Subsurface morphology of smear layer on cut dentin

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Abstract : The purpose of this study was to know the effects of tooth cutting on dentin collagen. The intact human teeth were cut with high-speed diamond burs and digested by the HCl-Collagenase method, and examined under the scanning electron microscope. Moreover, morphological changes of collagen in the superficial layer of cavity floor dentin were examined under the transmission electron microscope. These examinations indicated the presence of a layer containing denatured collagen underneath the smear layer.

抄録：本研究の目的は歯牙切削が象牙質コラーゲンに及ぼす影響を知ることである。健全歯を高速切削下ダイヤモンドバーで切削し，塩酸コラゲナーゼ法にて歯牙を消化したのちに走査型電子顕微鏡で観察した。さらに窩底象牙質表層部の象牙質コラーゲンの形態的変化を透過型電子顕微鏡で観察した。その結果，スメア層直下に変性したコラーゲンを含む一層が存在することが明らかとなった。

Introduction

High-speed tooth cutting in restorative treatments has become much more biologically tolerable as well as acceptable with regard to the time and cost effectiveness. Incidentally, the use of the sharp-edged bur, feather touch, and sufficient water cooling are indispensable to avoid the biological damage for the tooth . However, the coolant effect at the contact site with cutting burs on tooth substances has been evaluated by examining the responses of dental pulp distant from the cut surface. The ultrastructural concerns with the direct effects of tooth cutting on the dentin have been very scarce.

The cut debris layer formed on the cavity wall during cavity preparation with cutting instruments is called the smear layer. Since Boyde et al.[1] and Eick et al.[2] demonstrated this layer by scanning electron microscopy, whether it should be removed or not has been controversial in the field of restorative dentistry concerning the pulp irritation and dentin adhesion of the resin[3)-6)]. Iwaku[7] and Yamada et al.[8] digested the superficial layer of cavity floor dentin with HCl-collagenase by the method of Gunji et al.[9] and disclosed a layer resistant to HCl-collagenase under the smear layer.

The purposes of this study were to examine the relationship between this layer resistant to HCl-collagenase digestion and the smear layer by scanning electron microscopy (SEM) and to evaluate the effects of tooth cutting on the ultrastructure of the dentin collagen by transmission electron microscopy (TEM).

Materials and methods

HCl- collagenase digestion method

Freshly extracted intact human premolars were horizontally cut across the middle of crown using a flat-end tapered diamond bur (#103, Shofu, Japan) under the rotation speed of 330,000 r. p. m. and the water coolant of 27ml/min. The prepared teeth were immersed in 5%, 10%, and 20% dimethyl sulfoxide solutions for 20 minutes each, placed in liquid nitrogen, and cryofractured into two segments with a chisel perpendicularly to the cut dentin surface. The

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cut dentin surface of one of segments was treated with 50% citric acid for 60 seconds and cryofractured again as above perpendicularly to the treated dentin surface. Each specimen was then fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer for 24 hours and washed in 0.1M phosphate buffer for 24 hours. Half of specimens treated with 50% citric acid were subjected to HCl-collagenase digestion as follows. The specimens were demineralized with HCl (60℃, 5N) for 10 minutes, washed with distilled water for 10 minutes 3 times, and digested in 0.1% collagenase (Type II, SIGMA, USA) in 0.1M phosphate buffer (pH6.8) at 37℃ for 12 hours.

