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SCANNING ELECTRON MICROSCOPE STUDY OF THE HEALING MOLAR TOOTH EXTRACTION SOCKET IN THE RAT

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Abstract

Healing molar tooth extraction wounds in rats were examined by scanning electron microscopy from 15 minutes to 40 days following tooth removal. The wound epithelium, which was derived mainly from the gingiva but also from the cheek and hard palate, migrated beneath the superficial socket contents. The contents were lost between 5 to 11 days, thus leaving a central epithelial-lined depression. This decreased in width with time as the level of the wound epithelium approached that of the hard palate but was still present at 40 days. Between 5 and 7 days, the wound epithelium became more regular. However, from 11 days on, it became more irregular with increasing numbers of saucer-shaped depressions, circular defects and circular whorls of epithelial cells. The surface structure of the epithelial cells changed as it migrated and matured. The initially plump, then flattened cells mostly had smooth areas along with variable numbers of irregular microridges and microvilli, although cells derived from the cheek had only smooth surfaces. With further maturation, all cells developed a regular honeycomb surface pattern of interconnecting microridges similar to that on the hard palate. Why the wound epithelium became more uneven after 11 days is not known.

Key Words: Healing, tooth extraction socket, scanning electron microscopy.

Introduction

The extraction of teeth is still a common procedure, and the healing tooth socket is the most common surgical wound of the oral cavity. Tooth extraction is an unusual injury in that it creates a defect in the mucosa, the underlying bone and the periodontal ligament, all of which, potentially at least, may be exposed to saliva and the oral microflora. Many studies have reported on the healing process following tooth extraction in a variety of species in normal undisturbed sockets [12, 17, 19, 23, 34, 35, 40, 41], in sockets in which healing has been disturbed [2, 10, 24, 25], and in sockets that have had a drug or material placed on or in them [6, 43]. These and other studies, which have employed light microscopy (LM), radiography and occasionally biochemical techniques, have primarily been concerned with repair of the bony socket and have little detail regarding the structure of the migrating and maturing wound epithelium. Those that have reported on the epithelial changes in more detail all use the LM [40, 42, 43]. Those employing either transmission electron microscopy (TEM), scanning electron microscopy (SEM) or both confine their studies to an examination of the relationship of bacteria to the epithelial edge and surface of the wound [26], to a report on the presence of intracellular desmosomes in the wound epithelium [29], to an investigation of bone remodelling [39], to a brief mention in a review of oral wound healing [30] and to a brief mention in a study on the ecology of wound healing in the oral cavity [13].

A full understanding of the normal, uncomplicated healing of the tooth extraction wound is necessary before it is possible to understand the reasons for delayed healing and to prevent delay. The most common expression of this is the painful condition known as dry socket, a phenomenon which is reported in nearly 38% of cases following some types of tooth extraction [44]. Also, a fuller understanding of the mechanisms involved in uncomplicated healing following tooth extraction should contribute to the understanding of healing after preparation for and insertion of a dental implant.

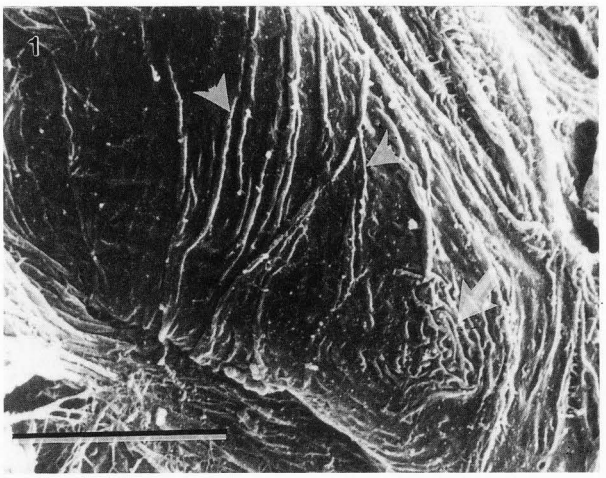

Figure 1. Fifteen minutes. Collapsed gingival epithelium with elongated microridges (arrowheads) that in places forms an irregular interconnected arrangement (arrow). Bar = 19 μm.

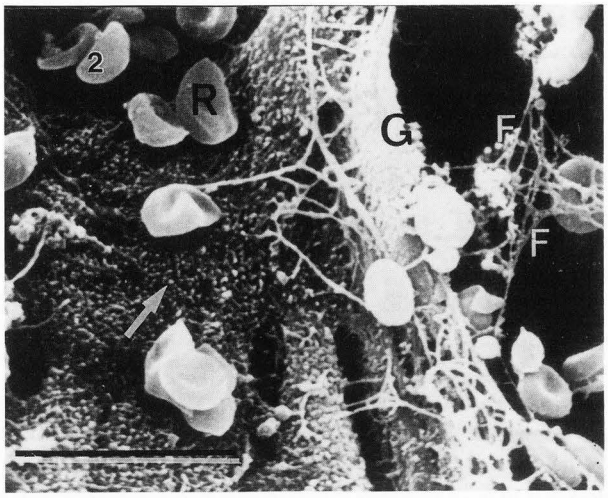

Figure 2. Fifteen minutes. Deepest edge of collapsed gingiva (G) with short microvilli (arrow) covering the epithelial cells. Red blood cells (R); fibrin (F). Bar = 14 μm.

The purpose of this study was to investigate, by SEM, the healing of the wound caused by the extraction of the first maxillary molars in rats with particular emphasis on changes in the migrating and maturing wound epithelium.

Materials and Methods

Eighteen male white Wistar rats, each 45 days of age, were anaesthetized with ether and placed on their backs in a jig which held their mouths open while maintaining anaesthesia. The maxillary left and right first molar teeth were removed. Following this, the animals were removed from the jig, placed in an empty clean cage and monitored for 10 minutes to ensure that they recovered from the operation. They were then returned to their normal cages and fed on a diet of stock pellets and water *ad libitum*. At 15 minutes and 1, 3, 5, 7, 11, 14, 24 and 40 days after the teeth were extracted, 3 anaesthetized rats were killed by cervical dislocation. The maxillae were rapidly dissected out, placed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3), separated into left and right segments, trimmed and left in the fixative at 4°C for at least 48 hours. All specimens were then rinsed in 0.1 M cacodylate buffer, washed in distilled water, dehydrated in graded concentrations of acetone and critical-point dried using liquid carbon dioxide. Following this, they were glued to aluminium stubs, sputter-coated with gold and viewed in the scanning electron microscope at 20 KV.

