The Periodontal Microvasculature
—A Morphological and Morphometric Study—

by

Kenneth K. K. LEW, BDS, MDS (Adelaide)

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Abstract

Various ultrastructural studies on the periodontal ligament (PDL) microvasculature have been reported. However, the lack of a systematic classification has led to much confusion in the terminology used to define vessel types. On the basis of morphological criteria, the author proposes that 3 vessel types are present in the PDL: terminal arterioles (Type A vessels), capillaries (Types B-1 & B-2) and postcapillary venules (Type C vessels). Morphometric analysis of the apical PDL revealed that the total vascular volume was 19.9%. Type C vessels predominated with a vascular volume of 16.4% while the other 2 vessel types made up the remainder of the vascular volume. The results of this study suggest that the apical PDL region is predominantly venous.

Introduction

By use of the SEM methacrylate corrosion cast technique[1] and transmission electron microscopy[2,3] in a variety of animal models, various types of microvascular segments have been described in the periodontal ligament (PDL). These include arterioles, venules, sinusoids, lymphatics and capillaries. However, a feature common to these studies was the lack of a systematic classification of the PDL blood vessels. Furthermore, the authors have not always defined their criteria for categorization of vessel types.

Estimates of the vascular volume in selected regions of the PDL range from 1–2%[4] to 22%[5]. It is generally agreed that the vascularization is densest in the apical region of the PDL[6,7]. However, the relative proportions of vascular types have not been reported.

The objective of this study was to derive an ultrastructural classification system for the PDL microvasculature and to determine morphometrically the vascular volume of different vascular types using the rat molar periodontal ligament as a model.
Materials and Methods

Six male Porton rats, aged 12 weeks and weighing 300–320 g each, were anesthetized with intraperitoneal urethane using a dosage of 30 mg/10 g body weight. Following intravenous heparinization with 46 I.U. via a tail vein, each animal was perfused through the left ventricle with 4% glutaraldehyde and 0.89% OsO₄ in 0.1 M cacodylate buffer at pH 7.4. The maxillae were then removed, hemisectioned and demineralized at 4°C with 0.1 M EDTA in 2.5% cacodylate buffer at pH 6.4. When radiographic examination indicated the completion of demineralization, each maxillary portion was divided mid-sagittally through the mesial root to provide complete occluso-apical blocks of the periodontal ligament. The blocks were post-fixed for one hour with 4% OsO₄ in cacodylate buffer and processed through an ethanol and propylene oxide series for embedding in Araldite.

For each animal, ten ultrathin sections (70 nm thick) of a defined apical PDL region were cut with a diamond knife, transferred to 200-mesh copper grids, and stained with modified Reynold’s lead citrate for viewing and photography with a JEOL 100-S transmission electron microscope. Magnification accuracy was checked against a cross-grating replica.

Results

Morphology

In the apical region, microvessels of three main types were found: terminal arterioles (Type A), capillaries (Type B) and postcapillary venules (Type C). Figure 1 illustrates the vessel types.

Type A vessels

Terminal arterioles (Fig. 2) usually varied between 18–24 μm in width and had 1–2 continuous layers of smooth muscle cells, each layer averaging 0.5–1.0 μm thick. The terminal ends of Type A vessels, commonly known as pre-capillary sphincters, varied from 10–14 μm in diameter.

The endothelial layer in both the precapillary sphincters and terminal arterioles was usually thicker (0.3–0.6 μm thick) than those in the capillaries and postcapillary venules and were attached to each other by an elaborate interdigitating junctional complex. Endothelial processes came into contact with smooth muscle cells (myo-endothelial junctions) in areas where the basal laminae of the endothelial and muscle cells were deficient. The subendothelium was thin and contained relatively few collagen fibrils. There was a thin adventitial layer comprising collagen fibrils, oxytalan fibrils, pericytes, unmyelinated and myelinated nerves and nerve bundles.

<table>
<thead>
<tr>
<th>Terminal arterioles (Type A)</th>
<th>Capillaries (Type B)</th>
<th>Postcapillary venules (Type C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>arterial (B-1)</td>
<td>venous (B-2)</td>
<td>non-fenestrated (C-α)</td>
</tr>
<tr>
<td>— continuous (B-1-α)</td>
<td>— continuous (B-2-α)</td>
<td>— non-fenestrated (C-α)</td>
</tr>
<tr>
<td>— fenestrated (B-1-β)</td>
<td>— fenestrated (B-2-β)</td>
<td>— fenestrated (C-β)</td>
</tr>
</tbody>
</table>

Fig. 1 PDL vessel types
Type B vessels

The endothelial lining of the capillaries in the apical PDL consisted of a single, continuous layer of simple squamous epithelial cells (0.05–0.5 μm thick). Each capillary profile usually comprised 2–4 of these endothelial cells. Capillaries usually had a luminal diameter of 6.8–7.8 μm. The single layer of endothelium in regions distant from the endothelial nuclei varied between 0.08–0.18 μm in thickness. Each endothelial cell was positioned with one face (luminal side) directly exposed to the blood and the opposite face (abluminal side) and its attached basal lamina was bathed by the interstitial fluid which mediated contact with the surrounding tissue.

