Revascularization Following a Combined Gingival Flap-Split Thickness Flap Procedure in Monkeys*

Simao Kon,† Raul G. Caffesse,‡ Walter A. Castelli§ and Carlos E. Nasjleti‖

Accepted for publication 1 August 1983

A COMBINED TECHNIQUE (gingival flap-split thickness flap) was performed in monkeys. The clinical, histological and microvascular aspects of healing were studied, mainly focusing on the gingival flap, since some of the vessels supplying this area were severed during the second phase of the procedure. The animals were perfused with a combined solution of Pelikan carbon black and 10% formalin solution and killed from 1 to 35 days postoperatively. Cleared specimens and regular histology were obtained. It was concluded that: (1) the remaining periosteal vessels and surrounding structures provided enough nutrition for the survival of the gingival flap, (2) the microvascularization was normal at the 14 days postoperative period in both the gingival flap and the apically positioned split areas and (3) the exposed connective tissue remained narrow and exhibited an irregular vascular arrangement throughout the experiment.

The treatment of periodontal pockets with associated mucogingival problems can be solved either by a two-stage procedure or by a combined gingival flap-split thickness flap performed in a single visit.[1] The microvascularization of various types of flaps has been described in several publications.[2-6] The purpose of this investigation was to study the clinical, histological and microvascular aspects of the combined procedure, mainly focusing on the process of healing of the gingival flap, since some of the vessels providing nutrition for this area were severed when the second phase of this procedure was performed.

MATERIALS AND METHODS

Two young adult Rhesus monkeys with an average weight of 12 pounds were used. General anesthesia with pentobarbital sodium (Nembutal®), 30 mg/kg of body weight and local anesthesia (lidocaine HCl 2%) were given to perform the surgeries. The combined procedures were performed at different time intervals on molars and premolars. The surgical technique has been described previously[1] (Fig. 1).

The monkeys were killed under general anesthesia at 1, 3, 9, 14, 21 and 35 days after surgery and exsanguinated via the femoral arteries. A combined solution of Pelikan carbon black and 10% buffered formalin solution was perfused through the exposed and cannulated external carotid artery to evaluate revascularization of the wound areas. After killing, each block, including the operated teeth and surrounding tissues, was divided in two and fixed in 10% formalin. One half was partially decalcified in EDTA and serially sectioned in buccolingual direction at about 1 mm and cleared by the Spalteholtz method.[7] The other half was dehydrated, embedded in paraffin and serially sectioned in the same direction at about 7 μm and stained using H & E and Mallory techniques.

RESULTS

The healing will be presented in three different areas in an occluso-apical direction: a, the full gingival flap; b, the exposed connective tissue and c, the apically positioned split-thickness flap (Fig. 2).

Clinical Aspects

From a clinical standpoint, areas a and c behaved as a closed wound while area b healed as an open wound. Immediately after the surgery, area b, where connective

* This work was supported in part by the Medical Research Division of the Veterans Administration.

† Visiting Associate Professor, Department of Periodontics, The University of Michigan School of Dentistry, Ann Arbor, MI 48109, and Professor Adjunto, Faculdade de Odontologia, Universidade de S. Paulo, S. Paulo, Brasil.

‡ Professor and Chairman, Department of Periodontics, The University of Michigan School of Dentistry.

§ Professor of Anatomy, The University of Michigan Medical School.

‖ Coordinator, Dental Research Program, Veterans Administration Medical Center.

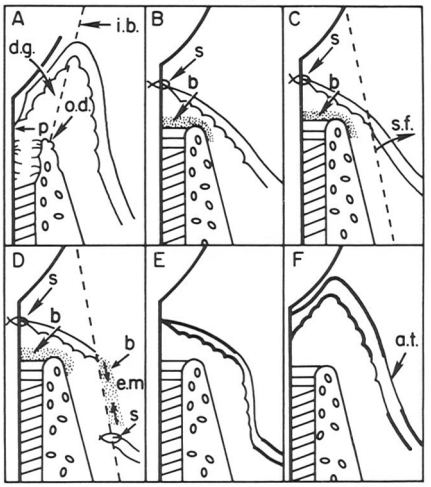

Figure 1. *Diagram of surgical procedure.* **A,** *reverse bevel performed;* **B,** *gingival flap sutured on crestal bone;* **C,** *split flap performed;* **D,** *sutured apically;* **E,** *immediately after surgery and* **F,** *final result. Key of abbreviations:* d.g., *dento-gingival area;* p, *pocket;* o.d., *osseous defect;* s, *suture;* b, *blood clot;* s.f., *split flap;* e.m., *epithelial migration;* a.t., *attached gingiva;* i.b., *reverse bevel.*