Results

There were no post-operative complications. A number of the extracted teeth had variable amounts of the buccal gingiva attached to them.

At 15 minutes, blood clot filled most of the sockets, although in 2 specimens the superficial blood clot was absent, exposing the deeper socket contents. The occasional bacteria was present on the exposed surface of the blood clot. The appearance of the gingiva that had collapsed into the socket varied. The superficial epithelium adjacent to the palate or cheek had a similar appearance to that on the undamaged hard palate. There were flat, polygonal cells with a honeycomb surface pattern of interconnecting microridges that surrounded depressions. Cell boundaries were formed by 2 parallel linear ridges separated by a narrow gap. Imprints of cells that had been desquamated were formed by single linear ridges. Deeper individual cells could not be seen, and the epithelium was covered by variable numbers of elongated microridges that were separated by smoother areas. In places, the microridges were arranged in an irregular interconnected pattern (Fig. 1). At the deep edge of the epithelium, cell boundaries were not seen, although there were some dilated intercellular spaces. The cells were covered by short blunt microvilli (Fig. 2). Below these were bundles of wavy and damaged collagen fibres, blood clot or both.

One day following tooth extraction, the superficial contents of the socket were made up of a mixture of

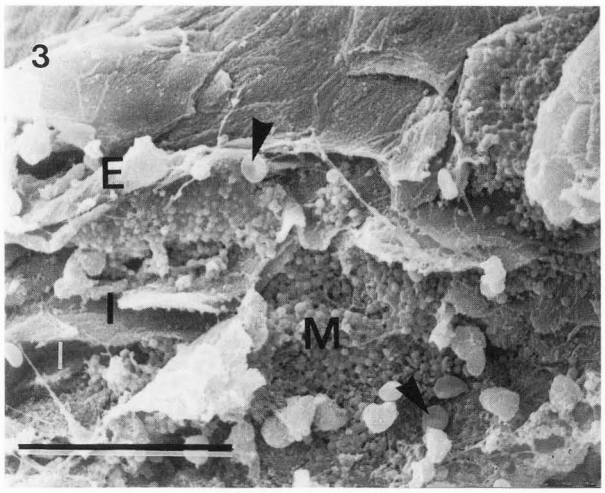

Figure 3. One day. Damaged palatal epithelium (E) with dilated intercellular spaces (I). Bacteria (M); red blood cells (arrowheads). Bar = 33 μm.

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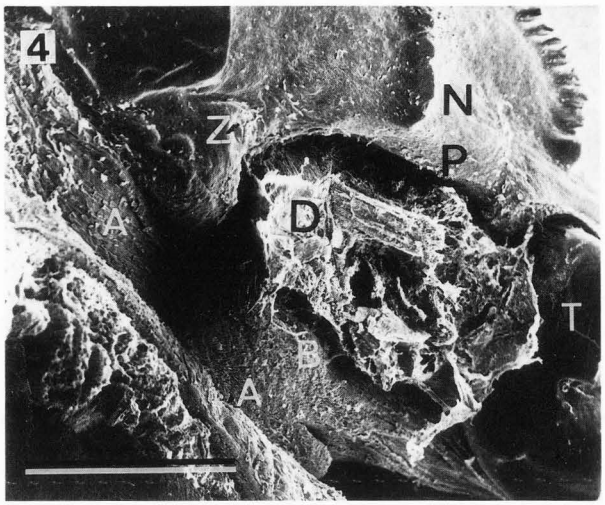
food debris, fibrin, damaged epithelial cells, leukocytes, bacteria, wood shavings from the cage bedding and occasionally, root remnants. The leukocytes were oval or spherical cells with variable number of folds and microvilli on their surface. The adjacent damaged epithelium had many desquamating cells and dilated intercellular spaces and was often heavily colonized by bacteria (Fig. 3). The surface structure of the most superficial epithelial cells of the old gingiva was similar to that in the 15-minute specimens. The deeper wound epithelium, which could be seen extending down beneath the superficial contents of the socket, was made up of plump elongated cells whose long axis was parallel to that of the socket. These cells were separated by distinct intercellular spaces. The surface of these cells was usually masked by bacteria, debris, and at times, leukocytes.

At 3 days, much of the palatal wound epithelium had a relatively smooth surface. Folded cheek-type epithelium extended into the socket buccally where the gingiva had been removed with the tooth (Fig. 4). The cells of this epithelium had a smooth surface devoid of either microridges or microvilli and lacked linear ridges corresponding to cell boundaries and cell imprints. The mucosa immediately rostral to the socket opening was irregular. Examination of the wound epithelium at a similar depth to that described for the 1-day specimens showed that although the elongated cells still had their long axis parallel to that of the socket, the cells themselves were much flatter (Fig. 5). Variable numbers of bacteria and leukocytes were present on the surface of this epithelium and associated with some of the dilated intercellular spaces. The surface of the epithelial cells was covered by an irregular pattern of interconnecting



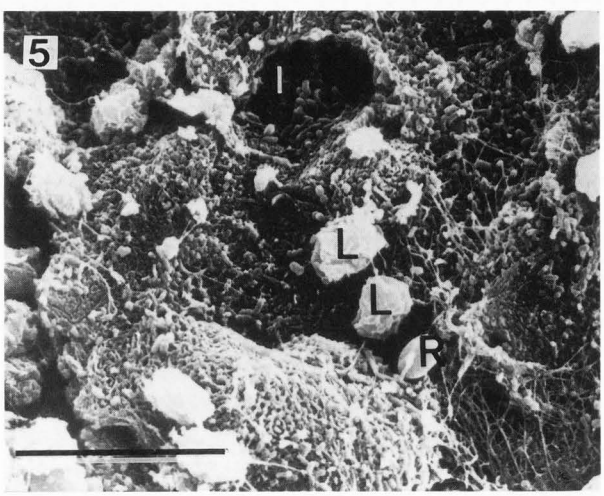


Figure 4. Three days. Palatal (P) and buccal (B) wound epithelium extending beneath the superficial debris (D). Cheek (A); hard palate (N); second molar tooth (T); irregular mucosa (Z) rostral to socket opening. Bar = 2 μm.