All the specimens were dehydrated with a graded series of acetone, dried in a critical point dryer

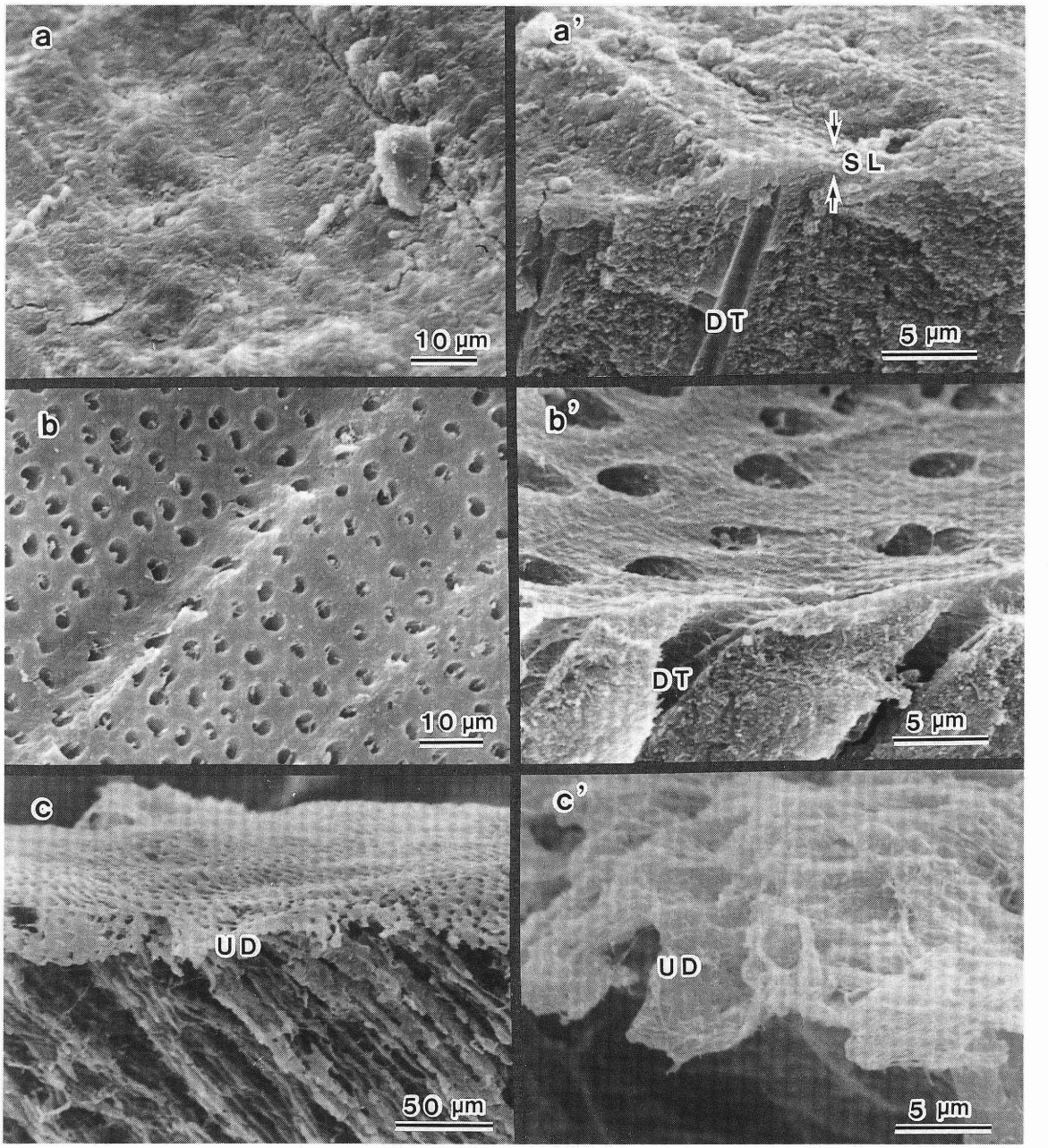

Fig. 1. **a :** SEM of the dentin surface prepared with a high-speed diamond bur. **a' :** The profile of smear layer(SL). DT : Dentinal tubule. **b :** SEM of the dentin surface after removing the smear layer with 50% citric acid for 60 sec.. **b' :** The aperture of dentinal tubules(DT) widely opened. **c :** SEM of the undigested layer(UD) developed by HCl-collagenase digestion following the surface treatment with 50% citric acid. **c' :** The higher magnification of undigested layer(UD).

