Effect of root surface alterations on periodontal healing. I. Surgical denudation

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Abstract. The present study was undertaken to evaluate the effect of root surface denudation on periodontal healing. Twelve teeth, distributed in four squirrel monkeys, were extracted and replanted after surgically denuding the coronal root surface of connective tissue fibers and cementum by reimplantation. The reimplantation schedule provided three teeth for histologic analysis at 1, 3, 7, and 21 days after reimplantation. One day after reimplantation the zone of fibrin enmeshing erythrocytes and inflammatory cells was interspersed between the root surface and the remaining periodontal fibers attached to the alveolar bone. Epithelium migrated rapidly along the denuded root, had reached the alveolar crest at 3 days, and was within the ligament space at 7 days. At 21 days, the epithelium was at the apical limit of root instrumentation, which corresponded to the level of attached connective tissue fibers on the root surface. No evidence of new connective tissue attachment was observed on the denuded root surface. It was concluded that the absence of fibers on the root surface resulted in apical migration of the epithelium, and precluded formation of new connective tissue attachment.

Increasing evidence has implicated the periodontitis affected root surface as being the principal factor preventing new connective tissue attachment (Ratcliffe 1966, Karring et al. 1980, Nyman et al. 1980, Polson & Caton 1981). Root surface alterations associated with periodontitis comprise denudation of fiber attachment (Grant et al. 1963, Glickman 1964), contamination of the root surface (Haffajee & Baumbhammers 1971, Aleo et al. 1974, 1975, Bravman et al. 1979), and alterations in mineral density (Selvig & Zander 1962, Herting 1967, Selvig & Landay 1972). It is essential to know the relative importance of these various root surface alterations in precluding new connective tissue attachment. Clarification of the situation would permit rational treatment of the exposed root surface to obtain new attachment.

A problem in elucidating the significance of selective root surface alterations upon new connective tissue attachment is that mere periodontitis results in a reduced periodontium. The reduction of a periodontium by marginal periodontitis may result in destruction of progenitor cells which have the potential to form the essential structural components of a periodontium, namely, cementum, periodontal ligament and alveolar bone (Melcher 1976). Consequently, evaluation of selective root surface alterations must take place in a periodontal environment which has the potential to form the essential structural components of the periodontal supporting apparatus.

It has been established that teeth which have been extracted and rapidly reimplanted or autotransplanted heal with the periodontium reestablishing a normal morphology (Lor & Waerhaug 1961, Fong et al. 1967, Andrews 1980, 1981). In these situations, therefore, the root surfaces permitted fiber reattachment.
the surrounding periodontium possessed the essential structural components for reformation of a new periodontium. In addition, the healing sequence regarding cell repopulation and fiber reattachment has been defined (Hurst 1972, Nasjleti et al. 1975, Prose & Poisson 1982). Consequently, it appears that tooth reimplantation may provide a suitable system for evaluating the influence of selective root surface alterations upon periodontal healing. In order to evaluate the effect of a denuded root surface upon periodontal healing, a root surface devoid of connective tissue fibers and cementum was reimplanted into a normal periodontium.

**Material and Methods**

The experimental animals consisted of four young adult female squirrel monkeys with all permanent teeth erupted, caries-free, and exhibiting minimal attrition. Six weeks prior to any experimental procedure, an oral hygiene regime was begun which consisted of mechanical plaque removal three times a week (Kantor et al. 1976, Perier & Poisson 1982). Teeth were extracted using previously described techniques (Prose & Poisson 1982), and the coronal third of the root surface was planed to remove the attached periodontal fibers and cementum. Each part of the instrumented root surface received 15 vertical strokes with a Gracey curette 13/14 (Hu-Friedy, Chicago, IL 60618), and this was supplemented at the cemento-enamel junction with five strokes using a Younger-Good 7/8 curette (Hu-Friedy, Chicago, IL 60618). During the root planing procedure, the tooth was held between the beaks of the extraction forceps, and the region of the root surface not instrumented was kept moist by applications of isotonic saline using a small paint brush. After completion of root planing, the instrumented surface was rinsed with saline, and the tooth reimplanted into its socket. The time between extraction and reimplantation was less than 15 min. No form of tooth stabilization or splinting was used. Post-operative diet and oral hygiene were as previously described (Prose & Poisson 1982).