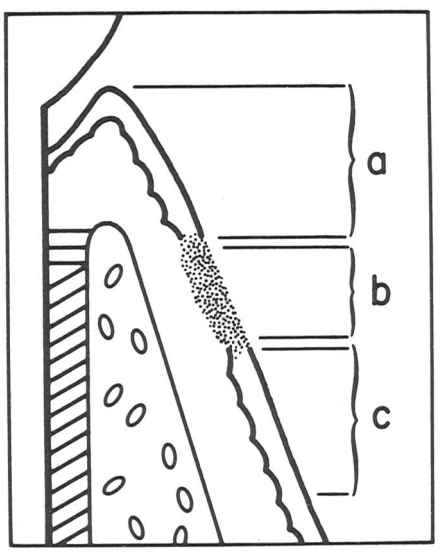

Figure 2. *Results are presented in three different areas:* a, *the full gingival flap;* b, *the exposed connective tissue and* c, *the apically positioned split thickness flap.*

tissue remained exposed, showed some oozing while the other two areas were slightly reddened at the incision sites.

Three days postoperatively the margins and releasing incisions in areas a and c were inflamed. Area b showed an intensive reddish color. This area appeared to be covered by a thin, immature epithelium at 9 days postsurgery. The color was more intense than in the other two areas where the clinical healing was almost complete.

Fourteen days after the procedure, a scar as a result of the connective tissue exposure was interposed between the two completely healed areas. Healing progressed uneventfully at 21, 28 and 35 days, but the scar placed apically to the mucogingival line remained up to the end of the experiment.

Histological-Microvascular Aspect

Full Gingival Flap Area. The 1-day specimen shows severed fiber bundles at the incision site with a flap well adapted to the tooth. The oral epithelium exhibited deep and regularly shaped rete pegs. Remnants of the crevicular epithelium were present. Scattered inflammation was present along the area of the incision, down apically to the bony crest and around the perfused vessels (Fig. 3). No appreciable change in the microvascularization of the oral aspect of the flap was seen. However, extravasated material along with dilated and increased numbers of perfused vessels were present facing the thin blood clot which separated the flap vessels from the capillary network of the periodontal ligament and the marrow spaces (Figs. 4A and 4B). In 3 days increased microvascularization and vasodilation were even more evident at those areas, mainly at the flap margin and at the apical border (which faced area b). Newly formed capillary buds connecting the wounded area were present within the clot and granu-

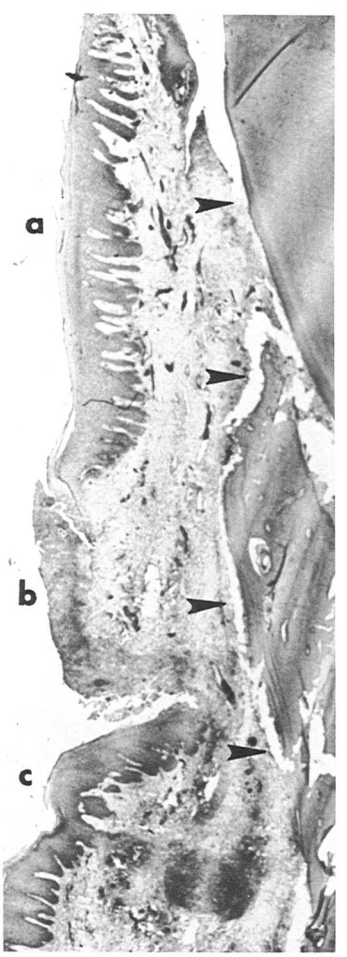

Figure 3. *One day after the procedure. Overall picture depicting the three areas* (a, b *and* c). *The arrows indicate the pathway of flap reflection (H & E, magnification* × *40).*