Figure 5. Three days. Palatal wound epithelium with dilated intercellular space (I). Leukocytes (L); red blood cell (R). Bar = 21 μm.

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microridges. The cells of the deeper wound epithelium were plump and elongated with their long axis parallel to that of the socket. They had relatively smooth areas and areas where there were variable numbers of irregular microridges and short microvilli. Often, numerous leukocytes almost totally obscured this epithelium.

By 5 days, the palatal-buccal width of the superficial socket contents was reduced. In 2 specimens, the superficial socket contents had been lost, exposing the deeper horizontal wound epithelium which lined the central

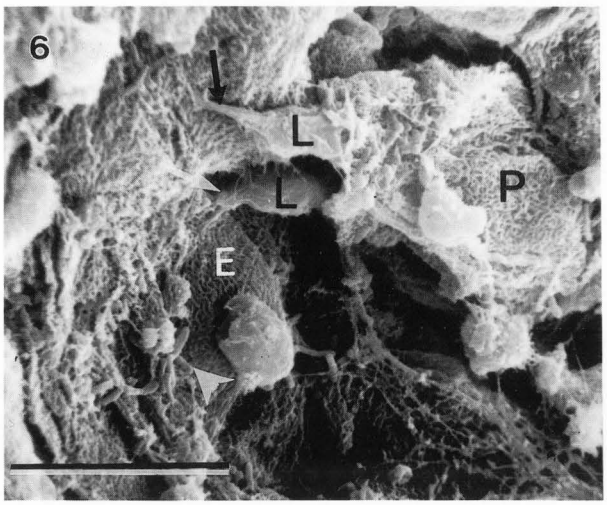

Figure 6. Five days. Deeper vertical (P) and horizontal (E) wound epithelium with a honeycomb surface pattern. Leukocytes (L) and pseudopodia (arrows); bacteria (arrowhead). Bar = 15 μm.

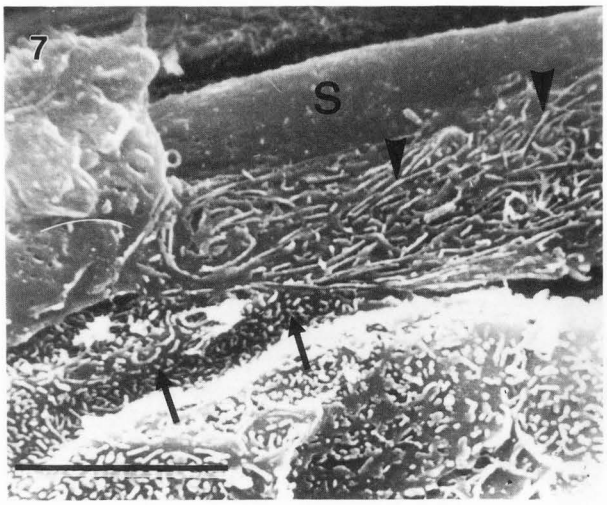

Figure 7. Five days. More central less mature horizontal wound epithelium with smoother areas (S), irregular microridges (arrowheads) and microvilli (arrows). Bar = 20 μm.

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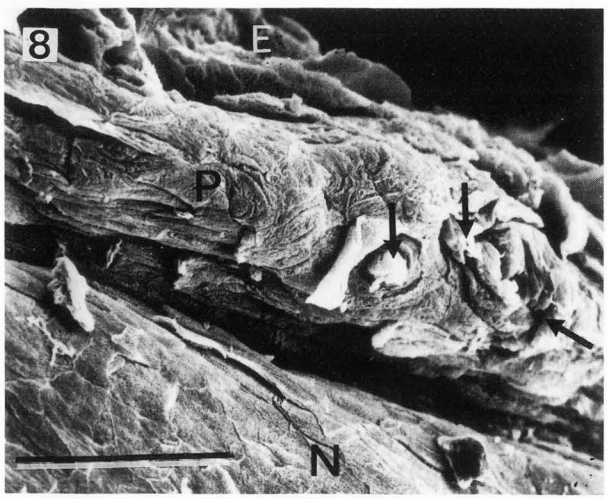
depression. The reduced width was due to a band of wound epithelium that was only at a slighter lower level than the surrounding mucosa. Although this band was most prominent on the palatal aspect of the sockets, it was also present anteriorly and to a lesser extent buccally. Much of this epithelium had an uneven folded surface and the junction between it, particularly the adjacent palatal epithelium, was abrupt. The surface of its cells had a distorted honeycomb surface pattern of interconnecting microridges; there were linear ridges corresponding to cell junctions and imprints of desquamated cells, and there were leukocytes. A similar structure was also found on the more superficial vertical wound epithelium lining the side of the central depression. On the deeper vertical wound epithelium and the peripheral horizontal wound epithelium the cells were flatter, usually had a more regular honeycomb surface pattern of interconnecting microridges and were often separated by dilated intercellular spaces. Leukocytes that often had pseudopodia, were found on this epithelium and in the dilated intercellular spaces (Fig. 6). The epithelial cells on the more central areas of the horizontal wound epithelium were also relatively flat but had a variable surface structure. In some areas, they were relatively smooth, while elsewhere there were irregular microridges, short blunt microvilli or both (Fig. 7). The intercellular spaces were often dilated, and there were leukocytes. Adjacent to the rostral aspect of the second molar teeth, the gingiva was developing. Varying numbers of bacteria were present in all areas.