(HITACHI, HCP-2, Japan), coated with gold (Eiko, IB3, Japan), and observed under a scanning electron microscope (HITACHI, S-430, Japan).

In addition, a class 1 cavity reaching the dentin was prepared in an intact human premolar using a cylindrical diamond burs (# 311,Shofu, Japan) at high-speed under water coolant before extraction for orthodontic treatment. After extraction, the tooth was vertically fractured in two segments. The cavity floor of one segment was observed under a scanning electron microscopy after HCl-collagenase digestion and the other was observed without HCl-collagenase digestion.

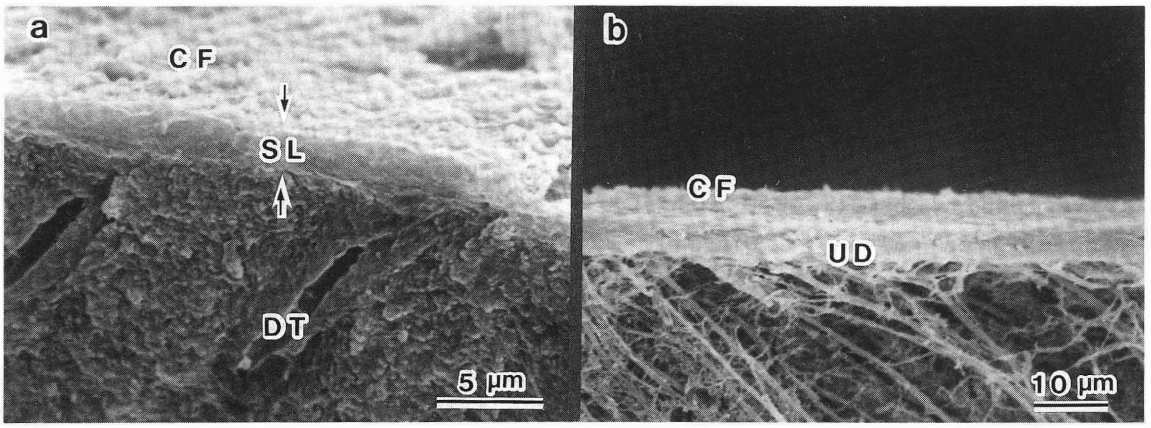

Fig. 2. a : SEM of Class 1 cavity floor dentin showing the profile of smear layer(SL). DT : Dentinal tubule, CF : Cavity floor. **b :** SEM of the undigested layer(UD) developed by HCl-collagenase digestion.

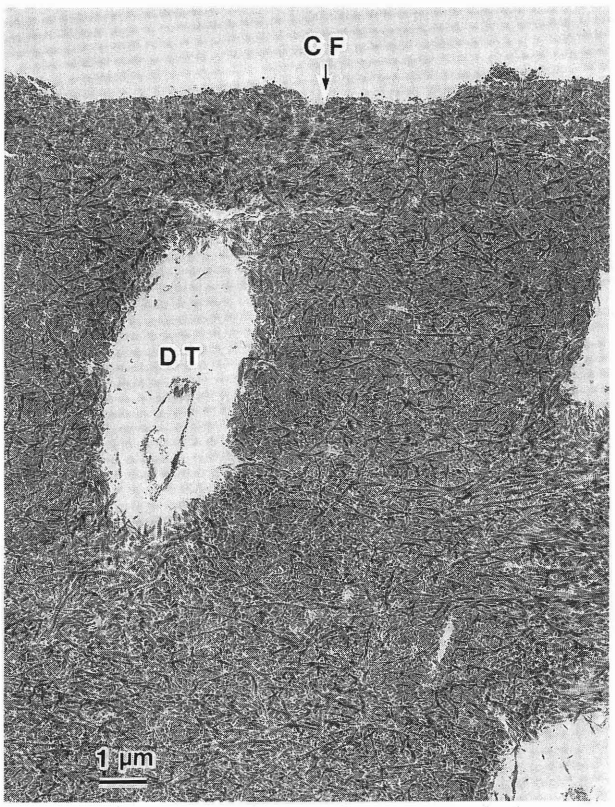

Fig. 3. Transmission electron micrograph of the cavity floor dentin. CF : Cavity floor, DT : Dentinal tubule.

