

Distribution of the epithelial rests of Malassez and their relationship to blood vessels of the periodontal ligament during rat tooth development

Background: There is some evidence that the epithelial cell rests of Malassez partition the root surface from the periodontal ligament blood vessels, and may protect the root from resorption.

Objective: The aim of the present study was to determine the distributions of the epithelial rests of Malassez (ERM) and blood vessels in the periodontal ligament (PDL) of the developing rat first molar before, during and after emergence.

Methods: Four Sprague-Dawley rats were sacrificed at two days, one week, two weeks, three weeks, four weeks and six weeks of age. After processing, the maxillae were embedded in paraffin, and sectioned longitudinally and transversely. The sections were stained with a double immuno-histochemical technique which utilised a keratin antibody AE1-AE3 (1:2,000) and an endothelial antibody Factor VIII (1:10,000) to enable simultaneous labelling of ERM and blood vessels. ERM and blood vessel counts were obtained from the mesio-buccal roots of three week, four week and six week-old rats, whilst qualitative observations were made for the earlier developmental stages.

Results: ERM cells and cell clusters were found in the tooth third of the PDL width at the three, four and six week stages. Cells and cell clusters increased in number with age, especially in the upper third of the mesio-buccal root. The largest numbers of cells and clusters were found on the distal surfaces of the roots in all age groups. Cells and clusters in all root surfaces increased from three to four weeks, but decreased from four to six weeks. The

greatest number of blood vessels was found in the bone-side third of the PDL. The distal surface had the highest proportion of blood vessels, and the palatal surface the least proportion. The number of blood vessels in all surface quadrants did not vary much from three to four weeks of age, but increased from four to six weeks of age, possibly as a reaction to tooth emergence and occlusal function.

Physiological root resorption was only observed after tooth emergence, and appeared to be related to loss of continuity of the ERM network and the incursion of blood vessels.

Conclusions: Orthodontic root resorption can be regarded as an exaggerated response to loss of PDL homeostatic control, possibly mediated by the epithelial rests of Malassez.

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Introduction

The epithelial rests of Malassez (ERM) were first fully described by Malassez in 1885¹ who found a network of epithelial cells around the tooth root, which appeared to persist into adulthood. In histological sections, the ERM appear as clusters or aggregates of four to 20 solid round to oval cells, or as strands of cells around the periodontal ligament (PDL) along the length of the tooth root.²⁻⁴ The ERM are derived from Hertwig's

epithelial root sheath (HERS),³⁻⁵ which is a sheet-like structure made up of a double layer of epithelial cells.⁷ It forms the outline of the root during root development and is made up of two parts: the sheath, which grows around the dental papilla, and the rim of the sheath, known as the diaphragm, which encloses the primary apical foramen. Root elongation occurs as a result of mitotic activity in the cells of the papilla adjacent to the HERS, which express the odontoblast phenotype and synthesize dentine.^{3,6} Early investigators⁸⁻¹⁰ believed that the cells of HERS then either migrated into the PDL where cell apoptosis occurred following mineralisation of the mantle dentine or became incorporated into forming cellular cementum. However, the fate of these cells has yet to be ascertained.

It is often believed that the surviving cells and fragments of HERS form the network of cells known as the ERM.^{3-5,11} Gurling and Sampson⁷ have postulated that with age HERS unfolds as the epithelial cells of the outer layer migrate apically, and add to the apical extremity of the sheath. The inner layer of cells abutting the dentine separate from the enamel organ, and ultimately from the surface of the root. They become the ERM and are carried occlusally within the developing PDL during tooth eruption. The ERM are generally found in the PDL in close proximity to the cementum.^{3,12,13} Reeve and Wentz¹⁴ noted that the distribution of ERM along the length of the root appeared to alter with age. The ERM were more prevalent in the apical third of the root in the first two decades of human life. In later years, the ERM were more numerous in the upper third of the PDL. Yamashiro *et al.*¹⁵ noted more ERM in the bifurcation of rat molars, although this finding was not confirmed by Reitan² in humans.

