A Scanning Electron Microscopic Observation of Microvascular Changes in Experimental Peri-implantitis

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実験的インプラント周囲炎下における微細血管構築変化の走査電子顕微鏡観察

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In this study, microvascular changes of periodontal and peri-implant tissue after experimental induction of inflammation were investigated, using a vascular resin cast method, to gain insight into the cause-and-effect relationship between inflammation and microcirculation.

A titanium-screw implant was inserted into one side of mandibular bone of beagle dogs, while the other side of the premolars served. In the experimental group, 90 days after implantation, dental floss was placed around the cervical area of an implant fixture and the premolars, to enhance the accumulation of plaque, for another 90 days, but the control group received no placement of dental floss. Plaque control, including brushing, rinsing, and cleaning, was prohibited during this period. To make a vascular resin cast model, synthetic resin was injected into the inferior alveolar arteries. Soft tissues of specimens were digested by a proteinase solution. All of the specimens were examined by scanning electron microscopy.

The vasculature beneath the sulcular epithelium (SE) formed vascular loops, while that of the junctional epithelium (JE) was arranged in a fishnet pattern.

In the case of periodontitis, the arrangement of the SE and JE both changed into glomerulus vascular loops. Vessels in peri-implant soft tissue (PIT) formed a dense network. Under that, a ring-shaped vascular network of connective tissue surrounded the implant neck. In the peri-implantitis, granulated blood vessels of PIT grew toward the apex, and the surrounding alveolar bone was resorbed.

These findings indicate that periodontal and peri-implant vasculature changed easily by experimental inflammation, and that it is more difficult to maintain the vascular structure of PIT than natural periodontal tissue. Protection of microcirculation from inflammation in PIT would ensure that periimplantitis would be amenable to treatment.

Key words: peri-implantitis, microcirculation, resin cast, SEM

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平成13年7月24日受付

13-569
Introduction

To a dental practitioner, the reconstruction of occlusion for a patient who has lost teeth may seem like a challenging dream. However, with recent progress in biological and biomedical engineering fields, this dream has become a reality. Yet an important issue that needs to be resolved is the prevention of inflammation around an osseointegrated implant. Peri-implantitis is defined as an inflammatory process whose function affects the tissues around an osseointegrated implant. The junctional epithelium of a natural tooth provides the actual attachment of the gingiva to the tooth, with the development of hemidesmosomes and a basal lamina that is known to seal a tooth from inflammation, as epithelial protection. In contrast, peri-implant soft tissue is a keratinized epithelium derived from an oral mucosal epithelium, which has no junctional epithelium. Therefore, the peri-implant soft tissue does not adhere to the implant fixture.

To study the vascular architecture of periodontal and peri-implant tissue with thin histologic sections, it is extremely difficult to grasp the vascular network in three dimensions, and it is impossible to make a distinction between the arteries and the veins. The microvascular resin cast method is a useful way to make three-dimensional observations of the vascular architecture. To observe the periodontal and peri-implant tissue, however, a structure to serve as a guide to understanding the relationship between blood vessels and bone is necessary. Thus, dissolving only the soft tissue using protease was devised. When this method is used, it is possible to observe the relationship between the vascular network and bone implants. In this study, microvascular changes of periodontal tissue and peri-implant soft tissue after experimental induction of inflammation were investigated, using a scanning electron microscope (SEM), with a view to gaining insight into the cause-and-effect relationship between inflammation and microcirculation.

Materials and Methods

In accordance with our institutional Animal Care Committee guidelines, three beagle dogs, weighing 10 to 12 kgs, were given general anesthesia of 25 mg intravenously (Nembutal®, Abbott, USA) for this series of experiments.

