjacent teeth) 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, creating an osseous excisional wound similar to that produced during periradicular surgery.

The soft and osseous tissues were frequently irrigated with sterile, physiological saline and, after a 15-min retraction period, the mucoperiosteal flaps were reapproximated (repositioned) and stabilized by interrupted plain gut sutures. To effect hemostasis, the flapped tissues were then compressed for 3 min by applying firm finger pressure to saline-soaked gauze pads placed over the surgical site.

Surgical procedures and sacrifices were scheduled to provide postsurgical periods of 1 to 4 days and 14 and 28 days, yielding four excisional wounds at each of the six intervals. Postsurgical care and preparation of experimental and control material for light microscopic examination have been detailed in part I of this study (2). Following fixation and decalcification, sections containing the excisional wounds were trimmed with a scapel blade, embedded in paraffin, and serial sections were made through the central one third of the wound at a thickness of 7 μm. At 20-section intervals, 4 sections were

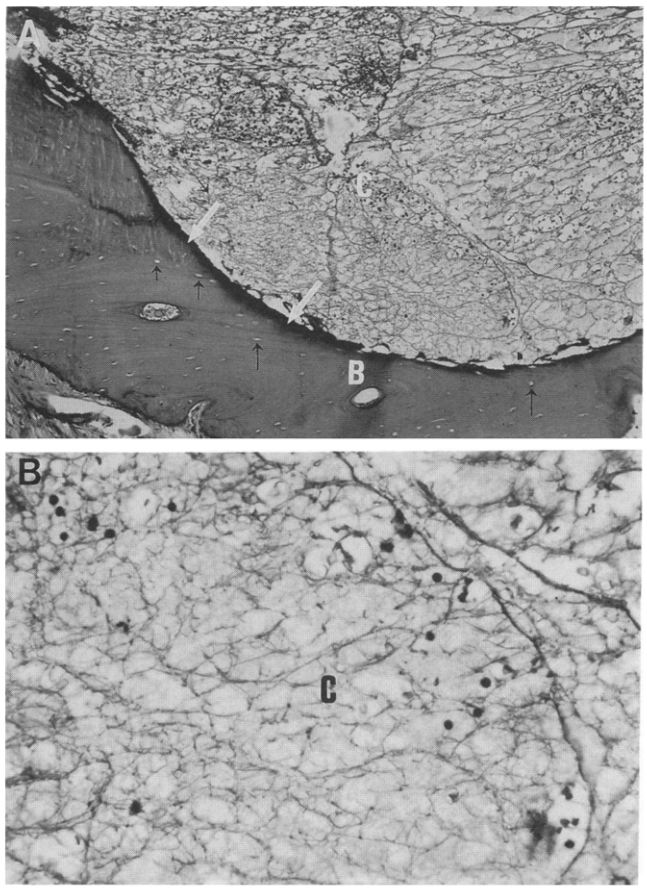

FIG 1. Coagulum occupying excisional osseous wound at 3 days after surgery. *A* and *B*, Coagulum (*C*) consists of disorganized, widely spaced fibrin strands with entrapped erythrocytes, degenerating cells, tissue debris, and scattered inflammatory cells. Empty lacunae (*small arrows*) and a hyperchromatic zone (*white arrows*), indicating bone devitalization, are seen along the periphery of the osseous wound edge (*B*) (hematoxylin and eosin; original magnification *A*, ×33 and *B*, ×132).

