Effect of Lyophilized Autologous Plasma on Periodontal Healing of Replanted Teeth*

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THE PURPOSE OF THIS HISTOLOGIC AND AUTORADIOGRAPHIC STUDY of replanted teeth was (1) to evaluate the beneficial effect, if any, of lyophilized autologous plasma (LAP) application on periodontal healing and (2) to re-examine rates of repair in different areas of the associated periodontium following replantation. Maxillary and mandibular incisors and premolars of three rhesus monkeys were used. Teeth were extracted with forceps and placed in sterile physiologic saline. After 5 minutes each tooth was returned to its socket and immobilized by interproximal acid-etch splints. Splints were removed after 1 week. Of the 48 replants performed, 24 (controls) were replanted as described. Of the 24 experimental teeth, during the 5 minute interval between tooth extraction and replantation, the root surface and the inner socket walls were bathed with 1 ml of the reconstituted LAP-saline solution (800 mg/ml). Replants and animal sacrifice were scheduled to provide observations at 1, 3, 7, 14, 28 and 45 days following replantation. One hour prior to sacrifice, each monkey received an intravenous injection of tritiated thymidine, 1 μCi/gm body weight. Tissue specimens were processed for evaluation following standard procedures. Eight replanted teeth were available for evaluation for each of the six time-points. Four teeth were treated with LAP and four without it. Histologically, tissue sections were examined for (1) epithelial proliferation and attachment, (2) periodontal fibers organization and maturation, (3) inflammatory cell types, (4) presence or absence of cementum resorption and dentoalveolar ankylosis and (5) degree of vascularity of the tissues. For autoradiographic evaluation, the periodontium associated with the replanted tooth was divided into nine spatial cell compartments. In each compartment, labeled tissue cells, epithelial or connective, were counted and recorded. Differences between the control (untreated) replanted teeth and the LAP-treated teeth, at each time-point and within each compartment, were analyzed for significance using the paired *t*-test. The findings of this study indicate that LAP use enhanced healing by (1) early replacement of the fibrin clot, (2) increased connective tissue cell proliferation, (3) reduction of the inflammatory response and (4) inhibition of root cementum resorption. Periodontal healing and repair occurred more rapidly in the supracrestal or transseptal connective tissue region than within the periodontal membrane space.

Polson and associates[1-4] studied tooth replants and postulated that connective tissue reattachment to the root surface depended upon a chronologic healing sequence related to fibrin and collagen interactions. They proposed a system called *fibrin linkage* to explain the initial mechanism for fibrin-collagen linkage involving fibronectin (FN).[5] They believe that FN may anchor a blood clot to surrounding collagen since it is covalently linked to fibrin and collagen by blood coagulation factor XIIIa. FN is adsorbed most strongly by collagen and this mechanism would facilitate the attachment of fibrin to collagen.[6] Recent *in vitro* studies indicated that: (1) the determinants for FN-collagen and FN-fibrinogen binding and crosslinking are similar or even identical,[7] (2) FN is a substrate for blood coagulation factor XIIIa and is found both in plasma and in the extracellular matrix of cultured cells[8,9] and (3) blood factor XIIIa mediates crosslinking of FN to collagen.[10] In cell cultures, studies of fibrillogenesis demonstrated that the attachment of human gingival and skin fibro-

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blasts to collagen is mediated by FN.[11,12] In this system FN is present in cell layers of substrate-attached cells and is organized into an extracellular matrix, whereas collagen (a second component of the matrix) binds to FN. FN serves as template or scaffolding for collagen fiber formation.

The relevance of these observations to the *in vivo* situation has not been established. However, the fact that patients with congenital coagulation factor XIIIa deficiency suffer from poor wound healing[13] indicates the importance of binding and crosslinking by blood factor XIIIa and is consistent with the involvement of FN, fibrinogen and collagen in *in vivo* wound healing and repair. Therefore the question arises as to whether, in tooth replants, blood plasma which contains FN,[14] fibrinogen[15] and blood factor XIIIa[16] could have a beneficial effect on wound healing, tissue repair and collagen fibrillogenesis.

There is extensive documentation of tooth replant studies[17,18] including reports both of frank failures and qualified successes in terms of periodontal reattachment.[19-29] Failures were usually associated with dentoalveolar ankylosis,[19] root surface resorption[20] and pocket formation.[21] The success of tooth replants has been reported to be highly dependent on (1) retaining and maintaining a viable, moist periodontal membrane;[22] (2) allowing a replanted tooth to remain out of the mouth for as brief a period as possible[23] and (3) preserving the vitality of the pulp tissue in immature teeth with wide-open apices[24] and treating endodontically mature teeth before or shortly after replantation.[25] Following replantation, the periodontium appears to undergo rapid repair with[26] or without splinting.[27]

Although most of these reports have established that a normal periodontium may result following tooth replantation, controversy persists regarding the rate of repair within the periodontium during healing.[1,28,29] Hurst[28] indicated that more rapid repair occurs in the periodontal membrane area beneath the crest of the alveolar bone than in the supracrestal area of the membrane above the crest, but Nasjleti et al.[29] found simultaneous rates of repair in these areas. Recently, Proye and Polson[1] reported that healing occurred more rapidly in the supracrestal region than within the periodontal membrane beneath the crest. Obtaining accurate information on the healing sequence following tooth replantation has important therapeutic implications, not only in tooth replants but also in periodontal surgery. Therefore, attempts to clarify the dynamics of tissue repair in these areas seem worthwhile.

As in previous studies,[29-32] the monkey was chosen for this investigation because the teeth and supporting tissues have similar patterns of growth and repair to human tissues and the size and shape of the teeth facilitate their extraction, replantation and splinting. The present study expands upon an earlier series of tritiated thymidine (^{3}H-TDR) autoradiographic studies in which cell proliferation was examined in periodontal tissues of surgically treated monkeys.[29-32] Since these monkeys were killed 1 hour following ^{3}H-TDR administration, labeling was restricted to cells in the premitotic stage. The number of labeled cells represents those cells that will subsequently enter mitosis; consequently, it provides a measure of the *proliferative activity* of the cell compartment under study.[33]

The purpose of this histologic and autoradiographic study of replanted teeth was (1) to evaluate the effect of lyophilized autologous plasma (LAP) application on periodontal healing and (2) to re-examine rates of repair in different areas of the associated periodontium following replantation.