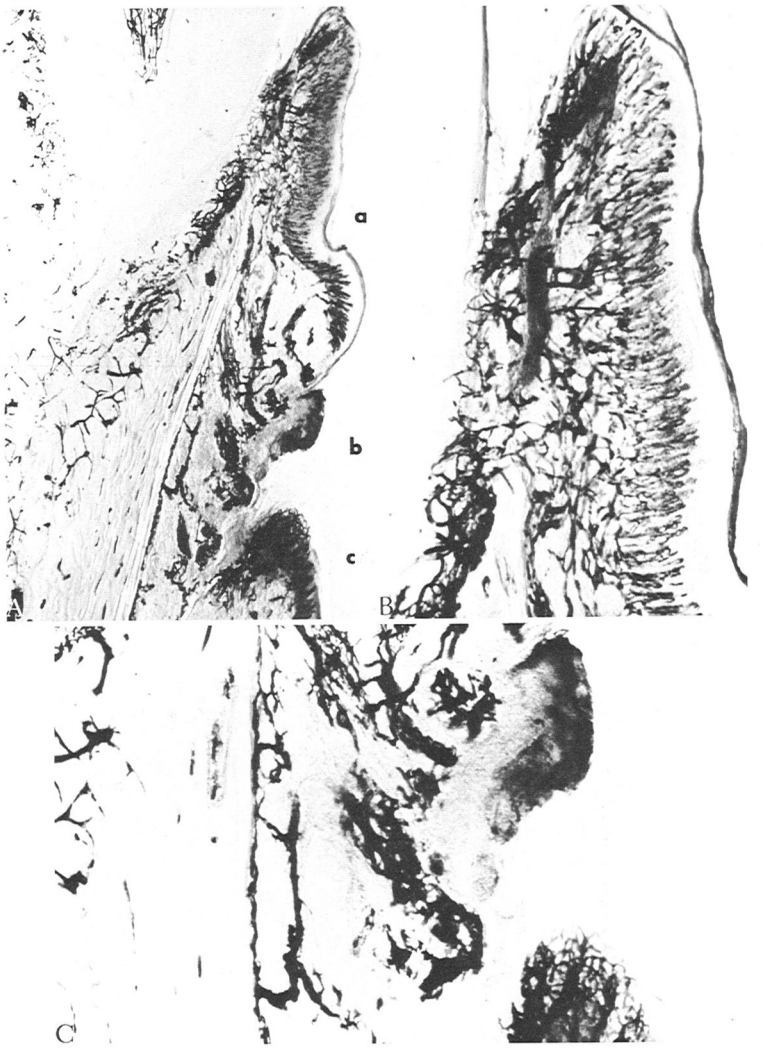

Figure 4. *One day after the procedure.* **A,** *overall picture. No appreciable change is present in the microvascularization of the oral aspect of the flap (area* a) *and the split flap (area* c). *A blood clot covers the exposed connective tissue. Periosteal vessels showed minimal changes.* **B** *and* **C,** *higher magnification of the previous picture showing areas* a *and* b *(Clear section. magnification* × *40 and* × *100).*

lation tissue. There was no change in the capillary loops along the oral epithelium (Figs. 5A and 5B). This epithelium exhibited some cell desquamation.

In 9 days small capillary buds continued to grow between the flap and the underlying area. Vascularization was still heavy at the marginal and apical borders of the flap and very heavy at the periodontal ligament space (Fig. 6). The crevicular epithelium was still immature and minor bone resorption was present at the tip of the alveolar crest. This was also observed in the 14-day specimens. At this time period, the microvascularization of the flap and the underlying tissue was almost normal (Figs. 7A and 7B).

The 21- and 35-day specimens showed bone apposition at the alveolar crest. Increased cellularity persisted. The junctional epithelium was located at the cementoenamel junction (Fig. 8). The microvascularization was similar to that observed in control specimens (Fig. 9).

Healing was delayed in one 14- and one 21-day flap, due to accidents. The sutures were lost and the flaps were dislodged apically. A new gingival margin developed with less mature oral and sulcular epithelia and a highly inflamed connective tissue. Increased vascularization filled the gap between the flap and tooth. The original sulcular epithelium, displaced from the tooth surface, showed keratinization, and the alveolar crest exhibited active bone resorption which was also present at 21 days (Fig. 10).