Reimplantation of teeth, and sacrifice of animals, were scheduled to provide observation periods of 1, 3, 7, and 21 days after reimplantation. A total of 12 teeth were extracted and reimplanted, consisting of maxillary central incisors, mandibular lateral incisors, bicuspids and molars. Three teeth were available at each of the time points for evaluation prior to tooth extraction, and during the observation period after reimplantation, clinical assessment was made of gingival inflammation and tooth mobility (Poisson et al. 1976).

Sacrifice of the animals, histologic preparation, and sectioning of blocks were as previously described (Kantor et al. 1976, Perier & Poisson 1982). Step-silver sections, representing intervals of 96 microns, were stained with hematoxylin and eosin. The sections on the slides immediately before the hematoxylin and eosin stained sections were stained using a silver impregnation technique (Lilla 1965) to delineate connective tissue fibers. Histologic analysis of the coronal periodontium was carried out using five step-silver sections from each specimen (Kantor et al. 1976, Poisson et al. 1979).

**Results**

**Clinical observations**

Immediately prior to extraction the teeth had no clinical mobility, and the gingival tissues exhibited zero or minimal signs of inflammation. The teeth had moderate mesio-distal and bucco-lingual mobility immediately after reimplantation which remained, or slightly increased, at later time points. In addition, the 21-day specimens had slight vertical mobility. The gingival tissues were well adapted to the reimplanted teeth, and showed only mild inflammation at all time points. No teeth were exfoliated.

**Histologic observations**

One day after reimplantation the coronal root surfaces were characterized by lack of attached
connective tissue fibers and cementum (Fig. 1). The transseptal fiber and coronal periodontal ligament regions were severely disrupted. Subcullar epithelium was present over the disrupted supracrestal connective tissue, and its apical extent approximated the original location of the cemento-enamel junction (Fig. 2).

A zone of fibrin containing numerous crythrocytes and polymorphonuclear leukocytes (PMNs) was adjacent to the denuded root surface. This zone extended approximately one-half of the width of the ligament area. The alveolar half of the ligament exhibited connective tissue fibers which were embedded in, and perpendicular to, the bone surface (Fig. 3). Areas of acellularity were present in this region. In the area of the periodontal ligament immediately apical to root denudation, connective tissue fibers were attached to the root surface (Fig. 4). A distinct break in the continuity between fibers attached to the root surface and fibers attached to the bone was apparent.
Fig. 3. Coronal periodontal ligament region 1 day after reimplantation. H and E stain, original magnification ×51.

Die Region des koronalen Desmodont. 1 Tag nach der Reimplantation. H und E-Färbung, Originalvergrößerung ×51.

Region du ligament parodontal coronaire 1 jour après reimplantation. Coloration H et E, agrandissement original ×51.

Three days after reimplantation, the apical end of epithelium was at the alveolar bone crest (Fig. 5). The transeptal fiber region showed areas of cell repopulation. The denuded root surface was covered by a zone of fibrin which extended apically from the end of the epithelium to the limit of root denudation (Figs. 6, 7). In the majority of specimens, an artifactitious split separated the fibrin from the underlying root surface. Viable cells were infrequent amongst periodontal ligament fibers attached to alveolar bone or root surface (Figs. 6, 7). The break in continuity between fibers attached to

Fig. 4. One day after reimplantation. Periodontal ligament at area of apical limit of root instrumentation. Coronal to the apical limit of instrumentation the root surface is without attached fibers. Apical to the instrumentation there is a distinct break in the continuity between fibers attached to the root surface and fibers attached to the bone. Silver impregnation stain, original magnification ×64.


Un jour après reimplantation. Lignement parodontal dans l'aré de la limite apicale de l'instrumentation radiculaire. Coronalement à la limite apicale de l'instrumentation, la surface radiculaire n'a pas de fibres attachées. Apicalement à l'instrumentation, il y a une nette rupture de la continuité entre les fibres attachées à la surface radiculaire et les fibres attachées à l'os. Coloration à l'impregnation d'argent. Agrandissement original ×64.
Fig. 3. Supracrestal region 3 days after reimplantation. H and E stain, original magnification X40.

