

Microvascular architecture of the buccal periosteum of the mandibular body in the dog

Akihiko Kuwabara, Mamoru Uemura, Isumi Toda, Fumihiko Suwa and Akimichi Takemura

Department of Anatomy, Osaka Dental University, 8-1 Kuzuhahanazono-cho, Hirakata-shi, Osaka 573-1121, Japan

We investigated the morphology of the microvascular architecture of the inner layer of the buccal periosteum of the mandibular body in the dog using the acrylic resin injection method for corrosion casts. These corrosion casts were examined from the bone side via scanning electron microscopy. The microvascular architecture of the periosteum comprised two layers : an inner and an outer layer. Infrequent vascular network formation was observed in the outer layer, whereas the inner layer contained venous networks. The microvascular architecture of this inner layer could be classified into four regions, according to differences in the morphology of the vascular networks : the attached gingival region, the alveolar mucosal region, the transitional region, and the basal region. The attached gingival, alveolar mucosal, and transitional regions corresponds to the alveolar portion of the mandible. The basal region corresponds to the basal portion, excluding the alveolar portion. Comparison of the microvascular architecture in the alveolar and basal portions of the mandible revealed that the vascular network in the alveolar portion consisted of finer vascular networks, relative to the basal portion. (J Osaka Dent Univ 2018 ; 52 : 129-137)

Key words : Microvascular architecture ; Mandible ; Periosteum ; Scanning electron microscopy ; Dogs

INTRODUCTION

Many studies of the microvascular architecture of the periosteum have described the long bones (the femur and tibia). According to Heřt and Hladíková¹ and Skawine *et al.*,² the morphology of the microvascular architecture of the periosteum varies with age, type of bone, and location. However, very few studies have examined the microvascular architecture of the periosteum of the mandible.³⁻⁶ Because the mandible supports the teeth, which are responsible for mastication, it exhibits a comparatively distinctive structure, relative to that of the long bones. In addition, the periosteum of the alveolar bone is covered by the oral mucosa, whereas the periosteum of the basal part of the mandible^{7,8} is covered with skin. Relative to the gomphosis of the teeth, the periosteum from the superior margin of the alveolar bone on both buccal and lingual slopes is the alveolar periosteum, with

the gingival fibers at its upper edge.^{7,8} The morphology of the microvascular architecture of the alveolar portion⁸ of the periosteum is affected by the gingival fibers ; in contrast, the base⁸ of the periosteum is not. We prepared resin injection corrosion casts of the microvascular architecture of the periosteum of the mandibular body in the dogs, from the superior margin of the alveolar portion to the base of the mandible ; we then observed these by scanning electron microscopy to identify the morphological characteristics of the microvascular architecture in these regions.

MATERIALS AND METHODS

Experimental animals

Four mixed-breed male dogs, weighing approximately 4.5 kg, with complete permanent dentition, belonging to the Department of Anatomy, Osaka Dental University, were used in this study. For three of the four dogs, specimens produced by the

acrylic resin injection method, described by Ohta *et al.*,⁹ were used as microvascular corrosion casts. The other animal was processed as a formalin-fixed specimen that had not previously been injected with acrylic resin. This animal study was approved by the Osaka Dental University Animal Research Committee and complied with the guidelines for the use of animals in research.

Preparation of microvascular corrosion casts

The mandibles of two of the three animals that had been used to prepare microvascular corrosion casts were split bilaterally to provide four specimens. To observe the periosteal microvascular architecture of these four specimens from the bone side, the mandible of each was removed in the area of the third premolar. The specimens were soaked in a 5% sodium hypochlorite solution in a 42°C water bath (Thermo Regulator, CTR-320; Iwaki, Tokyo, Japan) for 24 hours to remove soft tissue. They were then rinsed in water and immediately soaked in 5%

hydrochloric acid in a 42°C water bath for 24 hours to remove hard tissue. Each specimen was rinsed in running hot water at 42°C for 3 hours. They were then allowed to dry naturally and used as microvascular corrosion casts.

Preparation of microvascular corrosion cast-bone specimens

The mandible of the third animal was split bilaterally to provide two specimens. Each of these was removed in the area of the third premolar and transected buccolingually at the mesial root of the third premolar. These specimens were soaked in a 5% sodium hypochlorite solution in a 42°C water bath for 24 hours to remove only the soft tissue. They were then rinsed in running hot water at 42°C for 12 hours, after which they were cleaned using an ultrasound cleaning device (UT-105 HS; Sharp, Osaka, Japan) at 42°C for 10 minutes and allowed to dry naturally for 7 days before preparation of microvascular corrosion cast-bone specimens.

