A scanning electron microscope observation of gingival microvasculature after ultrasonic tooth preparation

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Abstract

Purpose: The aim of this study was to observe morphological changes in the gingival microvasculature after tooth preparation. The conventional dental air turbine and ultrasonic methods were examined as a way of tooth preparation. To visualize the changes in the gingival vasculature, a corrosion resin cast was used and visualization performed with a scanning electron microscope (SEM).

Materials and Methods: The preparations were performed on the premolars in Beagle dogs. A chamfered finishing line was created by a diamond instrument along the gingival margin with either a dental air turbine or an ultrasonic wave.

Results: Immediately after preparation using the dental air turbine, teardrop-shaped resin leakage was seen, indicating that bleeding had occurred in the gingiva. Fourteen days after the preparation, resin leakage was seen on the vascular resincast indicating an acceleration of vascular permeability. After 30 days, the blood vessels constructed glomerulus loops. Immediately after the preparation using ultrasonic waves, the vasculature appeared normal. Fourteen days after the preparation, new vasculature appeared along the finishing line. After 30 days, vascular regeneration was nearly complete.

Conclusions: Our observations suggest that the use of the ultrasonic wave instrument caused minimum damage compared to the use of the dental air turbine. Therefore, in terms of protecting the microcirculatory system in the gingival tissue during tooth preparation, the ultrasonic wave instrument is useful. [MVRC 3(1): 25-31, 2009]

Key words: gingiva, microcirculation, ultrasonic, preparation, resin cast, SEM
Introduction

Tooth preparation is an essential part of dental treatment, and its accuracy and the treatment outcomes are influenced by the instruments used. Ultrasonic wave technology is widely used in industrial settings for cutting and polishing for teeth. Recently, ultrasonic technology has received more attention because of its energy efficiency\(^1\). In the field of dentistry, ultrasonic wave technology has been used in periodontal and endodontic treatment and, more recently, has been utilized as a surgical knife in dental surgery\(^2,3\).

The gingival microvascular network has been previously visualized in the animal model. The Indian ink method allows for morphological observation of the microvasculature by the light microscope after injecting ink into the gingival tissue\(^4,5\). The corrosion resin cast method is another approach for observing the gingival microvasculature\(^6\). With this technique, low-viscosity synthetic resin is injected into the blood vessels, dissolving the peripheral tissue\(^7,8\). A clear vascular image, including capillaries, can be obtained by the complete infusion of synthetic resin and observation with a scanning electron microscope (SEM). The resin cast changes according to the state of the gingival blood circulation\(^9\). Hemorrhage at the gingival margin due to damage during tooth preparation provides evidence of gingival blood vessel destruction, which is a primary cause of gingival proliferation and recession.

The purpose of this study was to examine the impact of ultrasonic wave tooth preparation on the gingival microcirculation.

Materials and Methods

**Animals and tooth preparation:** Eight Beagle dogs with healthy periodontal tissue served as experimental animals. The subjects were treated in accordance with the Animal Care Committee guidelines of Kanagawa Dental College. Fourteen days prior to the surgery, a scaling and cleaning was performed. The lower premolars were prepared along the gingival margin. A chamfer finishing line was created (Fig. 1a,b). Four premolars in one side of the dentition was prepared by ultrasonic wave (Suprasson P-max, Satelec, Merignac, France. Fig. 1b) using a newly developed diamond tip (FLT tip, Hakusui Trading, Osaka, Japan. Fig. 1c, d), with water spray, and 33 Hz periodontal mode frequency (Fig. 1a-d). Four premolars in the other side of the dentition was prepared by a conventional diamond bur (SF102R, Syofu, Kyoto, Japan. Fig. 1g, h.) and dental air turbine (MIJET-T Yoshida, Tokyo, Japan. Fig. 1f) with water spray (Fig. 1e-h). The shape and density of the diamond particles on both instruments were the same; the width of the tip was 1.1 mm arrows and the angle was 4 degrees (Fig. 1c,g). Two subjects were not operated on to serve as the control group. Plaque control including brushing and cleaning was abolished twice a week.