ERM were thought to be functionless, but this has since been disputed. The ERM have been implicated in the formation of dental cysts,^{13,16} and the inflammatory response in the periodontal ligament in response to bacterial infection.¹⁷ They have also been shown

to be involved in bone resorption related to orthodontic tooth movement.^{18,19,21} In fact, Talic *et al.*²⁰ demonstrated that the ERM proliferated and increased in size during experimental tooth movement. Brice *et al.*²² noted ERM in resorption lacunae undergoing repair, whilst Kittel and Sampson²³ and Leedham²⁴ reported that blood vessels only occurred between the root surface and ERM in areas of active resorption. There appears to be a relationship between the ERM, the blood vessels of the PDL, and root resorption. The PDL has a rich vascular supply, whose primary role is to maintain the health and function of the periodontal tissues. The blood vessels have an important function during inflammation and repair of the PDL. Sims²⁵ and Douvartzidis²⁶ noted an increase in blood vessel volume from the upper to apical regions in the PDL of mice and monkeys. Furthermore, most of the vessels are found in the bone and middle third of the PDL rather than close to the tooth surface.^{27,28}

In the present study, immunohistochemistry has been used to visualise the ERM and PDL blood vessels, and to test the hypothesis that the ERM partition the root surface from the PDL blood vessels. The aims were: to evaluate the development and distribution of ERM and blood vessels around and along the length of the mesio-buccal root of the first molar in the rat, to investigate the relationship between the epithelial rests of Malassez and blood vessels and, to develop an immunohistochemical method for simultaneous demonstration of ERM and PDL blood vessels.

Materials and methods

Four Sprague-Dawley rat pups were sacrificed at two days, one week, two weeks, three weeks, four weeks and six weeks of age. A total of 24 rats was used. The maxillae were dissected out, fixed in 10 per cent neutral buffered formalin, decalcified in 4 per cent EDTA, and embedded in paraffin blocks. For each age group two maxillae were sectioned longitudinally, and the other two

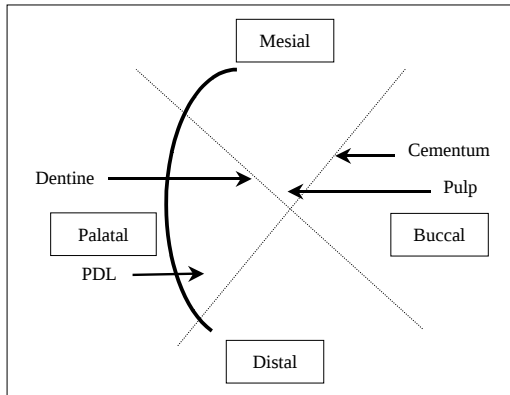


Figure 1. Root surface quadrants.

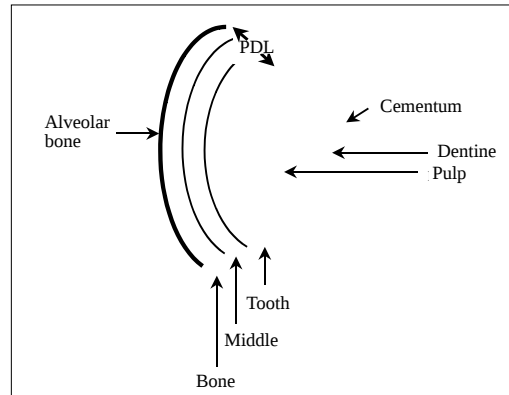


Figure 2. Periodontal ligament thirds.

maxillae were sectioned transversely into 5µm sections. Every twentieth section was selected for investigation.

After dewaxing, the sections were treated with 0.5 per cent hydrogen peroxide to block any endogenous peroxidase. Microwave antigen retrieval was then performed in a Sharp 700 watt oven for 2 minutes 15 seconds on high power, and 3 minutes on low power. Sections were further treated with 0.015 per cent trypsin II (Sigma Chemicals, USA) for 3 minutes. The sections were incubated with mouse anti-epithelial keratin AE1/3 (Cell Marque, USA) at a concentration of 1:2,000 overnight in a humid chamber. AE1 is a basic keratin and AE3 an acidic keratin antibody. The next day, after incubation with a secondary antibody (rabbit anti-mouse IgG) and streptavidin peroxidase (Pierce, USA), the sections were stained with the chromogen, Vector SG (Vector Laboratories, USA), which stained the ERM blue. After rinsing with phosphate buffered saline, the sections were incubated overnight with rabbit anti-mouse Factor VIII (Dako, USA) at a concentration of 1:10,000. The following day, after incubation with the secondary antibody (rabbit anti-mouse IgG) followed by streptavidin peroxidase, the sections were treated with DAB (Vector Laboratories, USA), which labelled the blood vessels with a brown stain. Finally, the sections were counter-stained with methyl green, and mounted on glass

slides for examination with a light microscope.