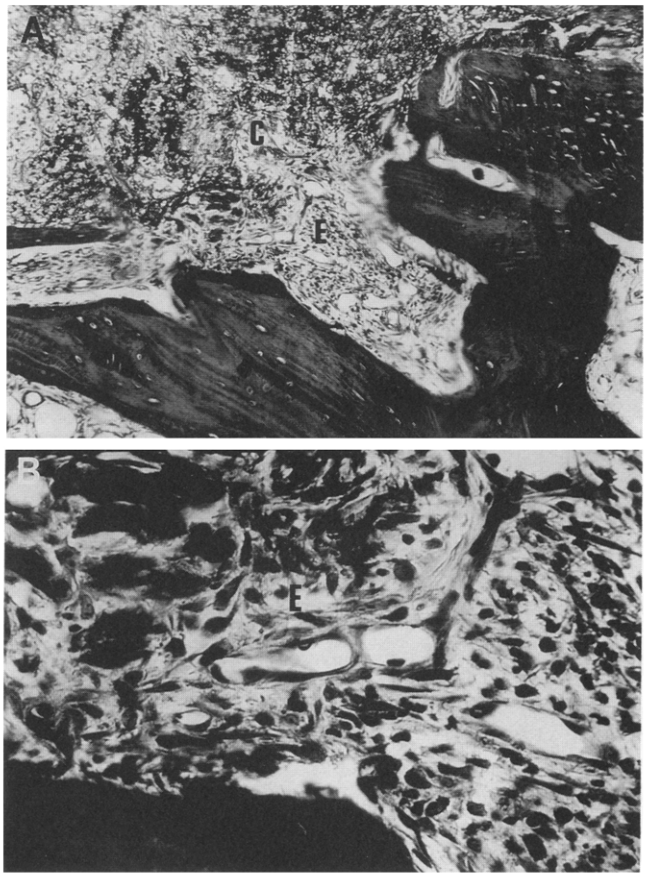

FIG 2. *A* and *B*, Proliferation of endosteal tissues at 4 days after surgery. Endosteal tissues (*E*) from the intertrabecular (medullary) spaces are migrating into the coagulum (*C*) occupying the osseous excisional wound site (Masson's trichrome; original magnification *A*, ×33 and *B*, ×132).

stained with hematoxylin and eosin, 4 sections with Masson's trichrome, and 4 sections with Snook's reticulin stain.

RESULTS

At 1 through 3 days after surgery, the osseous excisional wound remained filled with a coagulum comprised of disorganized, interrupted, widely spaced fibrin strands (Fig. 1). The central mass of the coagulum contained erythrocytes, degenerating inflammatory cells, and tissue debris. The surgically exposed cortical and trabecular (cancellous) bone forming the wound edges exhibited hyperchromatic staining and empty lacunae without vital osteocytes, indicating peripheral devitalization of bone. In the 3-day specimens, inflammatory cells (polymorphonuclear leukocytes and macrophages) and some reparative cells (undifferentiated ectomesenchymal cells, fibroblasts, and fibroblast-like cells) were seen migrating into the periphery of the coagulum from the endosteal tissues and, to a lesser extent, from the overlying mucoperiosteal tissues.

At 4 days after surgery, endosteal tissues occupying the medullary spaces between trabeculae in the deeper (more internal) excisional wound edges were proliferating into the coagulum of the excisional wound site (Fig. 2). This proliferative tissue retained the loose morphological and architectural pattern of endosteal tissues, varying only in its increased cellularity and vascularity. In advance of this proliferating front of tissues, inflammatory cells (macrophages and polymorphonuclear leukocytes) and reparative cells (fibroblast-like cells) were invading the coagulum. Few inflammatory cells were observed within the cellular population of the proliferating endosteal tissues which included undifferentiated ectomesenchymal cells, fibroblast-like cells, and cells which had morphological characteristics similar to those of osteoprogenitor cells and preosteoblasts. As compared with the 3-day specimens, an increase in the number of inflammatory and reparative cells entering the excisional wound coagulum from the overlying mucoperiosteal tissues was observed. However, the central core of the coagulum mass remained devoid of vital cells. Cortical and trabecular bone forming the excisional wound edges had the same light microscopic evidence of peripheral devitalization as described for the earlier postsurgical intervals.