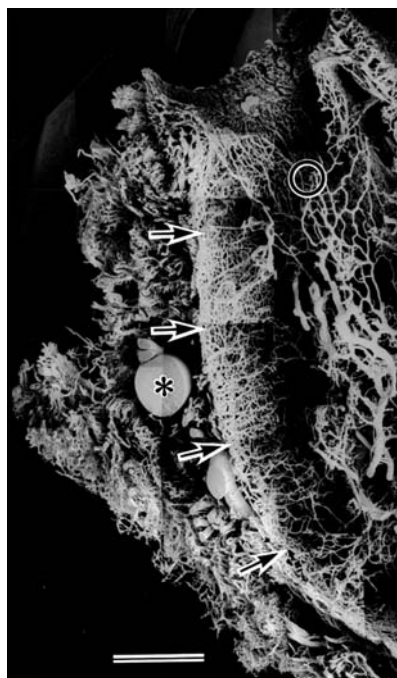


Fig. 1 Scanning electron micrograph of a microvascular corrosion cast with soft and hard tissues removed showing a cross-sectional view of a buccolingual section at the mesial root of the mandibular third premolar.

*Inferior labial artery, Arrows: Vascular network of the buccal periosteum, Circle: Alveolar socket, Bar=3 mm.

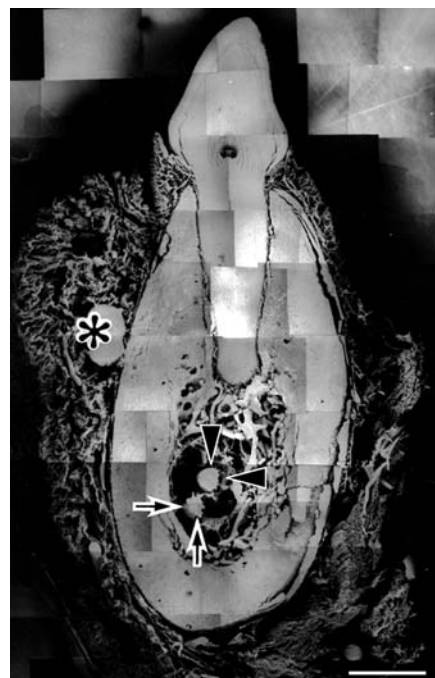


Fig. 2 Scanning electron micrograph of microvascular corrosion cast-bone specimens showing a cross-sectional view of a buccolingual section at the mesial root of the mandibular third premolar.

*Inferior labial artery, Arrows: Inferior alveolar vein, Arrowheads: Inferior alveolar artery, Bar=3 mm.

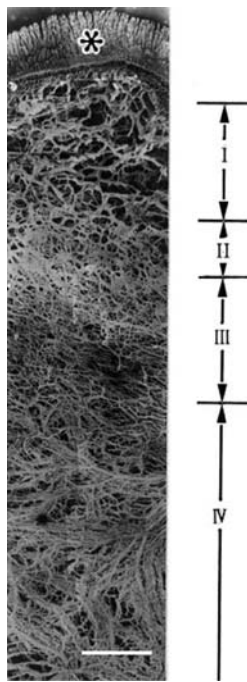


Fig. 3 Scanning electron micrograph of a microvascular corrosion cast of the periosteum showing the microvascular architecture of the buccal periosteum in the mandibular third premolar region, viewed from the bone side.

*Microvascular architecture of the free gingival area, I: Attached gingival region, II: Alveolar mucosal region, III: Transitional region, IV: Basal region, Bar=400 μ m.

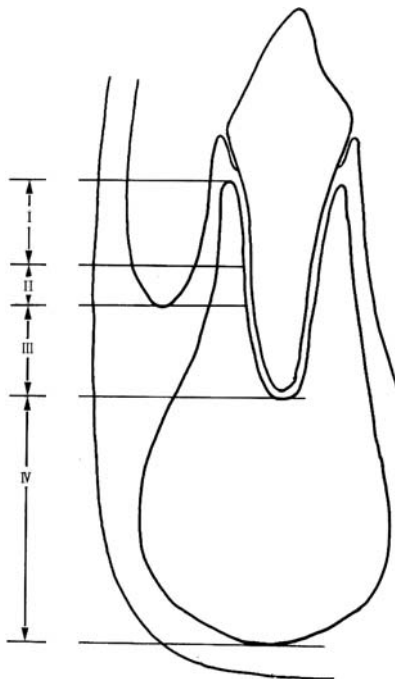


Fig. 4 Diagram of the four regions of the microvascular architecture of the buccal periosteum.