Surgical procedures (Fig. 1): To insert the implant fixture, after extraction of mandibular premolars we allowed 90 days for healing. After a gingivo-periosteal flap was created, a titanium-screw implant, 8 mm long and 3.3 mm wide (ITI system® TPS type, Straumann AG, Switzerland), was inserted. Enough saline was used during the drilling to avoid heat stimulation. In the experimental group, 90 days after implantation, dental floss was placed around the cervical area of an implant fixture (group d) and premolars (group b) on the right side, to enhance the accumulation of plaque,
for 90 days. In the control group, the implant (group c) and premolars (group a) served as controls, receiving no placement of dental floss. Plaque control, including brushing, rinsing, and cleaning, was prohibited during this 90-day period.

Vascular resin cast procedures: 90 days after the placement of dental floss, the animals were given a vascular perfusion through common carotid arteries with Ringer's solution containing 0.2% heparin, until the jugular veins were cleared of blood. Two-percent glutaraldehyde in a phosphate buffer was introduced into the carotid arteries, to fix. After fixation, the coronoid processes were resected, to expose the inferior alveolar arteries, into which synthetic resin (Mercox®, Dai Nippon Ink, Japan) was injected. The soft tissue was digested by incubating the specimens at 40°C for approximately 14 days, in a phosphate-buffered solution containing 20% proteinase (Prozyme 6®, Amano, Japan). All of the specimens were washed thoroughly with 40°C tap water and freeze-dried. After being ion-coated with platinum-palladium, the specimens were examined under SEM (JSM 6301 F, JEOL, Japan).

**Results**

Figure 2 shows intra-oral views of the 4 groups of the experiment. In the control group, a few dental calculuses are attached to the surface of the crown and implant. The gingival margin shows a slight red color in both groups (a: natural teeth, c: implant). On the other hand, in the experimental group (b: natural teeth, d: implant), dental plaque and calculus are attached to the surface of the teeth with the dental floss. Experimental induction of peri-implantitis was carried out, and suppuration from the peri-implant margin was noted around the implant (d).

The vascular network beneath the sulcular epithelium (SE) and junctional epithelium (JE) of natural teeth (Fig. 3) and the peri-implant soft tissue (Fig. 4) were observed under SEM. Figure 3a shows the periodontal vasculature of natural teeth (control group). The gingival epithelium consists of...
the SE and JE epitheliums. The vasculature beneath the gingival sulcus (SE) formed vascular loops, while that of the JE was arranged in a fishnet pattern. Vessels of this area can be seen anastomosing with the gingival and periodontal ligament (PDL) vessels. The bottom of the JE (arrows) corresponds to that of the epithelial attachment. The lower reaches of this picture represent the vascular network of the PDL, which formed in a polygonal ring shape. Vessels entered and existed the PDL by way of Volkmans canals of the alveolar bone wall. The area between the lower margin of the JE and the alveolar margin was the vascular network of the connective tissue layer (CT).

In the case of periodontitis (Fig. 3b), the arrangement of the SE and JE changed into glomerulus vascular loops. There is no distinction between the SE and JE vasculatures. Leakage of resin was observed along the placement of dental floss (*). The blood vessel of the lower margin of the JE (arrows) can still be seen. Also, the vascular networks of the CT and the alveolar bone (AB) were present.

Vessels in the peri-implant soft tissue (PIT) formed a dense network (Fig. 4a), and under that, a vascular network of CT was observed. The CT vessels ran circumferentially to surround the implant neck, causing a ring-shaped vascular network to form. There was no periodontal ligament around the implants. The blood vessels anastomosed with the gingival vessels (G). The vascular network of peri-implant soft tissue with peri-
Implantitis is shown in Fig 4b. Granulated blood vessels grew toward the apex and surrounding alveolar bone was resorbed remarkably.

The details of peri-implantitis were examined. X-ray film (Fig. 5) indicated a remarkable resorption of alveolar bone (arrowheads). Only the bottom part of the implant retained with the bone can be seen. Figure 6 reveals a vascular resin cast model with an implant fixture (IMP). Dental calculus (DC) accumulated on the dental floss (DF) around the neck of an implant fixture. PIT were naturally in close contact with the upper part of the implant, while a peri-implant pocket was found around the IMP, indicating the destruction of osseointegration (arrowheads). Figure 7 shows a higher magnification of the cervical area. Granulated vessels migrated to the destroyed peri-implant bone (PIB) and IMP. An SEM image of the bone

Fig. 4 Vascular changes beneath the peri-implant epithelium (resin cast model, SEM)
- a: Control group (implant). Vascular network of peri-implant soft tissue (PIT), vascular network of gingiva (G).
- b: Peri-implantitis group (implant with dental floss). Granulated blood vessels (*).