Exposed Connective Tissue Area. The 1-day specimen showed an acute phase, with the formation of a blood clot, edema, vasodilation, engorged vessels and neutrophilic infiltrate. This involved almost all the exposed supra-periosteal connective tissue but since bone remained protected by periosteum, the remaining periosteal vessels were unaltered. The blood clot filled the gap of exposed connective tissue, limited coronally by the attached gingiva and apically by the oral mucosa. This clot assumes the shape of the previous gingival wall. Perfused material released from cut vessels was

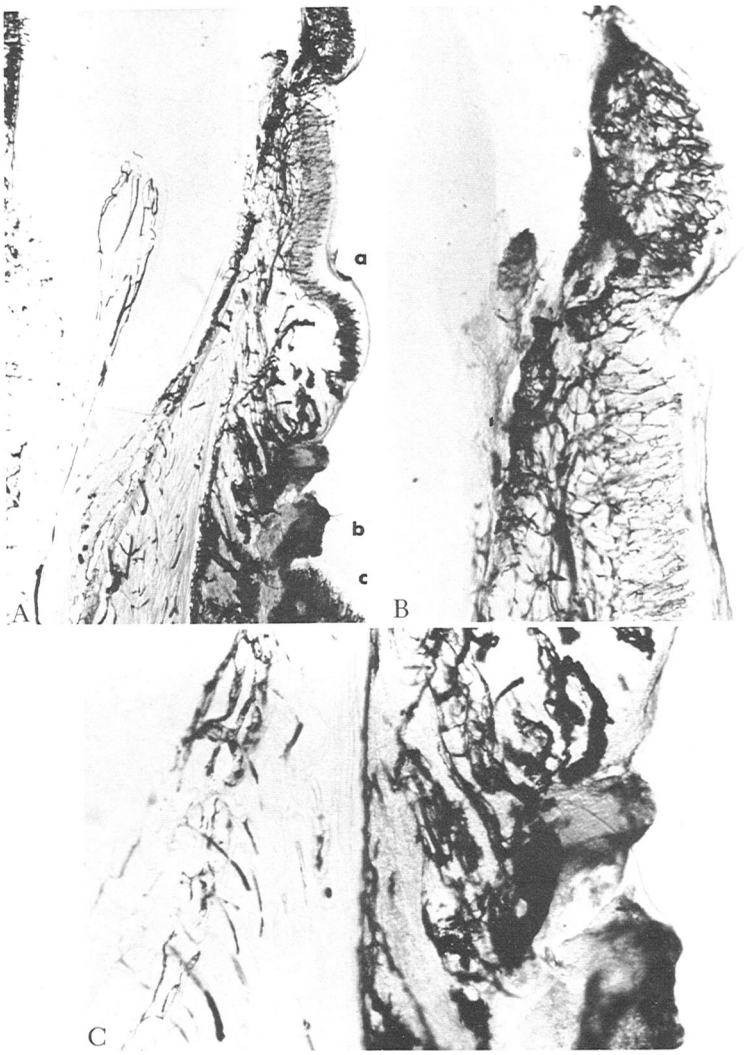

Figure 5. *Three days after the procedure.* **A,** *Overall picture. Well defined capillary loops on the oral aspect of the flap (area* a) *and split flap (area* c). *Increased microvascularization at the margins. The clot is reduced in size, and perfused material is present within it.* **B** *and* **C,** *are higher magnifications of the previous picture, showing areas* (a) *and* (b) *(Clear section, magnification × 40 and × 100).*

seen within the clot and on its surface (Figs. 3 and 4C). At 3 days the blood clot still filled the gap but was reduced in size. Dilated and perfused vessels were increased in number beneath the clot (Fig. 5C).

At 9 days, an immature epithelium with wide and irregular rete pegs covered the area. The microvascular aspect was different from areas a and c (Fig. 6). A vascular meshwork with no differentiation of capillary loops could be detected. The connective tissue was normal but narrower. This was even more evident in the 14-day specimens. Although the perfused periosteal vessels did not show the same diameter as the two other areas, they followed the same pathway. At the lamina propria, a capillary plexus growing from the two adjacent areas was present exhibiting a capillary loop-like configuration (Fig. 7C).

Even though more mature, the 21- and 35-day specimens exhibited the same irregularities. Capillary loops, periosteal vessels and the width of the connective tissue at this site differed from the control specimen (Fig. 9).

When healing was delayed due to the accidental loss of sutures, the periosteal vessels were even smaller in diameter and the connective tissue narrower.

