ORIGINAL ARTICLE

Atomic Force Microscopic Images of Acidic Conditioning Agents on Various Regions of Human Dentin

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SYNOPSIS
The purpose of this study is to investigate the morphological differences of human dentin after two types of acidic conditioning: etching reagent and self-etching primer using an AFM. The 6 upper first premolars were used in this study. The specimens were divided into three groups: group 1, No-pretreatment; group 2, Etching treatment with 10% citric acid-3% ferric chloride solution in Super-bond C&B system; group 3, an etching treatment with a self-etching primer, ED primer II, in Panavia Fluoro cement. These specimens were divided into the crown and root dentin and were observed in situ using an AFM. In the no-pretreatment specimen, the diameters of dentinal tubules in both the surface and middle layers of the crown dentin were narrower than in the deep layer of crown dentin. The diameters of dentinal tubules in the root dentin were narrower than that of crown dentin. Furthermore, the peritubular dentin was observed more clearly in the root dentin as compared with the crown dentin. After etching with a 10-3 solution of Super-bond C&B, the peritubular dentin was removed in both the crown and root dentin. The diameters of dentinal tubules were wider than that of no-pretreatment dentin. After ED primer II treatment, the peritubular dentin was removed in the crown dentin, but remained in the root dentin. It was concluded that differences in the dentin region after two types of acidic conditioning were influenced the arrangement of collagen fibers, the amount of collagen, and different sets of matrix proteins in the dentin.

Key words: atomic force microscopy, conditioning agents, human premolar, crown dentin, root dentin

INTRODUCTION
Nowadays adhesive dental resins are widely used in dental clinics, and give acceptable results. The tight bonding of dental resin to dentin is achieved by the acidic treatment such as etching or self-etching primer. Such treatments induce important changes such as removal or alteration of the smear layer and establishment of a microporous surface. The changed dentin surface may be penetrated by bonding agents to create a hybrid layer which was composed of partially demineralized dentin and bonding polymer 19.

A wide variety of acidic decalcifying
agents is utilized with various bonding systems. They induce different morphological effects and demineralization depth. Morphological conditions of dentin by etching or self-etching primer should influence the bonding ability of dental resins. To investigate these changes, a wide variety of sequential high vacuum imaging and analysis technique has been used, such as scanning or transmission electron microscopy and X-ray microanalysis 4, 8, 23, 24. Nevertheless, specimen dehydration, radiation damage and charging effects induced on dentin by high vacuum and high energy incident probes may lead to important artifacts 3, 9, 22. As dentin hydration is considered of primary important in preventing demineralized collagen collapse and maintaining tissue permeability, fixation.

Atomic force microscopy (AFM) is a versatile tool for the study of surface on material. The development of AFM was preceded by the development of the scanning tunneling microscopy and was developed in 1986 2. AFM successfully operates on non-conductive specimens in atmospheric conditions or under liquid and provides high resolution 3-D images of dentin. Nowadays, AFM become a valuable tool for studying microstructural changes, etching rates of peritubular dentin, and intertubular dentin recession during demineralization, the effect acids on dentin collagen as well as a variety of other properties on dentin 1, 5, 7, 10, 12-16, 21. Hybrid layer was also identified by AFM using diamond-knife microtomy technique 26.

Dentin has histologically a wide variation of structure at different regions, for example, the direction of dentinal tubules, the direction of dentinal tubules, the development of peritubular dentin, and the calcification degrees 16. The morphological changes by acidic treatment of etching reagent or self-etching primer will be varied in different regions. And such variation will influence the adhesion of dental resins to dentin.

The purpose of this study is to investigate the morphological differences of human dentin on various regions after two types of acidic conditioning, etching reagent and self-etching primer.

MATERIALS AND METHOD

1. Sample preparation
The 6 upper first premolar used in this study were stored in 10% neutral formalin at the Department of Histology, Cytology and Development, Nihon University School of Dentistry at Matsudo. They were extracted for the orthodontic reasons. They were sliced and were ground using the grinding stone and then were polished with the diamond pastes down to 0.25μm. After polishing, they were cleaned by the ultrasonic wave. The specimens were divided into three groups as following:

Group A: No-pretreatment before AFM observation
Group B: Etching treatment before AFM observation. The dentin was etched with 10% citric acid-3% ferric chloride solution in a Super-bond C&B system (Sunmedical Co., Ltd. Shiga, Japan). After 5 seconds, the etched dentin was rinsed under tap water and dried with compressed air.