MATERIALS AND METHODS

Three healthy young male rhesus monkeys (*Macaca mulatta*) weighing from 4.1 to 4.9 kg and of similar age and size were used. Each monkey had a nearly complete dentition and exhibited a mild to moderate marginal gingivitis with no loss of attachment. Four weeks before the experiment, all teeth were scaled and polished. Intraoral radiographs were also taken to check the degree of root development of the teeth and their supporting structures. The maxillary and mandibular incisors and premolars were replanted, thus providing a total of 48 replanted teeth for evaluation. Sedation was obtained by injecting ketamine hydrochloride intramuscularly (25mg/kg), and anesthesia was obtained by injecting pentobarbital sodium (Nembutal) intravenously (30 mg/kg). The method used for tooth replantation, without endodontics, was reported previously.[30] The teeth were extracted with forceps as gently as possible and placed immediately in sterile physiologic saline solution. After 5 minutes, and before replantation, any clotted blood within the socket was removed by suction, and the tooth returned to its original position using slight finger pressure. The marginal gingival tissues were carefully adapted and, for 3 minutes, moderate pressure with a moist sterile gauze was applied to increase their adaptation and also to promote a thin fibrin clot. No periodontal dressing or sutures were used. The teeth were temporarily immobilized with interproximal acid-etch splints.[34] Opposing teeth were ground when needed to relieve occlusal interferences. Splints were removed 1 week following replantation.

Of the 48 tooth replants performed, 24 (controls) were replanted as described above, whereas the others were treated with LAP. For this treatment, during the 5-minute interval between tooth extraction and its replacement in the corresponding socket, the root surface and the inner walls of the socket were bathed with 1 ml of the reconstituted LAP-saline solution (800 mg/ml), using a tuberculin syringe. For the preparation of LAP, samples of 40 to 50 ml of peripheral venous blood were obtained from each monkey and transferred to sterile

acid/citrate/dextrose tubes. These tubes then were centrifuged at 130*g* for 10 minutes, and the supernatant plasma transferred to sterile bottles for lyophilization. The resulting freeze-dried product was stored at −20°C until use. On the day of surgery, bottles were allowed to come to room temperature. Upon reconstitution in normal saline, a viscous, sticky solution was obtained.

Replantation of teeth and animal sacrifice were scheduled to provide observations at 1, 3, 7, 14, 28 and 45 days following replantation. One hour before sacrifice, each monkey received an intravenous injection of ^{3}H-TRD, 1 μCi per gram of body weight, Sp. Act. 6.7 Ci/mmol.* After the monkeys were sacrificed by exsanguination, tissue blocks corresponding to the replanted teeth were removed, fixed in 10% buffered formalin solution and decalcified in 20% formic acid. Paraffin-embedded tissue blocks were serially sectioned both buccolingually and mesiodistally at 6 μm. Every fifth section was placed on a glass slide and processed for autoradiographic study. Kodak's nuclear track emulsion, type NTB-3† was applied to the slides using the dipping technique of Joftes.[35] The slides were placed in light-proof boxes and exposed for 4 weeks at 4°C. These sections were stained with Harris's hematoxylin and eosin. Others were stained with Masson's or Mallory's connective tissue stain. For each of the six experimental time-points, eight replanted teeth were available for evaluation: four teeth treated with LAP and four without it.

Histologic Evaluation

Ten tissue sections from each of the 48 replanted teeth were examined for (1) epithelial proliferation and attachment, (2) periodontal fibers organization and maturation, (3) inflammatory cell types, (4) presence or absence of tooth root resorption and dentoalveolar ankylosis and (5) degree of vascularity of the tissues.

Autoradiographic Evaluation

Both epithelial and connective tissue labeled cells were counted in the periodontium associated with the replanted tooth. For this, the periodontium was divided into nine spatial compartments (Figs. 1 and 2). Compartments 1 and 2 encompassed the basal cell layers of the sulcular and the oral gingival epithelium, respectively. The latter included only the oral gingival epithelium portion extending from the free gingival margin to a line projected over the apical extent of the junctional epithelium. Compartment 3 comprised the connective tissue cells located in between and adjacent to Compartments 1 and 2, whereas Compartment 4 included the connective tissue cells situated below Compartment 3 and above the alveolar bone crest. Compartments 5, 6 and 7 comprised the connective tissue

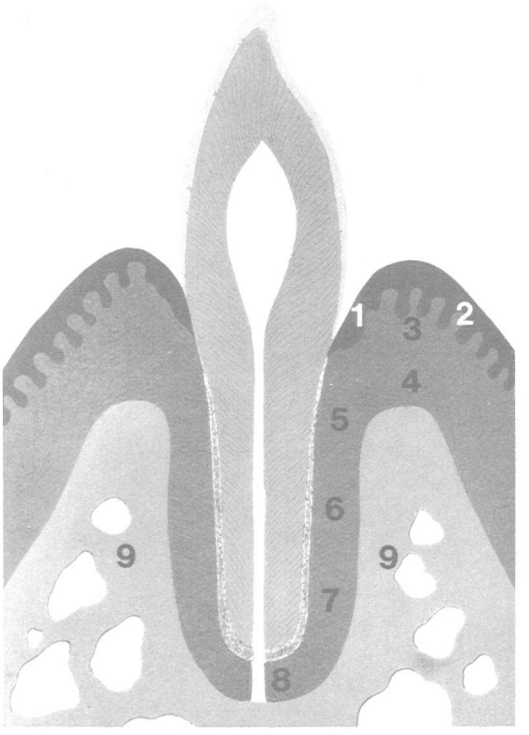

Figure 1. *Diagram showing location of the nine cell compartments where the histologic and autoradiographic evaluations were performed in buccolingual sections of replanted teeth.*

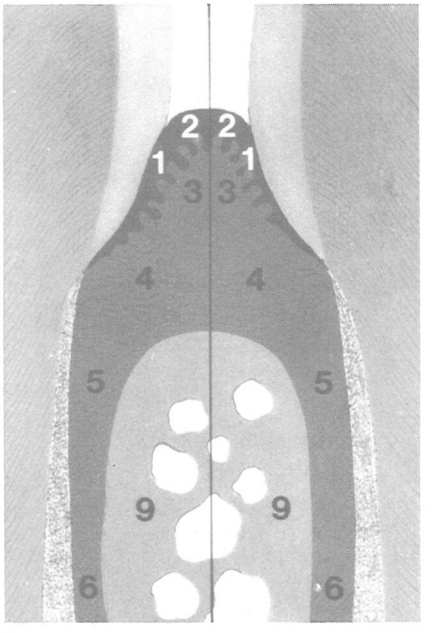

Figure 2. *Diagram showing location of the cell compartments in mesiodistal sections.*

cells of the periodontal membrane located beneath the alveolar bone crest and extending to the root tip. These three compartments corresponded to the coronal, middle and apical one third, respectively, of the periodontal membrane space. Compartment 8 included the connective tissue cells in the periapical region just below Compartment 7, and Compartment 9 included the

* New England Nuclear Corp, Boston, MA.