The microvascular corrosion casts (Fig. 1) and the microvascular corrosion cast-bone specimens (Fig. 2) were fixed to metal stages using electro-conductive tape (NEM tape; Nisshin EM, Tokyo, Japan) and silver paste (Dotite; Fujikura Kasei, Tochigi, Japan). They were coated with gold using an ion-sputter coating device (JFC-1500[®]); JEOL, Tokyo, Japan). The microvascular architecture specimens were observed using a scanning electron microscope (JSM-5500[®]); JEOL) and digital images were created. As shown in Figs. 3 and 4, for convenience the microvascular architecture was observed in four regions: the attached gingival, alveolar mucosal, transitional, and basal regions. We measured vessel and reticulation diameters with image analysis and measurement software (Image-Pro Plus[®]) 5.0 J; Nippon Roper, Tokyo, Japan).

Histological specimen preparation

The mandible of the formalin-fixed animal that had not been injected with acrylic resin was split bilaterally to provide two specimens; the mandibular bone of each specimen was removed in the area of the third premolar. Both specimens were decalcified for 3 days in a 10% nitric acid solution at room temperature, after which they were immediately soaked in 5% sodium sulfate for 1 day, then rinsed in running water for 2 days. They were then dehydrated in a series of ascending concentrations of alcohol. After dehydration, specimens were soaked in xylene for 8 hours at room temperature. The first specimen was then embedded in paraffin, whereas the second specimen was embedded in resin. The first specimen was immersed in paraffin at 63°C for 6 hours to yield a paraffin-embedded specimen, which was sliced into a continuous series of 6- μ m sliced specimens using a microtome (Microm HM 350[®]); Carl Zeiss Japan, Tokyo, Japan); then, Mallory Azan staining¹⁰ was performed. The second specimen was immersed in resin (Technovit[®]) 7200 VLC; Kulzer, Germany) for 24 hours in a refrigerator at 4°C to produce a resin-embedded specimen, which was sliced into 8- μ m sections using a microtome; then, hematoxylin-eosin staining was performed. Both sets of slices were examined and

photographed using an optical microscope (Eclipse®; Nikon, Tokyo, Japan) fitted with a digital camera (DS Rc1®; Nikon).

RESULTS

The microvascular architecture of the buccal periosteum in the mandibular premolar region was divided into two layers: an inner layer and an outer layer. We will describe our observations of the microvascular architecture of the outer and inner layers; within the inner layer, we will describe our observations of the attached gingival region, the alveolar mucosal region, the transitional region, and the basal region.

Microvascular architecture of the outer layer of the periosteum

The outer (fibrous) layer, shown in Figs. 5 and 6, contained venules with a thickness of 42-109 μm ; in most cases, arterioles with a thickness of 34-56 μm were flanked by 1-2 venules. There was no venular or arteriolar network formation (Figs. 7 and 8). The arterioles branched to form arterial capillaries with a thickness of 12-23 μm ; further, they formed anastomoses with the venules of the outer layer, as well as with the venules and venous capillaries of the inner layer.

Microvascular architecture of the inner layer of the periosteum

The microvascular architecture of the inner (osteogenic) layer consisted largely of a venular network, which enclosed a venous capillary network, excluding the attached gingival region (Figs. 3 and 7).

Attached gingival region

In the attached gingival region, the venules with a thickness of 45-67 μm were coarser than those of the alveolar mucosal region. There was no evidence of venous capillary network formation within the venular network (Fig. 9). The interstices of this network were 230-350 μm in diameter. The venules were flanked by 1-2 venous capillaries with a thickness of 21-30 μm (Fig. 9).

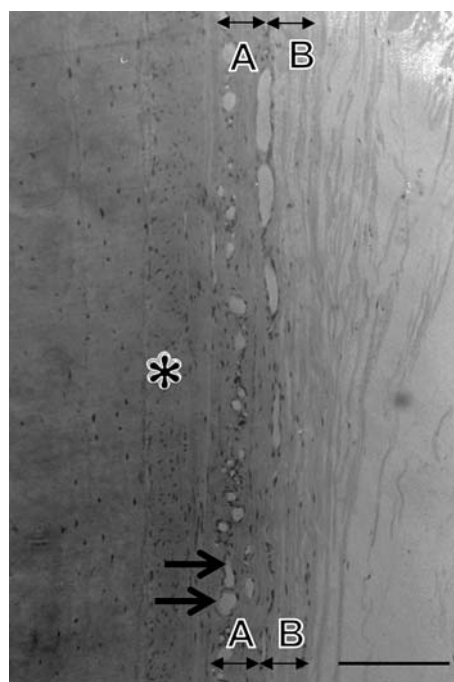


Fig. 5 Resin-embedded histological specimen (hematoxylin and eosin staining, low magnification).