Group C: Self-etching primer treatment before AFM observation. The dentin was treated with self-etching primer, ED primer II, in Panavia Fluoro cement (Kuraray Co., Ltd. Osaka, Japan). The composition of ED primer II is MDP (10-methacryloyloxyclylamino-salicylic acid), HEMA (2-hydroxyethyl methacrylate), 5-NMSA (5- methacryloxyloxy-2-(2-methyl)acrylate), polymerization initiator, and water. After 30 seconds, the primed dentin was directly dried with compressed air.

These specimens were divided into the crown and root dentin, and were observed in situ using an AFM.
2. AFM observation
AFM were performed using a JSPM-4200 scanning probe microscope (JEOL, Tokyo, Japan) under the atmospheric conditions at 25 °C. The imaging was performed in the AC mode using a silicon cantilever tips (NT-MDT, Silicon-MDT Ltd., Moscow, Russia) with a scanner having a maximum of 10 x 10 x 3 μm, x, y, z scale range. The loading force exerted on the sample was around 10⁻⁸ - 10⁻¹¹ N during scanning.

RESULTS
Figure 1 and 2 showed the untreated crown and root dentin (group A), respectively. Fig. 1a was the surface layer of crown dentin, Fig. 1b was the middle layer, and Fig. 1c was the deep layer. Fig. 1a and 1b showed the longitudinal sectioned dentinal tubules, and Fig. 1c showed obliquely crossed dentinal tubules. The diameter of dentinal tubules in the surface and middle layer was 0.6μm and 0.5μm (average), respectively. The diameter of dentinal tubules in the deep layer was 1.2μm (average). The diameters of dentinal tubules in both surface and middle layers were narrower than the deep layer (Fig. 1). The peritubular dentin was observed the middle and surface layers (Figs. 1a, b). The lateral branches of dentinal tubules were observed in the surface layers (Fig. 1a). Fig. 2a was the surface layer of root dentin, Fig. 2b was the middle layer, and Fig. 2c was the deep layer. Fig. 2 showed crossed dentinal tubules. The diameter of dentinal tubules in the surface, middle and deep layer was 0.7μm, 0.5μm, and 0.4μm (average), respectively. The diameters of dentinal tubules in the root dentin were narrower than that of the crown dentin (Figs. 1, 2). The peritubular dentin was observed clearly in the root dentin as compared with the crown dentin. There were more dentinal tubules per unit area in the crown than in the root.

Figures 3 and 4 showed the crown and root dentin obtained after etching with 10-3 solution (group B). Fig. 3a was the surface layer of crown dentin, Fig. 3b was the middle layer, and Fig. 3c was the deep layer. Fig. 3 showed the longitudinal sectioned dentinal tubules. The terminal branch of dentinal tubules was observed in the surface layer (Fig. 3a). The diameter of dentinal tubules in the surface layer was 1.9μm (average). The diameter of dentinal tubules in the middle and deep layer was 3.0μm and 3.1μm (average), respectively. Fig. 4a was the surface layer of root dentin, Fig. 4b was the middle layer, and Fig. 4c was the deep layer. Fig. 4a and 4b showed the longitudinal sectioned dentinal tubules, and Fig. 4c showed obliquely crossed dentinal tubules. The diameter of dentinal tubules in the surface layer was 1.9μm (average). The diameter of dentinal tubules in the middle and deep layer was 2.3μm and 2.6μm (average), respectively. The diameters of dentinal tubules of dentin in the group B (Figs. 3-4) were wider than that of the untreated dentin in the group A (Figs. 1-2). In the group B, the surface of the intertubular dentin was uneven (Figs. 3-4).

Figures 5 and 6 present the crown and root dentin obtained after ED primer treatment (group C). Fig. 5a was the surface layer of crown dentin, Fig. 5b was the middle layer, and Fig. 5c was the deep layer. Fig. 5 showed the longitudinal sectioned dentinal tubules. The secondary branch of dentinal tubules was observed in the surface layer (Fig. 5a). Fig. 5a showed the longitudinal sectioned dentinal tubules, and Fig. 5b and 5c showed obliquely crossed dentinal tubules. The diameter of dentinal tubules in the surface layer was 1.2μm (average). The diameter of dentinal tubules in the middle and deep layer was 1.8μm and 1.8μm (average), respectively. Fig. 6a was the surface layer of root dentin, Fig. 6b was the middle layer,
Fig. 1 No-preparation. Crown dentin. 1a: Surface layer. Diameter of dentinal tubules (dt): 0.6μm. The diameters of dentinal tubules in both surface and middle layers were narrower than the deep layer. 1b: Middle layer. Diameter of dt: 0.5μm. The peritubular dentin were observed both middle and surface layers. 1c: Deep layer. Diameter of dt: 1.2μm.

