Electron microscopic study of the junction between surgically denuded root surfaces and regenerated periodontal tissues

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The ultrastructural features of the junction between regenerated periodontal tissues and surgically exposed root surfaces were investigated in monkeys. Specimens were studied at intervals of 1 to 18 months. Specimens at all time intervals demonstrated regeneration of the junctional epithelium to both dentin, as well as cementum surfaces. Epithelial reattachment was often present on intact, as well as altered root surfaces. Cemental regeneration was also noted in all specimens, but appeared generally confined to the most apical portion of the wound. Cementum formed over dentin, as well as cementum. A dense, granular layer, possibly of a polysaccharide-protein nature, was frequently noted at the interface of new cementum and the surgically exposed dentin or cementum. Artifactual slits, apparently occurring during the demineralization phase of tissue processing, frequently involved this granular layer. It is suggested that these slits result from processing and do not reflect a lack of cohesion or adhesion within the regenerated periodontium.

Following the initial suggestion by Younger (1905) that curettage may result in reattachment of gingival tissues to tooth surfaces within periodontal defects, a number of reports were published on various techniques purported to achieve clinically successful reattachment (Good 1906, Smith 1915, Stillman 1917, Merritt 1918). However, Box (1924) was the first investigator to demonstrate histological evidence of periodontal tissue regeneration against a tooth-surface in a specimen obtained seven years after curettage (McCall 1926). Three years after Gottlieb's report on the existence of an epithelial attachment (Gottlieb 1927a and b), Box wrote "sections show a reattachment of the connective tissue to the cementum surface. In addition, there is to be observed the phenomenon of a tenacious adherence of the crevicular epithelium to the cementum. It will be noted that the connective tissue fibers run parallel with the curetted cemental surface; also that the principal fibers, cementoblasts and epithelial cords typical of the normal are absent." Two years later, Box (1928) reported on three additional short term cases, including a 9-day postoperative specimen in which...
connective tissue was closely apposed to the tooth surface. In addition, he included a micrograph of "old reattachment" on the basis of which he stated that new cementum may form during such reattachment. Beck-with, Willits and Fleming (1927) and Beckwith and Williams (1928) presented the first published reports of periodontal tissue regeneration in experimentally inflicted wounds in animals. However, their standard injuries did not communicate with the gingival crevice, and their brief description of the healing process was rather vague, though suggesting that repair began adjacent to the cementum surface with subsequent spreading toward the alveolar bone. Sippy (1927) produced injuries to the apical region of dogs' teeth with and without previous removal of the pulp. He concluded that reattachment takes place in either case. One photomicrograph showed new cementum over exposed dentin seven weeks after the injury. Thomas (1922) had reported previously that new cementum could form over a fractured dentin surface, as well as cementum in embedded root fragments from a human jaw. Stones (1934) surgically detached the gingiva from the root surface of monkey teeth and studied the epithelial and connective tissue repair against intact or ground root surfaces. His results were quite variable. He was able to obtain complete reattachment of epithelium and connective tissue to intact cementum with little difference from the normal. However, he stated that "this reattachment was found to be weak, even after thirty weeks, as it was much more frequently torn on cutting sections than the control side." Such tears were also noted in cases where the epithelial attachment had formed over an exposed dentin surface; an observation which Stones interpreted as evidence for a "weak union". Bodecker and LeFkowitz (1935) following tooth replantation experiments in dogs noted reattachment of epithelium to enamel, as well as cementum. Skillen and Lundquist (1937) later published some histological evidence of epithelial reattachment to human teeth. They also stated that in artificially created pockets in dogs, a connective tissue reattachment mediated by a thin cementum layer could be observed as early as two weeks post-operatively.


Most of the reports indicated the possibility of an epithelial attachment. The time required for this to occur varied from as little as 6 days in dogs (Kon et al. 1969), 9 days in monkeys (Caffesse et al. 1968) and 3 weeks in man (Dedolph and Clark 1958), to as much as 2 months (Jansen et al. 1955). Waerhaug (1955) noted a "complete readaptation of the epithelial cuff to the
denuded enamel surface" in dogs, 94-208 days after gingivectomy. In dogs, the reported times required for new cementum to form varied from approximately 3 weeks (Lingborne and O'Connell 1950) to 3 months (Jansen et al. 1955). Wilderman et al. (1970) reported that following periodontal surgery in humans, two months were required for new cementum to form.