*Mandible, A: Inner layer of periosteum, B: Outer layer of periosteum, Arrows: Blood vessels, Bar=250 μm .

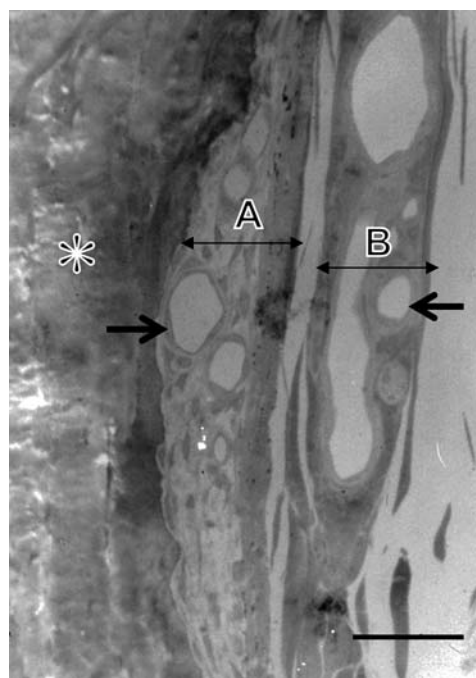


Fig. 6 Resin-embedded histological specimen (hematoxylin and eosin staining, high magnification).

*Mandible, A: Inner layer of periosteum, B: Outer layer of periosteum, Arrows: Blood vessels, Bar=75 μm .

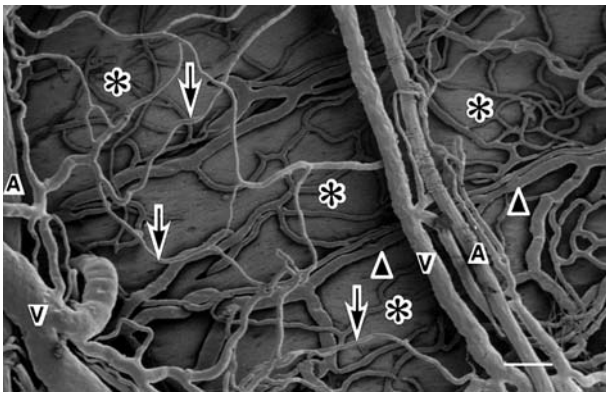


Fig. 7 Scanning electron micrograph of the microvascular architecture of the outer and inner layers of the periosteum, as seen from the outside.

*Venous capillary network of the inner layer, A: Arterioles in the outer layer, V: Venules in the outer layer, Arrows: Capillary vessels in the inner layer of the periosteum, Arrowheads: Venules in the inner layer of the periosteum, Bar=100 μ m.

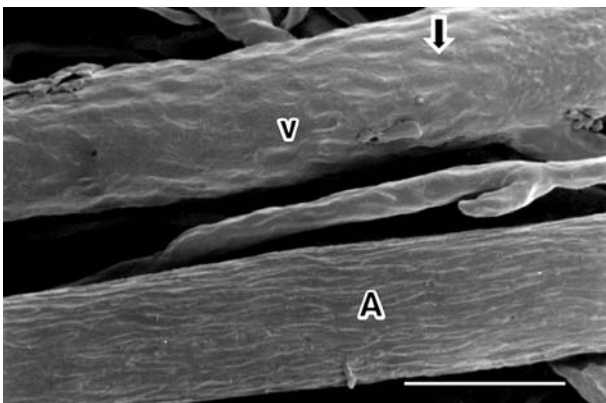


Fig. 8 Scanning electron micrograph of the microvascular corrosion casts of the arterioles and venules within the outer layer of the periosteum. Nucleus pressure scars (arrow) are visible on the surface of the corrosion casts of the venules.

A: Arteriole, V: Venule, Bar=100 μ m.

