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Morphology of the enamel-cementum microvascular junction of the mouse incisor

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Abstract

Scanning electron microscopy of methyl methacrylate vascular perfusion casts with stereopair imaging has been used to provide three-dimensional morpholgy of the mouse incisor periodontal ligament microvascular bed. Both the cementum and enamel related incisor vascular beds have a distinct vascular architecture demarcated by a "boundry zone" of anastomsing vessels extending along the length of the tooth. The microvascular bed overlying the enamel is separated from the alveolar wall by a dense venour system. This investigation provides the first description of the rodent incisor "boundry zone" and the related vasculature using plastic injection replicas.

Key Words: Microvascular bed, mouse incisors, methacrylate perfusion casts, SEM.

INTRODUCTION

Rodent incisors have been used for many studies including those of Beertsen and Everts (1977) and Berkovitz et al. (1981). Kindlova and Matena (1959) investigated the vascular morphology of the rat mandibular incisor using latex casts but the maxillary incisor was not described because it was said to be comparatively more complicated. Garfunkel and Sciaky (1971) using India ink perfusions published an account of the vascular supply of rat mandibular and maxillary incisors and recently Wakatsuki, Saitoh and Koyama (1980) traced the capillary networks of the rat mandibular incisor with a series of non-plastic perfusion techniques.

Although the mouse is extensively employed in dental research (Beertsen and Everts, 1977; McCulloch and Melcher, 1983), definitive information on the vascular patterns of the rodent incisor periodontal ligament has not been published. This investigation describes the microvascular bed of the coronal two thirds of the mouse maxillary and mandibular incisors opposite the enamel and adjacent to the enamel-cementum junction. Methyl methacrylate perfusion casts (Murakami, 1971; Inchikawa et al., 1976; Gannon, 1981) were prepared and recorded in stereopair photographs with the scanning electron microscope.

MATERIAL AND METHODS

Ten male LACA laboratory mice weighing 30 g and aged six weeks were used. Anaesthesia was produced with intra-peritoneal urethane (30.0 mg/10 g body weight) and each animal injected with 0.3 ml of a solution comprising 90 mg NaN02, 9 ml Rinser's solution and 10 IU heparin via a tail vein. The vascular system was washed out through the left ventricle using physiological saline containing 10 IU heparin, 1 x 10-7 g/ml papaverine and 58.7 g/l polyvinylpyrrolidone heated to 37 C. Casting medium for injection comprised 0.2 g benzoyl peroxide (initiator), 6.0 ml hydroxy-propyl-methacrylate monomer (plasticizer) and 0.3 ml n-n dimethyl-aniline (accelerator) dissolved in 14.0 ml of previously prepared, partly polymerized monomer (Gannon, 1981). The casting medium was injected into the aorta under constant pressure at 37 C. When the plastic had set the mouse was immersed in hot water to complete cast polymerisation. The heads were removed and processed through a repeating series of corrosive solutions including 20% KOH, 10% HCl and 5% NaOCl until cleared. The jaws were then frozen in water, sectioned, dehydrated, mounted on aluminium stubs with Dag and exposed to OsO4 and hydrazine hydrate vapour before sputter coating with sold at a pressure of 0.15-0.20 Torr and a 150 MA current. Plastic casts were examined in the scanning electron microscope and photographed in stereopairs at a 6° angle of tilt for three-dimensional imaging and assessment.

FINDINGS

The saggital view of the mesio-proximal aspect demonstrated distinct patterns of the vasculature corresponding to the enamel and cementum zones of the tooth (Figure 1). These zones were sharply delineated by a narrow boundaryformation made up of a thick, principal, longitudinal vessel with an internal diameter which varied from 100 microns to less than 70 microns (Figure 2). This vessel coursed parallel to the long axis of the incisor and extended from the gingival to the apical region. Shallow, irregularly oriented endothelial cell nuclei imprints along the vessel wall indicated its venous nature (Hodde and Nowell, 1980). Numerous anastomoses occurred with the smaller vessels of the cementum and enamel-related blood system.

