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REVASCULARIZATION OF AN EXCISIONAL WOUND IN GINGIVA AND ORAL MUCOSA. A SCANNING ELECTRON MICROSCOPIC STUDY USING CORROSION CASTS IN RATS

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Abstract

The purpose of this study was to examine microvascular regeneration associated with gingival wound healing. A full-thickness piece of gingiva and oral mucosa was excised along the palatal aspect of the right maxillary first and second molars in 20 young Wistar rats. The contralateral side served as unoperated control. After 2, 4, 7, 10 or 20 days of healing, microvascular corrosion casts were produced and examined by scanning electron microscopy. At 2 days, vessels surrounding the wound were dilated and impressions representing sites of leukocyte margination were prominent in the walls of venules. Capillary buds were emerging from venules and capillaries. At 4 days, the vessel buds had lengthened and connected in pairs to produce capillary loops. At 7 days, new vessels extended deeply into the wound space, mainly from the medial side, in a palisade-like pattern. At 10 days, the denuded bone surface was still not completely revascularized and Volkman's canals opening to the wound area were empty. At 20 days, the bone surface was covered by large, irregular vessels which originated mainly from the palatal mucosa. The periodontal ligament was less important in the tissue repair process, while the bony vasculature contributed little or not at all to revascularization of the healing gingiva and palatal mucosa.

Key Words: Angiogenesis, blood vessels, corrosion casting, gingiva, leukocyte margination, palate, revascularization, scanning electron microscopy, wound healing.

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Introduction

Angiogenesis is critical in the healing of incisional and excisional surgical wounds, as well as in the healing of free soft tissue grafts. Timing and pattern of angiogenesis as modified by a variety of growth factors, tumors, and tissue injury have been extensively studied, particularly in the rabbit cornea (Gimbrone *et al.*, 1974; Ausprunk and Folkman, 1977; Burger *et al.*, 1984). Although the cornea appears to be the most widely used *in vivo* model in histological studies of capillary formation, the results may not be generally applicable due to the normally avascular nature of this tissue. The gingiva and oral mucosa, on the other hand, are profusely vascularized, and may, therefore, provide a more suitable model for baseline studies of graft revascularization.

The extensive use of free gingival autografts in periodontal therapy has emphasized the need for improved knowledge of vascular reactions along the wound edge and of neovascularization at both the donor site and the recipient bed. A favorable recipient bed has been described as one that is capable of rapidly forming new capillaries and granulation tissue (Sullivan and Atkins, 1968; Sonntag and Paulini, 1977). Clinical (Dordick *et al.*, 1976) and histological findings (Bissada and Sears, 1978; James *et al.*, 1978) support the view that, when free gingival grafts are placed on denuded alveolar bone, a successful "take" is dependent on revascularization of capillaries passing through bone cortex. Other studies have shown that marked cell proliferation occurs from the cut margins of the recipient bed (Caffesse *et al.*, 1979) and that, in an excisional gingival wound, new vessels originate mainly from vessels in the periodontal ligament (PDL) (Nobuto *et al.*, 1987).

Recent developments in corrosion casting techniques (Murakami, 1971; Burger *et al.*, 1984; Selliseth and Selvig, 1993) have made it possible to study details of three-dimensional vascular architecture and to characterize the vessels in microvascular beds. Taking advantage of these techniques, the purpose of the present investigation was to determine the source most likely

responsible for revascularization of an excisional wound in the muco-gingival region by examining the timing and pattern by which vessels grow into a wound space and their maturation during the first twenty days of healing.

Material and Methods

Animals

The experimental protocol was approved by the institutional committee for animal experimentation.

Twenty male Wistar rats of 200 g body weight (BW) were given water and a hard diet *ad libitum* before and after surgery.

Surgical procedure

At the time of surgery, each animal was anesthetized (Hypnorm/Dormicum: Fluanisone 10 mg/ml + Fentanyl 0.2 mg/ml (Janssen Pharmaceutica, Beerse, Belgium) / Midazolam 1 mg/ml (Roche AG, Basel, Switzerland) 0.35 ml/100 g BW). A full-thickness mucoperiosteal flap, 2 mm x 4 mm in size, containing gingiva and palatal mucosa was removed from the medial side of the first and second right maxillary molars.

Perfusion procedure

At 2, 4, 7, 10 or 20 days after surgery, respectively, four animals were administered heparin (200 IU/kg BW) 5 minutes prior to general anesthesia. Both injections were given subcutaneously. The external carotid arteries were exposed bilaterally, and a catheter with 0.76 mm inner diameter inserted (Intramedic PE-60, Becton Dickinson & Co, Parsippany, NJ, USA). The jugular veins were opened to allow venous drainage. Eighty milliliters millipore-filtered (0.22 μ m filter, Millipore Corp., Cork, Ireland), 0.03% heparinized saline at 40°C was perfused through the catheters followed by 40 ml aqueous solution of phosphate-buffered 4% formaldehyde and 1% glutaraldehyde (McDowell and Trump, 1976) at the same temperature. The purpose of the fixation was to make the vessel walls tougher in order better to withstand later handling.

Preparation of corrosion cast specimens

Liquid acrylic resin with 2% hardener (Mercor cl RR, Vilene Hospital Inc., Tokyo, Japan) was perfused through each of the carotid arteries, applied by hand-controlled pressure through the inserted catheter.

Following polymerization of the resin at room temperature overnight, the maxilla was dissected out. Soft tissues were dissolved by maceration in 20% NaOCl. In some of the specimens, the teeth adjacent to the wound were removed. The specimens were rinsed in distilled water, briefly rinsed in graded ethanol baths and dried in air.

Scanning electron microscopy

Specimens were mounted on aluminium stubs using conductive silver paint and coated with gold for examination in a scanning electron microscope (SEM 515 Philips, Eindhoven, The Netherlands) operated at an acceleration voltage of 10 or 15 kV. Differences in endothelial cell nuclear impressions were used to differentiate arterioles from venules. These impressions have been reported to be elongated, spindle-shaped in arteries and ovoid in veins (Miodonski, 1976; Burger *et al.*, 1983; Kishi *et al.*, 1989).

Morphometric analysis

Measurements of terminal vessel diameters were performed on scanning electron micrographs with appropriate correction for magnification factors and image distortion. Vessel diameters in the experimental region medial and lateral to the wound were compared to those of unoperated gingiva and palate. In each animal, 20 terminal vessels were measured at each of the four sites; 1600 vessels were measured in all.

Data analysis

Means and standard deviations were tabulated from four animals at each site and each observation period. A two-way analysis of variance with repeated measurements was utilized to test differences between operation times (Minitab data analysis software, release 7.2). If a significant F-value was found, Tukey's multiple comparison test (Tukey, 1953) was applied to test differences between pairs of operation times. A one-sample t-test was utilized to test differences between sites. In statistical calculations, the animal was considered the experimental unit.