Alveolar mucosal region

The venular networks in the alveolar mucosal region were composed of the small (fine) interstices 40-130 μ m in diameter and large (rough) interstices 150-260 μ m in diameter. Interstices of the small venular network were smaller than interstices in other regions (Figs. 9 and 10). The large venular network lay scattered in the alveolar mucosal region. The venous capillary network with a diameter of 15-20 μ m was formed from venous capillaries in the large (rough) venular network. The gingival fiber bundles

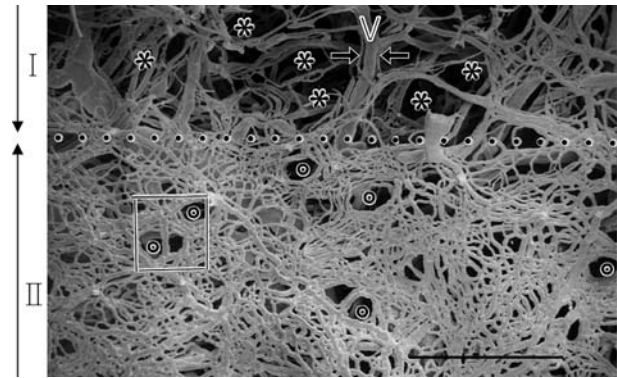


Fig. 9 Scanning electron micrograph of the microvascular architecture of the inner layer of the periosteum. Attached gingival region (I) and alveolar mucosal region (II). The dotted line shows the boundary between the attached gingival region and the alveolar mucosal region.

*Venular networks in the attached gingival region, Circles: Venular networks in the alveolar mucosal region, V: Venules, Arrows: Venous capillaries, Bar=1000 μ m.

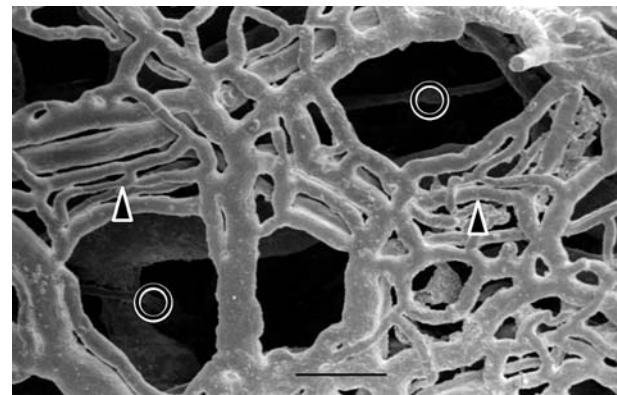


Fig. 10 Scanning electron micrograph of the venular network and venous capillaries in the alveolar mucosal region. Venous capillary networks (arrowheads) can be seen inside the venular networks (circles). Bar=100 μ m.

with a width of 140 μ m perforated into the periosteum in the histological specimens (Fig. 11). The thickness of the venules was 30-45 μ m, whereas the thickness of the venous capillaries was 15-20 μ m, and the thickness of the interstices of the venous capillary network was 30-56 μ m

Transitional region

The interstices of the venular network in the transitional region had a diameter 260-430 μ m and were larger than those of the interstices in the alveolar

mucosal region; a fine venous capillary network with numerous interstices with a diameter of 90-115 μm was present within the venular network (Figs. 12 and 13). The thickness of the venules was 38-70 μm , whereas the thickness of the venous capillaries was 9-13 μm .

Basal region

The interstices of the venular network in the basal

region,⁸ which had diameters of 530-830 μm , were larger than those of the interstices in any other region; moreover, a venous capillary network was present within the venular network. This venous capillary network exhibited larger interstices, which had diameters of 185-360 μm , relative to the venous capillary network in the transitional region (Figs. 12 and 14). The thickness of the venules

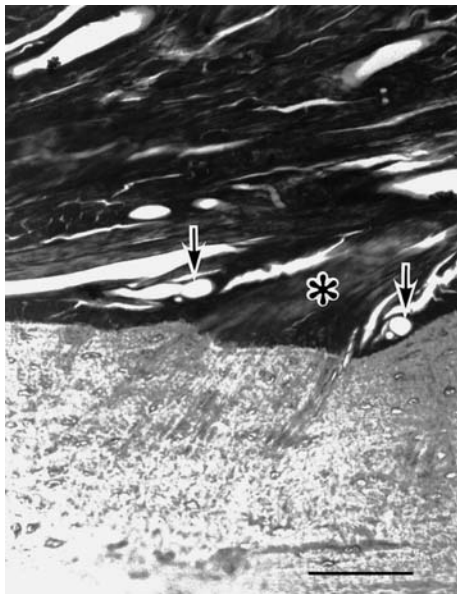


Fig. 11 Histological specimen showing optical microscopy image of gingival fibers becoming Sharpey's fibers in the bone of the alveolar mucosal region (Mallory Azan staining). *Gingival fiber bundle penetrating the periosteum, Arrows: Blood vessels, Bar=200 μm .

