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Histomorphometric Study of the Periodontal Vasculature of the Rat Incisor

N. BLAUSHILD, Y. MICHAELI, and S. STEIGMAN

Department of Anatomy and Embryology and 1Department of Orthodontics, Hebrew University-Hadassah School of Dental Medicine founded by the Alpha Omega Fraternity, P.O.B. 1172, Jerusalem 91010, Israel

This study assessed quantitatively the vascular system in the cementum-related periodontal ligament (PDL) along the rat incisor. The lower left incisors of six rats (±200 g) were subjected to routine histological procedures and cross-sectioned serially (2 μm), and the distance between each section and the apex was computed. The PDL of five sections at different levels along the tooth was divided into mesial, lingual, and lateral parts. The number and area of small and terminal arterioles, capillaries (C), sinusoids (S), post-capillary venules (PCV), and connecting venules, as well as the area of the PDL, were established. Blood vessels (BV) occupied 47 ± 2% of the PDL area in the apical half and 4 ± 2% at the incisal end. Of the total BV area, 41%, 32%, and 27% were located on the lingual, mesial, and lateral tooth sides, respectively. The majority of BV belonged to the venous system (98.5 ± 0.6% and 82.5 ± 3.0% in the apical and incisal parts, respectively). The apical venous system comprised 95.4 ± 1.6% S and 3.2 ± 1.0% PCV, reversing to 27.2 ± 14.2% S and 55.2 ± 11.3% PCV in the incisal half. The number of arterial profiles increased gradually from 6.8 ± 1.5 at the apex to 25.3 ± 2.4 in the incisal part and that of C from 9.0 ± 1.18 to 25.0 ± 4.3. The extensive vascularization in the apical half of the PDL is consistent with the high metabolic demands and with the need for protective cushioning of the constantly growing dental and periodontal tissues. The paucity of blood supply and the presence of numerous small BVs in the incisal end equate with the metabolic needs of the highly organized supporting tissue in this region.


Introduction.

In view of the extensive involvement of the vascular system in the majority of dental and periodontal functions, many research efforts have been directed toward the accurate description of its morphology in human and laboratory animal teeth (Edwall, 1982). With respect to the latter category, special attention has been given to rodent molars under normal and experimental conditions (Kindlava and Matena, 1962; Nakamura et al., 1986a,b; Weekes and Sims, 1986; Wong and Sims, 1987; Nemeth et al., 1989). Studies have shown that one of the morphological characteristics of the periodontal vasculature is the extensive fenestration of its capillaries (Fujimura et al., 1987; Moxham et al., 1987; Law et al., 1989; Clark et al., 1991).

Notwithstanding the widespread use of the rodent incisor as an experimental model, its vascularity surprisingly has received but scant attention. As far as we could ascertain, only four investigations of a descriptive nature address this topic (Kindlava and Matena, 1959; Carranza et al., 1966; Matena, 1973; Moe, 1981). Two main features distinguish the periodontal vascular system of the continuously erupting rat incisor from that of the rat molar: (a) The blood supply of the enamel-organ-bordering periodontal ligament (e-PDL) is distinct from that of the cementum-bordering PDL (c-PDL), and (b) all blood vessels (BV) of the c-PDL are concentrated in the anatomically differing bone-related part of the incisor ligament (b-PDL), with a total absence of BV in the tooth-related part of the ligament (t-PDL).

The purpose of this investigation was to obtain morphometric data pertaining to the vascularization of the b-PDL in the cementum-surrounding ligament proper of the normally functioning rat incisor. The results obtained in the present study form the baseline for future work on the influence of functional and mechanical stimuli on the periodontal vascular system.

Materials and methods.

The research was carried out on six young adult female rats of the New strain (weight, 200 ± 4 g). The animals were killed by an overdose of ether, their left mandibles were dissected, fixed in Bouin-Holland solution, subjected to lateral x-ray, and demineralized in 10% EDTA (pH 7.4) at room temperature. The bone-embedded part of the incisor was divided into six equal segments (A through F) (Fig. 1), by means of a previously reported method which eliminates the distortion caused by transverse sectioning of curved teeth (Steigman et al., 1983). The segments were embedded in glycol methacrylate (JB-4, Polysciences Inc., Warrington, PA), and serial transverse sections (2 μm) were cut perpendicularly to the long axis of the tooth. The distance of each section from the posterior alveolar wall (referred to in the present work as ‘apex’) was calculated. Segment A, which contained the alveolar crest, was discarded, because at that location the socket continuity around the tooth is frequently disrupted on the labial and lateral sides. From each of the remaining five segments, one section was chosen for examination by computerized image analysis (CUE-2 system, Galay Production Ltd., Israel). The most apical section investigated was located at 3.5 mm and the most incisal one at 17.5 mm from the apex. The combined data of all sections (a total of 30 sections) provided a representative view of the periodontal vascularization along the entire tooth. The c-PDL of each section was arbitrarily divided into mesial, lateral, and lingual parts, as follows: Starting at the respective cemento-enamel junctions (CEJ), and with the cemental line used as a landmark, the mesial part extended for 1500 μm, the lateral part covered 1000 μm, with the remainder of the PDL constituting the lingual part (Fig. 2). The inner perimeter of the PDL (i.e., the cemental line) as well as the t-PDL and b-PDL areas were measured. The mean widths of the PDL and t-PDL were