New bone formation in dogs was noted to begin at about 3 weeks (Lingborne and O'Connell 1950, Wilderman et al. 1960) and to continue for as long as 3 months (Jansen et al. 1955). Schaffer and Zander (1953) noted new bone formation up to 4 months post-operatively, following periodontal surgery in humans. Regeneration of the periodontal ligament was also reported by Beube (1947), Schaffer and Zander (1953) and Jansen et al. (1955).


Some investigators considered these tears to originate as a result of a weak tissue junction (Stones 1934, Bernier and Kaplan 1947, Jansen et al. 1955, Hiatt et al. 1970), notwithstanding their presence in specimens which had remained clinically satisfactory for as long as 15 years after gingival surgery (Beube 1960).

Despite the relatively large number of reports dealing with periodontal tissue regeneration, it is remarkable how little interest seems to have been expressed in the nature of the junction between the previously exposed tooth surface and the tissues that have become newly attached to it. With the exception of two reports dealing with the histochemical properties of the junction between the gingiva and the previously exposed tooth surface (Cimasoni et al. 1963, Edwards et al. 1969) and an electron microscopic study of epithelial reattachment (Listgarten 1967), very little information is available at this time on the nature of these tissue junctions.

This investigation was undertaken in order to study the ultrastructural features of the junction between experimentally exposed root surfaces of monkey teeth and regenerated periodontal tissues, at varying intervals following surgery.

The nomenclature used in the remaining portion of this report is based, in part, on that suggested in a recently published monograph dealing with the ultrastructural features of the dento-gingival junction (Schroeder and Listgarten 1971).

Methods and Materials

Seven adult rhesus monkeys were operated as follows: under general anesthesia induced with Sernylan (Bio-Ceutic Labs, St. Joseph, Mo., U.S.A.) and maintained with sodium nembutal, mucoperiosteal flaps were elevated on the buccal aspect of the right maxilla and mandible. With a watch-cooled No. 7 round bur, the alveolar bone was recontoured in order to expose approximately one third to one half of the anatomic root of the teeth in the operated quadrant. Third molars were not included. The root surfaces were purposely ground to expose dentin.

The exposed root surfaces were smoothed with curettes before suturing the flap back into place. The left side served as a control. Each animal received 400,000 units of penicillin intra-muscularly and was placed on a soft diet for two weeks. All animals recovered uneventfully. One animal was mor-
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In each of the following post-operative intervals, 1, 2, 3, 5, 6, 9, 12, and 18 months, the animals were sacrificed 9, 12, and 18 months post-operatively were scaled every six months, under general anesthesia. However, no animal was sacrificed within the two months period prior to sacrifice. The diet normally consisted of Purina monkey chow pellets supplemented with fruits and water ad libitum.

At the time of sacrifice, the animals were anesthetized as described above. Each animal received 5,000 units of heparin sulfate intravenously. The animal was then perfused through the left ventricle with a solution of 2.5% phosphate buffered glutaraldehyde (Warshawsky and Moore 1967). The bottle of fixative was suspended two feet above the heart and the solution allowed to enter the ventricle by gravity, through a gauge 13 hypodermic needle. Drainage was established by incision of the right atrium. Good perfusion resulted in almost immediate stiffening and blanching of the tissues. After 10-15 minutes following the beginning of perfusion, each incisor was excised in block with a Stryker saw and placed in ice-cold 2.5% glutaraldehyde buffered osmic acid. The central incisors were used to study the regenerated junction in a plane parallel to the longitudinal axis of the tooth. The lateral incisors were used for preparing sections in a plane perpendicular to the long axis of the tooth. The posterior teeth were excised as a single block and placed in 10% neutral buffered formalin. The first premolars were prepared for sections in a bucco-lingual plane; the remaining teeth were utilized for sections cut parallel to the occlusal plane.