![Fig. 2 Terminal arteriole. M: smooth muscle cell nucleus, E: endothelial cell nucleus, V: microvilli, L: lumen of adjacent post-capillary. Original magnification: ×4000. Bar: 2 μm](image1)

![Fig. 3 Typical capillary endothelial fenestra. K: central knob of diaphragm, V: albuminal vesicle, C: collagen fibrils. Original magnification: ×50,000. Bar: 0.1 μm](image2)

![Fig. 4 Intercellular junction of a capillary. OJ: occluding junctions. Original magnification: ×30,000. Bar: 0.2 μm](image3)

![Fig. 5 Fenestrated venous capillary. F: fenestra, M: microvillous projections, N: endothelial nucleus, P: pericyte. Original magnification: ×8,000. Bar: 2 μm](image4)
On the luminal side, the endothelial cell membrane displayed a fuzzy coat (10–20 nm thick) which often continued down the intercellular junctions.

Spherical vesicles (Fig. 3) of relatively uniform size (60–80 nm in outer diameter) were found, either free in the cytoplasm or opening onto one of the endothelial faces. The attached vesicles were generally flask-shaped, with a narrow neck and a stomatal opening (20–40 nm in diameter). Most of these stomata were closed by a thin diaphragm (6–8 nm thick), usually with a single central knob. Occasionally, single plasmalemmal vesicles, or chains of two or more fused vesicles, opened simultaneously on both the luminal and abluminal fronts to form a patent transendothelial channel. Adjacent endothelial cells were separated by a small intercellular space (4–6 nm wide) which narrowed at one or more points along the intercellular junction (Fig. 4). The endothelial junctions displayed regions of membrane fusion (zonula occludens).

Fenestrae (Fig. 3) were usually located away from the nuclear and organelle regions. A typical fenestrated capillary profile usually had 1 or 2 fenestrae, and the fenestral diameters were in the 49–58 nm range. A “sieve-plate” arrangement of fenestrations was rarely found, and no obvious polarity of fenestrae was noted. The fenestrae of the capillary endothelium were usually bridged by a thin diaphragm (6–8 nm) which appeared morphologically similar to the diaphragms of the vesicles and transendothelial channels. Each thin electron-dense diaphragm usually had a central knob (Fig. 3) 15–20 nm in diameter.

Unlike RHODIN[10], the present author found very few capillaries with complete pericytic coverings in the apical PDL. A few capillaries appeared to be completely devoid of pericytic coverings and could be termed “apericytic” capillaries. The pericytes were in juxtaposition with the abluminal surface of the endothelial cells, often separated only by the intervening basement membrane.

Type B vessels were mainly venous (Fig. 5) in morphology (Type B-2) although arterial elements could also be identified (Type B-1). Type B-1 vessels were characterized by a thicker endothelial layer (0.09–0.25 μm thick) and numerous microvillous projections along the luminal surface (Fig. 6). The nucleus protruded into the lumen and was generally thicker, shorter and more lobulated than its venous counterpart.

Both the venous and arterial capillary ends had vessel profiles of the continuous and fenestrated variety. Examination of serial sections revealed that 91% of the total capillary profiles present were of the continuous type while only 9% were fenestrated. 

Type C vessels

These vessels usually measured between 21–26 μm in internal luminal diameter. The endothelium was relatively thin, measuring 0.15–0.30 μm in non-fenestrated postcapillary venules and 0.05–0.25 μm in fenestrated postcapillary venules. Fenestral diameters were generally similar to those found in fenestrated capillaries and again, no obvious polarity of fenestrae was noted.

The endothelial cells rested on a continuous basal lamina. Interlocking and overlapping cell junctions tended to be more common than in capillaries. The pericytic layer was usually incomplete and some vessels were devoid of a pericytic cover-
ing. Occasionally, in some of the sections examined, larger venous vessels (luminal diameter 30–50 μm) were found. Myelinated and unmyelinated nerves were often identified in close proximity to the postcapillary venules (Fig. 7). Oxytalan and collagen fibers were found around the vessels in a close relationship with the endothelium and nerves.

**Morphometry**

For purposes of computation, the equation used for calculating the vascular volume was:

\[
\text{Vascular volume (as a percentage of apical PDL volume)} = \frac{\text{Total area of particular vessel segment} \times \text{section thickness}}{\text{Total grid area} \times \text{section thickness}}
\]

The results obtained for the vascular volume (as a percentage of apical PDL volume) are shown in Table 1.