Results

Unoperated gingiva and palatal mucosa

In gingiva and palatal mucosa, a highly organized vascular pattern containing arteries, arterioles, capillaries, venules and veins was seen. In the gingiva of unoperated control teeth, a plexus of blood vessels was present facing the oral epithelium (Fig. 1). When viewed from the tooth aspect, the free gingiva contained a flat plexus which formed elongated loops along the gingival margin. Near the bottom of the gingival sulcus, the vessels formed glomerulus-like structures. In the region of the dento-gingival junction, close to the tooth, a circumferential capillary structure surrounded the tooth (Fig. 2). In unoperated palatal mucosa, a deep plexus of arterioles and venules coursed sagittally, covered by a superficial, subepithelial capillary system. Clusters of capillary loops formed columns which reflected the presence of palatal rugae (Fig. 1).

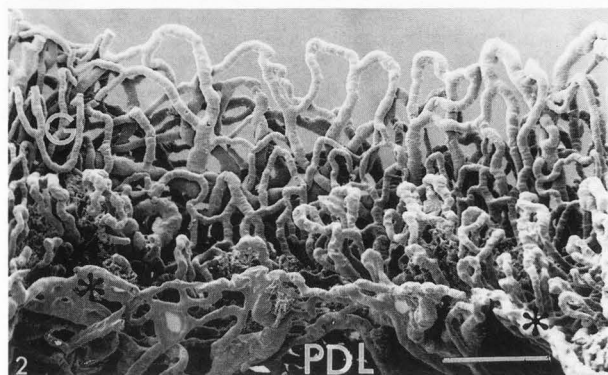
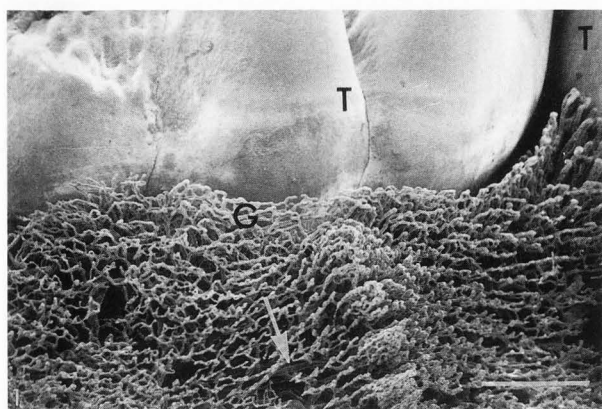


Figure 1. Scanning electron micrograph illustrating unoperated gingival and palatal microvasculature. Blood vessels of gingiva (G) form a dense capillary plexus with loops pointing towards the gingival surface. In the palatal mucosa, large vessels in a deep layer run sagittally (arrow), covered by superficial capillaries. Clusters of capillary loops represent palatal rugae. Maxillary molars (T). White magnification bar = 500 μm .

Figure 2. Blood vessels of gingiva (G) and cervical part of the PDL (PDL) as seen after removal of the tooth. Facing the tooth, the gingival plexus consists of a flat plexus which most cervically forms capillary loops. In the region of the bottom of the gingival sulcus, the vessels form glomerulus-like structures. A conspicuous vascular structure (*) surrounds the tooth. More widely spaced vessels connect the PDL with the gingival vasculature. Bar = 100 μm .

The superficial palatal capillary plexus consisted of trunk capillaries coursing sagittally, to which series of capillary loops presumably corresponding to connective tissue papillae and rete pegs were connected (Fig. 3). Individual vessels generally had a smooth luminal surface and exhibited relatively small variations in diameter.

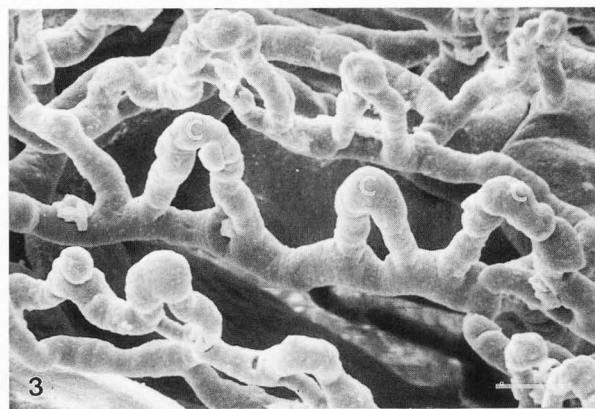


Figure 3. Palatal capillary network characteristically consisting of serially arranged capillary loops (C), connected to trunk vessels that course sagittally. Bar = 25 μm .

Two days

Two days postoperatively, wound shrinkage seemed negligible. In the corrosion cast specimens, the wound space was lined by PDL vessels laterally and by palatal vessels at the anterior, posterior and medial wound margins (Fig. 4). Capillary loops in the superficial layer medial to the wound differed from those in normal palatal mucosa by being dilated and exhibiting bulbous tips which tilted towards the wound. Closer to the bone surface, large, dilated, cut vessels from the profound palatal layer exhibited finger-like protrusions, often with a bulbous end, towards the margin of the wound. Scratches made by the scalpel were seen in the denuded bone surface indicating the location of the wound edge at the time of surgery (Fig. 5). At higher magnification, the large, profound vessels were identified as venules. These vessels had numerous impressions indicative of leukocyte margination at this stage and showed evidence of early sprouting (Fig. 6). Capillary sprouts fused to form new capillary loops (Fig. 7). Compared to control areas, the vasculature near the wound margin at 2 days as well as at later time points exhibited greater variations in size among vessels and frequent constrictions and out-pouchings along individual vessels.

At the cervical orifice of the PDL, vessels of large diameter extended just above the alveolar ridge. Some of these formed loops, others were characterized by finger-like protrusions with a bulbous or irregular end surface (Fig. 8). Like venules at the medial side, the venules at the lateral side of the wound had numerous impressions in their luminal surface indicative of leukocyte margination.

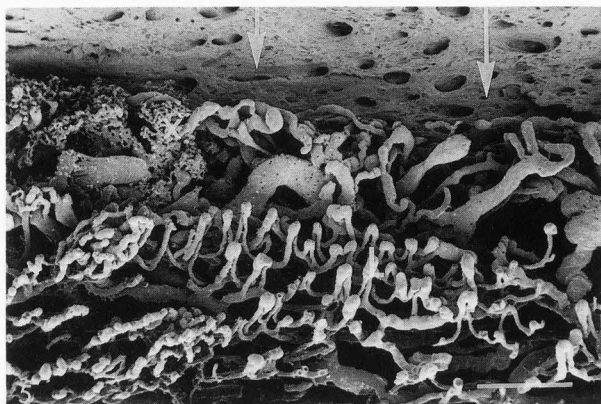
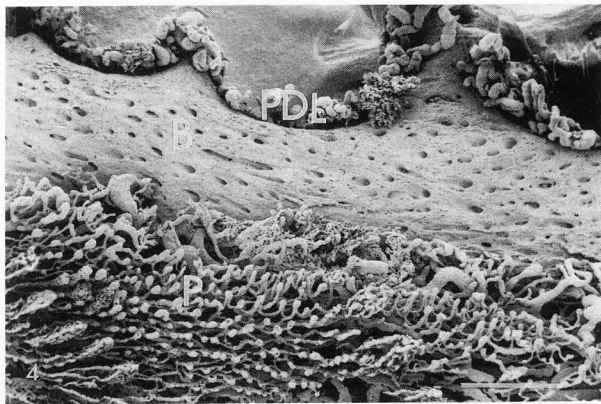


Figure 4. Low-magnification scanning electron micrograph illustrating vessels surrounding the wound 2 days postoperatively. From top to bottom: blood vessels at the margin of the PDL (PDL), bone surface (B) with empty Volkman's canals, and palatal vasculature (P). Bar = 500 μm .