Cell and blood vessel counts

Transverse sections of the mesio-buccal root of the first molar were used for the ERM and blood vessel counts. The longitudinal sections were used for orientation and descriptive purposes. The ERM and blood vessel counts were obtained for the three week-old, four week-old and six week-old rats only as there were no ERM present in the younger animals. A reference graticule was used to divide the cross-sections of each mesio-buccal root (on the left and right sides of the maxilla) into four quadrants – mesial, distal, buccal and palatal (Figure 1). The PDL in each quadrant was also divided into three sections: tooth third, middle third and bone third (Figure 2). Finally, from the sections obtained, the root was divided into upper, middle and apical thirds (Figure 3). Counting was commenced from sections where the furcation was noted, and results were not obtained at the level of gingival attachment. This produced an average of nine levels along the mesio-buccal root (except for the three week-old rats, which produced an average of five levels). The number of ERM single cells, cell clusters and the blood vessels in each root surface, PDL section, and root third were counted. The presence of root resorption lacunae on the root surfaces and along the length of the root

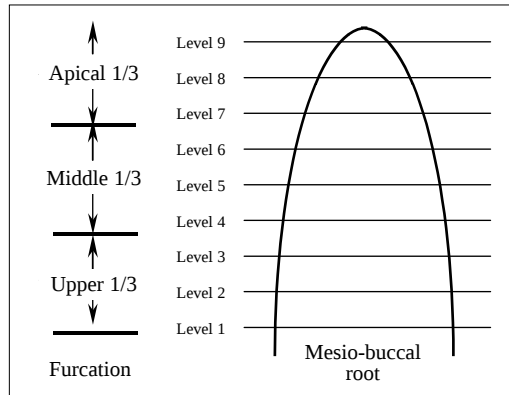


Figure 3. Mesio-buccal root thirds and levels at which sections were obtained for immunostaining and observation of ERM and blood vessel relationships. Counting was only commenced from sections where furcation was visible. Cervical tissue was not included in the counts, resulting in an average of nine levels per root for the older animals.

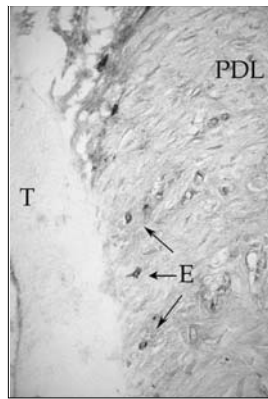


Figure 4. Longitudinal section of rat first molar depicting ERM single cells (E). T, tooth root. (Original magnification: 40x)

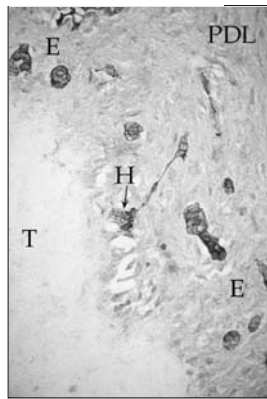


Figure 5. Cross-section of rat first molar depicting ERM clusters (E) and HERS (H). T, tooth root. (Original magnification: 40x)

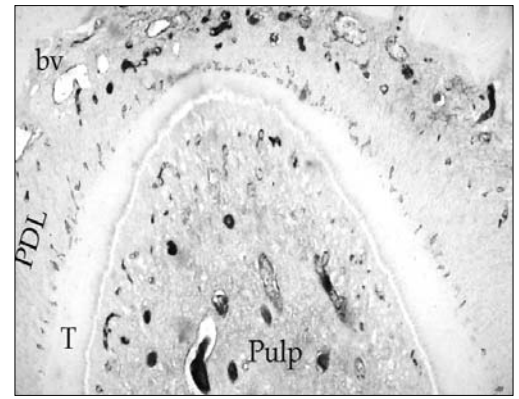


Figure 6. Distal surface of a three week-old rat mesio-buccal root in transverse section with a network made up of ERM and HERS cells in the tooth third of the PDL. HERS are the cells still adherent to the tooth root (T) surface. This also shows the distribution of blood vessels (bv) in the bone and middle thirds. (Original magnification: 20x)

were recorded also. The limited number of specimens precluded a robust statistical analysis.