Electron Microscopy: After 2 hours in osmic acid, the incisor blocks were removed and cut transversely into slices about 0.5-1 mm thick, on a rotary saw cooled with isotonic caccodylate buffer. After post-fixation for 2-4 hours in Karnovsky's fixative (Karnovsky 1965) the slices were demineralized in 0.25M EDTA buffered to pH 7.2 with sodium hydroxide pellets. When demineralization was complete, the slices were subdivided into smaller blocks suitable for electron microscopic study. These were fixed once again in 2% collidine buffered osmic acid, washed in buffer, stained in block in buffered uranyl acetate, and processed for embedding in epon (Luft 1961). Some blocks were dehydrated in acetone and embedded in an araldite-epon mixture. This resulted in no appreciable difference in the final preparation.

Light Microscopy: The posterior quadrants were trimmed to eliminate superficial tissue, and the first premolar separated from the remaining block which consisted of the second premolar and the first and second molars. These blocks were fixed in formalin for approximately one week, demineralized in a sodium citrate-formic acid mixture, and processed for paraffin embedding. Step-serial sections were prepared and stained with hematoxylin and eosin.

Results

The monkeys included in the experiment all developed mild to moderately severe gingivitis following the surgical procedure. The severity of the gingivitis and its distribution varied from animal to animal. The operated posterior segments generally appeared more severely inflamed than the control segments up to 6 months after surgery. Therefore, no difference was detectable in the severity of the gingivitis between the right and left side of the mouth. Periodic scaling alone did not succeed in controlling the ever present gingival inflammation. The anterior teeth demonstrated a similar degree of inflammation throughout the experimental period on both the experimental and control sides. No attempt to measure pocket depth was made in order to minimize any distor-
bance to the tissues adjacent to the experimen-
tally denuded root surface.

Histological evidence of both epithelial
and connective tissue reattachment was
found at all time intervals studied. Since
the experiment was designed primarily to
examine the structural features of the junc-
tion between the tooth and the regenerated
tissues, no attempt will be made to quan-
tify the healing response at the varying time
intervals. However, it was obvious, for ex-
ample, that thicker layers of new cementum
had formed after 18 months than after 1
month following surgery. Healing by an-
kylosis was noted in only one of the blocks
prepared for paraffin sections.

A. Regeneration of Junctional Epithelium
A new connection between the tooth sur-
face and the regenerated junctional epithe-
ilum (attachment epithelium) could be ob-
served in most specimens, at all time inter-
vals. The junctional epithelium had rege-
erated over denuded dentin (Figs. 1 and
3), as well as exposed cementum. The ultra-
structural features of the junction included
the presence of hemidesmosomes along the
cell membrane surface facing the tooth,
and a basement lamina, approximately 1000 A
thick, between the tooth surface and the
plasmalemma (Figs. 10, 11 and 12). This
attachment apparatus was morphologically
similar to that connecting the junctional
epithelium to the surrounding connective
tissue (Fig. 13). The epithelium was joined
to the tooth surface in this manner in speci-
mens which appeared relatively free of in-
flammation, as well as specimens demon-
strating a marked inflammatory cellular in-
filtrate within the adjacent connective tissue
(Fig. 2). Although the tooth surface under-
lying the junctional epithelium sometimes
resembled normal cementum or dentin,
with typical collagen fibrils of the mat-
rix extending up to the surface, the pre-
sence of superficial alterations in these
tissues was frequently noted. In cementum
these alterations took the form of a granu-
lar zone in which the collagen fibrils were
no longer distinguishable. In dentin, the su-
perficial tissue frequently consisted of a den-
sely staining, coarse, fibrillar material (Figs.
12, 14 and 15). At the light microscopic
level, this altered dentinal surface layer ap-
ppeared as a dense line, of somewhat ir-
regular width, which stained well with Azure
II in one micron thick sections of epon
embedded material (Fig. 3). A typical
dental cuticle was found in a single spec-
imen only, which was obtained one year
post-operatively. This cuticle was located
between the internal basement lamina of
the basement lamina facing the tooth and
the junctional epithelium and the cen-
tum. It was identified by its electron-
dense and relatively amorphous structure (Fig.
16). No dental cuticle was noted in the other
post-operative specimens examined. Control
specimens indicated that in non-operated
teeth, a dental cuticle could be more rou-
tinely detected in association with the junc-
tional epithelium than in the post-surgical
specimens.

The ultrastructural features of the dejux-
timal junction described above remain essen-
tially the same throughout the experi-
mental period. These features could be
noted at all time intervals and at speci-
mens taken from the region of dento-
epithelial junction.