Fig. 5 X-ray photograph of peri-implantitis. Resorbed bone with peri-implantitis (arrowheads), implant fixture (IMP), periodontal ligament (PDL) of mandibular third premolar (P3).
surface of an implant fixture removed from a resin cast model is shown in Fig. 8. The lacuna was filled with expanded, flat blood vessels (BV) of the PIB. The right side of the photograph shows a part that replaced granulated blood vessels (**) while bone resorption progressed. These granulated blood vessels were seen connected with those from alveolar bone marrow.

**Discussion**

The vasculatures of periodontal and peri-implant soft tissues were studied under a light microscope⁹ and a transmission electron microscope¹⁰. SEM observation, using a vascular cast method in rats, showed that the vasculature in peri-implant soft tissues was quite similar to that around natural teeth¹¹. Vascular patterns in the peri-implant soft tissues of dogs were also shown to be structurally identical to those in the gingival sulcus of natural teeth¹². A circular vascular network was noted surrounding the connective tissue layer of the implant fixture, from the bottom part up to the alveolar margin. Vessels were found between circular fibers and ran in a circumferential way parallel to the implant¹³. The junctional epithelium forms a
Fig. 7 Higher magnification of peri-implant pocket (Resin cast model with implant fixture, SEM). Granulated blood vessels (BV), Peri-implant bone (PIB), Implant fixture (IMP).

Fig. 8 Higher magnification of peri-implant bone surface (Resin cast model without implant fixture, SEM). Peri-implant bone (PIB), Lacuna filled with expanded blood vessels (BV), Granulated blood vessels (*)
collar around the natural tooth, which not only provides an attachment for the tooth to the jawbone but also seals the periodontal tissue off from inflammation. The gingival fluid discharged into the gingival sulcus through the junctional epithelial cells has a self-cleansing action, by washing pathogens out of the peri-oral tissue. Vascular networks arranged within the junctional epithelium to some degree play an important role as a barrier to inflammation. In the experimental group of natural teeth, the vasculatures of the SE and JE were changed. But the bottom of the JE was not destroyed. We think this phenomenon reveals that epithelial protection took place. However, the peri-implant soft tissues around an osseointegrated implant do not consist of a functional epithelium similar to that around natural teeth. The inflammatory infiltrate developed due to the bacterial stimulation. Polymorphonuclear leukocytes and monocytes from the circular vascular network were found passing through intercellular spaces in the gingival connective tissue to the gingival sulcus of natural teeth. An increase in the amount of gingival fluid may serve as a mechanism for washing pathogens out of peri-oral tissues and defending against inflammation. However, the sealing capacity around implants should not be overemphasized while comparing to the natural epithelial attachment of a tooth. Peri-implantitis also influenced vascular expression. The vessels formed in the inflamed area showed an apical migration with substantial bone resorption, resulting in the destruction of osseointegration.

The findings of this study indicated that gingival vasculature changed easily by experimetal inflammation, and that it is more difficult to maintain the vascular structure of peri-implant soft tissue than natural periodontal tissue. An implant has no junctional attachment, so epithelial protection on the part of blood vessels is not expected. However, vascular changes of peri-implant tissue incident to plaque control and the vasculature of PIT were changed to the regular arrangement of JE in natural teeth. Meticulous plaque control and adequate protection of microcirculation from inflammation in peri-implant soft tissues would ensure that peri-implant infection would be amenable to treatment.

The authors thank Dr. Takatsuna Nakamura (Implant Center Kyusyu) for clinical suggestions. This study was supported in part by The Japan Society for the Promotion of Science, under a Grant-in-Aid for Scientific Research (C) (No. 11671821).

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