At 14 days after surgery, endosteal tissue and multiple woven bone trabeculae occupied about four fifths of the excisional wound (Fig. 3). The newly formed trabeculae contained large lacunae with plump osteocytes and were surrounded by an osteoid layer and morphologically distinct,

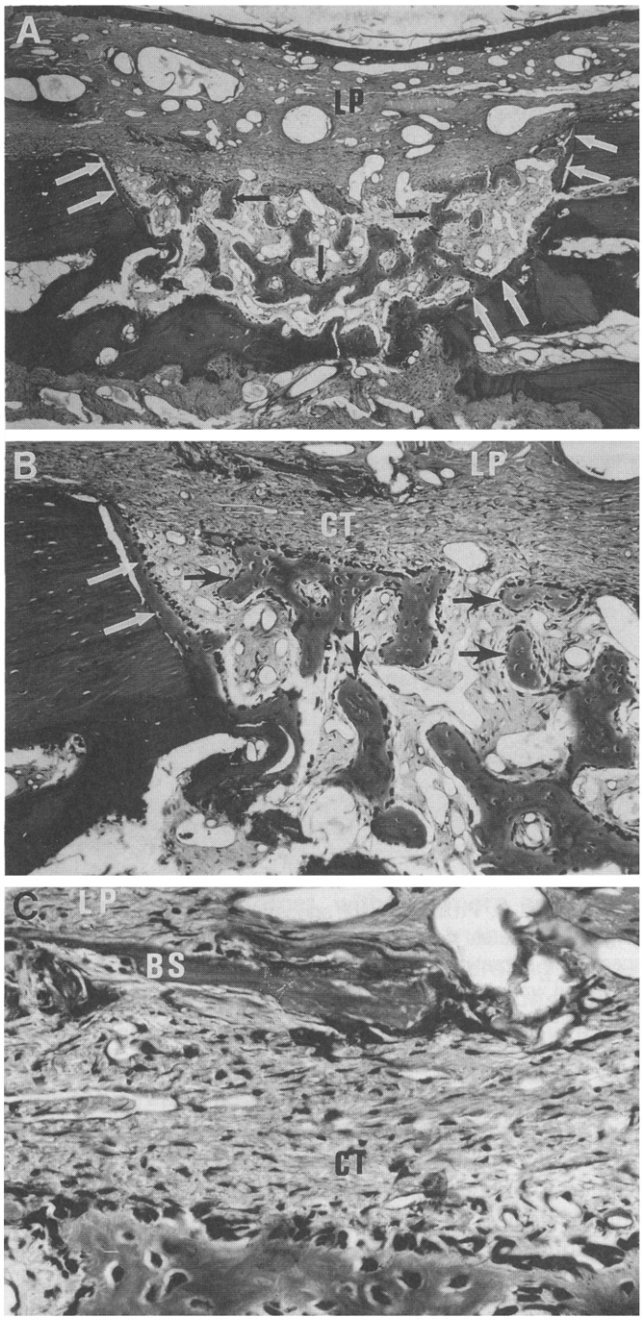

FIG 3. Endosteal tissue and multiple woven bone trabeculae in osseous excisional wound at 14 days after surgery. *A* and *B*, Coagulum in wound site has been completely replaced by endosteal tissue and new bone. Woven bone is apparent as trabeculae (*black arrows*) within the endosteal tissue and as appositional growth (*white arrows*) on the surface of devitalized cortical and trabecular bone of the wound edge. *LP*, lamina propria of alveolar mucosa. *B* and *C*, The surface (external) trabeculae are in contact with a thick band of dense fibrous connective tissue (*CT*), the reforming periosteum, which separates the excisional wound from the lamina propria of overlying alveolar mucosal tissues. Note the bone sliver (*BS*) lodged in the base of the flapped tissues (hematoxylin and eosin; original magnification *A*, ×13; *B*, ×33; and *C*, ×132).