Figure 5. Superficial, subepithelial capillary loops (L) medial to the wound space are enlarged, have bulbous tips, and are tilted towards the wound. Closer to the bone surface, cut vessels form finger-like protrusions. At right, extravasation of resin (X). A groove (arrows) in the bone surface carved by the scalpel marks the margin of the wound at the time of surgery. Two-day specimen. Bar = 200 μm .

Four days

Four days postoperatively, the wound appeared smaller than at 2 days. In the corrosion casts, the vasculature appeared more complex than at 2 days. Vascular proliferation was widely in evidence. In the superficial plexus at the medial wound margin, several loops originating from the same arterio-venous stalk were occasionally seen (Fig. 9). Vessel sprouts from

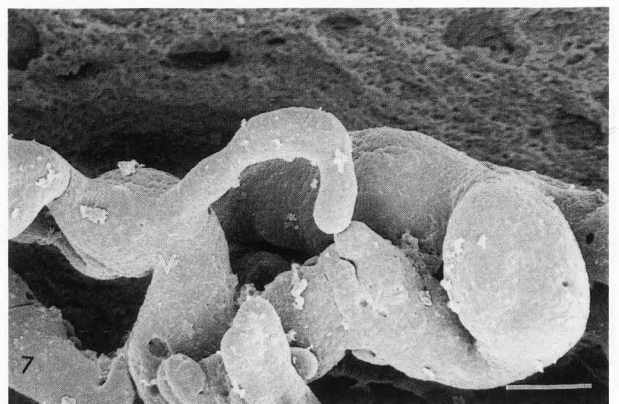


Figure 6. Cut venule (V) in profound palatal layer at the wound margin with impressions indicative of cellular margination and capillary sprouts (arrowheads). Two-day specimen. Bar = 100 μm .

Figure 7. Capillary sprouts about to form a complete capillary loop at the medial margin of the wound. Their originating vessels are venules (V). Two-day specimen. Bar = 25 μm .

venules in the profound palatal layer were a common observation. They were directed towards the wound space. Leukocyte impressions in the cast surface decreased numerically, while nuclear impressions appeared more distinct at 4 days than at 2 days. Palisade-like, dilated vessels arising from the PDL formed large, bulbous loops along the lateral margin of the wound. At this side, sprouts were rare, while leukocyte impressions were a more common finding than at the medial wound margin (Fig. 10). At some sites, resin had extruded from the vessels at the wound edge. These artifacts consisted of smooth-surfaced, multi-lobulated mounds. They were readily distinguished from the casts of blood vessels. This observation was especially common at 4 days.

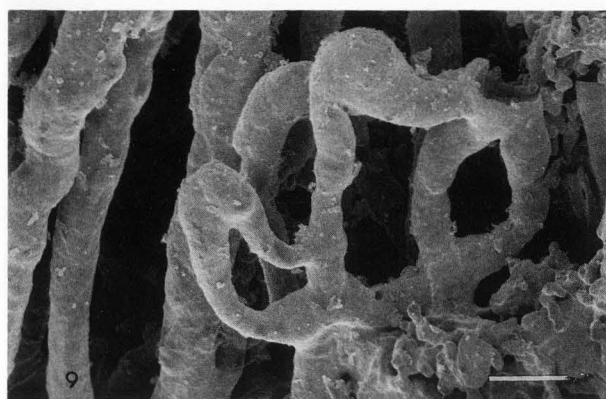
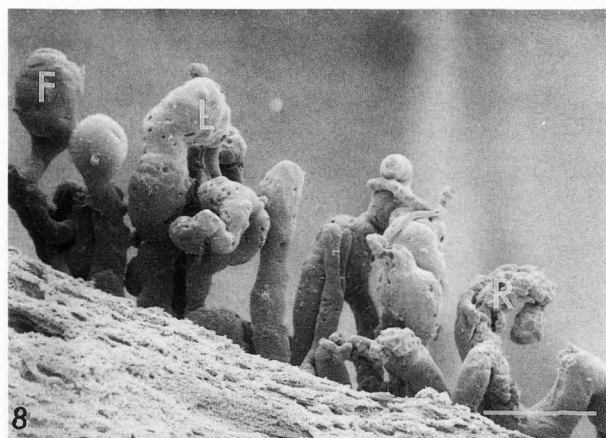


Figure 8. Cut vessels of the PDL just visible above the alveolar crest. These vessels are large in diameter and form loops (L), bulbous finger-like protrusions (F) or rough-surfaced finger-like protrusions (R). Impressions indicate leukocyte margination. Two-day specimen. Bar = 100 μm .

Figure 9. Detail from the superficial capillary plexus at the medial side of the wound 4 days postoperatively. Several loops originate from one arterio-venous stalk. Cellular depressions are less numerous than at 2 days. Bar = 25 μm .

Seven days postoperatively

New vessels from the medial side appeared elongated and mature, and extended into the denuded area parallel to the bone surface in a palisade-like pattern. Volkman's canals were empty (Fig. 11). New vessels at the wound edge at this side had several peculiar shapes including short and bulbous, long and slender, or loop-shaped configurations (Fig. 12). Laterally, loops extending from the PDL had numerous cellular impressions. At some sites, vascular sprouts were common, while other sites appeared inactive (Fig. 13).

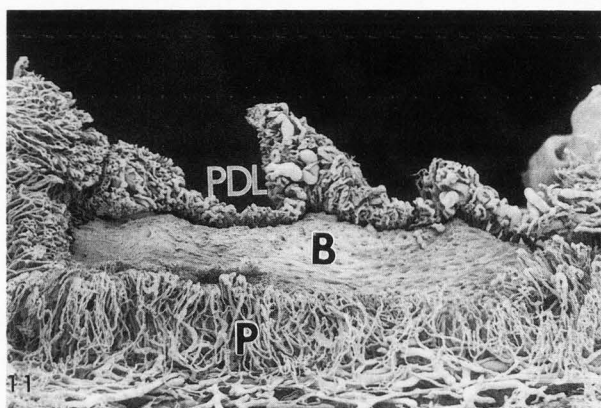
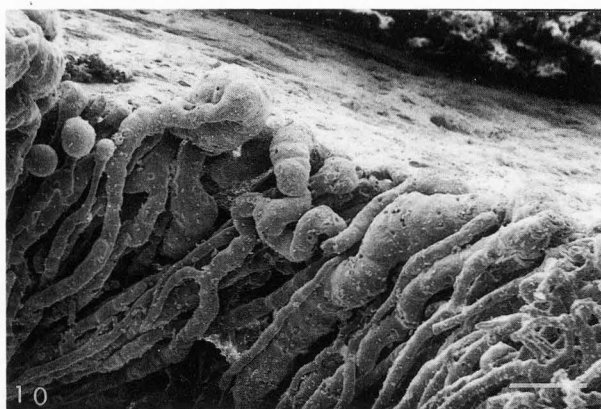


Figure 10. At the lateral side of the wound, a palisade-like system of PDL vessels form large, bulbous loops extending just above the alveolar ridge. Compared to the medial wound margin, depressions are more numerous and capillary sprouts less prevalent. Four-day specimen. Bar = 100 μm .