Of particular interest was the frequent
occurrence of artificial splits within the
epithelium and between the epithelium and
the tooth surface. These artifacts ap-
peared to affect specimens embedded in para-
form more frequently than those embedded
epon. They were more readily noted in
coronal portions of the junctional epithe-
ilum than in the apical region, although
in certain blocks the entire junctional epithe-
ilum had become separated from the
tooth (Figs. 6-9). These artifacts occu-
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In controls, as well as in experimental material with respect to epithelial tears.

R. Regeneration of Cementum
Cementum repair could be noted at all time intervals. In most of the specimens examined, new cementum formed over exposed cementum, as well as dentin. Its development was most advanced in the most apical region of the surgically created defect, somewhat apical to the termination of the junctional epithelium (Figs. 8 and 9). In the great majority of specimens examined, an artifactitious split separated the newly formed cementum from the underlying dental tissue. This artifactitious split was generally noted between the regenerated cementum and the surgically exposed tooth structure. Reactive cementogenesis, adjacent to the surgically created defect sometimes produced new cementum over the intact cementum layer adjacent to the defect (Figs. 8 and 7). In this situation, artifactitious splits occurred with lesser frequency than over surgically exposed cementum or dentin.

A distinct line which stained metachromatically with Azure II could be noted at the interface of new cementum and the surgical defect in the tooth (Figs. 4 and 5). This line appeared to correspond to a relatively dense granular layer in electron micrographs. This layer varied in width from a barely visible coat over the surgically exposed tooth surface to a substantial layer more than 1 micrometer wide (Figs. 17, 24, 29 and 30). The width of this layer did not seem to be related to the healing time or to the nature of the exposed surface. When artifactitious splits occurred, they always involved the dense granular layer in preference to the newly formed cementum on the surgically exposed tooth structure (Fig. 25). Whereas root cementum normally contains bundles of collagen fibers as a distinct component of its organic matrix, the newly formed cementum was often devoid of well-defined fiber bundles. The initial cementum deposited over the exposed tooth structure consisted of individual collagen fibers frequently running parallel to the tooth surface or otherwise arranged in a haphazard manner. In the specimens obtained at longer intervals following surgery, in which the new cementum formed a relatively thicker layer, the superficial cementum resembled typical root cementum, with recognizable fiber bundles in its structure. The deeper layers, however, retained the morphological characteristics noted above for newly formed cementum (Figs. 19–30).

When the dense granular layer was diffuse, if not completely absent, it was sometimes difficult to identify with certainty the exact location of the junction between new cementum and old tooth structure (Fig. 28). The presence of dentinal tubules at the dentino-cemental junction, and the different arrangement of dentinal and cemental collagen fibrils in these tissues provided some clues (Fig. 26). Similarly, at cemento-cemental junctions, the old cementum frequently exhibited well defined collagen fiber bundles, whereas the adjacent newly formed cementum did not (Figs. 18, 19). The orifice of surgically exposed dentinal tubules was frequently invaded by and completely filled with collagen fibers from reparative cementum. Collagen fibers also appeared within the cytoplasm of odontoblastic processes, within dentinal tubules near to the exposed dentin surface (Figs. 26 and 27).

Discussion
In 1967 the first report was published that 2–4 months following gingivectomy the regenerated junctional epithelium can become reconnected to the enamel or cementum surface by newly formed hemidesmosomes and a basement lamina (Listgarten 1967). Since then, it has been shown that this at-
tachment apparatus can form in considerably less time (Listgarten 1972). In the present report, evidence is presented that a similar dento-epithelial junction also can be created against surgically exposed dentin. It is of interest to note that morphological alterations at the dentin surface did not interfere with the regeneration against dentin of a new junctional epithelium. This is not altogether surprising, since epithelial reattachment to an altered cementum surface can also take place (Listgarten 1967). Furthermore, epithelial explants in vitro can be induced to produce a morphologically similar junction against artificial substrates (Flaxman, Lutzeuer and Van Scott 1968), as well as against enamel (Taylor 1970). It should perhaps be noted at this point that epithelial regeneration studied on simple gingival biopsies may be difficult to interpret, since the sampling technique will almost certainly tear the junctional epithelium. The epithelium remaining on the tooth side of the gingival specimen can no longer be readily identified as junctional, sulcular or pocket epithelium (Innes 1970, Stahl et al. 1969).