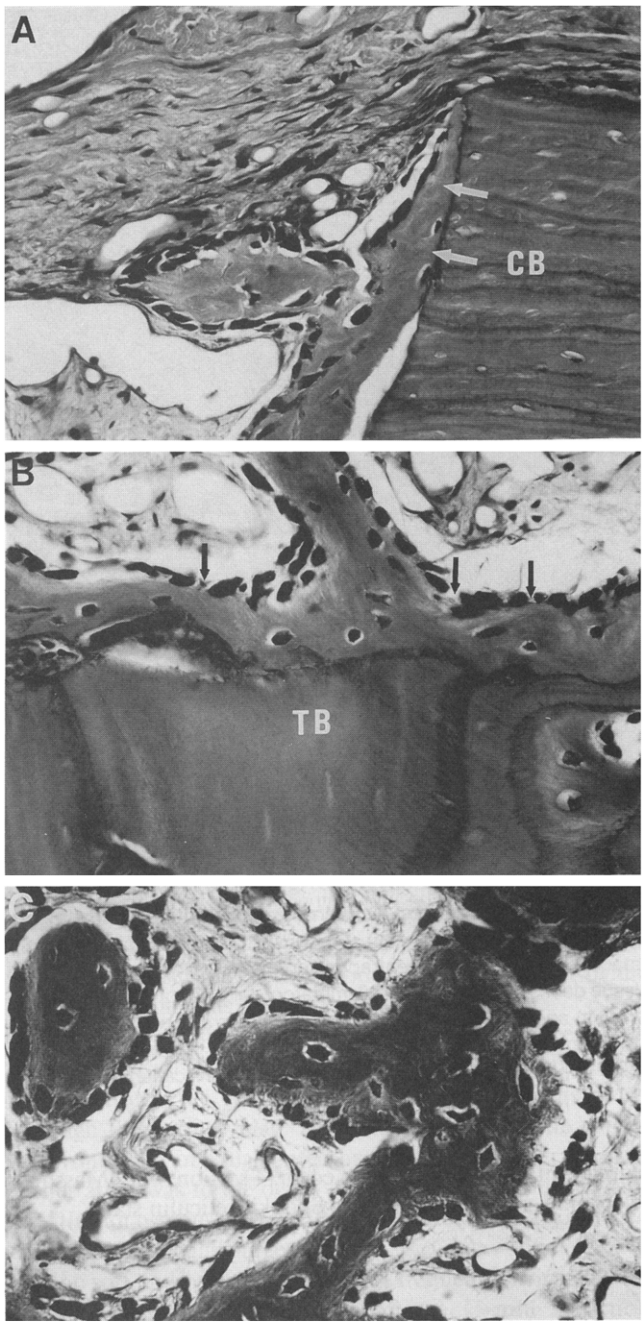

FIG 4. Fourteen days after surgery, same specimen as in Fig. 3. *A*, Apposition of woven bone (*arrows*) to the surface of devitalized cortical bone (*CB*) at coronal perimeter of the excisional wound. *B*, Apposition of woven bone (*arrows*) to devitalized trabecular bone (*TB*) deep in excisional wound. *C*, Woven bone trabeculae surrounded by uninflamed endosteal tissue. Note large osteocytes occupying lacunae and active osteoblasts at periphery of new trabeculae (hematoxylin and eosin; original magnification *A*, ×66 and *B* and *C*, ×132).

active osteoblasts (Fig. 4). The endosteal tissue was less cellular and less vascular than that seen in the earlier postsurgical periods. Osteoblastic activity was evident throughout the excisional wound site. Woven bone formation was seen as appositional growth on the surfaces of both cortical and trabecular devitalized bone forming the excisional wound edges (Fig. 4). The new woven bone trabeculae near the level of the cortical surface were in contact with a thick band of highly cellular, dense fibrous connective tissue separating the excisional wound from the overlying flapped tissues (Fig. 3).