Figure 11. Revascularization of the excisional wound 7 days postoperatively. Extending from the medial wound margin (P), new vessels appear elongated and matured. They course parallel to the bone surface (B) in a palisade-like pattern. Vessels originating from the PDL (PDL) show limited sprouting. Volkman's canals appear empty. Bar = 500 μm .

Ten days

The bone surface was almost, but not completely, covered by new tissue by ten days postoperatively. The main contributor to revascularization was clearly the vasculature medial to the wound space (Fig. 14). At this side, the larger, new vessels formed a transverse, palisade-like pattern parallel to the bone surface, in part covered by capillaries. At the edge of the wound, new capillary buds were rare, and vascular ingrowth consisted of elongation and proliferation of existing loops

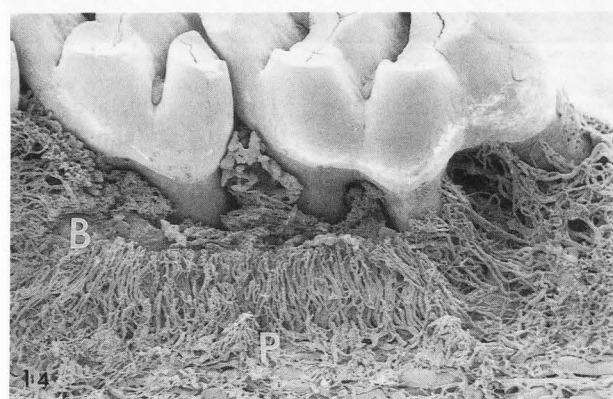
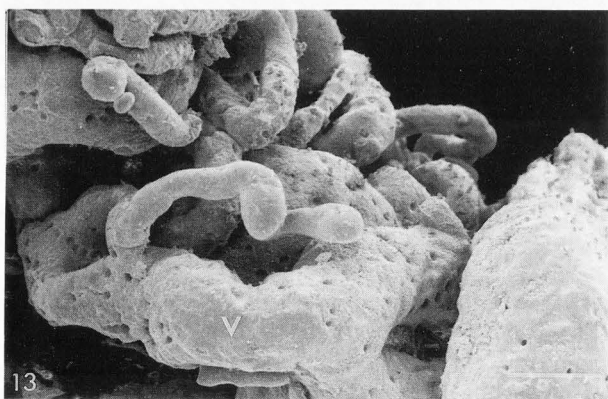
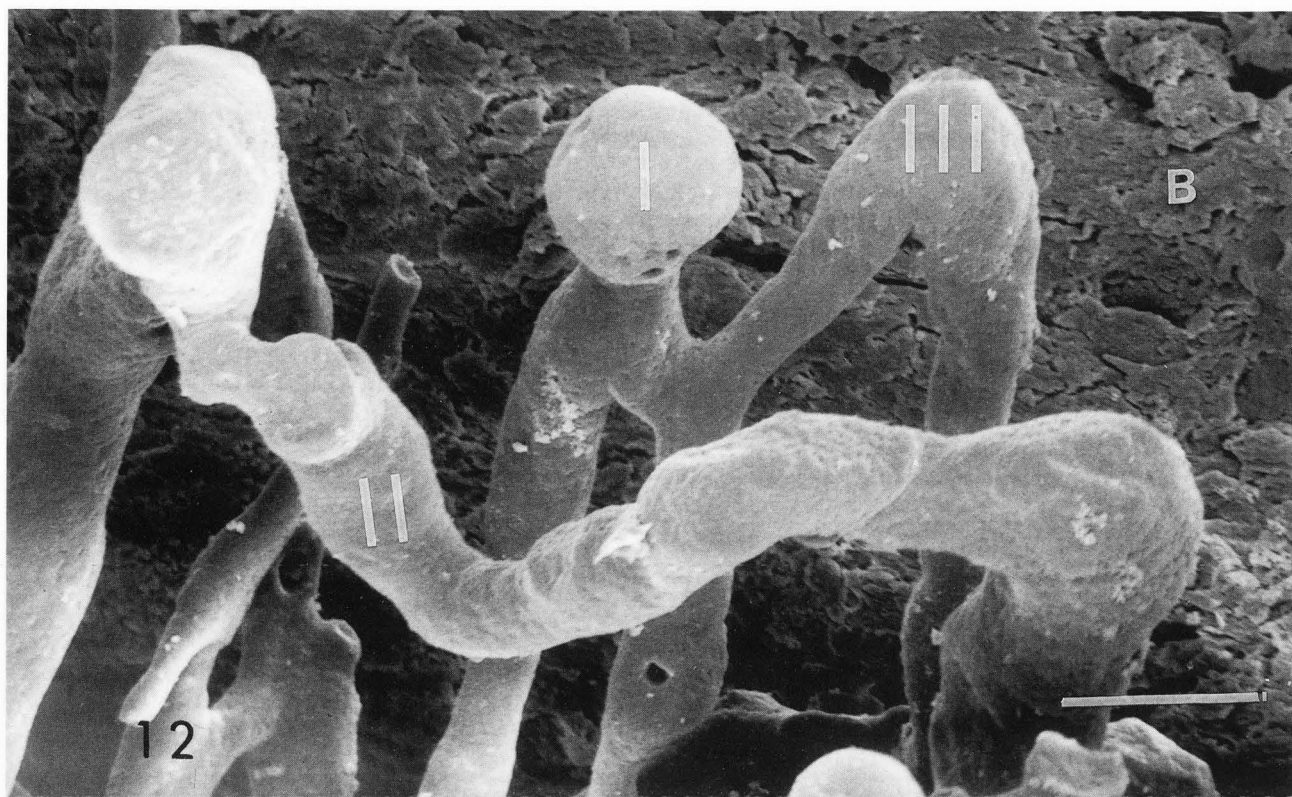


Figure 12. Medial wound margin illustrating three stages of capillary sprouting: short and bulbous (I), long and slender (II) and loop-shaped (III). Bone surface (B). Seven-day specimen. Bar = 25 μ m.

Figure 13. Lateral wound margin illustrating several capillary sprouts originating from PDL venules (V). The venules exhibit numerous cellular impressions. Seven-day specimen. Bar = 50 μ m

Figure 14. Low magnification scanning electron micrograph illustrating vessels surrounding the wound at 10 days. The new vessels are mainly originating from the palate (P), and the denuded bone surface (B) is not yet revascularized. At top, molars are seen. Bar = 500 μ m.