The inner layer of the microvascular bed corresponding to the enamel zone exhibited a dense complex network of vessels (Figures 1, 2). These vessels were short, smaller than their limiting boundary segments, and averaged 10 to 15 microns in internal diameter but did not extend beyond the mesial and distal margins of the enamel layer. They were arranged approximately parallel to each other and formed a compact anastomosing microvascular bed. Because of their principal mesio-distal alignment at right angles to the long axis of the tooth, and the short inter-connecting branches between them, this vessel system exhibited a ladder-like

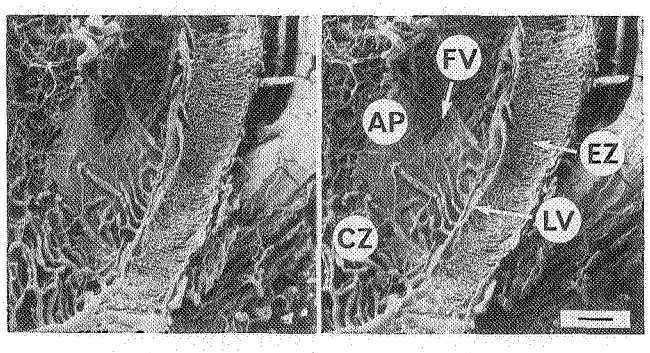

Figure 1 — Stereopair views of the mesio-proximal third of the maxillary incisor periodontal ligament vessels in the mid-root region. Sagittal section. AP, portion of alveolar wall: CZ, large vessels of the cementum zone; EZ, enamel zone vascular system; LV, longitudinal boundary zone vessels; FV, fine vessels of the cementum zone.
Magnification X30 Bar: 300 microns

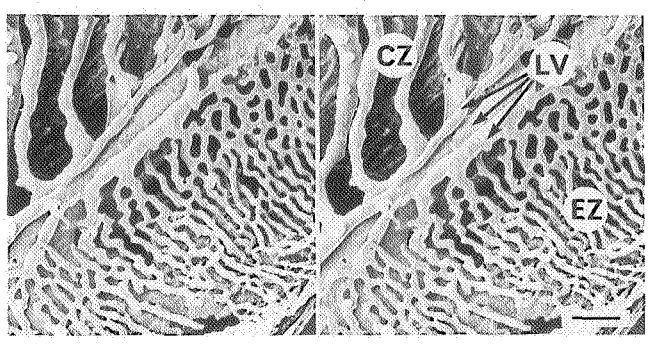

Figure 2 — Junction of the enamel and cementum microvascular beds at the boundary zone. The venous system extends between the enamel zone vessels and the alveolar wall. CZ, cementum zone vessels; EZ, enamel zone microvascular bed; LV, longitudinal anastomosing vessels of the boundary system.
Magnification X125 Bar: 100 microns

appearance. At its mesial and distal limits the enamel zone anatomoses coalesced to form an irregular, somewhat discontinuous longitudinal vessel, with an internal diameter approximating 30 microns, which periodically anastomosed with the principal venous structure.

A plexus of large venous vessels was positioned between the enamel zone microvascular bed and the alveolar wall (Figure 2). The arrangement and dimensions of the venous plexus vessels was not determined in detail although they drained into the major venous boundary vessel below the lateral margin of the enamel zone network. In the coronal two thirds of the ligament the venous system also had a mesiodistal orientation.

By contrast, the cementum zone exhibited a rather loose network of irregularly arranged vessels connected to the boundary system (Figure 1). The cementum related vessels were aligned approximately at right angles to the boundary venous vessel and appeared to be distributed at two layers in the occlusal third of the incisor but one layer in the middle third. The vessels of the layer adjacent to the tooth were large and interconnected freely with each other in the coronal region (Figure 1) whereas those adjacent to the alveolar wall were finer with fewer lateral anastomoses and could be seen more clearly towards the mid-root portion of the cementum zone (Figure 1). These fine vessels ran alongside and through the wall of the alveolar socket. The venous components of the cementum zone in the coronal third of the periodontal ligament appeared to be larger than the vessels in the middle third and ranged from 10-15 microns in internal diameter.

In the present investigation the periodontal ligament vessels of both the upper and lower incisors formed similar enamel and cementum microvascular beds enveloping the coronal two thirds of the root surface.

DISCUSSION

The mouse incisor microvascular zones demarcated by a system of boundary vessels have not been described previously. A feature of the maxillary and mandibular incisors was the plexus of large venous vessels which formed a separate layer between the enamel-related microvascular system and the alveolar wall.

Although Kindlova and Matena (1959) investigated the vascular morphology of the rat mandibular incisor, the maxillary incisor was not described because the authors were of the opinion that it was more complicated than the mandibular incisor. The present study found no significant differences between the mouse maxillary and the mandibular incisor vascular system in the periodontal ligament.