† Eastman Kodak Co, Rochester, NY.

bone marrow cells of the associated alveolar bone. Consequently, nine cell compartments were considered on each tissue section. Autoradiographs were examined using a binocular microscope at 100 × magnification. Tritiated thymidine labeled epithelial cells were counted in each of Compartments 1 and 2 and labeled connective tissue cells were counted in Compartments 3 through 8. Labeled bone marrow cells were counted within the alveolar bone of Compartment 9. For counting labeled cells a Veeder hand-tally counter* was used. Each tissue section was counted twice at intervals varying from 1 to several weeks to determine if errors in counting would influence the results. An exceedingly small discrepancy was found. A total of 480 tissue sections were evaluated, 240 treated with LAP and 240 without it. On each replanted tooth and at each time-point, labeled cells were counted in all nine cell compartments using 10 tissue sections; a mean for each tooth was then computed by averaging the individual values recorded.

Statistical Evaluation

Differences between the LAP-treated teeth and the control (untreated) replanted teeth, at each time-point and within each compartment, were tested for significance using the paired *t*-test.

RESULTS

Histologic

There were histologic differences between control teeth and LAP-treated teeth at 1, 3, 7, 14 and 28 days following replantation. There were distinct differences in terms of degree of surgically induced inflammation, rate and quality of healing and presence or absence of cementum resorption. No histologic differences were observed between the two groups in 45-day specimens.

One-Day Specimens. Each of the control teeth demonstrated an anchoring fibrin clot at the site of the wound. This clot consisted of a fibrin network into which were entangled many polymorphonuclear leukocytes (PMNs) and erythrocytes acting as a wound seal (Fig. 3). A partial loss of collagen fiber network was observed, as well as a reduced cellularity throughout the connective tissues. These tissues were infiltrated by a dense population of PMNs and macrophages. Epithelial attachment was lacking and the layer of cementoblasts was nonexistent. The outer and inner surface of the alveolar bone appeared normal and there was no evidence of change in the marrow spaces.

Experimental teeth treated with LAP before replantation showed similar histologic features, except for the degree of leukocytic infiltration. The connective tissue adjacent to LAP-treated teeth demonstrated a mild to moderate infiltrate consisting of PMNs and macrophages.

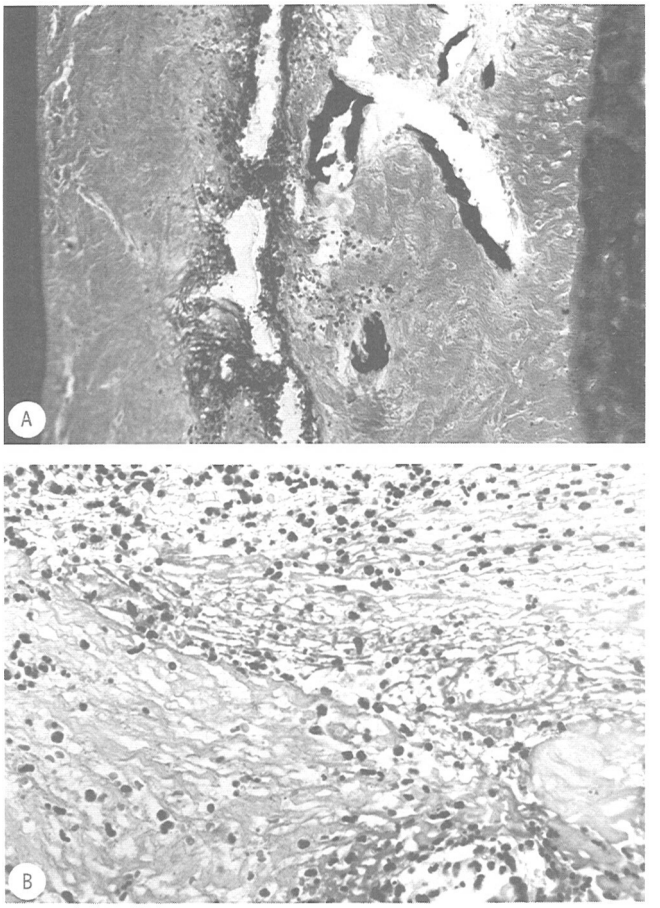

Figure 3. *One-day control specimens.* **A.** *Supporting tissues show torn periodontal fibers connected by a fibrin clot infiltrated by erythrocytes and PMNs (Mallory's stain, magnification × 40).* **B.** *Higher power photomicrograph of the fibrin stroma (Hematoxylin and eosin stain, magnification × 200).*

Three-Day Specimens. Each of the control teeth showed fusion to the supporting connective tissues mediated by the fibrin clot (Fig. 4A). A heavy inflammatory infiltrate made up of PMNs and macrophages was present both in the supracrestal region beneath the unattached crevicular and junctional epithelia and at the coronal one third of the periodontal membrane space. Loss of collagen and fibroblasts was observed in these tissues. Resorption of the alveolar bone crest had begun. The interface of break in continuity of the connective tissue fibers that resulted from tooth extraction was detectable.

In contrast, LAP-treated teeth showed that the fibrin clot was already being replaced by fine and delicate fibers which partially restored continuity between the severed ends of the connective tissue fibers (Fig. 4B). These tissues showed a moderate leukocytic infiltrate. Some small oval cells repopulated areas adjacent to the wound margins. No epithelial attachment was noticeable and cementoblasts were absent. Both the middle and apical areas of the periodontal membrane space

* Veeder Root Vue, Hartford, CT.