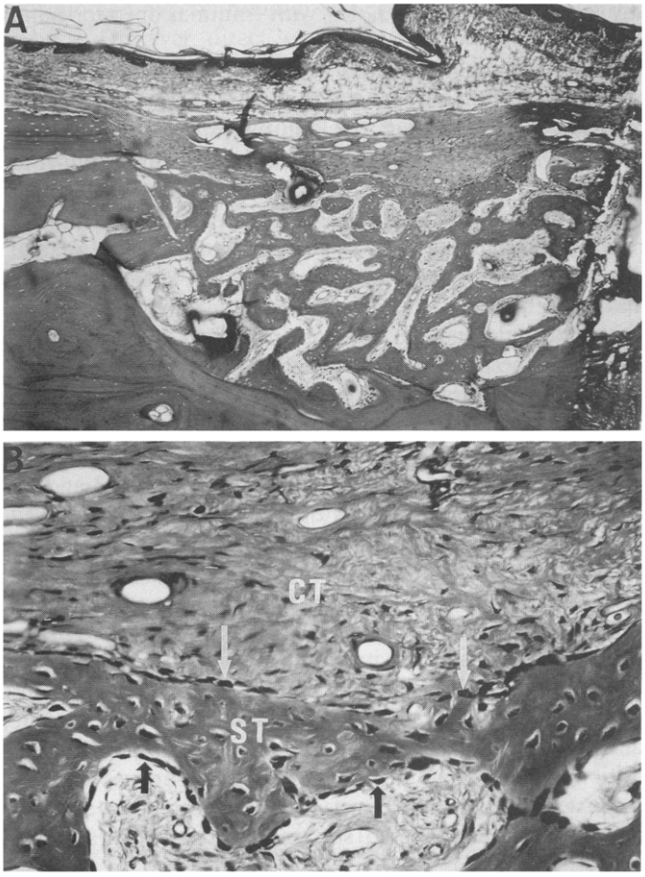

FIG 5. *A*, Endosteal tissue and maturing woven bone trabeculae in osseous excisional wound at 28 days after surgery. Trabeculae are coarser and are bordered by fewer active osteoblasts than at 14 days. *B*, The surface trabeculae (*ST*) have coalesced and are in direct contact with the dense fibrous connective tissue (*CT*) of the reforming periosteum. Note the difference in collagenation between the latter tissue and the loose connective tissue of the endosteum (below). Osteoid is being deposited by cells (*white arrows*) on the outer (periosteal) surface and cells (*black arrows*) on the inner (endosteal) surface (hematoxylin and eosin; original magnification *A*, ×13 and *B*, ×66).

This delimiting membrane, easily distinguishable from the overlying lamina propria by its collagen fiber orientation and architectural pattern, extended into the external fifth of the excisional wound defect. The collagen fibers were oriented essentially parallel to the plane of the cortical surface, joined the fibers of the reforming periosteum in the dissectional wound, and separated the internal osseous excisional wound site from the overlying connective tissue of the alveolar mucosa. This thick band of tissue has been identified in previous studies (4, 5) as the reforming periosteum which will subsequently function to repair the cortical plate destroyed in the excisional wounding.

At 28 days after surgery, the excisional wound site was characterized by maturing new trabecular bone (Fig. 5). The trabeculae had coalesced and now occupied a slightly greater total area of the osseous defect as compared with the endosteal tissue, which was the predominant tissue at 14 days. The new trabeculae were lined with flattened osteoblasts (morphological evidence of decreased cellular activity) and the lacunae encased smaller osteocytes than those observed at 14 days.

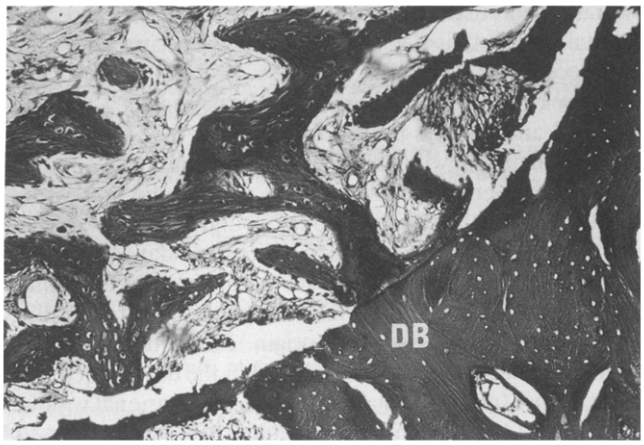

FIG 6. Maturing woven bone attached to devitalized bone (*DB*) at base of osseous excisional wound at 28 days after surgery. Note absence of osteocytes in lacunae of devitalized bone. As compared with the 14-day specimens, osteocytes in the maturing woven bone are decreased in size and the number of active osteoblasts at the periphery are reduced in number (hematoxylin and eosin; original magnification ×33).