Results

The inner and outer enamel epithelia of the enamel organ displayed good AE1/AE3 immunoreactivity at two days, one week and two weeks, particularly the cervical loop and initial HERS formation. The blood vessels surrounding the enamel organ were well demonstrated with Factor VIII. ERM were detected around the mesio-buccal root of the

first molar from three weeks of age. The ERM were observed to form a network around the root in both longitudinal and transverse sections. In the transverse sections the ERM presented either as single round or ovoid cells or as clusters of cells (Figures 4 and 5). Both forms of ERM cells and clusters were found in the tooth third of the PDL (Figure 6), and both cells and clusters increased in number with age. The vessels of the PDL were observed mainly in the middle and bone third of the PDL in all age groups. Furthermore, blood vessels located in the

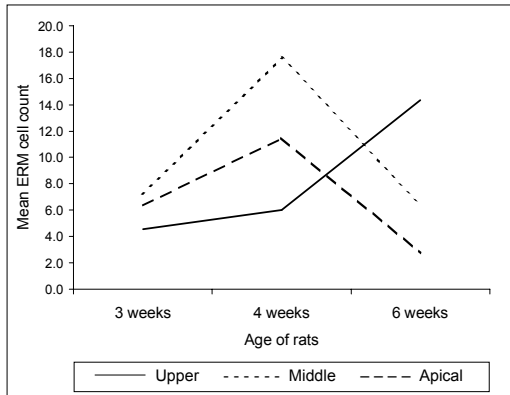


Figure 7. Line graph depicting mean ERM single cells along the length of the mesio-buccal root.

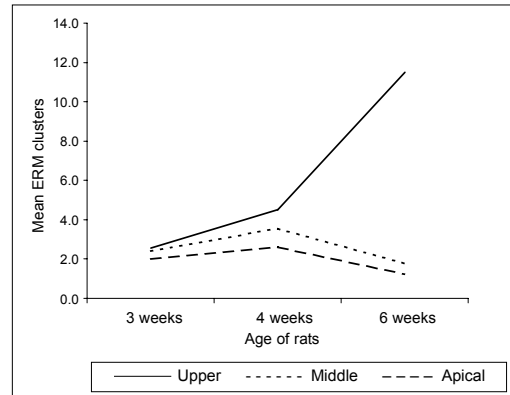


Figure 8. Line graph depicting mean ERM clusters along the length of the mesio-buccal root.

bone third appeared much larger than those in the middle third of the PDL. Physiological root resorption, in the form of resorption lacunae, was also present in the four and six week-old rats.

ERM counts

Transverse PDL thirds

At all the vertical levels and at all ages, ERM cells and clusters were noted in the tooth third of the PDL. No ERM cells or clusters were found in the middle or bone third of the PDL.

Vertical root thirds

At three weeks of age, single ERM cell counts were highest in the apical third of the forming root, and lowest in the upper third of the root. However, by six weeks of age, the cell counts in the middle and apical thirds of the root had decreased whilst counts in the upper third had increased (Figure 7). Generally, ERM cluster counts were highest in the upper third of the root at all ages. These counts continued to increase with age (Figure 8).

Root surface quadrants

In the upper third of the root, there was an increase in ERM cells and cell clusters with age. Although a higher proportion of cells was found on the buccal surface, most of the clusters were found on the buccal and distal

surfaces. In the middle third the number of ERM increased between three and four weeks of age, then fell between four and six weeks of age. Most of the cells and cell clusters in the middle third occurred on the distal surface. In the apical third, cells and clusters increased also between three and four weeks, and fell between four and six weeks of age. There was considerable variation in the distribution of cells on the various surfaces at all ages. Most of the clusters were noted on the mesial surface of the mesio-buccal root.