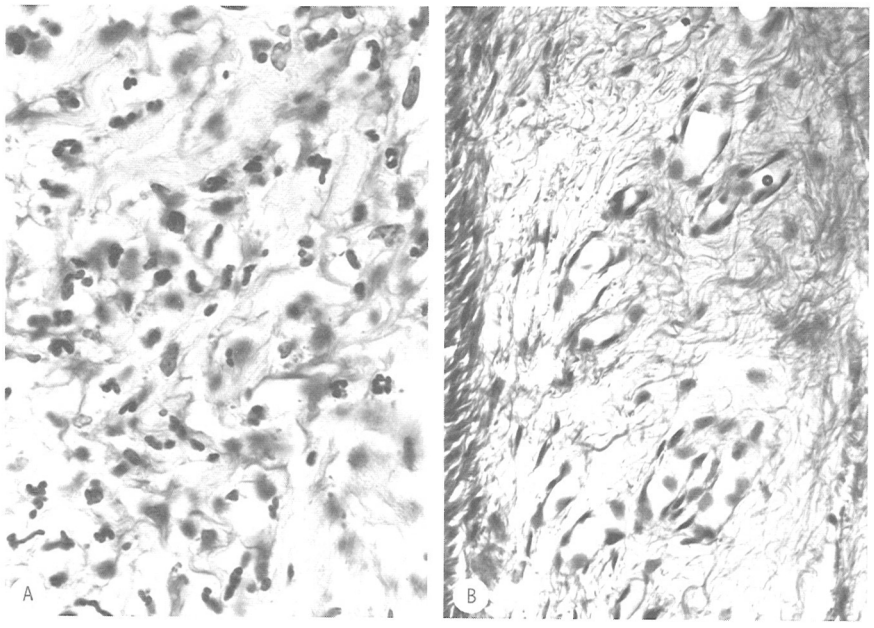

Figure 4. *Three-day specimens.* **A.** *Higher power photomicrograph of the fibrin stroma in a control specimen showing a dense inflammatory infiltrate made up of PMNs and macrophages.* **B.** *The fibrin stroma of an LAP-treated tooth was already being replaced by fine and delicate fibers which partially restored continuity between the severed ends of the connective tissue fibers (Masson's stain, magnification × 300).*

exhibited hyaline degeneration and the adjacent alveolar bone showed empty lacunae. No bone or root resorption was observed.

Seven-Day Specimens. In control teeth, the junctional epithelium was seen at the cemento-enamel junction. The extraction wound interface was not visible and the fibrin clot was replaced by loose fibrillar connective tissue blended with a small number of regenerating fibroblasts (Fig. 5A). There was a moderate accumulation of PMNs and macrophages in these tissues as well. Resorption of the alveolar crest was more pronounced. There were occasional and isolated areas of bone and cementum resorption at different levels of the periodontal membrane space. Cementoblasts were still absent.

In LAP-treated teeth, the supracrestal region and the periodontal membrane space demonstrated that the repair process had advanced by regaining cellularity. These areas became partially repopulated with connective tissue fibers which often showed functional orientation (Fig. 5B). The connective tissue fibers of the supracrestal region also showed a mild leukocytic infiltrate. Some spots of bone resorption were evident. However, cementum resorption was not seen in these specimens.

Fourteen-Day Specimens. In control specimens, the gingiva appeared normal, but the subgingival and supracrestal tissues had engorged vessels and a young and disorganized fibrillar stroma (Fig. 6A). In the periodontal membrane space, cementoclasts, osteoclasts and osteoblasts were aligned along the cementum and alveolar bone. There were areas of superficial cementum and bone resorption (Fig. 7).

In LAP-treated teeth, inflammation had subsided and the supracrestal connective tissue region demonstrated

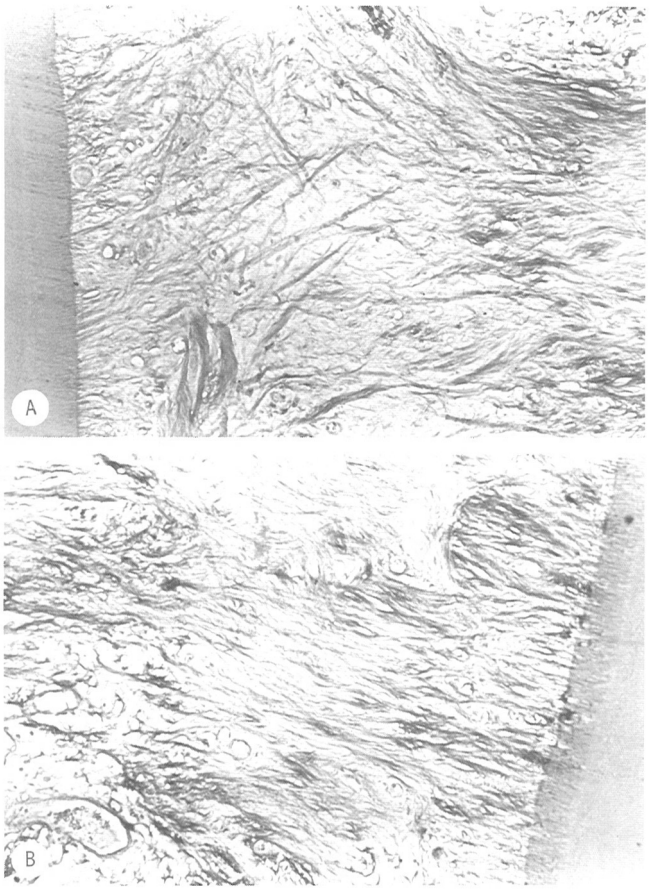

Figure 5. *Seven-day specimens. Supracrestal region.* **A.** *The extraction wound interface of a control tooth was not visible and the fibrin stroma was replaced by a loose fibrillar connective tissue blended with a small number of regenerating fibroblasts.* **B.** *LAP-treated tooth showing that the repair process had advanced by regaining cellularity. This region also became partially repopulated with connective tissue fibers which often showed functional orientation (Masson's stain, magnification × 100).*