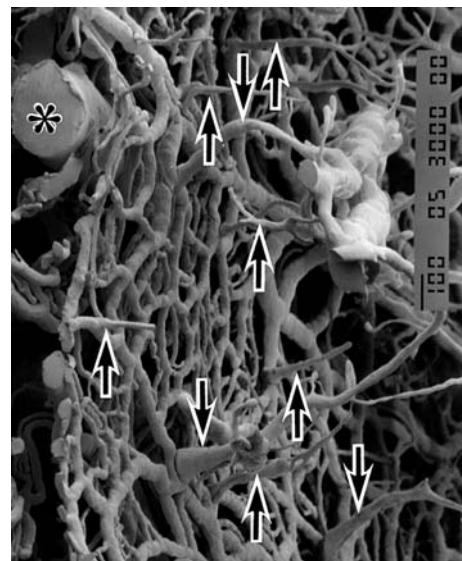


Fig. 13 Scanning electron micrograph of the microvascular architecture of the inner layer of the periosteum in the transitional region in an image taken from above the bone side of the microvascular architecture. Emissary veins (arrows) form anastomoses with the venules and venous capillaries in the inner layer of the periosteum. *Venules, Arrows: Emissary veins, Bar=100 μm .

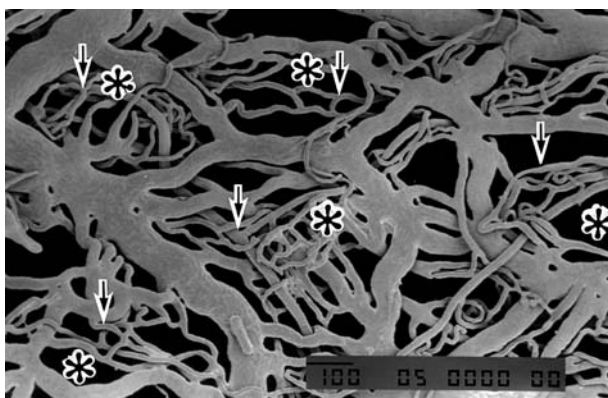


Fig. 12 Scanning electron micrograph of the venular network and venous capillaries in the transitional region. *Venular networks, Arrows: Venous capillaries, Bar=100 μm .

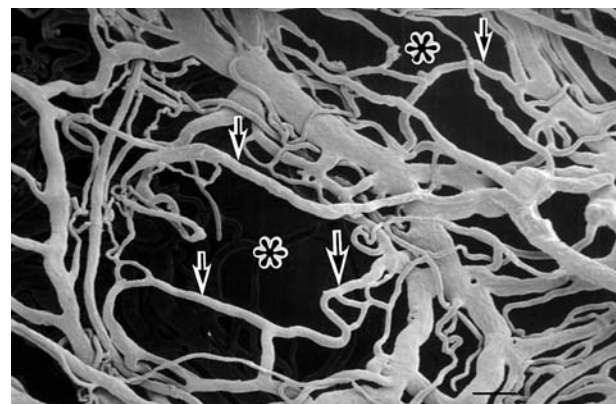


Fig. 14 Scanning electron micrograph of the venular network and venous capillaries in the basal portion of the mandible. A venous capillary network (*) consisting of venous capillaries (arrows) can be seen inside the venular network. Bar=100 μm .

Table 1 Comparison of interstices of the venular and venous capillary networks, and thickness of venula and venous capillaries in each region

Region	Interstices of venular network	Thickness of venula	Interstices of venous capillary network	Thickness of venous capillaries
I	230-350	45-67	—	21-30
II	40-130* 150-260**	30-45	30-56	15-20
III	260-430	38-70	90-115	9-13
IV	530-830	45-65	185-360	20-25

(μm)

*Venular network of small (fine) interstices, **Venular network of large (rough) interstices, I: Attached gingival region, II: Alveolar mucosal region, III: Transitional region, IV: Basal region.

was 45-65 μm and the thickness of the venous capillaries was 20-25 μm. Table 1 shows the diameters of the interstices of the venular networks, the thicknesses of the venules, the diameters of the interstices of the venous capillary networks, and the thicknesses of the venous capillaries in the four different regions.

Histological findings

Hematoxylin and eosin staining

The buccal periosteum was divided into two layers: an inner layer and outer layer. Many vessels were observed in the inner layer (Figs. 5 and 6).

Mallory Azan staining

Within the periosteum in the alveolar mucosal region, the gingival fiber bundles, which were approximately 140 μm, penetrated the periosteum and became Sharpey's fibers in the bone (Fig. 11).