Fig. 1—Schematic drawing of a dissected rat mandible demonstrating the division of the incisor into six segments.

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calculated by dividing the pertinent areas by the length of the cemental perimeter. The widths of the mesial, lateral, and lingual PDL parts were established in the same manner. The data obtained for all the parameters were grouped according to tooth segment (F-B), and their mean values were calculated.

The blood vessels in the e-PDL were classified on the basis of their histomorphological properties (Lew, 1987; Simionescu and Simionescu, 1988), as follows (Fig. 3):

1. **Small arterioles (SA)**, having an inner diameter of 20-80 μm, a thick wall (1-2 layers of smooth muscle cells), scalloped contour of the intima, and endothelial cells with small, round nuclei protruding into the lumen.

2. **Terminal arterioles (TA)**, having a capillary-like inner diameter of 5-15 μm, thin adventitia showing few fibroblasts, 1-2 smooth muscle cells containing clear prominent nuclei and located around the endothelium, smooth contour of the intima, and endothelial cells with elliptical nuclei protruding into the lumen.

3. **Capillaries (C)**, having an inner diameter of up to 10 μm, a thin wall without media or adventitia (occasionally showing a few pericytes around the endothelium), a single continuous layer of flat endothelial cells with elongated thin nuclei, and elliptical contour of the lumen.

4. **Post-capillary venules (PCV)**, of the same histological structure as C, except for a larger diameter of 10-40 μm.

5. **Sinusoids (S)**, of the same histological structure as C and PCV, but larger than either, their diameter exceeding 40 μm.

6. **Collecting venules (CV)**, characterized by an inner diameter of 20-100 μm, a relatively thick wall containing a smooth muscle layer and adventitia, a continuous layer of flat endothelium, and regular elliptical contour of the lumen.

In each section, all types of blood vessels were counted (excluding S) and their area measured. The mean values for each of the three PDL parts separately, as well as for the total PDL, were calculated for each tooth segment. The reproducibility of the measurements was assessed by statistical analysis of the difference between double determinations performed at a time interval of three months with use of 20 cross-sections taken from different incisors. The error was 9% for the total PDL and BV areas, and 12% for the t-PDL and B-PDL areas. All data were plotted according to their distance from the apex and fitted with second-order polynomials (Steigman et al., 1983). The adequacy of the polynomial fit was checked by the $x^2$ test. The polynomials did not vary significantly from the observed points. One-way ANOVA was used for analysis of both the variability among segments F to B and the differences among the mesial, lateral, and lingual PDL parts for all parameters. The significance of the differences was established by means of the Schefte F test (level of significance, 95%).

**Results.**

**Morphometry of the periodontal ligament.**—The cross-sectional PDL area was not uniform in size, in that it expanded along the middle third of the tooth (Table and Fig. 4). The difference in size between the central (D) and the outermost (B or F) periodontal segments was statistically significant ($p < 0.01$).

The variations in PDL width were consistent with those occurring in PDL area, i.e., the ligament was widest along the middle tooth third (statistically significant for the mesial and lingual sides; $p < 0.05$; Fig. 5). Comparison among the mesial, lingual, and lateral parts showed the lingual side to be consistently widest ($p < 0.05$ for all segments). There were no significant differences in width between mesial and lateral PDL, except for segment B, where the lateral width was twice that of the mesial one ($p < 0.05$).

The division of the total PDL area into t-PDL and b-PDL is presented in Fig. 6. The b-PDL formed the bulk of the ligament, the two parts being approximately the same size only near the alveolar crest.

