Ultrastructure of Regenerating Junctional Epithelium in the Monkey.*

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It has been established that after gingivectomy the junctional epithelium is reformed from the oral epithelium but there is little information on the regenerative potential of residual junctional epithelium. In this study reformation of the epithelial attachment in the monkey was followed ultrastructurally after surgical removal of all of or part of the original junctional epithelium by internal or external bevel techniques respectively. In both circumstances a new epithelial attachment developed from the adjacent gingival oral epithelium and residual junctional epithelium appeared to persist as small nests of cells adjacent to the cemento-enamel junction. Epithelialization of the gingival wound was rapid, taking place in as little as 5 days after the partial removal of the junctional epithelium by the external bevel technique and by 10 days in wounds in which the junctional epithelium had been completely excised. This difference in the rate of epithelialization seemed to be primarily related to the quantity of coagulum and cell debris present; the greater amount remaining after the internal bevel technique tending to retard epithelial migration and reattachment.

After more than 50 years of controversy, the nature of the epithelial attachment to the tooth surface was finally resolved by the ultrastructural studies of Listgarten¹ Frank and Cimasoni,² and Schroeder and Listgarten³ who were able to demonstrate a unique junctional epithelium joining the oral epithelium to the enamel surface.

Interest naturally focused on whether the epithelial attachment could be reformed after mechanical interruption, and a number of workers¹,⁴,⁶ showed that after gingivectomy, cells from the oral epithelium were capable of migrating to the tooth surface and forming a new attachment. In his initial study Listgarten¹ described complete healing at 2 months but subsequently¹¹ found that an epithelial attachment was complete 12 days after surgery. Kon, et al.⁶ and Taylor and Campbell⁷ have reported even shorter times but the former workers used only light microscopy in their study which limits identification of structures defined principally in ultrastructural terms, while Taylor and Campbell⁷ only made an incision through the epithelium so as to separate it from the tooth rather than removing it completely. Thus we can conclude that epithelial reattachment takes place rapidly but the minimum time necessary still remains uncertain.

The region of the gingival attachment comprises three types of epithelium: keratinized gingival oral epithelium, nonkeratinized (or parakeratinized) oral sulcular epithelium and junctional epithelium, which are all located in close proximity yet show distinct differences at the histological and ultrastructural level. The observation¹,⁷ that such a variety of tissues can arise from gingival epithelium normally committed only to forming keratinized oral epithelium has prompted much speculation as to the mechanisms that might influence epithelial differentiation in this region.

The work to be described here examines two points that arise from a consideration of the literature cited above: what is the minimum time necessary for a new epithelial attachment to form and does regeneration of this new attachment inevitably occur from gingival oral epithelium, even when portions of junctional epithelium remain after surgery? To answer these questions a longitudinal study of periodontal wound healing was undertaken using macaque monkeys.

MATERIALS AND METHODS

In order to ensure a clinically healthy periodontium before starting surgery, three adult macaque monkeys were subjected to a mechanical prophylaxis three times a week during an initial 4-week period followed by a weekly prophylaxis for a further 3 weeks. During the latter 3-week period the animals received drinking water containing 0.05% of chlorhexidine gluconate, which was continued for a further 1-week period prior to surgery.

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during which time no mechanical prophylaxis was carried out. At the end of this 8-week period, clinical assessment of the central incisor, second premolar and molar of each quadrant indicated a gingival index of zero and a plaque index close to 1 for each animal. Prophylaxis and surgery were carried out under general anesthesia using ketamine hydrochloride injected intramuscularly.

The teeth selected for surgery were the second pre-

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Figure 1. Diagram to show the surgical technique: a represents an external bevel incision to remove all the oral-vacuolar epithelium but leave a portion of the junctional epithelium. b is an internal bevel used to completely remove the junctional and oral-vacuolar epithelium. E = enamel, C = cementum, c.e.j. = cemento-enamel junction.

Figure 2. Histological section of control unoperated gingival tissues to show the effectiveness of the prophylactic regime. The oral aspect of the gingiva is covered by a thick keratinized layer and there are very few inflammatory cells evident beneath the junctional epithelium (Magnification, × 30).

Figure 3. Diagram summarizing the changes observed during periodontal healing after surgery. The upper series represents events around maxillary teeth, where some junctional epithelium was left. The lower series represents events around mandibular teeth, where all the junctional and subjunctional epithelium were removed. E = enamel, C = cementum, c.e.j. = cemento-enamel junction, j.e. = junctional epithelium.

molar and first and second molars. Different surgical techniques were carried out on the buccal aspect of the maxillary and the mandibular quadrants of the same side (Fig. 1). An external bevel incision was used to
Figure 4A. Light micrograph of an open embedded section from a maxillary specimen at 1 day. A thin layer of epithelium covers the wound and has migrated as far as the cemento-enamel junction (arrows). The areas outlined in the boxes are shown enlarged in the electron micrographs in B and C (Magnification, × 1,110). B. Electron micrograph of migrating epithelium close to the gingival crest. The epithelium is 3 to 5 cells thick and a mitotic figure is evident. The epithelium is separated from the connective tissue by a basal lamina BL on the left and from the enamel cuticle C by debris on the right (Magnification, × 2,000). C. Electron micrograph of migrating epithelium close to the cemento-enamel junction. The junctional cells contain numerous free ribosomes but few filaments. The debris on the right can be seen to consist of degenerating cells including erythrocytes and polymorphs. BL = basal lamina, C = enamel cuticle (Magnification, × 6,400).
remove all the oral sulcular epithelium but to leave a portion of the junctional epithelium of the maxillary teeth (Fig. 1, line a); this procedure is similar to a common gingivectomy. On the mandibular teeth an internal bevel incision was used to completely remove the junctional and oral sulcular epithelium (Fig. 1, line b). As it was not possible to tell clinically whether the junctional epithelium had always been removed as intended, the excised surgical tissue was fixed and processed to check our assumption by histological examination.