### Table 1 Vascular volumes of each of the vessel types

<table>
<thead>
<tr>
<th>ANIMAL</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>4</th>
<th>6</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terminal arteriole</td>
<td>1.61</td>
<td>0.68</td>
<td>0.70</td>
<td>1.10</td>
<td>1.20</td>
<td>0.99</td>
<td>1.05</td>
<td>0.35</td>
</tr>
<tr>
<td>Arterial capillary</td>
<td>0.35</td>
<td>0.48</td>
<td>0.36</td>
<td>0.40</td>
<td>0.41</td>
<td>0.38</td>
<td>0.40</td>
<td>0.05</td>
</tr>
<tr>
<td>Venous capillary</td>
<td>1.67</td>
<td>1.46</td>
<td>2.81</td>
<td>2.00</td>
<td>2.10</td>
<td>2.09</td>
<td>2.02</td>
<td>0.46</td>
</tr>
<tr>
<td>Postcapillary venule</td>
<td>16.26</td>
<td>19.40</td>
<td>13.95</td>
<td>15.51</td>
<td>16.64</td>
<td>16.89</td>
<td>16.44</td>
<td>1.79</td>
</tr>
<tr>
<td>Total vascular volume</td>
<td>19.89</td>
<td>19.92</td>
<td>22.02</td>
<td>17.82</td>
<td>19.08</td>
<td>20.90</td>
<td>19.94</td>
<td>1.39</td>
</tr>
</tbody>
</table>
Discussion and Conclusion

The existing literature is remarkable if only for the paucity of microanatomical information on the types of vessels found in the PDL. Most investigations into PDL vasculature have been histological in nature and have either reported on arterial supply and ignored venous drainage, or have cursorily mentioned “blood vessels” with no further categorization.

Bevelander and Nakahara provided the first ultrastructural description of human ligament vessels and, although they did not categorize the vessels definitely, they reported thin-walled vessels of varying caliber that resembled capillaries. Ryghe mentioned the existence of arterioles, capillaries and venules in the rat molar ligament but did not discuss his criteria for classification nor the architectural arrangement of the vessels except to say that they were observed alone or in groups.

The average vascular volume in the apical PDL, as determined in the present study, was almost 20%. If the ligament is regarded as a connective tissue, it would only require a 5% vascular volume to provide for nutritional needs. The high vascular volume in the apical PDL could be related to other metabolic needs such as dissipation of occlusal force and bone and cementum resorption/repair.

The results of the present study suggest that the predominant vessel type in the apical PDL is the postcapillary venule (Fig. 8), comprising 82.4% of the total

![Fig. 8 Histogram of the vascular volumes of the different vessel types](image)
vascular volume. Morphologically, many of these venules differ from those of Rhodin's classification\cite{10} with respect to their scarcity of pericytes, the presence of fenestrae\cite{8} and the existence of nerves alongside the endothelium. Furthermore, in the sections examined, a substantial number of venous vessels were wider than 30 \( \mu \text{m} \), which dimensionally could be classified as collecting venules according to Rhodin's classification\cite{10}. However, these larger venous vessels did not have the complete layers of pericytes and veil cells said to be typical of such venules. As such, these "apericytic" venules were therefore classified as large postcapillary venules\cite{9}. It is possible that the large proportion of postcapillary venules, coupled with the presence of large "apericytic" postcapillary venules, is indicative of the special operant characteristics of the PDL in the apical region which enables it to withstand heavy intermittent loading during mastication.

Avery et al.\cite{12}, in their examination of terminal blood vessels in the mouse periodontium, stated that fenestrations were present in type A-1-alpha capillaries, although no information regarding vessel dimensions and their exact location within the PDL was given. The greater frequency of fenestrae in the venous segments as found in the present study is in accord with the observations of Casley-Smith\cite{11}, who demonstrated that fenestrae were several times more common in venous than in arterial limbs of capillaries.

In other microvascular systems, fenestrae are considered to enhance normal capillary permeability and to play a role in rapid fluid and protein transfer through the interstitial tissues\cite{13}. Although the exact role of PDL fenestrations is not known, it is possible that the presence of fenestrations in the apical PDL may help in dissipating axial loads, play a role in bone or cementum metabolism or assist in coping with the high metabolic rate of the PDL\cite{14}.

The findings of the present study lead to speculation that venous capillary and postcapillary wall structure facilitates the rapid passage of fluid between the vessels and interstitial tissue\cite{13}. This fluid movement could alter the biochemistry of the collagen and ground substance in a manner appropriate to the needs of tooth support\cite{15}.

Future studies will focus on the microanatomical and microphysiological features of periodontal endothelial junctions, vesicles, fenestrae and lymphatics, thereby bringing into clearer perspective the structural attributes of the capillary barrier. It is possible that periodontal capillaries may possess similar properties under loading, and function as tube and tunnel capillaries for fluid exchange between the intravascular and extravascular space\cite{16}. However, it should be emphasized that at the present time, there are no published studies on alterations in capillary fine structure and the properties of ground substance with variations in tissue activity.

Acknowledgements

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References