Two of the 7-day specimens had substantial amounts of superficial socket contents and in these, much of the buccal wound epithelium was derived from the cheek. This wound epithelium had a folded surface with many deep furrows running parallel to the long axis of the socket. The surface of the cells was covered by an elongated pattern of interconnecting microridges, and there was only the occasional cell boundary. In the other 4 specimens, which contained little superficial socket contents, a rounded, relatively smooth buccal margin separated the wound epithelium from the cheek. In these, the central depression, which extended for a variable distance in the caudal-rostral direction, was narrower than at 5 days. Most of the buccal and palatal wound epithelium peripheral to this was relatively flat and was covered by flat polygonal cells with cell boundaries and imprints of desquamated cells similar to those on the uninvolved hard palate. The cells had a relatively regular honeycomb surface pattern of interconnecting microridges that surrounded depressions. Immediately adjacent to the central depression, on both the buccal and palatal aspects, the appearance of the wound epithelium varied. In some areas, it was similar to that derived from the cheek, although elsewhere the epithelium was thrown into a fold. There were isolated circular whorls of epithelial cells (Fig. 8). The arrangement of microridges on the cells was more regular in these smoother folded areas than in the areas of cheek-type wound epithelium, and there were linear ridges corresponding to cell boundaries and imprints. The gingiva on the rostral aspect of the second molar was better formed than at 5 days. The appearance of the epithelium lining the vertical walls of the central depression was similar to that at 5 days. The epithelial cells covering the



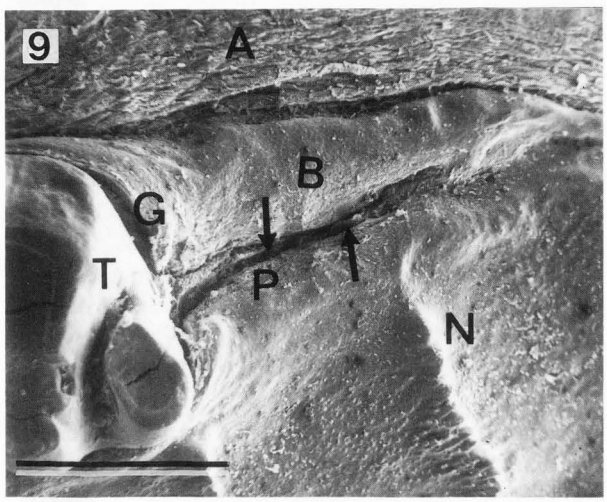


Figure 8. Seven days. More mature palatal wound epithelium (P) with circular aggregations of epithelial cells (arrows). Deeper palatal wound epithelium (E); edge of hard palate (N). Bar = 46 μm.

Figure 9. Eleven days. Socket with central depression (arrows) and buccal (B) and palatal (P) wound epithelium. Cheek (A); hard palate (N); gingiva (G); second molar tooth (T). Bar = 1 mm.

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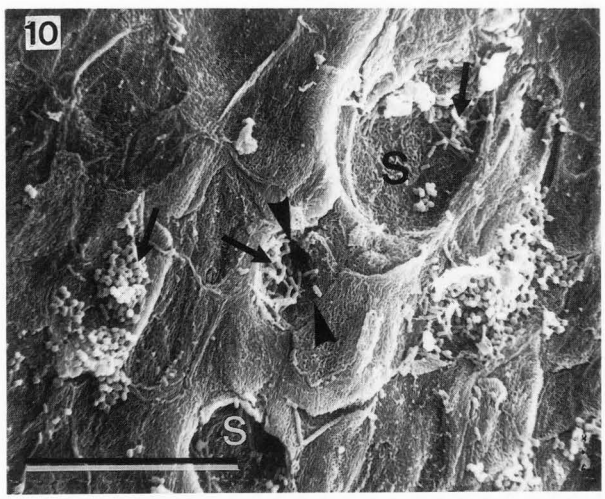
horizontal floor of the depression were often flat and covered by interconnecting microridges of varying regularity. Bacteria and leukocytes were present on all of the wound epithelium that lined the central depression.

In many respects, the 11-day specimens were similar to the 7-day ones that contained little superficial socket contents. However, the central depression was usually much narrower, and unless bone or tooth remnants were being sequestrated, it was lined by epithelium that had a similar structure to that seen at 5 to 7 days.



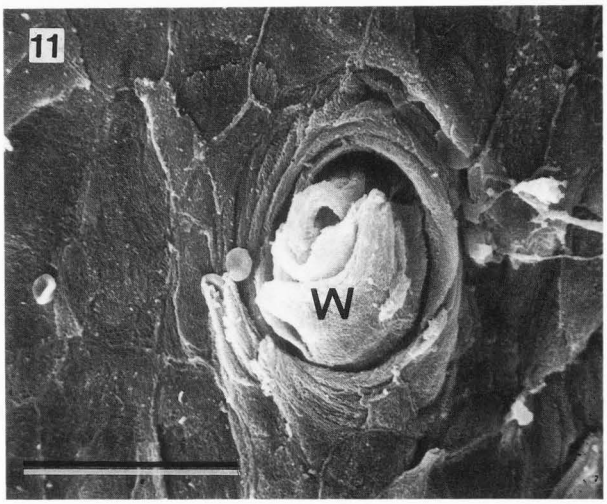
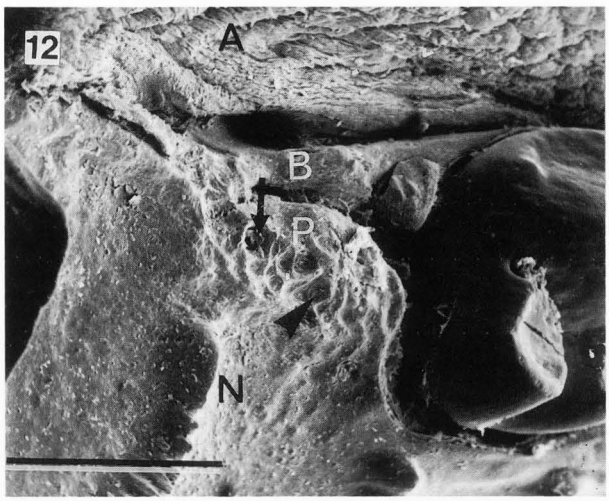


Figure 10. Eleven days. Palatal wound epithelium with saucer-shaped depressions (S), circular defect (arrowheads) and bacteria (arrows). Bar = 36 μm.