Biopsies were taken from both maxillary and mandibular teeth at 5, 10, 15 and 20 days after surgery, control biopsies also being taken from two unoperated teeth. Tissue was removed by separating the gingival tissue from the adjacent soft tissue with a scalpel and a cooled knife blade was used to divide the crown in a mesiodistal direction and to cut through alveolar bone and cementum in a horizontal plane. Undermined in this fashion the tooth fragment together with attached soft tissue was removed by inserting a chisel occlusally and gently prying.

The entire biopsy was fixed in cacodylate buffered 2% formaldehyde-2.5% glutaraldehyde at pH 7.4 for 4 hours and then decalcified in a 0.1 M solution of EDTA (sodium ethylene-diamine tetra acetic acid) containing 4% glutaraldehyde and 0.2 M sucrose. The solution was changed every 24 hours and decalcification was normally accomplished in 3 to 4 days. After a buffer wash the biopsies were divided into smaller blocks by slicing the gingival tissues coronal-apically. A few blocks were processed for paraffin wax embedding and histological examination; the rest were osmicated, dehydrated in alcohol and embedded in epoxy resin for electron microscopy.

Sections of the resin-embedded tissue cut at 1 μm on an ultramicrotome were stained with toluidine blue for light microscopic examination. Ultra-thin sections were cut and stained with lead citrate and uranyl acetate and examined in a Siemens 101 electron microscope.

RESULTS

Control Tissue (Fig. 2). The oral surface of the gingival epithelium consisted of a well developed orthokeratin-
ized layer which became parakeratinized at the gingival crest. The sulcus was lined by nonkeratinized epithelium. The junctional epithelium had proliferated slightly apically beyond the cemento-enamel junction, but there was little evidence of inflammation in the epithelium and only a few inflammatory cells in the connective tissue, testifying to the efficacy of the prophylactic measures.

**Experimental Tissue.** The major events observed are summarized in Figure 3. At day 5 the specimens from the maxilla, in which an external bevel technique had been performed, showed a continuous layer of epithelial cells extending from the gingival oral epithelium to the tooth surface in the region of the cemento-enamel junction (Fig. 4A). This layer consisted of one or two rows of squamous cells, which ultrastructurally showed little evidence of differentiation, possessing few desmosome attachments between cells and very few filaments within the cells. There were, however, considerable numbers of free ribosomes and some rough endoplasmic reticulum (Fig. 4C). The epithelium appeared to be migrating beneath a heterogeneous layer consisting of a small amount of fibrin, some inflammatory and red blood cells, and occasional degenerating cells (possibly representing residual junctional epithelial cells) all of which were situated adjacent to the dental cuticle (Figs. 4B and C). Numbers of mitotic figures were evident in the epithelial layer, in a region closer to the gingival crest (Fig. 4B).

An interesting finding in the maxillary specimens at both 5 and 10 days was the presence of a nest of epithelial cells adjacent to the tip of the advancing epithelial layer, at the cemento-enamel junction (Fig. 5A). These cells appeared to be completely surrounded by connective tissue, for serial sections at the light microscope level revealed no connection with the advancing epithelial layer or with papillae of the gingival oral epithelium. Ultrastructurally (Fig. 5B) the cells were clearly epithelial, possessing desmosomes and filaments and being enclosed by a continuous basal lamina. Occasional mitotic figures were also seen in these nests of cells.

The 5-day specimens taken from the mandibular teeth, where a complete removal of junctional epithelium had been attempted, presented a different picture. There was considerable debris between the tooth surface and the migrating epithelium (Fig. 6A), which had not advanced very far, only reaching the cemento-enamel junction in two out of three specimens. The epithelium showed a thick advancing front, instead of the thin layer seen in maxillary specimens and contained numerous inflammatory cells, extravasated red blood cells and fibrin remnants (Fig. 6B).

At 10 days after surgery the maxillary biopsies showed
a junctional epithelium some five to six layers thick, the surface cells contacting the enamel cuticle and attached to it in places by hemidesmosomes. In one specimen a nest of epithelial cells again was observed near the cemento-enamel junction. The 10-day mandibular specimens all showed migration as far as the cemento-enamel junction, although there was little evidence of intimate attachment to the enamel, possibly because of the considerable amount of cellular debris which still intervened.

By 15 days both maxillary and mandibular specimens looked very similar, with a junctional epithelium tapering down from 6-12 cell rows coronally to one to two rows apically. The cells adjacent to the tooth surface possessed hemidesmosomes inserted into a basal lamina. At 20 days the appearance of both maxillary and mandibular specimens resembled that of the control. The junctional epithelium was slightly wider than at 15 days, tapering to a tip two to three cells deep (Fig. 7A). The cell layer contacting the tooth surface bore numerous hemidesmosomes which were inserted along a basal lamina in an almost continuous row (Fig. 7B). The basal lamina was situated on dental cuticle or ameloblastic enamel of the cementum usually appearing as three to four appositional strata (Fig. 7A). Parakeratotic or nonkeratinized cells lined the oral sulcular region and keratinization started at the gingival crest.

**DISCUSSION**

Two points of interest arise from this study: (1) after surgical excision of the epithelial attachment, new junctional epithelium originates from the oral epithelium even when a portion of the original junctional epithelium remains, and (2) the rate of formation of this new epithelium is very rapid, a new attachment being formed in some circumstances in as little as 5 days.

It seems well established from the work of Listgarten and others that after gingivectomy a new epithelial attachment is formed by the cells that migrate from the cut edge of the gingival oral epithelium. This was evident in our study for at all time periods the epithelium cov-