DISCUSSION

The microvascular architecture of the periosteum has been studied by Heřt and Hladíková,¹ and by Skawine *et al.*² in human long bones (the femur and tibia), as well as by various investigators (e.g., Irino *et al.*,¹¹ Draenert and Draenert,¹² Ohtani *et al.*,¹³ and Iwaku *et al.*¹⁴) in rat long bones. Studies of the microvascular architecture of the mandibular periosteum include those of Aharinejad³ in guinea

pigs, Ri⁴ in rats, Nobuto *et al.*⁵ in dogs (mandibular ramus), and Makigusa *et al.*⁶ in monkeys. However, those studies only assayed the microvascular architecture of the periosteum of alveolar bone; thus, there has been no report of the periosteal microvascular architecture from the superior margin of the alveolar region to the inferior margin of the mandible. Therefore, we include here a discussion of observations regarding the microvascular architecture of the buccal periosteum in the premolar region of the mandibular body in the dog.

Microvascular architecture of the inner and outer layers of the periosteum

Skawine *et al.*² reported that the dense capillary network in the periosteum of human long bones is in contact with and nourishes the cortical bone. Trueta¹⁵ described an association between bone remodeling and blood vessels, and Takahashi^{16, 17} addressed resorption of the mandible and the vasculature, noting that the alveolar bone is constantly deformed by occlusal pressure and thus undergoes repeated remodeling. In their description of the vascular architecture of the diaphyseal periosteum of rat long bones, Iwaku *et al.*¹⁴ stated that the inner layer forms a vascular bed of capillary vessels that serve as a nutrient supply system, whereas the outer layer contains an irregular venous network that serves as a drainage system. In response to periods of osteogenesis, resorption, and quiescence, state changes may occur in the network of the inner layer. Previous reports suggest that the mandibular periosteum is intimately linked with mandible remodeling; a similar relationship may be true for the microvascular architecture of the periosteum.

The structure of the mandibular periosteum in dogs, as in the periosteum of the long bones, consists of an outer and an inner layer. The outer layer does not contain a venular or arteriolar network, as these are present in the inner layer. This suggests that the nutrient supply system, formed by the inner layer of the mandibular periosteum and its venular and arteriolar capillary networks, may be affected by bone remodeling.

Microvascular architecture of the attached gingival region (alveolar portion of the mandible)

The size of the interstices of the venular network in this region was larger than in the alveolar mucosal region. The attached gingival region contains numerous alveologingival fibers^{7,8} and cementoperiosteal fibers,^{7,8} which pass through this part of the periosteum and affect the morphology of the periosteal microvascular network. The presence of alveologingival and cementoperiosteal fibers may affect the morphology of the vascular network in this region, such that it comprises a coarse venular network with large interstices, without the formation of a venous capillary network; notably, this interaction is unconfirmed.

Microvascular architecture of the alveolar mucosal region (alveolar portion of the mandible)

The interstices of the small venular network in this region were smaller than interstices in other regions. Moreover, the large venular network lay scattered in the alveolar mucosal region. We regarded this as a feature. We reviewed the large (rough) venular network: the cement periosteum fiber bundle, which was adhered with cementum to the lateral wall of the alveolar bone, existed in this region. Additionally, the large (rough) venular network has large (rough) interstices that are larger than the width of fiber bundles perforating from the periosteum. Therefore, it appeared that the large (rough) venular network involved the existence of fiber bundles perforating from the periosteum.

Microvascular architecture of the transitional region (alveolar portion of the mandible)

The venous capillary network within the venular network became coarser from the alveolar mucosal region to the transitional region, as well as from the transitional region to the basal region. This may occur in relation to a decrease in the number of venous capillaries.

Microvascular architecture of the basal region (basal part of the mandible)

The venular network was coarser in the basal re-

gion⁸ than in the transitional region; moreover, there were coarser venous capillaries forming a network within the venular network, relative to the transitional region. The interstices of the venular network in this region were thus larger than those in other regions, and the venous capillary networks within the venular network also exhibited coarser interstices.

Microvascular architecture of the emissary veins

The vessels of Volkmann's canal, as well as those from the bone marrow and the periodontal membrane, may flow into the microvascular architecture of the inner layer of the periosteum.^{16,17} As this subject was not the purpose of our present study, however, the matter remains unclear. As shown in Fig. 13, a very large number of vessels are present, which is very important. The relationship between these vessels and the microvascular architecture of the inner layer of the periosteum serves as a topic for future research. In this paper, all venous vessels transporting blood from the bone side to the periosteum were regarded as emissary veins.