Figure 11. Eleven days. Buccal wound epithelium with an isolated whorl of epithelial cells (W). Bar = 33 μm.

Varying numbers of bacteria and leukocytes were present within the depression. At low power, much of the maturer buccal and palatal wound epithelium appeared relatively flat and blended in with the adjacent uninvolved epithelium (Fig. 9). The flattened polygonal cells of this wound epithelium had a similar structure to those on the uninvolved hard palate. However, closer examination of the maturer wound epithelium showed that in some places the surface was more uneven. There were shallow saucer-shaped depressions, circular defects (Fig. 10) and isolated whorls of epithelial cells that projected above the surface of the surrounding epithelium (Fig. 11). Leukocytes were present in a number of the



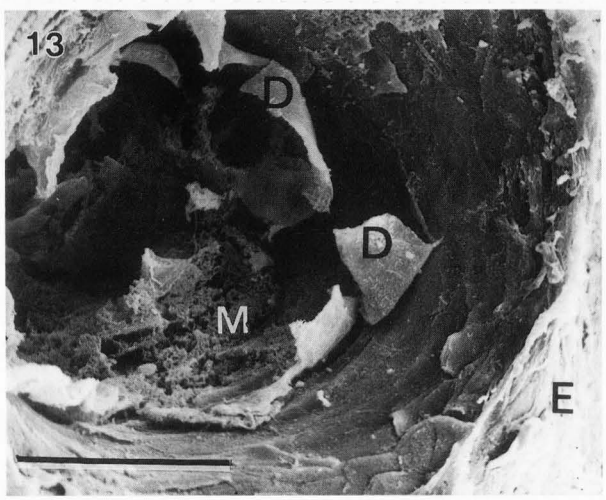


Figure 12. Forty days. Socket with uneven wound epithelium containing saucer-shaped depressions (arrowhead) and circular defects (arrow). Buccal (B) and palatal (P) wound epithelium; cheek (A); hard palate (N). Bar = 1.2 mm.

Figure 13. Forty days. Circular defect with desquamating epithelial cells (D). Bacteria and amorphous material (M); adjacent wound epithelium (E). Bar = 38 μm.

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surface defects. The epithelium covering all of these irregularities also had a similar appearance to that on the uninvolved hard palate, although at times the honeycomb surface pattern was not as regular, and the ridges forming cell boundaries and cell imprints were more pronounced.

A central depression of variable length was still present in all of the 14-, 24- and 40-day specimens. The structure of the epithelium lining this depression

Figure 14. Twenty four days. Saucer-shaped depression (S) on the buccal wound epithelium with desquamating cells (arrows). Bar = 100 μm.

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contents were similar to what was found in the 11-day specimens. The most obvious change with increasing age was the heightened unevenness of the buccal and palatal wound epithelium (Fig. 12), particularly at 24 and 40 days post extraction. This unevenness was caused in part by increasing numbers of surface defects, epithelial lined depressions (Fig. 12) and isolated whorls of epithelial cells.

Some of the surface defects were similar to those in the 11-day specimens, and at times, contained bone or tooth remnants. However, the majority were larger and more conical in shape. Variable numbers of bacteria, amorphous material, and sometimes, leukocytes were present on the surface defects' epithelial lined walls (Fig. 13). The epithelial cells were arranged in a radial fashion around the defect. The surface structure of this epithelium was similar to that on the uninvolved hard palate, except that many of the cells were being desquamated. The undersurface of the desquamating cells was covered by short microvilli and linear depressions, the latter being the cell boundaries or the imprints of the boundaries of the underlying cells. The microvilli and linear depressions complemented the honeycomb surface pattern of interconnecting microridges and linear ridges, respectively, on the superficial surface of the deeper cells.

Although some of the epithelial lined depressions in the 14-, 24- and 40-day specimens were similar to the saucer-shaped ones present at 11 days, most were of greater diameter and depth. They were lined by epithelial cells, of which some were desquamating (Fig. 14). Also, in the older specimens, most of the isolated whorls of epithelial cells were of larger diameter and were

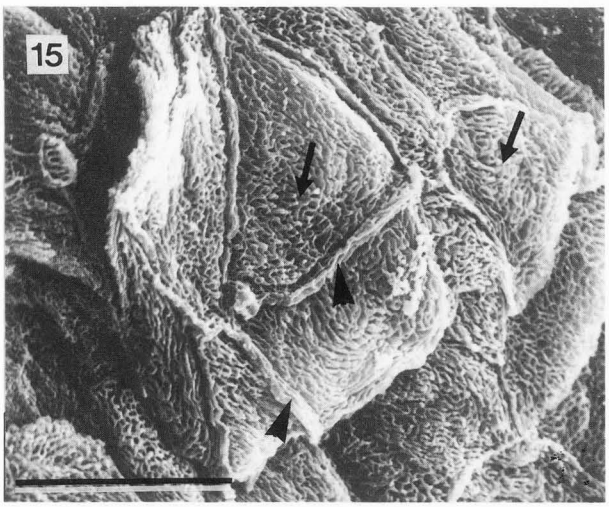

Figure 15. Fourteen days. Isolated whorl of epithelial cells (arrows). Bar = 100 μm.

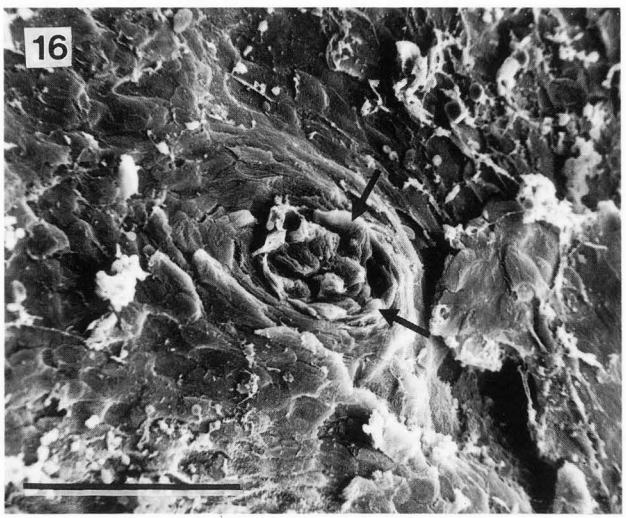

Figure 16. Twenty four days. More uneven wound epithelium with more irregular microridges (arrows). Broad linear ridges (arrowheads), that correspond to the imprints of overlying cells that have been desquamated, onto which the honeycomb surface pattern extends. Bar = 10 μm.