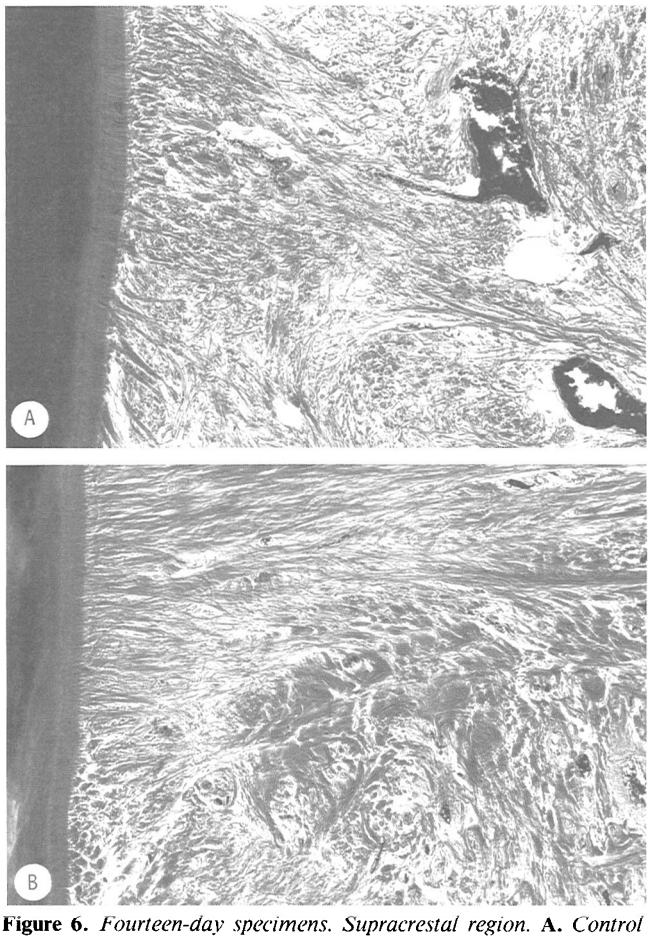

Figure 6. *Fourteen-day specimens. Supracrestal region.* **A.** *Control specimen showing engorged vessels and a young and disorganized fibrillar stroma.* **B.** *LAP-treated tooth showing a clear tendency of its collagen fibers toward orientation (Mallory's stain, magnification × 100).*

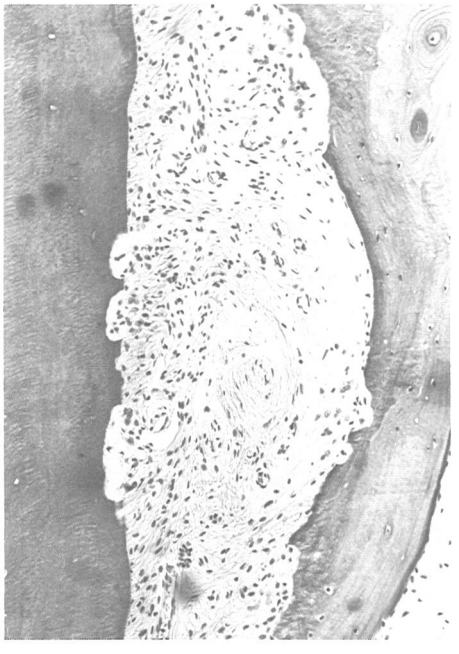

Figure 7. *Fourteen-day control specimen. The periodontal membrane space shows areas of superficial cementum and bone resorption (Hematoxylin and eosin stain, magnification × 80).*

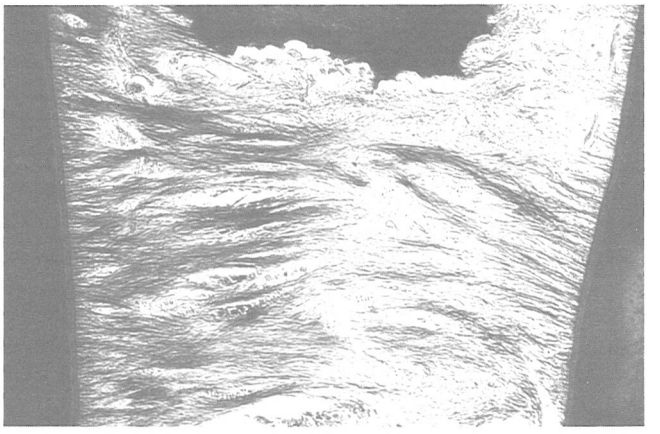

Figure 8. *Twenty-eight-day specimen. Interdental area, supracrestal level. The tooth on the left was LAP-treated and the tooth on the right was the control. A band of transseptal collagenous fibers extends from the cementum of one tooth to the cementum of the adjacent one over the crest of the interdental alveolar bone. However, connective tissue maturity had not yet been achieved at the control tooth side (Mallory's stain, magnification × 50).*

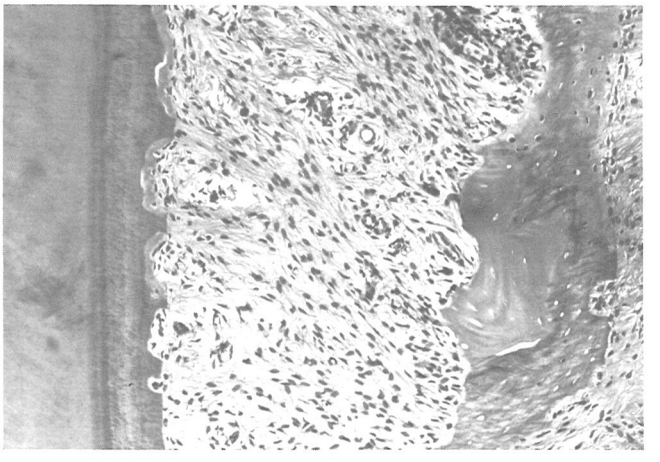

Figure 9. *Twenty-eight-day control specimen. Many areas of cementum and bone resorption as well as cementum and bone repair were observed (Hematoxylin and eosin stain, magnification × 30).*

a clear tendency of its collagen fibers toward orientation (Fig. 6B). Definite attachment of the collagen fibers was also observed in the periodontal membrane space. No areas of cementum resorption were observed, but there were many areas of bone resorption and repair.

Twenty-Eight-Day Specimens. Control specimens demonstrated that both the supracrestal region and the periodontal membrane space had an almost normal appearance, but connective tissue maturity had not yet been achieved (Fig. 8). Many areas of cementum and bone resorption as well as cementum and bone repair were observed (Fig. 9). Cementoblasts were present, but no inflammatory cells were depicted in these specimens.