Blood vessel counts

Transverse PDL thirds

Most of the blood vessels were observed in the bone and middle thirds of the PDL. In the four and six week-old animals, several blood vessels were found in the tooth third.

Vertical root thirds

At three weeks of age, no blood vessels were found in the tooth third of the PDL. The largest number of vessels was found in the middle third of the root length and the least in the upper third. At four weeks of age, the upper third of the root length had the largest number of vessels. Similar counts were noted in the middle and apical thirds of the root length. Some blood vessels were noted in the tooth third of the PDL. However, their numbers were small compared with the

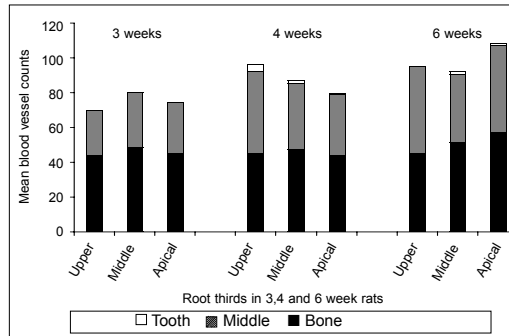


Figure 9. Mean blood vessel distribution along the root thirds.

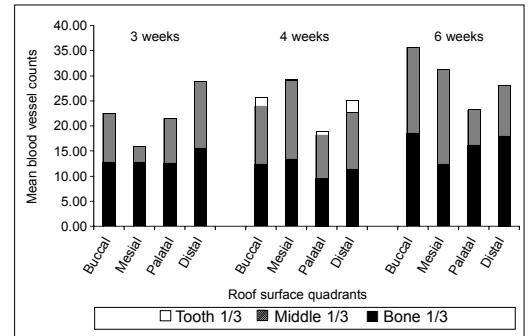


Figure 10. Mean blood vessel distribution around the root surfaces (upper third).

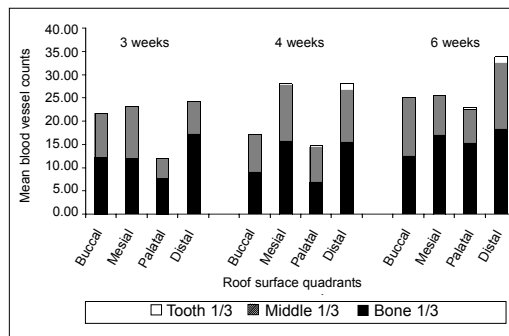


Figure 11. Mean blood vessel distribution around the root surfaces (middle third).

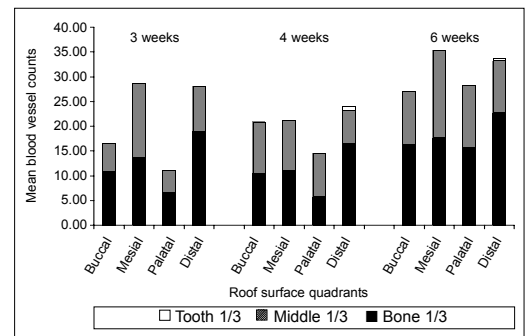


Figure 12. Mean blood vessel distribution around the root surfaces (apical third).

numbers of blood vessels found in the other thirds of the PDL. Similar numbers of blood vessels were found in the vertical root thirds at six weeks of age (Figure 9).

Root surface quadrants

Upper third

At three weeks of age, the largest number of blood vessels was noted on the distal surface. No blood vessels were found in the tooth third of the PDL. By four weeks of age, the largest number of blood vessels was observed on the mesial surface. Some blood vessels were also found in the tooth third of the PDL. At six weeks of age, the number of blood vessels increased slightly. The largest number of blood vessels was noted on the buccal surface of the root followed by the distal surface of the root. There were almost no blood vessels in the tooth third of the PDL (Figure 10).