Increased maturation of the trabeculae was further confirmed by polarizing light microscopy. As in the 14-day specimens, some new trabeculae were in direct contact with devitalized cortical and trabecular bone forming the excisional wound edges (Fig. 6). At the level of the cortical surface, the new trabeculae had coalesced and a layer of cells of the delimiting membrane (morphologically similar to the cells of the cambium layer of the periosteum) was actively depositing osteoid on the outer (periosteal) surface of the trabeculae. These cells were immediately subjacent to the fibers of the delimiting membrane, which now had an architectural pattern similar to the fibrous layer of the periosteum. Osteoid was also being deposited on the inner (endosteal) surface of these trabeculae (Fig. 5).

DISCUSSION

In periradicular surgery, the excisional wound involves the removal of bone by a rotary instrument to gain surgical access to radicular tissues. This investigation confined its objective to an excisional wound which did not include radicular tissues in order to concentrate on the osseous wound-healing response without superimposing potential radicular and intraradicular variables. It is also important to note that these observations are not morphometric, but are based on a descriptive review of sections through the central portion of the wounds.

Unlike the incisional and dissectional wounds, the wound edges of the excisional wound cannot be reapproximated because tissues have been intentionally removed. Because of the internal location of the osseous excisional wound, postsurgical soft tissue compression does not affect the blood that fills the excisional wound site. As a result, a *coagulum* rather than a thin, well-defined fibrin clot occupies the excisional wound site. This coagulum acts as a barrier instead of a migratory pathway for inflammatory and reparative cells because of the irregular, interrupted, widely spaced fibrin strands (6) (Figs. 1 and 2). The barrier must be removed and replaced

by granulation tissue migrating from the endosteal (and periodontal ligament) tissues before repair of the excisional wound can proceed (7).

The selected postsurgical observation periods of 1 through 4 and 14 and 28 days proved fortuitous in investigating the healing events following incisional and dissectional wounding (2, 3). However, these observation periods did not prove adequate for the osseous excisional wounds because key biological events obviously occurred between day 4 and 14. During this period, the coagulum occupying the excisional osseous wound site was removed and replaced by proliferating granulation tissue of endosteal origin. The initial outgrowth of this granulation tissue was observed in the day 4 specimens in the deeper (more internal) portion of the excisional wound. In the day 14 specimens, the coagulum had been removed, and a loose fibrous connective tissue, morphologically identical to endosteal tissue, containing multiple woven bone trabeculae occupied the excisional wound site. An investigation of osseous excisional wound healing which includes several postsurgical intervals between 4 and 14 days has been completed and will be reported at a later date (5).

The formation of new bone within the excisional osseous wound apparently proceeded from the deeper internal surfaces and progressed toward the external surface to the level of the former cortical plate. The more external new trabeculae were formed in contact with a thick band of overlying dense fibrous connective tissue termed the delimiting membrane, as it separates overlying mucosa from the excisional wound site (3, 5). This tissue was morphologically similar to the reforming periosteum described in the dissectional wound (3), and the fibers of the former were attached to the fibers of the latter. In the 28-day specimens, cells associated with the base of the periosteum (delimiting membrane) were depositing osteoid on the coalesced, external trabecular surfaces. On the inner surfaces of these trabeculae, endosteal tissue was evident and active osteoblasts were also depositing osteoid on these surfaces (Fig. 5). This was interpreted as the beginning of reformation of cortical bone, with bone deposition on the external surfaces now under control of a functioning periosteum and bone deposition on the internal surfaces under control of endosteal tissues. These findings are in agreement with Melcher and Irving (4) who reported that endosteal tissues must fill the excisional wound defect with new bone before overlying connective tissues can take part in the wound healing process as a functioning periosteum. This suggests that there is an inductive influence from the new bone trabeculae at the external excisional wound surface that is necessary for the connective tissue of the delimiting membrane to develop osteogenic potential and become a functioning periosteum.