LAP-treated teeth demonstrated normalcy in their cellular and collagen fiber components both in the supracrestal region and in the periodontal membrane space (Fig. 8). Areas of bone resorption and repair were depicted in these specimens, but no areas of cementum resorption were seen.

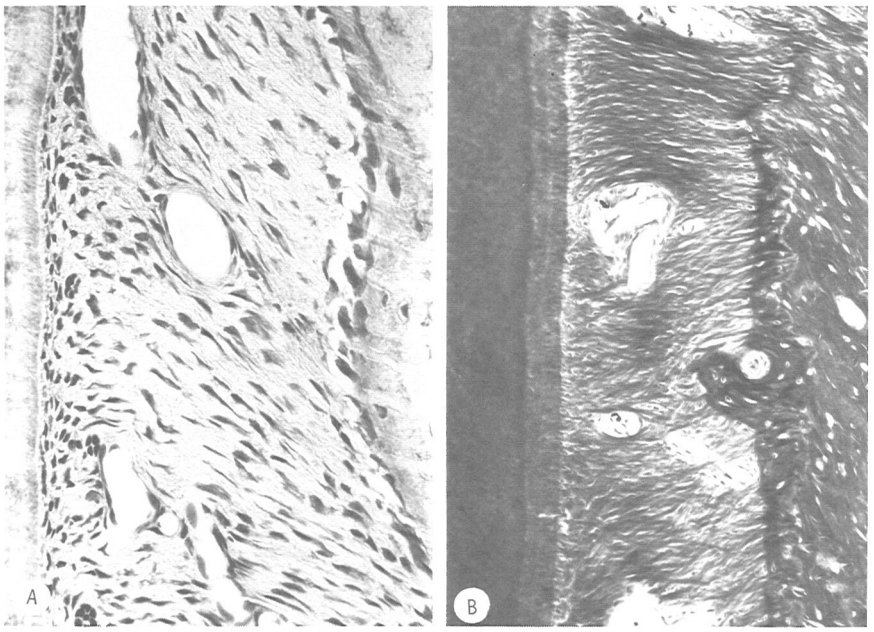

Figure 10. *Forty-five-day specimens. Both LAP-treated and control replanted teeth showed normalcy of their connective tissue components.* **A.** *Cellular (Hematoxylin and eosin stain, magnification × 50).* **B.** *Periodontal fibers (Mallory's stain, magnification × 40).*

Table 1
Labeled Cells in the Periodontium of Teeth Treated with and without LAP, Observed Following Replantation

C*	Mean difference,† standard deviation of difference,‡ and significance					
	1 Day	3 Days	7 Days	14 Days	28 Days	45 Days
1	17.22† (2.28)§	27.90 (4.97)‖	24.37 (1.58)§	6.60 (1.46)‖	13.85 (1.87)§	1.75 (1.12) NS
2	5.85 (1.03)‖	19.35 (2.12)§	19.07 (0.89)§	18.72 (1.20)§	2.75 (1.38)¶	0.62 (1.12)¶
3	4.10 (1.48)¶	3.57 (0.92)‖	2.67 (0.34)§	1.25 (0.72)¶	3.52 (0.90)‖	0.85 (0.46)¶
4	1.62 (0.95)¶	18.20 (2.26)§	20.12 (2.37)§	11.95 (2.03)‖	2.95 (0.89)‖	1.05 (0.19)‖
5	1.45 (1.19) NS	4.07 (1.03)‖	12.87 (1.32)§	11.30 (0.24)§	2.57 (0.59)‖	0.75 (0.30)¶
6	2.27 (1.09)¶	4.07 (1.41)¶	5.42 (1.74)‖	3.77 (1.61)¶	4.12 (0.72)‖	1.00 (0.28)‖
7	0.77 (0.90) NS	8.07 (1.16)§	15.42 (1.81)§	4.65 (0.50)§	7.15 (1.54)‖	0.57 (0.28)¶
8	1.20 (0.88) NS	7.87 (1.91)‖	11.95 (2.10)‖	5.75 (1.58)‖	2.90 (0.64)‖	0.70 (0.34)¶
9	0.65 (0.25)¶	8.17 (0.85)§	6.72 (0.88)§	2.60 (1.27)¶	1.05 (0.36)¶	1.05 (0.36)¶

* Abbreviations used: C = Compartment. NS = not significant.
† Mean difference between LAP-treated and control teeth.
‡ Standard deviation between parentheses.
§ $P < 0.001$.
‖ $P < 0.01$.
¶ $P < 0.05$.

Forty-Five-Day Specimens. Both control and LAP-treated replanted teeth showed normalcy of their connective tissue cellular (Fig. 10A) and fiber (Fig. 10B) components. In these tissues no inflammatory cells were present; however, some round cells were detected in proximity to the crevicular tissues. Areas of new bone formation and cementum repair were evident in control specimens.

Autoradiographic

Table 1 shows the mean differences in the labeled cell counts obtained for the nine spatial cell compartments in teeth treated with and without LAP at 1, 3, 7, 14, 28 and 45 days following replantation. Since the mean values of each of the nine compartments, obtained in buccolingual and mesiodistal sections, were very close, they were averaged and the results were used for statistical analysis of the data. Statistically significant differences were found between LAP-treated teeth and control untreated replanted teeth, within each compartment and at each time-point. For the most part, over the 45-day investigation period, the epithelial, connective tissue and bone marrow cell proliferative activity for LAP-treated teeth were significantly different from control teeth. Figures 11–13 illustrate epithelial and connective tissue cell labeling at various times after tooth replantation.