(Fig. 15). The superficial capillaries at the medial side often formed complex, glomerulus-like patterns (Fig. 16). This produced a dense capillary plexus, making this region extensively vascularized. At the lateral side of the wound, adjacent to the tooth, the wound margin re-

mained at the alveolar ridge. However, the PDL venules were sprouting extensively at some sites, while other sites appeared passive. At higher magnification, cellular depressions and endothelial cell hypertrophy were observed, especially at sites where vessel sprouts

SEM of wound healing in the rat palate

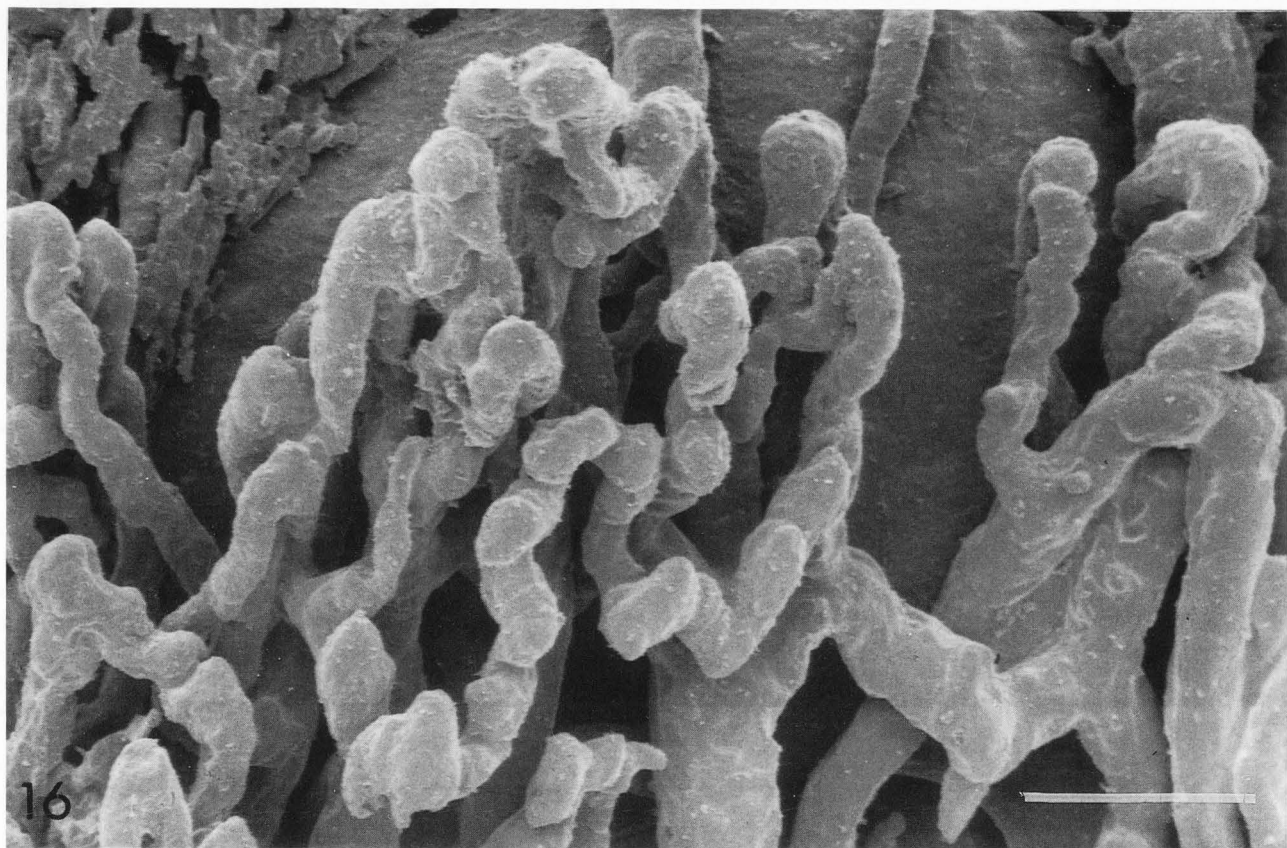
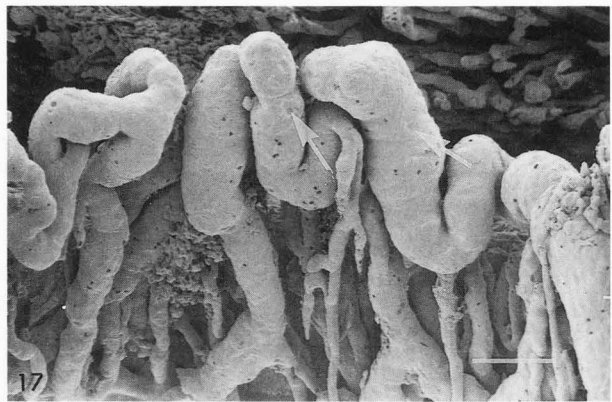
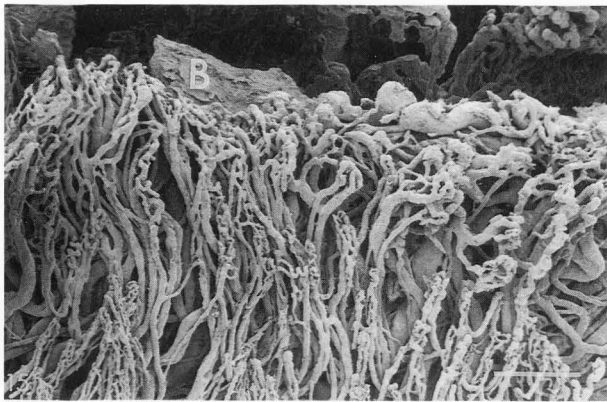


Figure 15. Palisade-like transverse vessels parallel to bone surface (B), covered in part by capillaries. Numerous capillary loops illustrate extensive vascularization at the wound margin. Ten-day specimen. Bar = 250 μm .

Figure 16. Detail from Figure 15 at higher magnification. Capillaries covering large vessels in the healing tissue forming glomerulus-like structures. Ten-day specimen. Bar = 50 μm .

Figure 17. Incomplete vascular regeneration at the margin of the PDL illustrating that PDL vessels contribute little or not at all to revascularization of the wound. Leukocyte depressions are commonly present, and endothelial nuclear impressions (arrows) are enlarged. This site shows hardly any capillary sprouts. Palatal vessels in the background. Ten-day specimen. Bar = 100 μm .