Middle third

The number of blood vessels increased gradually with age. At three weeks of age, most of the blood vessels were observed on the mesial and distal surfaces of the root, and no blood vessels were found in the tooth third of the PDL. At four weeks of age, the largest number of blood vessels was once again noted on the mesial and distal surfaces. Some blood vessels were found in the tooth third of the PDL from four weeks of age. Similarly, at six weeks of age, more blood vessels were noted on the distal surface (Figure 11).

Apical third

At all ages, the largest number of blood vessels was found on the mesial and distal surfaces of the root. Blood vessels in the tooth third of the PDL were only identified on the distal surface in the four and six week-old rats. More blood vessels were found in

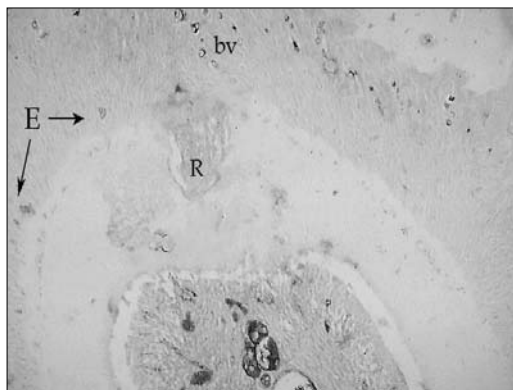


Figure 13. A resorption lacuna (R) on the distal surface of a mesio-buccal root in a six week-old rat. Note the break in the ERM network (E) and the invasion of blood vessels (bv) across the PDL towards the root surface. (Original magnification: 20x)

the six week-old specimens than in the three and four week-old specimens (Figure 12).

Root resorption

No resorption was noted in three week-old rats. The highest mean number of resorption lacunae occurred in the upper and middle thirds of the root in four and six week-old animals. A few resorption lacunae were observed in the apical thirds of the mesio-buccal roots in the four week-old rats. All the resorption lacunae were found on the distal surface of the mesio-buccal root. Both active and repairing lacunae were noted. Although ERM cells and clusters were found adjacent to the resorption lacunae, no ERM cells or cell clusters occurred directly over a lacuna. Rather there was a break in the ERM network wherever resorption was present. Many of the blood vessels present in the tooth third of the PDL were associated with resorption lacunae. No blood vessels were found directly between the ERM and the root surface, instead the blood vessels were either on the bone side of the ERM or inside the resorption lacunae (Figure 13).

Discussion

At three weeks of age, the epithelial diaphragm of HERS was present at the formative end of the mesio-buccal root of the rat first molar tooth. However, following frag-

mentation of the sheath the ERM could be seen in the PDL. The ERM formed a network around the root, presenting as either single cells or clusters of cells. According to Wentz,²⁹ the different presentations of ERM represent either different stages of development or trauma-induced proliferation. Thus, it could be postulated that the single ERM cells and clusters of cells noted in this study are ERM in different stages of proliferation. The activity, or inactivity, of the cells in the PDL was not investigated in the present study.

The number of ERM cells and cell clusters increased with age, and by six weeks of age the largest number was found in the upper third of the root. This is in agreement with Hamamoto *et al.*³⁰ and Wentz *et al.*²⁹ who used older rats than the rats used in the present study. Wentz *et al.*²⁹ were of the opinion that some of the ERM may be derived from either the epithelial attachment or the gingival epithelial cells, which could account for the larger number of ERM cells and cell clusters in the upper third of the root. Gingival inflammation, following emergence of a tooth into the oral cavity, may also contribute to the high number of ERM in the upper third of the root. The results of the present study are similar, but not identical, to Reeve and Wentz¹⁴ who investigated the distribution of ERM in human periodontal ligament. They reported more ERM were found in the apical third of the root in adolescents, whereas the greatest concentration of ERM in adults occurred in the upper region of the root. The authors postulated that the difference in the distribution of ERM was due to the presence of persistent gingival inflammation in the adults. Gingival inflammation may stimulate proliferation of the ERM in the upper region of the root, especially in older animals. Gurling and Sampson⁷ proposed that HERS “unfolds” during root development breaking up into the ERM, which are then carried occlusally during tooth eruption, thus increasing the number of ERM in the upper region of the root.