DISCUSSION

The present study was carried out in accordance with the notion that in tooth replants new connective tissue attachment to the root surface appears to be dependent upon a chronologic healing sequence related to fibrin

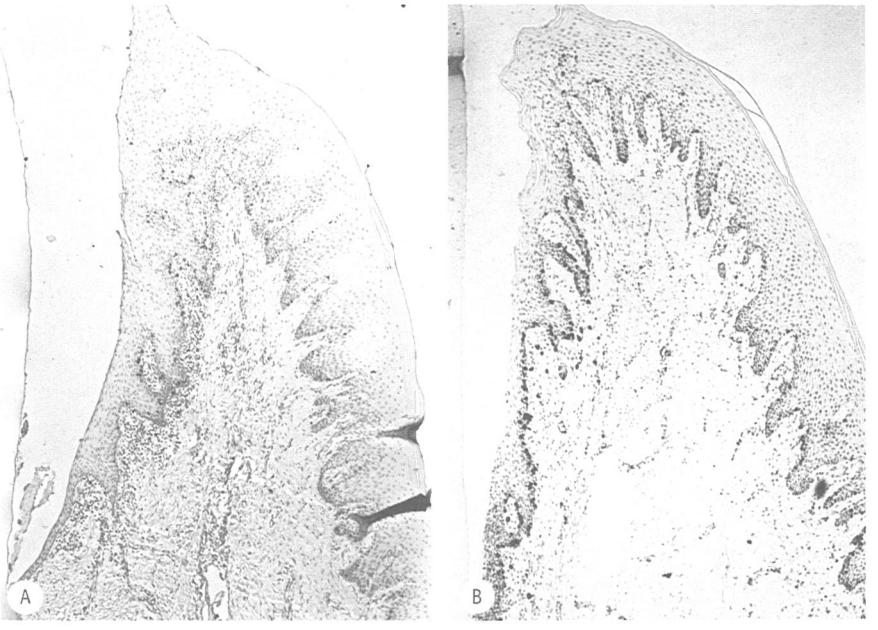

Figure 11. *Three-day specimens. Autoradiographs of marginal tissues: Compartments 1, 2 and 3. Epithelial labeling reaches its peak both in control and LAP-treated replanted teeth.* **A.** *Control specimen.* **B.** *LAP-treated specimen (magnification × 25).*

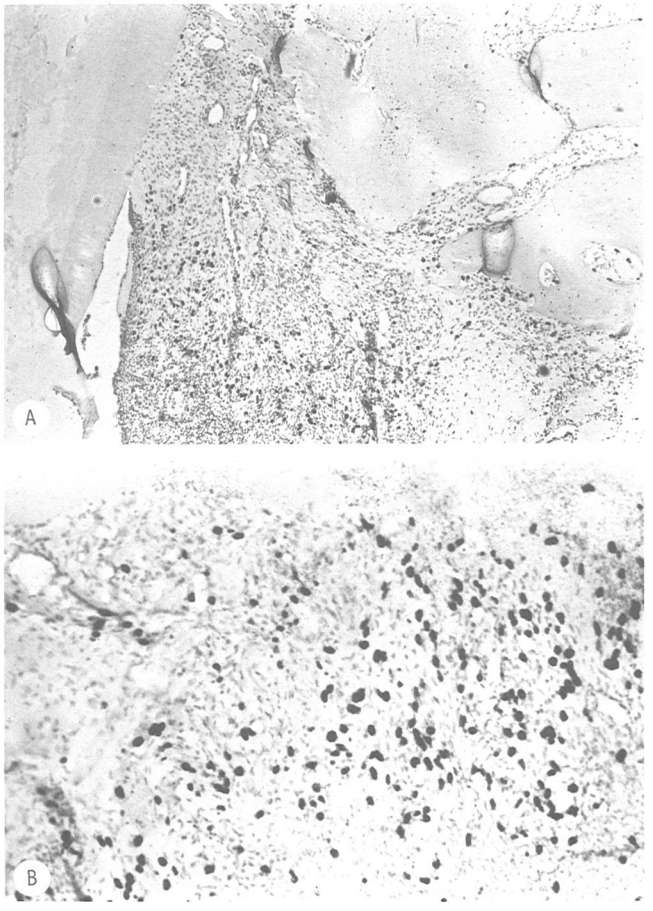

Figure 12. *Seven-day specimens. Autoradiographs of the supracrestal region: Compartment 4. Connective tissue cell labeling reaches its peak in control and LAP-treated teeth.* **A.** *Control specimen shows that cell proliferative activity occurred more rapidly in the supracrestal region than within the coronal portion of the periodontal membrane space (magnification × 25).* **B.** *Higher power photomicrograph of an LAP-treated tooth illustrating increased labeling of cells in Compartment 4 (magnification × 80).*

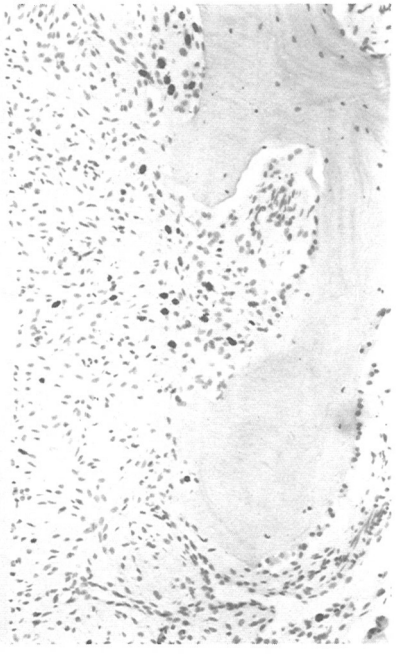

Figure 13. *Twenty-eight-day specimen. Coronal portion of the periodontal membrane space in a control specimen: Compartment 5. Cell-labeling indicates that the repopulation of this area appeared to be derived from sources both within the periodontal membrane space and the adjacent bone marrow spaces (magnification × 80).*

and collagen interactions involving FN.[5] For the present study, however, an alternative approach was taken involving the use of LAP which contains FN, fibrinogen and plasma factor XIIIa. Fibrinogen was converted into fibrin by the action of thrombin at the tooth extraction site, thus producing clotting of the blood.[10] The main objective was to determine whether applying autologous plasma to root surfaces and the inner walls of the tooth extraction socket would enhance wound healing, tissue repair and collagen fibrillogenesis, by modifying

or modulating the early surgically induced connective tissue breakdown.

In this study the healing process of the periodontal tissues was uneventful, following a pattern similar to that described previously.[29] Histologic tissue sections of 1- and 3-day specimens, from both LAP-treated and untreated teeth showed a zone of fibrin containing PMNs and macrophages occupying the interface created by the disrupted epithelial and connective tissues. In 7-, 14- and 28-day specimens, the supracrestal areas and the periodontal membrane spaces were repopulated with connective tissue cells, and collagen fibers replaced the fibrin. It seemed that the rapid healing of the gingival and connective tissues was due mainly to the minimal trauma and the close adaptation of the marginal gingival tissues, even without support of sutures or surgical dressings. The thinness of the fibrin clot allowed its quick replacement, and the subsequent connective tissue repair.