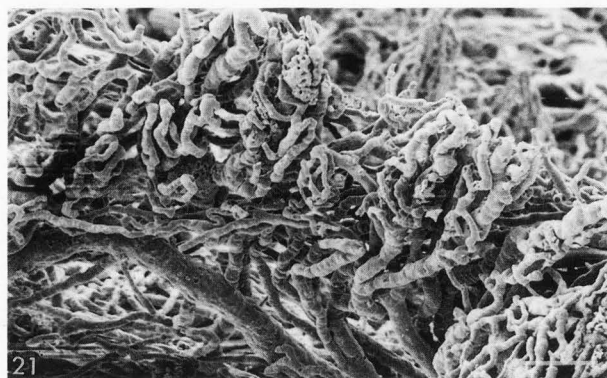
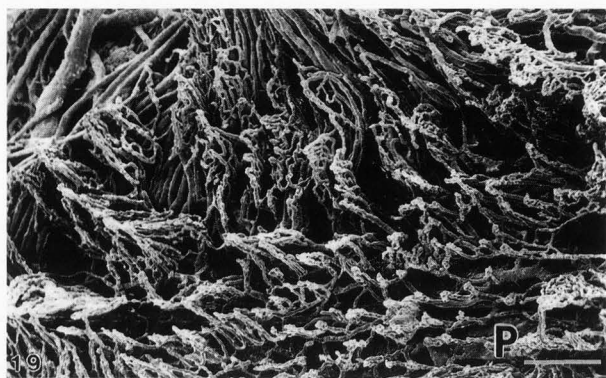
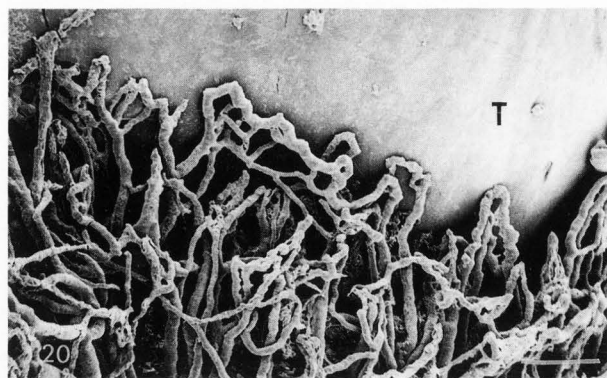


Figure 18. Wound space filled with new vessels at 20 days, mainly extending from the palate (P). Palatal rugae are not present. Molars (T). Bar = 500 μ m.

Figure 19. Large vessels coursing transversely from the medial side close to the bone surface (top left) are covered by a capillary plexus which is also oriented transversely. This newly formed capillary plexus consists of glomerulus-like structures laterally, and capillary loops medially, giving the impression of a remodeling progressing from the medial side of the wound. Unoperated palate (P) at the bottom. Twenty-day specimen. Bar = 250 μ m.

Figure 20. New vessels at the gingival margin are enlarged and have a varying diameter, but the general architecture is similar to that of unoperated gingiva. Molar (T). Twenty-day specimen. Bar = 100 μ m.

Figure 21. The healing gingiva is mainly revascularized from the palate and contains a dense plexus of dilated capillary loops which is supplied and drained by large vessels. Twenty-day specimen. Bar = 100 μ m.

were less prevalent (Fig. 17).

Twenty days

Twenty days postoperatively, the wound space was filled with new vessels, but palatal rugae were not apparent (Fig. 18). Adjacent to the teeth, large vessels coursed transversely from the medial side close to the bone surface. These vessels were covered by a capillary plexus which also coursed transversely. This plexus consisted of dense glomerulus-like structures laterally and more disperse capillary loops medially, giving the

impression that remodeling and vascular regression was progressing from the medial side of the wound (Fig. 19). The new gingival vessels had an irregular architecture, and a variable diameter with frequent constrictions and outpouchings along most vessels. The general appearance, however, was similar to that of unoperated gingival vessels (Fig. 20). At some sites, the wound area appeared to have been revascularized entirely from the palate. At these sites, the microvasculature consisted of a dense plexus of dilated capillary loops, supplied and drained by the large vessels which coursed transversely from the medial side close to the bone surface (Fig. 21).

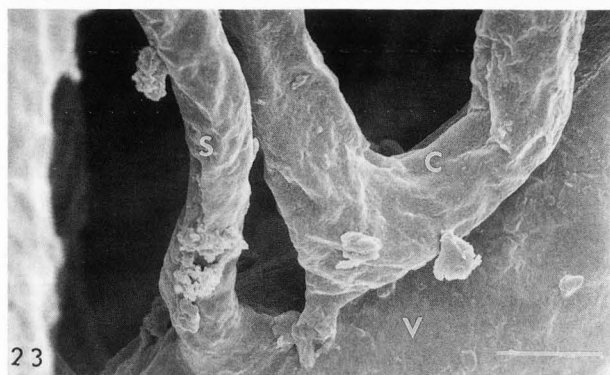


Figure 22. Gingival microvasculature as seen from the tooth side at 20 days post-surgery. At site, the PDL vessels seem to have contributed to the revascularization. The new gingival vascular plexus consists of arterioles (A) and venules (V) which are located deep in the tissue, and capillaries (C) which course close to the tooth surface. The capillary loops point coronally and are interconnected in a complicated, arcade-like pattern both in their ascending and descending parts. Twenty-day specimen. Bar = 100 μm .

Figure 23. Detail illustrating splicing of a capillary sprout (S) and a capillary loop (C) from the medial side of the wound with a venule (V) from the PDL. Twenty-day specimen. Bar = 10 μm .

At other sites, the PDL vessels seemed to have contributed to some extent to the revascularization. When viewed from the tooth side, the new gingival vascular plexus at these sites consisted of profound arterioles and venules which were directed occluso-apically, and of a capillary system close to the tooth surface. Capillary loops pointed coronally and were connected to a comarcade-like capillary network both in their ascending and descending parts (Fig. 22). The newly formed capillary sprouts had an irregular luminal surface. At this stage, a functional blood flow was being established between

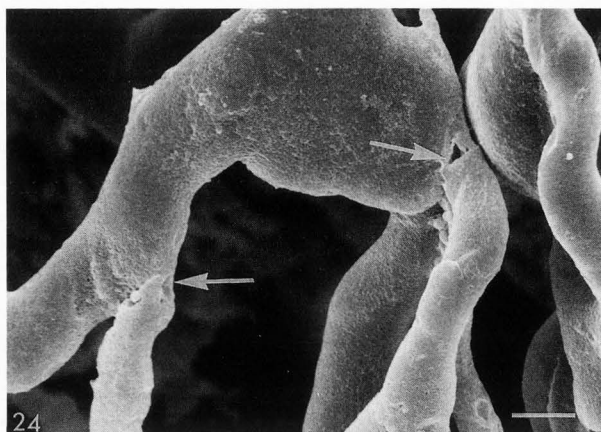


Figure 24. Detail illustrating fusion of vessel sprouts (arrows). At the stage of connection, the vascular lumen at the site of fusion may momentarily be too small to allow passage of erythrocytes. Twenty-day specimen. Bar = 10 μm .

granulation tissue from each side of the wound. At some sites, splicing of vessels could be observed. This process seemed to occur in three ways: (1) A capillary sprout from one side spliced tip-to-tip with a sprout from the opposing side. (2) A capillary sprout spliced with a capillary loop. (3) A capillary loop spliced with an arteriole or venule (Fig. 23).

Morphometric analysis of vessel diameters

Table 1 shows variations in luminal diameter of terminal vessels in the different areas studied. In unoperated palatal gingiva and mucosa, the vascular diameters remained constant at all observation periods. Also, the terminal vessels in the normal tissues exhibited remarkably small variations in diameter, as indicated by small standard deviations. Gingival vessels were larger in diameter than vessels of the palatal mucosa ($p < 0.001$).

Vessels adjacent to the wound were enlarged in diameters compared to those on the unoperated control side throughout the experimental period ($p < 0.001$). This difference was most pronounced at 2 days and was gradually reduced during the experimental period, although mean vessel diameter did not reach control levels within 20 days. At the lateral aspect of the wound, vessel diameters at 2, 4 and 7 days were larger than at 10 and 20 days ($p < 0.005$). Also, these vessels were larger than those on the medial side at 2, 4, and 7 days postoperatively ($p < 0.005$). At ten and twenty days postoperatively, the difference in vessel diameters between the medial and the lateral side of the wound was insignificant. The vascular bed in the unoperated side of the palate and gingiva did not exhibit any changes in diameter throughout the experimentation period.