Die Region über der Knochenleiste, 3 Tage nach der Reimplantation H und E-Färbung, Originalvergrößerung X40.

Region supracrestale 3 jours après reimplantation. Coloration H et E, agrandissement original X 40.

Fig. 6. Coronal periodontal ligament 3 days after reimplantation. An artifactual space is present between the fibers network and the denuded root surface. H and E stain, original magnification X40.


Ligament periodontal coronaire 3 jours après reimplantation. Un artefact est présent entre le filet de fibres et la surface radiculaire dénudée. Coloration H et E, agrandissement original X 40.

Fig. 7. Periodontal ligament involvement of the area of E stain, original magnification X40.

Desmodont im coronalen Randschnitt, Originalvergrößerung X 40.

Ligament periodontal coronaire, 3 jours après reimplantation. Verrue de la limite radiculaire. Coloration H et E, agrandissement original X 40.

The root surface and those attached to bone was still present. Several osteoclasts were present in the narrow spaces adjacent to the periodontal ligament, and rear resorption was occurring.

Seven days after reimplantation the epithelium along the root surface had migrated apically a considerable distance into the periodontal ligament space (Fig. 8). The precise extent of epithelial migration was sometimes difficult to distinguish since the epithelium tapered to form a monolayer in its most apical part.

Cell repopulation appeared complete in the transseptal region. A zone of highly cellular and vascular granulation tissue, which appeared to be in continuity with the transseptal region, was present in the periodontal ligament area, intersected with various tissues or demarcated root and the ends of the connective tissue fibers attached to the alveolar bone surface (Fig. 10). The granulation tissue appeared to have greater cell density than adjacent connective tissue fiber regions attached to alveolar bone and where still exhibited areas of reduced cellularity.

Cellular areas in the connective tissue fiber region were located primarily adjacent to the...
Fig. 7. Periodontal ligament 3 days after reimplantation at area of apical limit of instrumentation. H and E stain, original magnification X51.


Ligament periodontal 3 jours après réimplantation dans l'aire de la limite apicale de l'instrumentation. Coloration H et E. agrandissement original X51.

Fig. 8. Supracrestal region and coronal periodontal ligament 7 days after reimplantation. H and E stain, original magnification X51.


Region supra-crescale et ligament periodontal coronaire 7 jours après réimplantation. Coloration H et E. agrandissement original X51.
openings from narrow spaces. Many multinucleated cells were present at the interface between the granulation tissue and the fiber region. Numerous osteoclasts were present within the narrow spaces or lining the periodontal ligament surface of the alveolar bone, and active bone resorption was present.

Correlation of observations from the different histological staining techniques indicated that fibers were present in the zone of granulation tissue (Figs. 9, 10). These fibers were thinner than the remaining ligament fibers associated with the alveolar bone surface, and were oriented parallel to the root surface. In the region of the ligament apical to the limit of root instrumentation, the break in continuity between fibers attached to the root surface and those attached to alveolar bone was still present.
In the 21 day specimens, there was no evidence of connective tissue attachment to any portion of a denuded root surface (Fig. 11). The apical termination of epithelium on the root surface coincided with either the limit of root instrumentation or at the level of a resorption lacuna (Fig. 11). Fibers reattachment and root resorption were present on each surface apical to the end of the epithelium. Many regions exhibited active resorption characterized by the presence of multinucleated cells lining the root surface.

There had been considerable loss of crestal alveolar bone compared with earlier time points. Although the narrow spaces in the alveolar bone appeared larger, and the ligament wider, than at 7 days, osteoclasts were less frequent. Areas of new bone formation were evident lining the narrow spaces, and in several regions of the periodontal ligaments. Ankylosis was present in one of the ligaments examined.