In periodontal surgery, if the gingival fibers attached to cementum are retained, as proposed by Ratcliff[36] and demonstrated by Levine and Stahl,[37] there is little delay in healing and no apical migration of the epithelial attachment. Levine and Stahl stated that, when supracrestal gingival fibers were retained on the root, reattachment to the retaining fibers could occur by "interdigitation" of the incised collagen fibers on the root with fresh collagen from the healing flap.[37] This process of healing "by scar" implies the deposition of an enclaving connective tissue matrix. Similar occurrences were noted in tendon,[38] and skin repair.[39] Klein and Weiss[40] reported that the existing collagen may be broken down into subunits which are reorganized and contribute to new collagen formation serving as a link between the existing connective tissues. Further, this molecular rearrangement may be significant in the collagen reattachment potential of root surfaces treated by fiber retention procedures.[37] Thus, in the present study the gingival and periodontal tissues healed "by scar." According to Levine and Stahl,[37] an advantage of healing by scar is that it may provide a more secure connective tissue barrier against the invading inflammatory elements that cause migration of the junctional epithelium.

A closer analysis of results reveal that replanted teeth treated with LAP attained an earlier and significantly greater connective tissue attachment, with less inflammation and no cementum resorption. The differences seen in Table 1 document the efficacy of the reconstituted LAP. The components in LAP such as FN, fibrinogen and plasma factor XIIIa probably provided a cumulative effect on periodontal healing, which would account for the very favorable results obtained. However, other components in LAP, although present in small amounts, such as platelet-derived growth factor (PDGF) and fraction Cohn IV_1 may have influenced the results. Recently, it has been postulated that in wounds, PDGF aids tissue repair by attracting fibroblasts into the clot and by inducing their subsequent proliferation.[41] The effect on gingival fibroblasts attachment of treating diseased root surfaces with a solution of plasma fraction Cohn IV_1 has been reported.[42] That study demonstrated that more fibroblasts adhered to the fraction-treated root surfaces than to surfaces that were not treated with it.

Of particular interest were the findings in LAP-treated replanted teeth showing early replacement of the fibrin clot and increased connective tissue cell proliferation. Both processes started 3 days after replantation. It therefore appears that the application of LAP may have stimulated the connective tissue proliferation by the removal of fibrin. In view of these findings, LAP could serve as a useful adjunct in the surgical treatment of periodontitis, particularly following acid conditioning of scaled root surfaces. It would be interesting to see if with the use of citric acid, the addition of LAP significantly enhanced reattachment of the connective tissue fibers. Such studies are underway.

Other findings in this study merit comment. Early histologic specimens, both from untreated and LAP-treated replanted teeth, showed a close association between PMNs and macrophages, a connective tissue composed of degenerating fibroblasts and a scarcity of collagen fibrils. Thus, after tooth replantation, PMNs, macrophages and fibroblasts were the major cell components involved in the surgically induced acute inflammation. It is well established that PMNs and macrophages are the first infiltrating cells to arrive and to participate in the defense and repair aspects of this process.[43] Phagocytosis of tissue debris is the main function of these cells in healing wounds.[44-46] However, PMNs and macrophages can also cause tissue damage. These cells undergo phagocytosis, releasing lysosomal enzymes, including collagenases and acid hydrolases, and other substances such as prostaglandins that have the capacity to destroy collagen and other connective tissue substances, thereby potentiating the inflammatory response and mediating additional tissue injury.[47-49] Moreover, recent evidence demonstrated destruction of extracellular FN from human gingival fibroblasts by neutral proteases present in PMNs.[50] PMNs contain one or more fibrinolysins which can mediate fibrinolysis through the release of proteases that digest fibrin directly, and also inactivate tissue fibrinogen.[51] Connective tissue fibroblasts are known to produce collagen, ground substance and by mitotic division, more fibroblasts.[52] However, fibroblasts are not only collagen-secreting cells, they are also dynamic entities capable of migration, phagocytosis and degradation of polymerized collagen. Migration of fibroblasts across collagen-coated filters has been observed in response to products from PMNs and activated monocytes,[53] as well as collagen and peptides produced by the enzymatic degradation of collagen.[54] Therefore, the

tissue damage associated with a PMN and macrophage-dominated infiltrate may well be a function of not only a degradation of existing extracellular collagenous matrix, but also of cytotoxic mechanisms affecting fibroblasts.[55] Furthermore, fibroblasts are attracted to FN,[56] and to the portion of the FN molecule which contains the cell-binding region, but not to that containing the collagen-binding region.[57] Only small amounts of FN were necessary to promote fibroblast migration and cell adherence.[58] Fibroblasts were able to phagocytize extracellular collagen fibrils and degrade them within their vacuolar apparatus.[59] These cells synthesize and degrade collagen at similar rates in order to maintain homeostasis.[60]

It was also an objective of this study to re-examine rates of repair in different areas of the periodontium following tooth replantation. Autoradiographic examination of early specimens from control, LAP-untreated tooth replants showed a more rapid cell proliferation within both supracrestal and transseptal connective tissue fibers region than in the periodontal membrane space. Repopulation of the supracrestal region appeared to be mediated by local paravascular cells. Cells repopulating the periodontal membrane space appeared to be derived from sources both within the membrane and the adjacent bone marrow spaces. These findings agreed with results of a recent study by Proye and Polson,[1] dealing with repair in different zones of the periodontium after tooth replantation. They also showed that healing occurred more rapidly in the supracrestal or transseptal region than within the periodontal membrane region. In contrast, in a previous similar study, Nasjleti et al.[29] reported simultaneous rates of repair in these regions. In that previous study,[29] however, replanted teeth were treated endodontically, were exposed to a 1-hour extraoral period and were splinted for a longer time. Any of these factors or some combination of them may account for differences in the results.

Finally, despite the promising results of LAP in tooth replants, studies are needed to determine the long-term effects on root cementum resorption and ankylosis. The optimal concentration of LAP also needs to be clarified.

CONCLUSIONS

Within the limits of this study it may be concluded that:

- With the use of lyophilized autologous plasma (LAP), an enhanced periodontal healing resulted by (1) early replacement of the fibrin clot, (2) increased connective tissue cell proliferation, (3) reduction of the inflammatory response and (4) inhibition of cementum resorption.
- Following tooth replantation, healing and repair occurred more rapidly in the supracrestal or transseptal region than within the periodontal membrane space.

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