The Apically Positioned Split Thickness Flap Area. The acute phase observed in the 1-day specimens showed exactly where the split incision was performed. Although increased cellularity was evident around the perfused vessels, the oral aspect of the split flap (epithelium and connective tissue) exhibited very minimal changes. The periosteal vessels were normal up to the most coronal part of this area where the microvascularization was not only increased but also disorganized (Figs. 4A and 4C). This was even more dramatic at 3 days. However, capillary loops remained normal along the oral mucosa area. By 14 days the area was less vascular (Fig. 7C). The periosteal vessels were already connected to those from area b. This condition had matured at 21 and 35 days after surgery (Fig. 9).

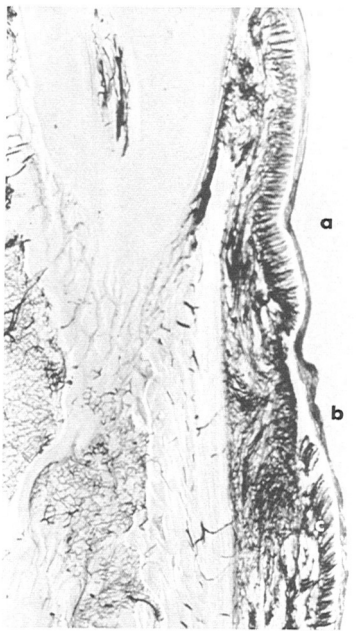

Figure 6. *Nine days after the procedure. Capillary buds continue to grow between the flap and the underlying tissues. Vascularization is very heavy at the periodontal ligament space. An immature epithelium, with wide and irregular rete pegs, covers area* b. *Capillary loops are normal at areas* a *and* b *(Clear section, magnification* × *40).*

DISCUSSION

The advantage of this combined technique performed in one visit has been discussed in a previous paper.[1] The histological and microvasculatory aspect of the surgery, however, remained to be studied, and particularly that of the gingival flap (area a), since the vessels at its apical border (facing area b) were severed by the procedure. The microvascular and histological aspect of the gingival mucoperiosteal flap, the exposed connective tissue and the apically positioned split thickness flap techniques were adequately studied by several authors.[2-4,6,8-10] The combined procedure allowed for study of the healing process of these three situations at the same time.

From a clinical point of view, the closed wounds exhibited signs of inflammation at the margins but were healed 9 days postoperatively. The open wound was also epithelialized and a scar, localized apical to the mucogingival line, was present at 14 days and remained throughout the experiment.

No appreciable change in the microvascularization of the oral aspect of the gingival flap was seen. Vessels comprising this area maintained their patency after the

Figure 7. *Fourteen days after the procedure.* **A,** *microvascularization of the flap (area* a) *and the split flap (area* c) *are normal. Periosteal vessels of reduced diameter are seen in area* b. **B** *and* **C,** *are higher magnifications of the previous picture, showing areas* a, b *and* c *(Clear sections, magnification* × *40 and* × *200).*

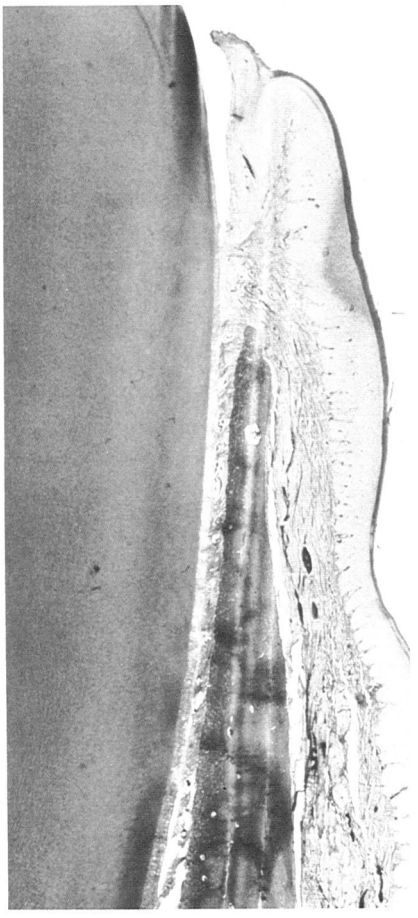

Figure 8. *Twenty-one days after the procedure. Healing is completed (H & E, magnification × 40).*