**Discussion**

The present study was designed to evaluate the effect of a denuded root surface upon periodontal healing. Independent evaluation of this variable can be made only if the healing occurs in an environment known to possess the potential for periodontal ligament regeneration. The design used in our study met this criterion since reformation of the periodontium occurs after rapid reimplantation of an extracted tooth (Loe & Aarberg 1961, Andreasen 1980, 1981, Proye & Polson 1982).

Removal of the periodontal ligament fibers attached to the root surface by the instrumentation procedure created a potential space between the instrumented root surface and the periodontal ligament fibers attached to the alveolar bone surface. One day after reimplantation this space was occupied by a zone of

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**Fig. 11.** Coronal periodontium 21 days after reimplantation. H and E stain, original magnification X 40.
fibrous remeshing reticulum and PMNs. Formation of a fibrin network represents an initial event in the wound healing response, and has been reported interposed between root surface and gingival connective tissue 1 day after subgingival curettage (Ramfjord & Kroetz 1954, Stone et al. 1966, Gillman 1964, Ross 1968). The presence of an artificial split between the fibrin layer and the denuded root surface implies limited adhesion, and perhaps represented zones of weak connection between the healing periodontal tissues and the surgically denuded root surface. This impression was strengthened subsequently by the observation of epithelial migration occurring at the interface between the fibrin and root surface. Strands of fibrin, oriented parallel to the root surface, could have provided a direction for epithelial cells during their apical migration through the phenomenon of contact guidance (Weiss 1958, 1961).

The epithelium which lined the gingival sulcus 1 day after reimplantation had migrated to the level of the alveolar crest at 3 days, and was within the ligament space well apical to the crest at 7 days. The rapid epithelial migration correlated with previous findings that epithelial tissues began to proliferate immediately after reimplantation, and reached a peak at 3 days (Nasjleti et al. 1975). It appears that the initial events determining the ultimate apical extent of epithelium on the root surface occur very soon after healing has been initiated.

Although reports investigating the periodontal response after reimplantation of a denuded root have not reported epithelial migration (Løe & Waerhaug 1961, Hammer et al. 1970), it is apparent in their illustrations. Similar epithelial migration along a denuded root surface down to the immediate vicinity of the apical limit of instrumentation has been reported recently after root planing on monkeys (Caton & Zander 1979), flap surgery on rats (Stahl 1977a), dogs (Nieves et al. 1978, van Dijk 1979), monkeys (Ramfjord & Lutgarten 1972, Caton & Zander 1976, Yuen et al. 1980, Nyman et al. 1981), humans (Frank et al. 1974, Steinert et al. 1981), and treatment of intrabony defects with or without bone grafts (Lutgarten & Rosenberg 1979, Moskow et al. 1979, Caton et al. 1980). These studies conflict with reports of absence of, or very limited, apical epithelial migration along a root planed surface (Ling 1980, O'Connell 1980, Wolt & Wentz 1965, Hlaat et al. 1968, Ririe et al. 1980). It is perhaps noteworthy that these latter studies had in common the experimental design (removal of buccal alveolar plate, surgical denudation of the root and replaced flap), and the experimental animal (dog). The nature of the experimental animal could explain these results since it has been documented that spontaneous regeneration of experimental periodontal defects occurs in dogs (Jansen et al. 1977, 1979, van Dijk 1979).

The eventual apical termination of epithelium on the root surface was either at the level of connective tissue fibers attached to the root surface immediately apical to the limit of root instrumentation, or coronal to a resorption bay. Similar topographical relationships between epithelium and resorption bays have been described when root resorption occurs following fresh autogenous iliac bone graft surgery (Drago & Sullivan 1973). Cessation of migration when epithelium contacts connective tissue fibers attached to a root surface has been repeatedly demonstrated following tooth reimplantation (Løe & Waerhaug 1961, Nasjleti et al. 1975, Proye & Poisson 1982), and flap surgery when fibers were left attached to the root surface (Kohler & Ramfjord 1960, Stahl 1977, a,b). The present results thus support the increasing body of evidence demonstrating that removal of fibers at the connective tissue-root interface is followed by migration of the epithelium along the denuded root surface, and that this migration is stopped when epithelium contacts fibers attached to the root. Furthermore, it appears most unlikely that the cementum surface per se exerts an inhibitory influence upon epithelial migration over the root surface. It may be hypothesized that a gingival connective tissue component, which occurs due to intercelleular interaction, prevents the migration of epithelial cells across the root surface.
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epithelial migration since migration was observed over regions of cementum remaining after the root planing, and which no longer had any attached connective tissue fibers. It has also been hypothesized that a splicing between gingival connective tissue fibers and exposed collagen fibers after attrition demineralization which occurred 7-14 days after healing was the factor preventing epithelial migration (Crigger et al. 1978). However, since the epithelium in our investigation had migrated into the ligament space within 7 days, it infers that the critical interactions which inhibit epithelial migration occur within the first week after initiation of healing.