Garfunkel and Sciaky (1971) examined the rat maxillary and mandibular incisors and found large vessels at the apex of the maxillary incisors supplying the pulp and the periodontal ligament. As with the mouse the periodontal vessels of the cementum region were reported to be generally larger than those found alongside the enamel region. The luminal size of the mouse incisor vessels overlying the cementum side also increased in diameter towards the coronal end which corresponded with the observations in rats by Garfunkel and Sciaky. However, detailed features of rat periodontal vessels regarding type, size, shape, number and orientation were not depicted in their publication.

Iwaku and Ozawa (1979) studied rat incisors but only gave a vague description of the mandibular incisor vasculature simply stating that the periodontal vessels of the enamel zone were supplied by a ladder-like capillary network. They did not elaborate further on the arrangement and type of these capillary vessels nor their relationship to the rest of the microcirculatory bed. Furthermore, the vessels opposite the cemenum zone were totally ignored.

Using the light microscope Gangler and Merte (1979) described the pattern of arteriovenous anastomoses and the direction of blood flow in the periodontal ligament of the rat incisor, but there was no evidence to support their interpretations in the present study. It could be that the pattern reported by Gangler and Merte was species specific, or the result of overlapping images of the vascular networks located in the enamel zone as illustrated in Figure 2. Such structures could easily be misinterpreted, when viewed under vital light microscopy with its inherent disadvantage of a shallow depth of field.

In a stereoscopic evalution of non-plastic perfusions of rat mandible blood vessels, Wakatsuki et al. (1980) illustrated similar vascular arrangements to the mouse over the emanel and cementum portions which they described as artery nets. On the contrary, the mouse microvascular bed is essentially venous. No mention of the boundry layer was made by Wakatsuki et al.

The use of injection replica casts recorded in stereopairs has revealed new aspects of the intricate microvascular architecture of the mouse incisor periodontal ligament. As an extension of this investigation transmission electron microscopic studies are being undertaken to provide complementary qualitative and quantitative information on this vascular system and determine if the incisor microvascular bed is essentially venous like the mouse molar ligament (Sims 1983).

REFERENCES

BEERTSEN, W. & EVERTS, V. (1977)
Site of remodelling of collagen in periodontal ligament of mouse incisor.
Anat.Rec. 189:479-498.
BERKOVITZ, B.K.B., WEAVER, M.E., SHORE, R.C. & MOXHAM, B.J. (1981)
Fibril diameters in the extracellular matrix of the periodontal connective tissues of the rat.
Connect.TissueRes. 8:127-132.
GANGLER, P. & MERTE, K. (1979)
Die vitalmikrOskpische Untersuchung der periodontalen Blutzirkulation und Ratteninzisivus.
Zahn-, Mund- und Kieferheilka. 67:129-136.
GANNON, B.J. (1981)
Preparation of microvascular corrosion casting media: procedure for partial polymerization of methyl methacrylate using ultraviolet light.
Biomed.Res. 2:227-233.
GARFUNKEL, A. & SCIAKY, I. (1971)
Vascularization of the periodontal tissues in the adult laboratory rat.
J.dent.Res. 50:880-887.
HODDE, K. & NOWELL, J. (1980)
SEM of micro-corrosion casts.
SEM/Part II, 88-106.
SEM Inc., AMF O'Hare (Chicago). Il. 60666
ICHIKAWA, T., WATANABE, O. & YAMAMURA, T. (1976)
Vascular architecture in oral tissues by vascular casts. Method for Scanning Electron Microscopy.
9th Europ.Conf. Microcirculation,Antwerp,Bibl.anat. 15:544-546.
IWAKU, F. & OZAWA, H. (1979)
Blood supply of the rat periodontal space during amelogenesis as studied by the injection replica SEM method.
Arch.histol.Jap. 42:81-88.
KINDLOVA, M. & MATENA, V. (1959)
Blood circulation in the rodent teeth of the rat.
Acta.Anat. 37:163-192.
McCULLOCH, C.A.G. & MELCHER, A.H. (1983)
Cell density and cell generation in the periodontal ligament of mice
Amer. J. Anat. 167:43-58.
MURAKAMI, T. (1971)
Application of the scanning electron microscope to the study of the fine distribution of the blood vessels.
Arch.histol.Jap. 32:445-454.
SIMS, M.R. (1983)
The microvascular venous pool and its ultrastructural associations in mouse molar periodontal ligment.
Submitted to Aust.Orthodont.J.
WAKATSUKI, E., SAITOH, S & KOYAMA, K. (1980)
Stereoscopic observations of the blood vessels of rat mandibles.
Bull.Tokyo dent.College 21:111-125.

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