**Morphological procedure:** The dogs were anesthetized with 25 mg intravenous Nembutal (Abbott Laboratories, North Chicago, IL, USA) prior to all dental and experimental procedures. The each 2 dogs received vascular resin injections on either the day of the procedure (Day 0) or 14 or 30 days after the procedure. During these period, gin-
gival recession was not clinically observed. The common carotid arteries were cannulated, and Ringer's solution containing 0.2% heparin was perfused until the jugular veins were cleared of blood. After perfusion, 2% glutaraldehyde phosphate buffer solution (pH 7.4) was injected into the carotid arteries for fixation. The mandible was removed after fixation to expose the inferior alveolar arteries for the manual injection of synthetic resin (Mercox®, Dai Nippon Ink, Tokyo, Japan). The peripheral tissue was decalcified using 10% hydrochloric acid, followed by 20% potassium hydroxide to dissolve the tissue. All specimens were washed thoroughly with 40 degree centigrade tap water and freeze dried. After ion coating with platinum palladium, the 16 specimens (total 8 premolars) on each side were examined under an SEM (JSM6301F, JEOL, Tokyo, Japan). Buccal side of third premolars was shown in the figures (Figs. 3-9). Differences in the vascular structure of each tooth in the buccal side and lingual side were not observed.

**Results**

Upon SEM observation, tooth surfaces prepared by the dental air turbine appeared uneven, and lateral stripes, which seemed to have been created by the rotation of the diamond bur, were visible. Erythrocytes (EC) were attached to the tooth surface, indicating that the turbine bur had damaged the marginal gingival blood vessels (Fig. 2a). On the other hand, tooth surfaces prepared by ultrasonic waves appeared flat and smooth (Fig. 2b).

**Control group:** Figure 3 shows the gingival vascular network in control animals. The resin cast images reveal the vasculature beneath the sulcular epithelium (SE) and the junctional epithelium (JE) adjacent to the tooth surface (Fig. 3a). Comprised of capillaries, U-turn vascular loops capillaries converge in a network beneath the SE (Fig. 3b). The blood vessels beneath the JE (Fig. 3c) are arranged in a two-layered vascular network; the surface layer is comprised...
of 10-15 µm capillaries (CA), and the deeper layer is comprised of the 30-50 µm venous plexus (VN).

**Dental air turbine group**: On day 0 of the dental air turbine preparation, the architecture of the vascular network was buried in leaked resin (Fig. 4a) and resin leakage from the vessels was observed beneath the SE (Fig. 4b). In the SE area, blood vessels had been destroyed and teardrop-shaped resin leaking from the vessels was recognizable. Resin leakage was also observed in the vascular network beneath the JE (Fig. 4c).

Fourteen days after tooth preparation, the blood vessels were covered with leaked resin (Fig. 5a). The expanded vessels of the vascular network had arranged themselves around the JE area. Resin leakage was also observed within the vascular network (Fig. 5b, c).

Thirty days after tooth preparation, the blood vessels were thicker in diameter (30-90 µm) and formed glomerular loops (Fig. 6b). In the JE, a dense vascular network was observed (Fig. 6c), and resin leakage was observed among the thick (20-40 µm) vessels.

**Ultrasonic group**: On day 0 of the ultrasonic tooth preparation, the vascular networks of the SE and JE appeared to be quite similar to those of the control group (Fig. 7a). Although U-turn loops were observed in the marginal gingival blood vessels (Fig. 7b), teardrop-shaped resin leakage was not observed. The vascular network was intermittent, indicating that a disturbance has occurred in the microcirculation (Fig. 7c).

Fourteen days after tooth preparation, two types of vasculature were observed (Fig. 8a); one was the newly created SE network, which appeared to be of the dense mesh type, and the other was new JE vascularization consisting of tall vascular loops. The vascular network in the marginal region formed short vascular loops (Fig. 8b). The border between the vascular networks in the SE and JE was formed by newly created 40-50 µm-diameter blood vessels (Fig. 8c). The vessels continuously developed in the horizontal direction along the finishing line. The mesh-like network in the upper portion of the gingiva and the tall loops in the lower portion were anastomosed.