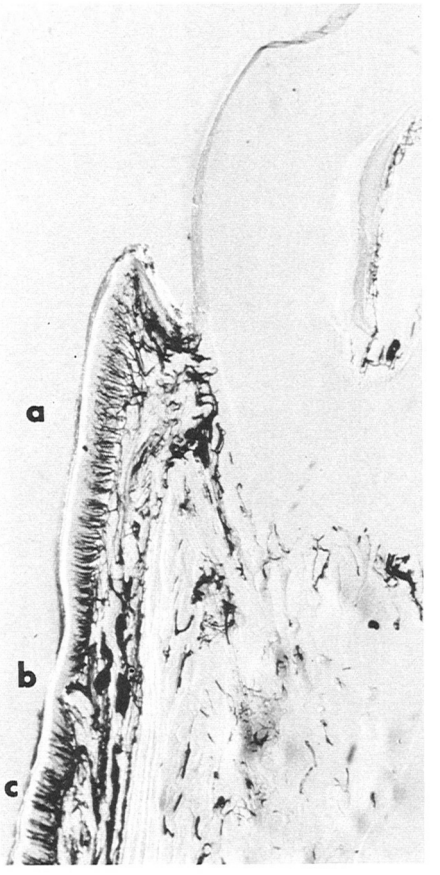

Figure 9. *Twenty-one days after the procedure. Healing is completed. The microvascular aspect present in areas* a *and* c *is similar to that observed in controls. Area* b *depicts narrower connective tissue, periosteal vessels with less diameter and the absence of capillary loops (Clear section, magnification × 40).*

surgery, thus contributing to the healing process. These vessels plus those from the remaining periosteal, periodontal and bone microvascular network are all important sources in providing nutrition for the gingival, mucoperiosteal flap to survive.

The capillary loops within the full and split thickness flap, although dilated at the 3- and 9-day postoperative periods, maintained their normal pattern of distribution throughout the experiment. These areas showed a normal histological and microvascular appearance at 14 days.

The revascularization of the mucoperiosteal flap is very similar to that described for the modified Widman flap.[6] In this experiment, the periodontal ligament was deliberately exposed. Continuity between the periodontal and the flap vascularization was seen at 9 days. This finding supports the report by Nielson et al.[11] where perfused blood vessels exited from the periodontal ligament 1 week after the procedure, providing a primary source for the healing process.

The blood clot covering the exposed connective tissue (area b) was reduced in size in 3 days. It assumed a shape similar to that of the previous tissue contour and was permeable in the perfused material, in agreement with other studies.[3,4] The 9-day specimens showed immature epithelium covering the area with irregular capillary loops. This irregularity, the smaller diameter of the periosteal vessels and the narrow band of supraperiosteal connective tissue at this site remained throughout the experiment as a clinical scar. The exposed connective tissue exhibited, nonetheless, a faster epithelial coverage than that reported by Staffileno et al.[9] and by Novaes et al.[3] This may be due to the smaller size of the connective tissue exposure and to the fact that it was surrounded by mature epithelium from areas (a) and (b).

Healing was delayed 7 to 24 days when sutures were accidentally lost. The gap between the flap and the tooth was filled by a well vascularized granulation tissue mainly derived from the periodontal ligament, as shown by Karring et al.[12] The two areas where this accident happened showed bulky gingival margins with some recession. As previously reported[13] the displaced sulcular epithelium was keratinized.