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formed by a greater number of more flattened cells (Fig. 15) than in the 11-day specimens. The structure of the epithelium lining the depressions and forming the whorls was similar to that lining the conical surface defects, as was the structure of the deep surface of desquamating cells

In the 24- and 40-day specimens, much of the epithelial surface between the defects, depressions and whorls was more uneven than in the 11- and 14-day specimens. The epithelial cells were not as flat, and the interconnecting microridges on their surface formed a more irregular pattern than elsewhere. Also, although cell boundaries were formed by parallel linear ridges, the gap between them was often of increased width, and the ridges that corresponded to the imprints of the overlying cells that had been desquamated were broader and more pronounced than in other specimens. Often, the honeycomb surface pattern extended onto them (Fig. 16).

Discussion

Overall, the changes seen by SEM up to 7 days following the removal of the teeth were what would have been expected from previous LM studies [12, 17, 19, 23, 34, 35, 40, 41]. However, by using SEM rather than the LM, a much broader and more detailed understanding was obtained of the superficial changes that occurred. In addition, the increased irregularity of the surface of the wound epithelium over the 11 to 40 day period would not have been expected from the LM studies.

The great majority of the leukocytes would be neutrophilic polymorphonuclear leukocytes, as they had an appearance similar to that described in previous SEM studies of such cells [20, 38]. Also, they are the main type of inflammatory cell associated with the superficial aspects of the healing tooth extraction socket [17, 24, 25].

Because of the anatomical configuration of the healing tooth extraction socket, much of the wound epithelium, at least for the first 5 days, was hidden by the superficial socket contents. Because of this, it was not possible to examine the migrating front of the epithelium. What could be examined, however, were changes in the surface structure of the wound epithelium during its maturation when separation occurred between the superficial socket contents and the epithelium, when the level of the wound epithelium approached that of the adjacent mucosa, or when the superficial socket contents were lost. Although it is known that maturation of the wound epithelium commences just behind its migrating front [17, 24, 25, 40, 41] rather than following epithelial union, this process has not been studied in any detail.

Most of the wound epithelium was derived from the buccal and palatal aspects of the socket. It was usually derived from the remaining gingiva but in specimens where segments of gingiva had been removed with the tooth, it was derived from the epithelium of the cheek, or occasionally, the hard palate. The structure of the epithelium on relatively undamaged areas of the collapsed gingiva in the 15-minute specimens was similar to that of the normal functioning gingiva [28]. The epithelium which had a similar structure to that on the uninvolved hard palate was either the buccal or palatal

gingival epithelium, while the epithelium where the epithelial cells had smooth surfaces interspersed with variable numbers of microridges was similar to crevicular epithelium. The epithelium that was covered by microvilli and had dilated intercellular spaces was a deeper junctional and crevicular epithelium exposed by separation of the epithelium during tooth extraction. Cells in the stratum granulosum and stratum spinosum of stratified squamous epithelium from different sites in a number of species have microvilli on their surfaces [7, 9, 11, 16].

By combining what was seen in different areas at the same time period with what was seen at different time periods, a distinct sequence of changes in the surface structure of the wound epithelium as it progressively covered the socket and matured could be determined. Although some of the changes were similar irrespective of the origin of the wound epithelium, others were not.

When the wound epithelium was derived from the gingiva and hard palate, the least mature areas of the epithelium consisted of plump, elongated cells whose surface structure varied. In some areas, the surface was smooth but elsewhere there were variable numbers of irregular microridges, microvilli or both. In the slightly more mature areas, the cells were flatter, although their surface structure was similar to that of the less mature cells. In both areas, the intercellular spaces were often dilated. In more mature areas, the surface of the cells was covered by an irregular pattern of interconnecting microridges and linear ridges corresponding to cell junctions; imprints of desquamating cells were found, as were some dilated intercellular spaces. With further maturation, dilated intercellular spaces became uncommon and the pattern of interconnecting microridges on the surface of the cells became more regular and formed a honeycomb pattern similar to that on the normal hard palate in the rat [27, 28], irrespective of the regularity of the epithelial surface as a whole.

Wound epithelium derived from the cheek differed in some respects from that derived from the gingiva and hard palate. Although in the least mature areas, it did resemble wound epithelium derived from the latter two areas, in the slightly more mature areas, it resembled that of the normal cheek in the rat [28]. The surface of the cells was smooth; linear ridges corresponding to cell boundaries and cell imprints were only occasionally found, and the wound epithelium was folded. In more mature areas, however, although cell boundaries and cell imprints were only occasionally found and the epithelium remained folded, the surface of the cells was covered by an elongated pattern of interconnecting microridges.

By 11 days, most of the epithelium derived from the cheek was indistinguishable from that derived from the gingiva and hard palate. This occurred as the level of the wound epithelium approached that of the hard palate. The elevation of the wound epithelium started buccally and palatally and progressed towards the midline, although a central depression persisted up to 40 days. This elevation appears to be brought about by an ingrowth of granulation tissue, mainly from the adjacent lamina propria and submucosa, which matures into the lamina propria and submucosa that covers the healed bony socket [17, 24, 35, 40, 41]. It is known that the development and maintenance of epithelial differentiation is controlled, in part at least, by the underlying connective tissue [5, 15, 22]. Thus, the change from cheek type to hard palate type of wound epithelium could have been brought about by the change in the underlying connective tissue, rather than by external stimuli such as function, friction [1, 23] or degree of stretch [31].