Like its area, t-PDL width increased incisally (from 41.7 ± 1.9 μm to 130.1 ± 7.9 μm on the lingual side, and from 37.3 ± 5.9 μm to 112.4 ± 9.7 μm on the lateral side), the increase being statistically significant ($p < 0.05$) between the succeeding tooth segments. The width of the mesial t-PDL was nearly uniform at 60 μm along the

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**TABLE**

**MEAN (± SE) VALUES FOR THE VARIOUS CROSS-SECTIONAL AREAS OF THE PERIODONTAL LIGAMENT AND VASCULATURE ACCORDING TO DISTANCE FROM APEX**

<table>
<thead>
<tr>
<th>Tooth Segment</th>
<th>Distance from Apex</th>
<th>Total PDL Area (mm²)</th>
<th>Blood Vessel Area (mm²)</th>
<th>Connective Tissue Area (mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>3.62 ± 0.18</td>
<td>0.66 ± 0.02</td>
<td>0.33 ± 0.03</td>
<td>0.33 ± 0.03</td>
</tr>
<tr>
<td>E</td>
<td>6.92 ± 0.18</td>
<td>0.90 ± 0.05</td>
<td>0.43 ± 0.04</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>D</td>
<td>10.29 ± 0.10</td>
<td>0.91 ± 0.04</td>
<td>0.39 ± 0.02</td>
<td>0.52 ± 0.03</td>
</tr>
<tr>
<td>C</td>
<td>14.08 ± 0.19</td>
<td>0.76 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.63 ± 0.02</td>
</tr>
<tr>
<td>B</td>
<td>17.44 ± 0.11</td>
<td>0.63 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td>0.61 ± 0.03</td>
</tr>
</tbody>
</table>

*Difference from preceding segment statistically significant:

$p < 0.01$; *p < 0.05. PDL = periodontal ligament.*
entire tooth, except for segment F, where it narrowed significantly to 36.3 ± 2.5 µm (p < 0.05).

Vascularity of the periodontal ligament.—All periodontal blood vessels were concentrated near the bone, leaving the t-PDL avascular. BV area initially increased in the apical half, but dropped sharply in the incisal half (Table). The net area of the periodontal connective tissue (CT), on the other hand, increased uniformly in the incisal direction, coming to a plateau in segments C and B. Starting at the apex, the BV/CT ratio decreased consistently (from 1.07 ± 0.21 in segment F to 0.04 ± 0.02 in segment B).

The BV area constituted about 50% of the total PDL in the apical tooth half (50.2 ± 3.9%, 47.5 ± 2.3%, and 42.7 ± 1.7% in segments F, E, and D, respectively), declining steeply in the incisal half (16.1 ± 3.8% and 3.8 ± 1.5% for segments C and B, respectively; p < 0.05) (Fig. 7). When evaluated separately, the data obtained for the mesial, lingual, and lateral PDL parts exhibited patterns resembling those of the total PDL. Circumferentially, in the incisal half, the percentage of the lingual BV area of the PDL was higher than that on the mesial and lateral sides (p < 0.05 in segment C). Of the total BV area, 41% was concentrated in the lingual PDL, with only...
32% being located in the mesial and 27% in the lateral PDL.

**Types of blood vessels in the periodontal ligament.**—The relative proportions of the six BV types were similar around the tooth, with area and amount for each BV type always being largest on the lingual aspect of the tooth. The venous system constituted the major part of the total cross-sectional BV area, 98.5 ± 0.6% in segments F-C, and decreasing to 82.5 ± 3.0% in segment B (p < 0.01). Among the three types of venous vessels, the sinusoids were the most prevalent, followed by the post-capillary venules, while the contribution of the collecting venules to the vascular area was negligible (not more than 27 of the latter vessels were observed in the entire sample). Along the F-C segments, S occupied 95.4 ± 1.6% of the total BV area, with PCV forming only 3.2 ± 1.0%. In the most incisel segment (B), the situation was reversed, with S decreasing to 27.2 ± 14.2% and PCV increasing to 55.2 ± 11.3% (p < 0.01).

Since the routine histological procedures may have caused strong contraction of the arteriolar vessels, these were evaluated according to number and not to area. The mean number of arteriolar profiles per section rose gradually along the tooth, from 6.8 ± 1.5 in the apical segment (F) to 25.3 ± 2.4 in the most incisel segment (B), the difference in number between the apical (segments F-D; mean, 8.5 ± 1.1) and the incisel (segments C and B; mean, 22.4 ± 2.2) tooth halves being statistically significant (p < 0.05). When evaluated separately, the two constituents of the arteriolar vasculature exhibited the same trend, albeit at a more pronounced degree for TA (Fig. 8). The difference in number of TA profiles between apical and incisel segments was statistically significant (p < 0.05).

The mean number of capillaries per section was uniform along the apical tooth half (9.0 ± 1.2), rose to 15.7 ± 2.0 in segment C, and reached 25.0 ± 4.3 in the incisel segment (B). The difference in number of capillaries between apical and incisel tooth half segments was statistically significant (p < 0.05).

**Discussion.**

The present morphometric study complements the existing morphological descriptions of the periodontal blood supply in the rat incisor (Kindlova and Matena, 1959; Matena, 1973). The quantitative data afford comparison between the PDL vasculature of the continuously growing tooth and that of teeth with limited eruption.