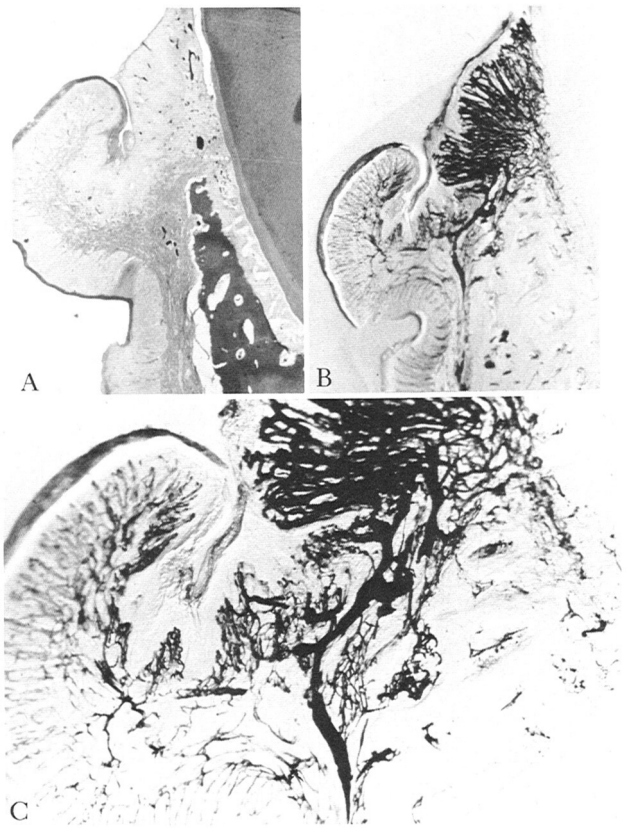

Figure 10. *Fourteen days after the procedure. Healing was delayed due to the accidental loss of sutures.* **A,** *the displaced sulcular epithelium depicts keratinization (H & E, magnification × 40).* **B,** *granulation tissue with increased vascularization fills the gap between the flap and the tooth. The displaced flap remains well vascularized.* **C,** *Higher magnification of* **B** *(Clear section, magnification × 40 and × 100).*

REFERENCES

1. Kon, S., Garcia, V. G., Pustiglioni, F. E., and Ruben, M. P.: Gingival flap-split thickness flap, a combined procedure. Clinical study in humans and dogs. *J Periodontol* **50:** 427, 1979.

2. Kon, S., Novaes, A. B., Ruben, M. P., and Goldman, H. M.: Visualization of the microvascularization of healing periodontal wound. IV. Mucogingival surgery: full thickness flap. *J Periodontol* **40:** 441, 1969.

3. Novaes, A. B., Kon, S., Ruben, M. P., and Goldman, H. M.: Visualization of the microvascularization of healing wound. V. Periosteum retention. Technique of mucogingival surgery. *J Periodontol* **41:** 685, 1970.

4. Novaes, A. B., Kon, S., Ruben, M. P., and Novaes, A. B., Jr.: Rebuilding of microvascularization following surgical gingival elimination by split flap. Study by perfusion and diaphanization. *J Periodontol* **47:** 217, 1976.

5. Ruben, M. P., Smukler, H., Schulman, S. M., et al.: Healing of periodontal surgical wounds in Goldman and Cohen. *Periodontal Therapy,* ed 6, pp. 640–754. St. Louis, C. V. Mosby Co, 1980.

6. Caffesse, R. G., Castelli, W. A., and Nasjleti, C. E.: Vascular response to modified Widman flap surgery in monkeys. *J Periodontol* **52:** 1, 1981.

7. Spalteholtz, W.: *Die Arterien der Herzwand,* p. 13. Leipzig, S. Hirzel, 1924.

8. Staffileno, H. F., Wentz, F., and Orban, B.: Histologic study of healing of split thickness flap surgery in dogs. *J Periodontol* **33:** 56, 1962.

9. Staffileno, H., Levi, S., and Gargiulo, A.: Histologic study of a cellar neofilization and repair following a periosteal retention operation via split thickness mucogingival flap surgery. *J Periodontol* **37:** 117, 1966.

10. Kon, S., Pustiglioni, F. E., Novaes, A. B., et al.: Split thickness flap. Apically displaced, with protected linear periosteal fenestration: a clinical and histologic study in dogs. *J Periodontol* **49:** 174, 1978.

11. Nielsen, I. M., Ellegaard, B., and Karring, T.: Kielbone in healing interradicular lesions in monkeys. *J Periodont Res* **15:** 328, 1980.

12. Karring, T., Cumming, B. R., Oliver, R. C., and Löe, H.: The origin of granulation tissue and its impact on postoperative results of mucogingival surgery. *J Periodontol* **46:** 577, 1975.

13. Caffesse, R. G., Nasjleti, C. E., and Castelli, W. A.: The role of sulcular environment in controlling epithelial keratinization. *J Periodontol* **50:** 1, 1979.

Send reprint requests to: Dr. Raul G. Caffesse, Department of Periodontics, The University of Michigan, School of Dentistry, Ann Arbor, MI 48109.

Announcement

BAYLOR COLLEGE OF DENTISTRY DEPARTMENT OF PERIODONTICS

Baylor College of Dentistry Department of Periodontics announces the 13th annual Arthur H. Merritt Memorial Lecture in Advanced Periodontics

SPEAKER: Robert J. Genco, DDS, PhD, Director, Periodontal Disease Clinical Research Center
State University of New York at Buffalo

DATE: 11 August 1984

PLACE: Baylor University Medical Center
Dallas, TX

For further information contact: Dr. James E. Makins, Baylor College of Dentistry, 3302 Gaston Ave., Dallas, TX 75246.