The generation of frictional heat is the most damaging result of producing an excisional wound in bone with a rotary instrument (8). The degree of osseous damage resulting from excisional wounding with a rotary instrument is influenced by a range of variables including the type, shape, and sharpness of the rotary instrument; the speed, technique, depth, and time of cutting; and the effectiveness of the use of coolants (9). Studies indicate that the least damage to osseous tissues during excisional wounding is accomplished through the use of round burs (10, 11) at high speeds (10, 12) with water or saline coolant (13, 14), and a superficial shaving (or brush stroke) technique (11, 13, 15) with minimal operator hand pressure (16).

The present investigation followed these documented guidelines with the exception of the use of a superficial shaving technique. Instead, the #10 round bur was used to directly penetrate bone to the depth of its cutting head in an effort to create osseous wounds of similar dimensions and to avoid contact with adjacent roots. This direct penetration technique may have resulted, at least in part, in the zone of peripheral devitalization observed along the surgically created cortical and trabecular wound edges. The latter technique likely prevents adequate cooling and flushing effects of the coolant, increasing the frictional heat generated by the bur and, thus, the degree of injury to bone.

It is interesting to note that new woven bone was appositioned directly onto devitalized trabecular and cortical bone during the excisional wound healing process (Figs. 3 and 4). This was in direct contrast to the osteoblastic activity observed in the dissectional wound where osteoclastic activity always preceded osseous deposition, as evidenced by the presence of Howship's lacunae and reversal lines (3, 7), and new bone was not deposited on devitalized bone. In the present investigation, osteoclastic activity on the devitalized trabecular and cortical wound edges was not observed in any of the postsurgical intervals. This suggests that there is apparently some difference in the mechanisms inducing or inhibiting the deposition of new bone onto devitalized bone surfaces between the external (periosteal) surface of cortical bone and the internal (surgically exposed) surfaces of cortical and trabecular bone.

In reviewing the pathogenesis of periapical lesions, Stashenko (17) provided evidence of the complex interaction of immunological, inflammatory, and hormonal mediators influencing osteoclastic activity. These include bacterial components (lipopolysaccharides), prostaglandins (prostaglandin E_2), cytokines (interleukins, tumor necrosis factor, lymphotoxin), parathyroid hormone, and calcitriol. Based on studies with fractionated bone cells, it is the osteoblast, rather than the osteoclast, that possesses the receptors for resorptive mediators. Upon activation by these mediators, osteoblasts release a low molecular weight mediator (Factor X) that provides the resorptive signal to osteoclasts. Thus, in the functional absence of osteoblasts, osteoclasts are unable to respond to resorptive mediators. In the dissectional wound, the cells of the cambium layer of the periosteum are destroyed by the reflective forces used to elevate the flap (3). The absence of functional osteoblasts on the cortical surface may explain the observation that osteoclastic activity was not observed on this surface until the 14th postsurgical day. However, this does not explain the observation in the excisional wound that osteoclastic activity did not occur on devitalized bone surfaces where there were functional osteoblasts, as evidenced by the apposition of new bone to these surfaces. Further research is indicated to determine whether this results from an alteration in the complex interaction of resorptive mediators, some of which also influence osteoblastic activity.

A hyperchromatic (or basophilic) zone at the periphery of osseous excisional wounds produced with high-speed rotary instruments has been previously reported as an indication of osseous devitalization (14, 18). Mazorow (18) suggested that the basophilic zone was resistant to both bone resorption and bone apposition. The present study found no evidence of

resorption on hyperchromatic surfaces of cortical or trabecular bone. However, direct woven bone apposition to hyperchromatic surfaces was observed in all 14- and 28-day specimens. The latter observation agrees with the findings of Spatz (14), who reported apposition of new bone to hyperchromatic surfaces of cut bone in the mandibles of dogs. Horton et al. (19) also observed new bone formation directly on the cut surfaces (wound edges) of both vital and devitalized bone in excisional wounds in the maxillas and mandibles of dogs.