The changes in the structure of the wound epithelium in the present study have some similarities to what is found during healing of suction blisters in the skin of rats [33] and during healing of incisional and excisional wounds in the palate of rats [18]. In the former site, the surfaces of the migrating and regenerating epithelial cells are similar to those in this study in that some are smooth and others are covered by microvilli. In the latter site, there are microvilli on the surface of the cells immediately following epithelialization, while with maturation, ridge-like microridges are first seen, followed by a network-like arrangement of microridges. Finally, the microridges form a regular honeycomb surface pattern similar to what is found on the normal hard palate in the rat.

Complex interconnecting microridges are equated with a high degree of epithelial cell maturation while less organised microridges are equated with less mature cells. Microvilli are said to be present on even less mature cells than such microridges [42]. The findings in the present work and in another wound healing study mentioned previously [18] would tend to support this concept. However, such a generalization does not always hold. For example, the cells on the cheek epithelium in the rat, which is orthokeratinized, have a smooth surface [28]. The fact that migrating and regenerating epithelial cells often have microvilli on their surface is an important observation since such a feature has been equated with epithelial dysplasia and malignancy [14, 32, 37].

Most of the LM studies of the healing tooth extraction wound that comment on the structure of the maturer wound epithelium, present following complete epithelial coverage of the socket, describe a normal keratinized stratified squamous epithelium [17, 19, 34, 35, 40]. In one instance, this epithelium is described as being covered by a smoother layer of keratin than that on the surrounding uninvolved epithelium [35]. However, it is

clear from the present study that, for this experimental system at least, the surface of the maturer wound epithelium was more uneven than that of the surrounding uninvolved epithelium and that this irregularity increased from 11 to 40 days. This was in spite of the fact that the surface of the epithelial cells themselves retained, in the main, a regular honeycomb surface pattern of interconnecting microridges similar to that of the normal hard palate of the rat [27, 28]. Cell boundaries and imprints of desquamated surface cells on the maturer wound epithelium also had an appearance similar to those on the normal hard palate, although intercellular spaces and cell imprints on the maturer epithelium were sometimes wider. These two variations would eventually be interrelated, and were most commonly found in the 24- and 40-day specimens. The complementary nature of the deep or undersurface of desquamating cells and the superficial surface of the adjacent deeper cells is also similar to what is seen in the normal hard palate of the rat [27]. The similarities in these features to observances in the normal hard palate of the rat in most areas of the maturer wound epithelium at 11, 14, 24 and 40 days following tooth removal supported the LM finding that this maturer wound epithelium is a normal keratinized stratified squamous epithelium [17, 19, 34, 35, 40]. They also showed that the surface irregularities and defects were lined with normal mature epithelium, similar to that on the hard palate, although the nature of the epithelium lining the deeper aspects of the circular defects could not be determined.

The nature and origin of the saucer-shaped depressions is not known. However, because of their regularity, they were probably formed in the epithelium rather than being caused by irregularities in the underlying connective tissue. It is known that the basal cells of many stratified squamous epithelia are heterogeneous in nature [3, 4]. It has been proposed that all types of stratified squamous epithelia have a functional organization of cell growth similar to that of the epidermal proliferative unit (EPU) found in non-undulating thin epidermis, even though an obvious pattern of ordered structure is absent [8]. Each EPU is based on about 1 or 2 centrally situated stem cells, the progeny of which make up the rest of the EPU [36]. The stem cells have been equated with similarly situated cells that retain radioactivity from 3H-TfR for long periods; such cells are found in mouse oral epithelia [3], hamster palatal and tongue epithelia [4]. If a functional organization of cell growth does exist in the maturer wound epithelium, it could be postulated that, compared with that in adjacent areas, a decreased rate of cell division, in either the stem cell(s) or their immediate progeny which are also able to divide [8] could give rise to the saucer-shaped depressions. The depressions could also be the result of an increased rate of cell loss in the area or of a combination of both of these changes. However, even if such mechanisms are responsible for the saucer-shaped depressions, they do not explain how such localized differences might be initiated.

Isolated whorls of epithelial cells of similar appearance to those seen in the present study have been described in developing human palatal shelves [45] and during healing of deep excisional palatal wounds in rats [18]. In the former study, it is suggested that such whorls are associated with surface remodeling of the epithelium, while in the latter study, they are shown to be associated with epithelial down growth and keratin plug formation. It is also possible that they represent healing circular defects.

Although some of the circular defects contained bone or tooth remnants, many did not. They did contain varying numbers of bacteria and leukocytes along with amorphous material. It is thought that these defects were the oral openings of the epithelial lined sinus tracts which surround superficial fragments of necrotic bone and tooth and facilitate their sequestration during healing of the tooth extraction wound [2, 25, 34]; this process of the epithelial lined sinus tract is associated with varying numbers of neutrophilic leukocytes and bacteria along with necrotic material [2, 25, 35]. Similar sinus tracts are seen by SEM during the healing of deep excisional wounds in the hard palate of rats [18].

Changes in the underlying connective tissue and bone could also have contributed to the increased unevenness of the wound epithelium seen over the 11 to 40 day period. During this time, resorption, deposition and maturation of bone occurs not only in the more superficial aspects of the socket itself, but are also associated with the bone of the former alveolar crests. In addition, the connective tissue that separates the wound epithelium from the bone is being remodelled and is becoming more collagenous and less thick; although at 40 days, it is still much thicker than that in the adjacent hard palate [12, 25, 35, 41]. However, the changes taking place in the bone and connective tissue at 40 days are much less than those occurring at 11, 14 and 24 days, at least as far as can be determined in the LM [12, 35, 41].

It is apparent from the present study that changes in the wound epithelium continue up to at least 40 days following extraction of the first maxillary molar teeth in rats. These changes could be related to changes in the underlying bone or connective tissue, to changes in the epithelium itself, perhaps in part due to masticatory trauma, or to changes in some or all of these tissues. This latter alternative would be the most likely. The reason that the unevenness of the wound epithelium should increase rather than decrease from 11 to 40 days is not known and requires further investigation.