The highest numbers of single ERM cells and cell clusters were noted on the distal surface in the upper and middle thirds of the root. In the apical third of the root, the highest count was found on the mesial surface. Wentz *et al.*²⁹ and Yamashiro *et al.*¹⁵ reported that the highest ERM counts were found in the root bifurcation area, a finding which corresponds to the distal surface in the higher part of the mesio-buccal root in the present study. The lowest cell and cluster counts for all root thirds were on the palatal surface. The distribution of ERM around the root surface may be related to tooth eruption. Dreyer and Sampson³¹ noted that rodent teeth erupt occlusally and distally. Thus, as the crown tips distally, the root tips mesially which corresponds to the ERM distribution around the root surface along the vertical root thirds and correlates with presumed pressure zones in the rat PDL, particularly once occlusal function is attained.

The highest number of resorption lacunae was found on the distal surfaces in the upper thirds of the roots in the four week-old and six week-old rats (this may also be related to the eruption pattern). The first molar usually emerges into the oral cavity at four weeks of age. This corresponds to the larger number of ERM cell and cluster counts found at this age.

In the apical third of the root, lower ERM counts were found on the distal surface, corresponding to fewer resorption lacunae. Paradoxically, the distribution of resorption lacunae appeared to correlate with increased ERM counts, which might support a role for ERM in the initiation of resorption. However, in areas of active resorption there was a break in the ERM network, which suggests a possible protective or reparative role for the ERM. Although seemingly contradictory, a greater likelihood of resorption may result in proliferation of ERM to either protect against further resorption or to commence repair once active resorption has ceased. This agrees with Brice *et al.*²² and Leedham²⁴ who only noted the presence of

ERM in repairing resorption bays and not active ones.

The greatest number of vessels was found in the bone third of the PDL, followed by the middle third of the PDL. Very few vessels were found in the tooth third of the PDL, and these were only found at four weeks and six weeks of age. Blood vessels in the tooth third of the PDL were only observed where root resorption was noted, and where the ERM cells were inconspicuous. The highest numbers of vessels were noted in the upper third and secondly, in the apical third of the PDL. Generally, the number of vessels increased between three and four weeks of age, and decreased slightly at six weeks of age. This may be due to inflammation occurring during active eruption or penetration of the crown into the oral cavity.

The highest number of vessels was generally found on the distal and mesial surfaces of the root in all vertical root thirds, whilst the lowest number was noted on the palatal surface. This may be due to anatomical variation and the physiological imperatives of tooth support or it may be related to the incidence of root resorption. Vessels were never found between the ERM and the root surface, even in areas undergoing resorption. This agrees with the findings of Kittel and Sampson,²³ and supports the notion of a protective function for the ERM network against root resorption. Physiologic resorption is common in rat teeth. It begins as soon as teeth come into occlusion, around four weeks of age, and can be provoked by any trauma to the teeth including occlusal attrition.³² However, root resorption is not rampant, which suggests that a protective mechanism must exist within the PDL, some measure of which may lie within the ERM network.

Conclusions

An interpretation of the observations may be that in areas where physiological resorption occurs, a breach is produced in the apparently protective ERM network. At the same time, adjacent and surrounding PDL vessels

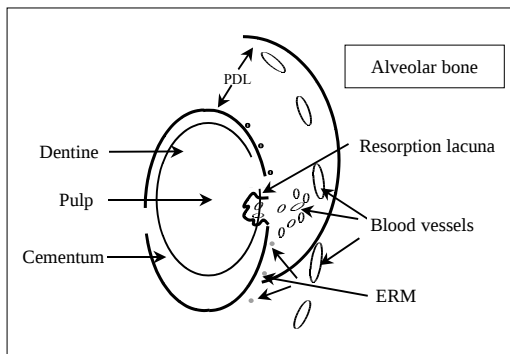


Figure 14. Possible interactions which may occur during physiological root resorption during rat root development.

are stimulated to proliferate until ERM reconstitution occurs in synchrony with repair of the resorption lacunae. This may be related to tooth emergence and occlusal contact of the molars as no resorption was seen prior to four weeks of age. These putative interactions are summarized in Figure 14. Orthodontic root resorption can be regarded as an exaggerated response to loss of PDL homeostatic control, possibly mediated by the remnant embryonic epithelia known as the epithelial rests of Malassez.

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