Fig. 2 Group A. No-preparation. Root dentin. 2a: Surface layer. Diameter of dt: 0.7μm. The peritubular dentin was observed clearly in the root dentin as compared with the crown dentin. 2b: Middle layer. Diameter of dt: 0.5μm. 2c: Deep layer. Diameter of dt: 0.4μm.

Fig. 3 Group B. 10-3 solution. Crown dentin. 3a: Surface layer. Diameter of dt: 1.6μm. The terminal branch of dentinal tubules was observed in the surface layer. 3b: Middle layer. Diameter of dt: 3.0μm. 3c: Deep layer. Diameter of dt: 3.1μm. The diameters of dentinal tubules of dentin were wider than that of the untreated dentin in the group A.

Fig. 4 Group B. 10-3 solution. Root dentin. 4a: Surface layer. Diameter of dt: 1.9μm. The surface of the intertubular dentin was uneven. 4b: Middle layer. Diameter of dt: 2.3μm. 4c: Deep layer. Diameter of dt: 2.6μm.

Fig. 5 Group C. ED primer treatment. Crown dentin. 5a: Surface layer. Diameter of dt: 1.2μm. The peritubular dentin was removed. 5c: Deep layer. Diameter of dt: 1.8μm.

Fig. 6 Group C. ED primer treatment. Root dentin. 6a: Surface layer. Diameter of dt: 1.2μm. 6b: Middle layer. Diameter of dt: 1.6μm. 6c: Deep layer. Diameter of dt: 1.2μm. The peritubular dentin was removed in the root dentin as compare with the dentin of the group B.

and Fig. 6c was the deep layer. Fig. 6a and 6b showed obliquely crossed dentinal tubules. Fig. 6c showed the longitudinal sectioned dentinal tubules. The diameter of dentinal tubules in the surface, the middle and deep layer was 1.2μm, 1.6μm and 1.2μm (average) respectively. The After ED primer II treatment (group C), the peritubular dentin was removed in the crown dentin, but remained in the root dentin as compare with the dentin of the group B.
DISCUSSION
There were more dentinal tubules per unit area in the crown dentin than in the root dentin. Schikle et al. described that the differences in the dentistry of tubules between the middle and deep dentin were more marked in the bovine crown than the root. Similar findings were observed for human dentin. The hardness of the crown dentin differs from that of root dentin. Collagen fibers were irregularly arranged in the crown dentin, but were arranged regularly and perpendicular to the dentinal tubules in the root dentin. Masuda et al. reported tooth crown dentin showed greater adhesion than root dentin in all 4 types of resin cements. AFM showed removal of smear layer and the opening of dentinal tubules both on the crown and root dentin surface after treatment with the Super-Bond C&B. Wider opening of the dentinal tubules was observed on the crown dentin surface than root dentin surfaces, and residual peritubular dentin was observed on the root dentin after the ED primer treatment.

After the ED primer treatment, the peritubular dentin was remained in the root dentin as compared with the dentin of treatment the 10-3 solution of Super-Bond C&B. The peritubular dentin was observed clearly in the root dentin as compared with the crown dentin. The peritubular dentin was harder than the intertubular dentin at all sites. The hardness of fully hydrated peritubular dentin did depend upon location, and was significantly greater near the dentin-enamel junction than near the pulp. Peritubular dentin is composed mainly of crystals of carbonated apatite together with a small amount of collagen. Peritubular dentin contained an assemblage of unique acidic proteins. It was suggested that the differences in the dentin region, after receiving two types of acidic conditioning were influenced the arrangement of collagen fibers, the amount of collagen, and different sets of matrix proteins in the dentin.

CONCLUSION
1. The diameters of dentinal tubules in the root dentin were narrower than that of the crown dentin. The peritubular dentin was observed clearly in the root dentin as compared with the crown dentin. There were more dentinal tubules per unit area in the crown dentin than in the root dentin.
2. AFM showed the removal of smear layer and the opening of dentinal tubules both on the crown and root dentin surface after treatment with the 10-3 solution of Super-Bond C&B.
3. The dentinal tubules of the crown dentin were wider than that of root dentin, and the residual peritubular dentin was observed on the root dentin after the ED primer treatment.
4. After the ED primer II treatment, the peritubular dentin was remained in the root dentin as compare with the dentin of treatment the 10-3 solution of Super-Bond C&B.
5. It was concluded that differences in the dentin region after two types of acidic conditioning agent were influenced the arrangement of collagen fibers, the amount of collagen, and different sets of matrix proteins in the dentin.

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REFERENCES


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