Thirty days after tooth preparation, most of the vascular networks presented with a normal arrangement (Fig. 9a). The SE network formed a loop. Under higher magnification, we observed a regenerated SE network (Fig. 9b). The JE...
Fig. 6. Thirty days after dental air turbine preparation (resin cast)
Vascular network was winding and dense, and resin leakage could be observed all over the area (a). In SE, thick vessels formed glomerular loops in SE (b). In the JE, Resin leakage was also observed among those expanded vessels (c). Scale bars=100μm

Fig. 7. Day 0 after ultrasonic preparation (resin cast)
Gingival vascular network showed quite similar image to those of the control (a). The U-turn loops in SE were observed (b). Impressions of erythrocytes (#) were observed on the cast surface. Scale bars= 100 μm

Fig. 8. Fourteen days after ultrasonic preparation (resin cast)
Vascular network of new sulcal epithelium (SE) and new junctional epithelium (JE) were observed (a). U-turn loops were observed in SE (b). The border between SE and JE was formed by newly created blood vessels (arrowhead; c). Scale bars=100μm
vascular network (Fig. 9c) was rearranged into a mesh-type capillary network.

**Discussion**

Various experimental methods have been used to investigate microcirculation. To observe the vasculature, injection methods are typically used[10,11]. The arteriole that runs through the inner part of the gingiva gives rise to a capillary network in the SE and JE. The marginal gingiva around the cervical line of tooth, and its blood vessels, must be protected during tooth preparation. The presence of EC on the tooth surface indicates bleeding within the gingiva, which occurred after preparation by the dental air turbine. The vascularization of the gingival margin had been destroyed. In addition, the existence of teardrop-shaped resin leakage provided additional evidence that the preparation had damaged blood vessels. However, resin also leaked from vessels in the JE, which had not been in contact with the diamond bur. An earlier study found that, when the tooth was prepared without water, blood vessels showed signs of acute inflammation due to heat[12]. During acute inflammation, the venular capillaries are extended and a gap forms in the epithelium. The resin cast model demonstrated that, although no vessel destruction had occurred, synthetic resin had leaked from the vessels due to accelerated vascular permeability[13]. Though water was used in the present study, the dental air turbine generated heat and resulted in a gap in the endothelium from which resin leaked[14].

Almost all of the capillaries in the gingival vascular network are fenestrated blood vessels with pores in the vascular wall[15]. These vessels exist in the renal glomeruli, the salivary glands, and the articular synovial membrane. These are areas in which a frequent exchange occurs through the vascular wall supported by the pores in the vascular wall. After 30 days, regenerated blood vessels formed glomerulus loops, but they clearly differed from the normal structure, suggesting that additional time was needed for recovery[16].

After ultrasonic preparation, no evidence was found to indicate that bleeding had taken place. However, some irregularities appeared along the surface of the resin cast. It is known that the presence of EC indicates a microcirculatory disturbance. Ultrasonic vibration affected gingival vessels when EC was stagnant in the blood vessels[17], but teardrop-shaped resin was not seen. These results suggest that ultrasonic preparation does not destroy blood vessels or generate heat during its use. New vascular generation began within 14 days of tooth preparation and a normal vascular arrangement was visible along the finishing line after 30 days.

**Conclusions**

It is known that vascularization is the key to initiating tissue regeneration, and that it is critical to protect the marginal gingiva around the cervical line during tooth preparation. Hemorrhage at the gingival margin due to vascular destruction is the primary cause of gingival proliferation or recession. The JE protects the tooth from external stimulation and inflammation, suggesting that the destruction of the JE leads to periodontal disease. In this study, we confirmed that using ultrasonic waves for tooth preparation minimizes the damage to gingival tissue and is effective for gingival protection.

**References**