Table 1. Luminal diameters of terminal vessels in palatal gingiva and mucosa according to post-surgical healing period.

Days	Unoperated		Wound area	
	Gingiva	Mucosa	Lateral	Medial
2	9.1 ± 2.7*	8.2 ± 2.0	46.3 ± 19.5	22.2 ± 9.0
4	9.9 ± 3.8	8.4 ± 1.9	44.9 ± 23.8	21.9 ± 9.1
7	9.9 ± 3.5	8.1 ± 2.4	37.6 ± 23.1	19.2 ± 7.6
10	9.8 ± 3.4	7.1 ± 2.1	24.0 ± 13.7	18.2 ± 7.1
20	9.9 ± 4.0	7.3 ± 1.7	15.7 ± 5.2	15.9 ± 7.9

*Vessel diameter in μm (mean of 4 animals \pm standard deviation). Twenty vessels measured per site per animal; 1600 measurements in all.

Discussion

The SEM observations of revascularization of an excisional wound in the rat palate were consistent with accepted mechanisms of inflammatory response and wound healing (Gillman *et al.*, 1955; Staffileno *et al.*, 1962; Whipple, 1963; Ross, 1969; Hunt and Dunphy, 1974; Fine, 1976). Two days postoperatively, vessels adjacent to the wound space seemed to have responded to the injury by acute inflammation as expressed by vasodilatation and presence in the vascular casts of impressions consistent with marginating and, presumably, emigrating leukocytes. Four and 7 days postoperatively, capillary buds extended into the wound space, while the wound was incompletely revascularized at 10 days. At 20 days, the wound space was filled with new vessels. The architecture of this vasculature was different from that found in unoperated palate and gingiva in that vessels were mainly oriented in a frontal plane instead of sagittally and by the presence of seemingly randomly dispersed clusters of capillary loops in the superficial capillary plexus.

Vessel diameters were enlarged along the wound margin as compared to vessels in unoperated palate and gingiva. This feature was most pronounced at two days, but vessel diameters did not reach control levels even at 20 days postoperatively. It has been argued that the perfusion procedure may affect vessel diameters and that microvascular casts, therefore, do not perfectly reflect the situation *in vivo*. However, the size of the vessels measured by this method was similar to those found by light and transmission electron microscopy in tissues processed by conventional histological techniques (Movat and Fernando, 1964; Rhodin, 1967, 1968). The dilated vessels seen adjacent to the wound space were similar in diameter to dilated vessels adjacent to a site of chemical cautery when examined in histological sections of unperfused eyes at comparable time intervals (McCracken *et al.*, 1979; Burger *et al.*, 1983). The casts, therefore,

appear to provide a relatively accurate representation not only of the microvascular architecture, but also of vessel size during wound healing. At 2, 4 and 7 days postoperatively, vessel diameters were larger at the lateral aspect of the wound than at the medial side. This might be caused by mechanical irritation, which, due to mastication of hard pellets, is probably a larger problem near the teeth than in the palate (Tabak *et al.*, 1982).

Hemispherical impressions in the surface of the casts were frequently observed at 2 days, decreasing to a rare occurrence at 10 days at the medial side of the excisional wound, while PDL vessels at the lateral side of the wound exhibited depressions in large numbers even at later time points. These impressions are considered to reflect the presence of marginating leukocytes, as they were not present in control specimens. The localization of such impressions to venules and veins and not to capillaries is characteristic of marginating leukocytes (Marchesi, 1961; Ryan and Majno, 1977). Also, perfusion by saline probably flushed out other unattached elements, leaving only leukocytes to account for these impressions. Vascular casts may, therefore, be useful in defining the chronology and sites of leukocyte margination and emigration (Burger *et al.*, 1983). The fact that impressions of marginating leukocytes were seen on the lateral side of the wound space as late as 10 days postoperatively indicates a prolonged inflammatory response at this site. As leukocytes do not normally marginate and emigrate through capillaries (Marchesi, 1961; Ryan and Majno, 1977), nor from the walls of new vessels, new vessels probably share, or lack, some common features with capillaries (Burger *et al.*, 1983).

The rate of wound closure was surprisingly slow, and the wound was not completely closed at 10 days postoperatively. In wounds of similar size to those studied here, where the wound healed by second intention, a cellular inflammatory response has been reported to be intense between 6 and 48 hours (Edwards *et al.*, 1957; Viljanto and Kulonen, 1962) or 3 and 5 days (Lamont,

1989). Rapid decline in marginating white cells has been reported at 4 to 8 days postoperatively (Boucek and Noble, 1955). These seemingly inconsistent results may indicate that a large excisional wound on a rigid tissue bed, such as in the palate, heals at a slower rate than a skin wound where tissue contraction is an important initial factor in healing. The effect of wound contraction on healing in the palate was probably limited, owing to the strong attachment of soft tissue to the palatal bone (Bodner and Dayan, 1990). This may explain why the wound space was not filled with granulation tissue by 10 days. A denuded bone surface is open to infection and necrosis. The absence of capillary budding in the Volkman's canals also indicated a slow revascularization of the wound.

New vessels have been observed histologically 4 days after implantation of cellulose sponges (Viljanto and Kulonen, 1962). By measurements of clearance rate of ^{133}Xe injected into implanted polyether polyurethane sponges, new capillaries have been demonstrated by day 8 (Mahadevan *et al.*, 1989). Increase in clearance rate was interpreted as increased vascularization. However, it is likely that ^{133}Xe clearance increases only when functional circulation is present and not during the phase when capillary buds are formed. In the present study model, initiation of new vessel buds was observed as early as 2 days. Thus, the time during which new vessels formed overlapped the phase of acute inflammation, in contrast to previous observations (Viljanto and Kulonen, 1962; Mahadevan *et al.*, 1989). This reflects an advantage of the corrosion cast method, which allows detailed examination of the entire vascular bed in each specimen. In studies of tissue sections, on the other hand, capillary buds are difficult to distinguish from normal capillaries or venules passing in or out of the plane of section (McCracken *et al.*, 1979; Burger and Klintworth, 1981). The casting material appeared to fill newly formed buds despite stagnant liquid flow and produced recognizable, directionally-oriented structures which would not be expected from an artifact. Venues were the predominant source of capillary sprouting, while few, if any, buds originated from arterioles. These observations are in accordance with previous studies of retinal neovascularization (Burger and Klintworth, 1981; Tano *et al.*, 1981; Burger *et al.*, 1983).