The vascularity of the rat incisor proved much richer than that of the rat molar, in that, in the apical tooth half, the BV volume constituted about 50% of the total PDL, as opposed to the reported 20% in the molar (Lew, 1987). All incisel BVs were located in the b-PDL, and it is interesting to note that, in the rat molar, where the BVs are dispersed throughout the width of the PDL—the capillaries even being situated near the tooth (Freezer and Sims, 1987)—the major source of blood supply is nevertheless concentrated in the bone-adjacency ligament, peaking at 16-24 μm from the bone (McCulloch and Melcher, 1983). This particular vascular distribution may be attributed to either the close association between the periodontal and medullary vascular systems (Carranza et al., 1966; Wong and Sims, 1987) or the significant role of the blood supply in the bone remodeling processes (Nemeth et al., 1989), or both.

The quantitative variations in the periodontal vasculature around the circumference of the rat incisor are consistent with the observations by Matena (1973), in that the incidence of BVs on the mesial and lateral tooth sides was lower than that on the lingual side. In rodent molars, on the other hand, both arterial and venous vessels have been shown to be larger and more abundant in the mesial and distal ligament (Wong and Sims, 1987). Longitudinally, the quantitative increase in BV area in the incisor in an apical direction matched the descriptive presentations of "a great number of capillaries," "abundance of wide vessels," or "large blood spaces" in the basal region (Kindlova and Matena, 1959; Matena, 1973; Chiba and Komatsu, 1988). A similar incisel-apical gradient has been observed for rodent molars (Kindlova and Matena, 1962; Nakamura et al., 1986a; Wong and Sims, 1987). However, the difference in BV area between the incisel and apical parts was considerably more pronounced in the incisors; whereas in the molar the BV area amounts to 7.25% (McCulloch and Melcher, 1983) and 19.9% (Lew, 1987) of the incisel and apical PDL areas, respectively, for the...
incisor the values for the corresponding areas showed a ten-fold rise in vascularization in the apical region. This extremely steep gradient may explain why the regional differences in mechanical properties of the PDL are reportedly more marked in the rat incisor than in teeth of limited growth (Chiba et al., 1990).

As in the rodent molar PDL, the vascular system of this tissue in the rat incisor was primarily venous (Weekes and Sims, 1986; Lew, 1987; Wong and Sims, 1987; Lew et al., 1989). However, structurally, the venous systems differed: While in the molar the PCV’s comprise 88% of the total vascular PDL volume (Freezer and Sims, 1987), in the incisor these venules predominated only in its incisal half, with the apical displaying mainly large sinusoids. No such comparison could be made for the arterial system, since we could find in the literature no pertinent data for the molar. The increasing amount of SA and TA in an incisal direction observed in the present study may be explained by the topography of these vessels. The arterioles enter the PDL along the apical two-thirds of the socket via numerous orifices in the lingual alveolar bone, where they bend and run incisally, parallel to the long axis of the tooth (Kindlova and Matena, 1969; Carranza et al., 1966; Matena, 1973), thus creating a cumulative effect in the incisal part.

In contradistinction to teeth of limited eruption, rat incisors do not possess a dense circumferential capillary network in the marginal PDL (Kindlova, 1965). We therefore attribute the two- and three-fold capillary increase in segments C and B, respectively, to branching off of the larger vessels coursing in the PDL in order for them to anastomose with the extensive capillary net of the enamel organ.

The present work shows that the vascular system of the rat incisor PDL may be divided into two distinctive portions: an apical part, which is characterized by its extremely large volume and huge sinusoids, and an incisal part, which resembles both in quality and quantity the periodontal vasculature of the rodent molar. This division is closely connected with the different functions of the apical and incisal parts of the PDL in the continuously growing tooth. Thus, the dental and periodontal progenitor compartments are contained within the apical PDL, the nutritional demands of their proliferating and differentiating cells being much higher than those of adult, fully functioning, cells. Furthermore, the former are also exceptionally susceptible to pressure/tension stimuli (Steigman et al., 1992). The extensive vascular volume found in the apically located, thin-walled, sinusoids meets these nutritional demands and provides protection against the heavy functional loads exerted on the sensitive generating tissue (Robins, 1977; Picton and Wills, 1978). The tooth-to-bone anchorage, on the other hand, is effected mainly by the incisal half of the PDL. Consequently, the chief component (97%) of this part of the ligament consists of highly organized connective tissue which requires only minimal blood supply to fulfill its nutritional needs (Lew, 1987). The functional analogy between the incisal PDL in the rat incisor and total PDL in the rodent molar explains the similarity of the two respective vascular systems.

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