Since a new junctional epithelium is able to originate from adjacent gingival epithelium, this suggests that the absence of keratinization in junctional epithelium is due to environmental factors rather than to a genetically predetermined epithelial characteristic. This is supported by the observation that junctional epithelium sealed in millipore chambers implanted subcutaneously will behave in a manner similar to keratinizing gingival epithelial explants, with both types of epithelium becoming keratinized (Innes 1971).

The chemical nature of the dentino-epithelial junction is not known, although a summary of its histochemical characteristics has been published recently (Rehstein 1967). In a similar experiment to that reported here, Cimasoni et al. (1963) indicated that the dentino-epithelial junction, as observed with the light microscope, contained a layer of material which reacted positively with the periodic acid-Schiff stain and with Alcian blue and colloidal iron. This suggested to them the presence of a polysaccharide material, which may or may not be closely associated with a protein moiety. These investigators also noted that these staining qualities resembled those of the lining of the dentinal tubules. From the information obtained in this study, it is quite possible that the stained layer described by these investigators corresponds in part to superficially altered dentin, as well as to the actual demineralized junctional epithelium.

The metachromatic line noted at the junction of new cementum and the surgically exposed tooth surface, described by other workers at the University of Michigan, has been by Innes and Stahl (1962) and Eriksen (1966) demonstrated to be a layer of reticular elastic fibers and by Beswick (1966) and Eriksen (1967) to be well vascularized. In our experiments, the importance of the presence of a reticular elastic layer at this junction is emphasized.

The tenacity of the junctional epithelium against detachment was noted by both Beswick (1966) and Eriksen (1967). The ability of the epithelial layers to prevent reattachment of the tooth structure of interest is obtained by the use of a reticular elastic layer. This would provide a true reattachment would still hold. The presence of this layer has also been reported by the
Balaza, Bloom and Swann (1966) suggested that the fine fibrillar layer noted over the articular surface represented an unusual form of collagen fibers, a proteoglycan (glyco-protein) or a glycosaminoglycan of the hyaluronic acid type, probably created by the adsorption of a macromolecular layer of the hyaluronic acid type to the cartilage surface. In that situation, of course, the layer remained as a surface layer.

McGaughey and Stowell (1967) reported the selective adsorption of mucins from saliva by hyaluronidase surfaces. The material stained strongly with periodic acid-Schiff reagent and Alcian blue. Staining was reduced by neuraminidase digestion. Selective protein adsorption by hyaluronidase has also been reported by Hay (1967, 1969).

It is conceivable that the mechanism of formation of the granular layer observed over surgically denuded root surfaces may be related to the hyaluronidase content of the mineralized tissue. Furthermore, since calcium ions appear to be required for the binding of proteins to hyaluronidase (McGaughey and Stowell 1967), it is also possible that chelation of EDTA during the demineralization process may have contributed to the formation of artefactual splits in relation to the surgically denuded surface.

The tendency for artificial splits to occur in association with the dense granular layer has led certain investigators to consider this region as a weak connection between the regenerated cementum and the exposed tooth structure (Hiatt 1970). It is, therefore, of interest to note that in a human specimen obtained 15 years after a clinically successful reattachment procedure, such a split could still be demonstrated (Beube 1966). This would suggest that the split was not present prior to histological processing, but was probably the result of it. This is supported by the observation, that during the subdivision of freshly obtained specimens, the junction between old and new tooth structure remained intact, whereas after demineralization these tissues could be readily separated. If the regenerated cementum and the existing tooth structure shrink to different extents during the demineralization procedure, such differential shrinkage might promote the formation of artefactual splits. Furthermore, such shrinkage artifacts would be more likely to occur in larger tissue blocks, such as those embedded in paraffin. The small size of the epon embedded tissue blocks may be partly responsible for the better preservation of the interface between new cementum and the underlying tooth structure.

On the other hand, as indicated previously, it is possible that the demineralizing process may have a direct influence on the junction between old and new tooth structure, thereby contributing to the formation of splits by directly affecting that interface. In any case, it would be inappropriate to relate the effect of histological processing on this tissue layer to its cohesiveness in the intact state, and consequently to its consideration as a potentially weak link within the regenerated periodontium.

References