Our observations of the mandibular periosteum showed that the attached gingival, alveolar mucosal, and transitional regions of the periosteum corresponded to the alveolar portion of the mandible, whereas the basal region corresponded to the basal portion⁸ of the mandible. The alveolar bone is subjected to high biomechanical stress (masticatory pressure) as a result of gomphosis teeth; however, the base of the mandible is less affected by this stress. The alveolar portion may also undergo higher rates of bone remodeling than the base,^{16,17} which may contribute to the finer structure of the vascular network of the periosteum in the alveolar portion of the mandible, relative to the base. However, the presence of alveologingival and cementoperiosteal fibers may affect the morphology of the vascular network in the attached gingival region, such that it comprises a coarse venular network with large interstices, without the formation of a venous capillary network.

This study was presented at the 72nd Annual Meeting of the Japanese Stomatological Society (Nagoya, Japan) on May 13, 2018. We would like to thank the staff of the facilities of Osaka Dental University for their support with the animal experimentation and image processing. We are indebted to the staff of the Department of Anatomy for their advice and encouragement. The authors have no conflicts of interest to declare with respect to this paper.

REFERENCES

1. Heřt J, Hladíková J. Die Gefäßversorgung des Haversschen Knochens. *Acta Anat* 1961; **45**: 344-361. (German)
2. Skawine A, Litwin JA, Gorczyca J, Miodoński AJ. The vascular system of human fetal long bones: a scanning electron microscope study of corrosion casts. *J Anat* 1994; **185**: 369-376.
3. Aharinejad S, Franz P, Firbas W, Fakhari M. Mandibular and molar vascularization in guinea pigs – Scanning electron microscopic study of corrosion casts –. *Anat Rec* 1990; **228**: 471-477.
4. Ri S. Age-related changes in the periosteal vasculature – Microvascular architecture and endothelial permeability –. *J Jpn Soc Periodontol* 1991; **33**: 799-823. (Japanese with English abstract)
5. Nobuto T, Yanagihara K, Teranishi Y, Minamibayashi S, Imai H, Yamaoka A. Periosteal microvasculature in the dog alveolar process. *J Periodontol* 1989; **60**: 709-715.
6. Makigusa K, Toda I, Suwa F. Microvasculature of the mandibular periosteum in Japanese monkeys. *J Jpn Soc Periodontol* 2001; **43**: 227-239. (Japanese with English abstract)
7. Avery JK. Histology of the gingiva and epithelial attachment. In: Avery JK, Steel PF, eds. Oral development and histology, 3rd ed. New York: Georg Thieme Verlag, 2002; 263-267.
8. Amano O. Anatomy in the vicinity of oral cavity. In: Maeda T, Endou K, Hatanaka Y, eds. The newest series of textbook for dental hygienists – Oral anatomy, oral development and histology. Oral physiology. Tokyo: Ishiyaku, 2011: 10-17, 244. (Japanese)
9. Ohta Y, Okuda H, Suwa F, Okada S, Toda I. Plastic injection method for preparing microvascular corrosion casts for SEM and its practical application. *Okajimas Folia Anat Jpn* 1990; **66**: 301-312.
10. Ban T, Oka H, Miyazaki Y, Takizawa N, Ogata T, Tokoro Y. In: Ogata T, ed. Method of making pathology and histology microscopic specimen. 8th revised ed. Tokyo: Nanzando, 1954: 157-160. (Japanese)
11. Irino S, Ono T, Watanabe K, Toyota K, Uno J, Takasugi N, Murakami T. SEM studies on microvascular architecture, sinus wall, and transmural passage of blood cells in the bone marrow by a new method of injection replica and non-coated specimens. Proceedings of the eighth annual scanning electron microscopy symposium 1975; 267-274.
12. Draenert K, Draenert Y. The vascular system of bone marrow. *Scan Electron Microsc* 1980; **IV**: 113-122.
13. Ohtani O, Gannon B, Ohtsuka A, Murakami T. The microvasculature of bone and especially of bone marrow as studied by scanning electron microscopy of vascular casts – A review. *Scan Electron Microsc* 1982; **I**: 427-434.
14. Iwaku F, Ozawa H. Microvasculature of bone, 1 The three dimensional corrosion cast microvasculature on the periosteum of the diaphysis of the long bone. *J Niigata Dent* 1985; **15**: 11-18. (Japanese)
15. Trueta J. The role of the vessels in osteogenesis. *J Bone Joint Surg* 1963; **45B**: 402-418.
16. Takahashi K. Changes in the vasculature of the alveolar bone and its resorption. *Jpn Dent Sci Rev* 1983; **2**: 76-109. (Japanese)
17. Takahashi K. Microcirculations of the periodontal tissue. *J Jpn Med Soc Biol Interface* 1997; **28**: 22-28. (Japanese)