Lurie et al. (20) made standardized bone cuts with low-speed, cross-cut fissure burs in the mandibles of 24 adult vervet monkeys. These investigators reported that a hyperchromatic zone was not present in all bone defects and was not more prevalent in defects made without irrigation, as compared with those made with water or saline irrigation. The present investigation agreed with their findings that (a) new bone matrix was formed directly on devitalized bone surfaces; (b) osteoclastic activity was not observed within the excisional wound site; (c) the endosteal tissues play the major role in osseous excisional wound healing; and (d) the periosteum (delimiting membrane) does not function in bone repair until the excisional wound is almost filled with woven bone trabeculae of endosteal tissue origin.

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References

1. Harrison JW. Healing of surgical wounds in oral mucoperiosteal tissues. J Endodon 1991;17:401–8.
2. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery. I. The incisional wound. J Endodon 1991;17:425–35.
3. Harrison JW, Jurosky KA. Wound healing in the tissues of the periodontium following periradicular surgery. II. The dissectional wound. J Endodon 1991;17:544–52.
4. Melcher AH, Irving JT. The healing mechanism in artificially created circumscribed defects in the femora of albino rats. J Bone Joint Surg [Br] 1962;44:928–36.
5. Craig KR. The evaluation of radicular and periradicular wound healing following demineralization of apically resected root ends [Thesis]. Waco, Texas: Baylor University, 1990.
6. Melcher AH. Healing of wounds in the periodontium. In: Melcher AH, Bowen WH, eds. Biology of the periodontium. London: Academic Press, 1969:499–529.
7. Gutmann JL, Harrison JW. Surgical wound healing. Surgical endodontics. Boston: Blackwell Scientific Publications, 1991:320–3.
8. Eriksson A, Albrektsson T, Grane B, McQueen D. Thermal injury to bone. A vital-microscopic description of heat effects. Int J Oral Surg 1982;11:115–21.
9. Gutmann JL, Harrison JW. Surgical access: hard tissue management. Surgical endodontics. Boston: Blackwell Scientific Publications, 1991:183–202.
10. Argen E, Arwill T. High-speed or conventional dental equipment for the removal of bone in oral surgery. III. A histologic and microradiographic study on bone repair in the rabbit. Acta Odontol Scand 1968;26:223–46.
11. Moss R. Histopathologic reaction of bone to surgical cutting. Oral Surg 1964;17:405–14.
12. Boyne P. Histologic response of bone to sectioning by high-speed rotary instruments. J Dent Res 1966;45:270–6.
13. Tetsch P. Development of raised temperatures after osteotomies. J Maxillofac Surg 1974;2:141–5.
14. Spatz S. Early reaction in bone following the use of burs rotating at conventional and ultra speeds. Oral Surg 1965;19:808–16.
15. Hall RM. The effect of high-speed bone cutting without the use of water coolant. Oral Surg 1965;20:150–3.
16. Costich ER, Youngblood PJ, Walden JM. A study of the effects of high-speed rotary instruments on bone repair in dogs. Oral Surg 1964;17:563–71.
17. Stashenko P. The role of immune cytokines in the pathogenesis of periapical lesions. Endod Dent Traumatol 1990;6:89–96.
18. Mazorow H. Bone repair after experimentally produced defects. J Oral Surg Anes Hosp D Serv 1960;18:107–15.
19. Horton JE, Tarpley TM, Wood LD. The healing of surgical defects in alveolar bone produced with ultrasonic instrumentation, chisel, and rotary bur. Oral Surg 1975;39:536–46.
20. Lurie R, Cleaton-Jone P, Vieira E, Sam C, Austin J. Effects of water and saline irrigation during bone cutting on bone healing. Int J Oral Surg 1984;13:437–44.