A periodontitis affected root surface has been identified as a principal factor preventing new connective tissue attachment (Karring et al. 1980, Lopez et al. 1980, Nyman et al. 1980, Polson & Caton 1981). It has been suggested that it is the contamination of the periodontitis affected root surface which is the primary factor precluding new connective tissue attachment (Alae et al. 1975, 1975, Bravman et al. 1979).

Although root planing is advocated and performed to remove the bacterial contaminants, and render the root surface compatible with the periodontal tissues (Nishimine & O'Leary 1979), new connective tissue attachment does not occur along such a surface (Yukna 1976, Caton & Zander 1979). Our present results indicated that a non-exposed and non-contaminated but denuded root surface, placed in an environment known to be conducive to regeneration was no more receptive to new attachment. It is possible that the healing potential in this situation could not be expressed because epithelium migrated apically, and became interposed between the denuded root surface and healing periodontal connective tissues. The potential importance of preventing epithelial migration into the healing periodontal wound has been emphasized (for review, see Stahl 1977b, Wirzlin 1981). It has been proposed that absence of the epithelial layer interposed between the periodontal connective tissues and root surface would permit new connective tissue attachment to the denuded surface (Bjorn 1961, Bjorn et al. 1965, Hatt et al. 1964). The subsequent attachment relationship between root surface and periodontal tissues may be related to the phenotype expressed by the cells populating the wound (Melcher 1976, Nyman et al. 1980, Besko et al. 1981). Studies are needed to clarify the periodontal healing response in different situations where epithelium has been effectively prevented from migrating between the root surface and adjacent gingival and periodontal ligament connective tissues.

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Zusammenfassung
Die Auswirkung von Veränderungen der Wurzeloberfläche auf die periodontale Heilung. I. Wurzelplanung
kalawanderung restitue, was die Bildung eines erneuten Bridgewebsattachment verhinderte.

**Résumé**

Effets des altérations de la surface radiculaire sur la cicatrisation parodontale. I. Déséquilibres chirurgicaux. Le but de la présente étude a été d'évaluer l'effet de la déséquilibre de la surface radiculaire sur la cicatrisation parodontale. Douze dents provenant de quatre sujets ont été extraites et réimplantées après une déséquilibre chirurgicale de la surface radiculaire coronale des fibres du tissu conjonctif et du cément par ligature radiculaire. Le plan de la réimplantation prévoyait trois dents pour l'analyse histologique 1, 3, 7 et 21 jours après la réimplantation.

Un jour après la réimplantation, une zone de fibrose remplie d'extrémités de cellules inflammatoires s'est interposée entre la surface radiculaire et les fibres parodontales restantes attachées à l'os alvéolaire. L'épithélium avait migré rapidement le long de la racine dénudée, atteint le sommet alvéolaire après 3 jours et se trouvait dans l'espace ligamentaire après 7 jours. Après 21 jours, l'épithélium se situait à la limite apicale de l'implantation radiculaire correspondant au niveau des fibres du tissu conjonctif attachées à la surface radiculaire. Aucune preuve de nouvelle attachement conjonctif n'a été observée sur la surface radiculaire dénudée.

Il a été conclu que l'absence de fibres sur la surface radiculaire entraînait une migration apicale de l'épithélium et prévenait la formation d'une nouvelle attachement conjonctif.

**References**