The pattern of angiogenesis has been extensively studied in the rabbit cornea (Gimbrone *et al.*, 1974; Ausprunk *et al.*, 1977; Burger *et al.*, 1983, 1984). The cornea, however, is avascular by nature, and the findings, therefore, cannot immediately be extrapolated to oral angiogenesis, since oral tissues are profusely vascularized in a rather complex manner. The vasculature of the unoperated palate in the rat consisted of two layers: a superficial capillary plexus and a more deeply situated

system of larger vessels. The pattern of angiogenesis appeared to be different in the two layers. The superficial plexus showed enlarged vessels at 2 days, forming simple hairpin-loops with bulbous tips. At 4 days, capillaries consisting of several loops originating from one arterio-venous stalk were seen, and by 10 days even more complex, glomerulus-like structures had developed. Such complicated microvascular patterns were more abundant towards unoperated palate, while simple capillary loops predominated towards the margin of the wound, indicating that different stages of wound healing were present at the same time at different sites. The profound layer, on the other hand, proliferated into the wound by sprouting towards the wound space. The observations indicate that the formation of new capillaries extending into the wound space may be divided into four stages: (1) formation of protrusions or bulbous sprouts from a venule; (2) elongation of these sprouts to form finger-like capillaries with a bulbous tip; (3) fusion of two capillary sprouts to form a capillary loop; and (4) fusion of several capillary tips to form more complex vessels. This is in accord with observations of neovascularization in previous studies (Burger *et al.*, 1983; Phillips *et al.*, 1991). Vessels originating from the PDL at the lateral side of the wound seemed to proliferate by the same pattern as the profound vessels in the palate, although at a slower rate.

The origin of new vessels growing into an excisional wound in the gingiva has been discussed in the past. Clinical and histological findings have suggested that, when grafts are placed on denuded bone, a successful "take" is dependent on revascularization of capillaries passing through the bone cortex (Dordick *et al.*, 1976; Bissada and Sears, 1978; James *et al.*, 1978). In the present study, Volkman's canals appeared empty, indicating that revascularization through bony channels is not crucial to wound healing. It should be kept in mind, however, that blood vessels subjacent to exposed, necrotic and infected bone, as in the present study, may react differently from vessels in a situation where a graft is placed on bleeding, healthy bone. Other investigations have shown that the vasculature of the marginal PDL may take a predominating part in the neovascularization of a gingival wound (Karring *et al.*, 1975; Nobuto *et al.*, 1987). This is in contrast to the findings in the present study, where the palatal vasculature was the main contributor, and PDL vessels contributed little, if at all, to revascularization of the wound. This variance may, to some extent, be explained by differences in experimental design and postoperative wound management.

Remodeling of the superficial palatal vascular plexus seemed to include simplification of the complex, glomerulus-like structures seen at 10 days towards simple capillary loops at 20 days postoperatively. This process

advanced from unoperated palate towards the area of wound closure. But even at 20 days, the palatal capillary plexus was oriented in a sagittal direction, in contrast to the longitudinal arrangement seen in unoperated palate (Kajiwara, 1989). At the lateral side of the wound, most sites seemed to be devoid of capillary sprouts even at 10 days, while at some sites, PDL vessels seemed to contribute more distinctly to the revascularization process. At 20 days postoperatively, new-formed gingiva seemed to have a vascular arrangement dependent on the source of revascularization. Most often, the palatal vascular plexus was the source responsible for revascularization. The gingival vessels at these sites formed a dense plexus of capillaries. At some sites, however, contribution by PDL vessels had resulted in a vascular arrangement consisting of vertically-oriented arterioles and venules and anastomosing capillary loops, similar to vessels found in unoperated PDL (Selliseth and Selvig, 1994).

Functional circulation between the medial and the lateral side of the wound was established by 20 days. Splicing of newly formed capillaries from each side or splicing of capillaries from one side with venules on the other side seemed to be the predominating mechanism in this process.

The vascular bed in the unoperated side of the palate was indifferent throughout the study and similar to that of unoperated rats (Selliseth and Selvig, 1994). Thus, the control side seemed to be unaffected by the angiogenic activity on the contralateral side.

In conclusion, this study has provided information on the initiation, timing and pattern of angiogenesis in an excisional wound in gingiva and oral mucosa, and may form a basis for experimental studies on the influence on revascularization of different mediators of healing applicable to periodontal surgery.

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Discussion with Reviewers

D.E. Schraufnagel: Several different forms of angiogenesis have been reported, including broad based budding, sprouting, long thin tube formation and hairpin loop growth. We reported a lateral capillary unfolding from small veins and hair-like growth in the trachea. Did you see similar structures? Could you comment on what were the most common forms of new capillary growth in your model?

Authors: We did not quantify the different forms of new capillary growth. However, it is our impression that the superficial, subepithelial palatal capillary plexus contributed to revascularization of the wound mainly by lengthening and proliferation of loops. The profound palatal vessels, however, proliferated by sprouting. At the lateral side of the wound space, vessels from the PDL proliferated by sprouting, but at a slower rate than profound vessels at the medial side of the wound. Lateral capillary unfolding from small veins and hair-like growth was not a common observation in our material.

D.E. Schraufnagel: Did you notice new capillaries that were too small to allow the passage of erythrocytes?

Authors: Capillaries with inner diameter down to 2 μm are commonly seen in unoperated oral mucosa. In a healing wound, the vessels are enlarged, and such small vessels are not common. Obviously, at a stage where two sprouts are about to connect, the vascular lumen at the site of fusion may momentarily be too small to allow passage of erythrocytes (Fig. 24).

C.W. Kischer: Do you expect that these healing conditions in the rat approximate those in humans?

Authors: Principally, we see wound healing as a fundamental biologic process that is similar in all mammals, and much of our knowledge of physiological and biochemical reactions is based on observations in rats which have been extrapolated to humans. This study showed that the rate of wound healing also is tissue dependent. Obviously, differences between species depending on size and mechanical differences in the tissue to be investigated, will influence healing conditions. We feel that the results obtained in this study might improve our understanding of wound healing in humans.

Y. Ohta: In the Abstract, could the newly formed vessels be called "sinusoidal capillaries"?

Authors: First, all new vessels were not sinusoidal. Secondly, all sinusoidal vessels were not necessarily capillaries. Therefore, a more general term was chosen.

S. Aharinejad: Why are the newly formed vessels around the wound area larger in diameter than those in the same area of control animals? It is hard to understand that the diameter of some newly formed vessels was more than four times larger than the control vessels!

Authors: Wound-healing is a state of inflammation, and a cardinal feature of inflammation is dilatation of blood vessels.

S. Aharinejad: Why does vasculogenesis originate from venous vessels and not from arterial ones?

Authors: This study supports the findings of others with respect to the origin of new vessels. We do not know why this is so.

S. Aharinejad: Although the periosteal layer of bone comprises of several feeding vessels, I was not surprised that angiogenesis did not occur from the bone toward the soft tissue. This might have the following reason: Periosteal vessels mainly enter the bone to feed the deeper layers, from where capillary sprouts invade the more superficially located bone layers. Please discuss this point.

Authors: In oral surgery, vessels in bone are believed to be important in revascularization of a graft or flap when placed on bone. In addition to the references in the Introduction, this has recently been discussed by Yanagihara (1991). However, the relative importance of bony vessels in revascularization of a graft or flap can only be assessed when the bone is covered by soft tissue. When the bone surface is left uncovered, it is open to infection and becomes necrotic. This is probably the reason why, in this study, bony vessels seemed unimportant in revascularization of the wound.

Additional Reference

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