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Discussion with Reviewers

C. Piacentini and C. Marchetti: Why was post-fixation in OsO_4 not used?

Author: We have never found such post-fixation to be necessary for a wide range of SEM specimens with glutaraldehyde alone giving excellent results. This is so even for delicate specimens such as ethylenediaminetetraacetic acid (EDTA) separated epithelium from the hamster cheek pouch. Such epithelium that has been reprocessed for the LM confirms the excellence of the fixation [see: McMillan MD, Kerr MA (1990) A light and scanning electron microscope study of epithelial thickenings and rete-ridges in the adult hamster cheek pouch. Archs oral Biol **35**: 235-240]. Also, if OsO_4 is used, the tissues are stained black, this means that sections from specimens that have been reprocessed for the LM cannot yield useful information following routine staining because the black colour masks cellular and tissue detail.

Reviewer V: Without using OsO_4 in your experiments, how can we know that all macromolecules are properly fixed and the surface anatomy really represents the original detail?

Author: Please see above. In addition, when I first started looking at the structure of rodents oral mucosa in the early 1970's, I compared the effects of 12 different fixative/preparative regimes for SEM which included using just glutaraldehyde and glutaraldehyde followed by OsO_4. There were no differences in the structure of oral mucosa from the cheek, hard and soft palate, and gingiva, between those specimens fixed in glutaraldehyde alone and those fixed in glutaraldehyde followed by OsO_4. Most of this investigation has not been published although some has [McMillan MD (1974) A scanning electron microscopic study of keratinized epithelium of the hard palate of the rat. Archs oral Biol **19**: 225-229]. The structure of the mucosa of the normal hard palate adjacent to the healing tooth sockets in the present study is similar to what was described in my 1974 study. Also, I have reprocessed some of the SEM specimens from the present study for LM and the tissues and cells appear similar to what is seen when specimens are processed routinely for LM following formalin fixation.

P.D. Chemello: In your description of the healing molar socket of rats, do you think the healing would be

different (quicker or slower) if the sockets had primary approximation of the epithelial edges versus allowing healing by secondary intention?
Author: Because of the size of the wound created by the removal of the tooth and the fact that there is a defect within the bone, healing will always be by secondary intention, irrespective of whether the epithelial edges are approximated or not. Because of the small amount of gingiva compared with the width of the socket, I do not believe that true approximation of the epithelium could be obtained without surgery to reduce the width and height of the bony socket. It may also be necessary to raise a mucosal flap to achieve approximation. However, the partial coverage of the socket opening by suturing the gingiva would help ensure that normal healing took place because it would help haemostasis and prevent premature loss of the blood clot.

Reviewer V: For proper orientation, can a scanning electron micrograph at low magnification be provided?
Author: The accompanying low magnification micrograph (Fig 17) should help in orientation; it should be viewed along with Figures 4, 9 and 12. It is taken from the buccal or cheek side of the socket.

Reviewer V: Were light microscopic examinations performed and with what results?
Author: The present study is part of a much broader ongoing study into various aspects of experimental tooth extraction wound healing employing LM, SEM and TEM. Some of the LM studies used animals that had their teeth extracted at the same time as those used in the present SEM study. In addition, one SEM specimen from each time period in the present study has been reprocessed for LM; the changes in the LM seen were similar to what has been described previously for a number of species [12, 17, 19, 21, 34, 35, 40, 41].

Reviewer V: What part of the gingival epithelium does Figure 1 represent?
Author: Figure 1 represents the collapsed gingival epithelium on the palatal aspect of the socket from a similar area to that marked with the white arrow on Figure 17. Its position and surface structure is consistent with that of the epithelium that lines the gingival crevice. It is known from LM studies and from SEM examination of the extracted teeth from the present study (not reported) that nearly all of the palatal gingival tissues remain in the animal and are not removed with the teeth.

Reviewer V: In Figure 2, can those cell-like elongated structures with microvilli-like projections be cellular exudates or secretions (these are commonly seen if specimens are not properly washed)?

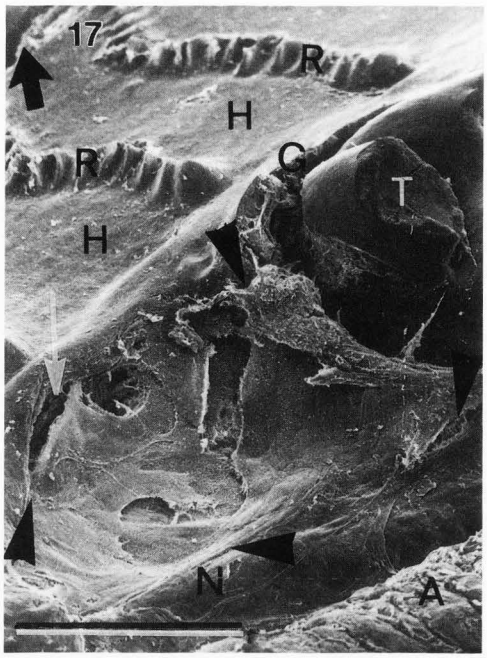

Figure 17. Low magnification micrograph. Fifteen minutes after extraction, the socket opening (arrowheads) is full of blood clot. The palatal gingiva (G) adjacent to the second molar tooth (T) has been damaged during tooth extraction. Deeper collapsed palatal gingiva (white arrow); buccal gingiva adjacent to socket (N); cheek (A); normal hard palate (H) with rugae (R); trimmed edge of specimen (black arrow) which was the mid-line of hard palate. Bar = 1 mm.

Author: I am unsure what type of cellular exudate or secretion in the present situation could give rise to such an appearance. As the specimen was removed 15-minutes following tooth extraction, the main exudate present, if such a term can be used, was blood clot. This consisted of an interconnecting network of fibrin fibres and red blood cells, along with some smaller structures that were thought to be platelets. Another secretion that could have been present was saliva. However, in my experience, when saliva remains on the surface of oral mucosa, it has a more film-like homogenous appearance in the SEM although sometimes it may have a fibrous or stringy appearance. As the fixative used was an aqueous solution, most of the mucus component of the saliva would be dissolved. Also, TEM studies are also being performed on similar material. When areas, such as that depicted in Figure 2 are examined, the surface of the epithelial cells are covered by small surface projections that are consistent with short microvilli.