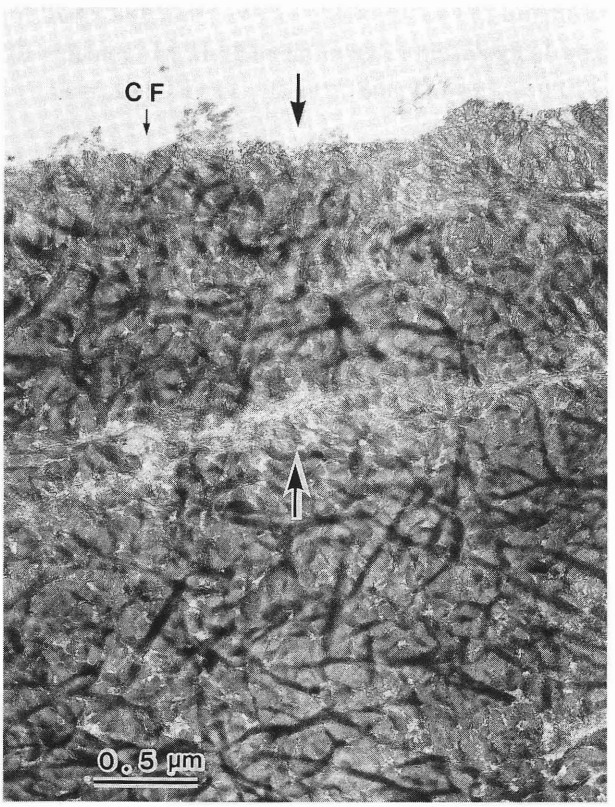

Fig. 4. The superficial layer of cavity floor dentin. Collagen fibrils partially deformed into the filamentous structures (between arrows). CF : Cavity floor.

Preparation of specimen for TEM

The same class 1 cavity preparation as for SEM observation was carried out in intact human premolars before extraction. Immediately after extraction, the teeth were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH7.2) for 24 hours, demineralized with 5% EDTA for 30 days, postfixed with 1% osmium tetroxide for 2 hours, dehydrated through a graded series of ethanol, and embedded in Epon 812. The ultrathin sections perpendicular to the cavity floor were made, stained with tannic acid, uranyl acetate and lead citrate, and observed under a transmission electron microscope (HITACHI, HU-11DS, Japan).

Results

HCl-collagenase digestion

Fig. 1-a and a' show a cut dentin surface and its profile, respectively. The dentin surface was covered with the cut debris layer, so-called smear layer of 1 to 2 μm in thickness and no apertures of dentinal tubules were observed. The cut debris layer was removed by surface treatment with 50% citric acid for 60 seconds, and the apertures of dentinal tubules were widely opened (Fig. 1-b and b'). Moreover, HCl-collagenase digestion after the removal of cut debris revealed an undigested layer 1 to 2μm in thickness in the superficial region of cut dentin (Fig. 1-c and c'). This undigested layer was also observed in Class 1 cavity floor dentin with the thickness of 1 to 2μm (Fig. 2).

Transmission electron microscopic findings

Because the dentin specimen was completely demineralized by EDTA, all inorganic substances including the smear layer disappeared and the collagen network of dentin matrix could be observed (Fig. 3). In a magnification of the superficial region (Fig. 4), the collagen fibrils beneath the cavity floor were deformed into filamentous structures, and such a deformed collagen fibrils were coexisted with normal collagen fibrils of 1 to 2μm in thickness (Fig. 4, between arrows). In a greater magnification (Fig. 5), the col-

Fig. 5. Higher magnification of the filamentous structures in the most superficial region of cavity floor dentin.

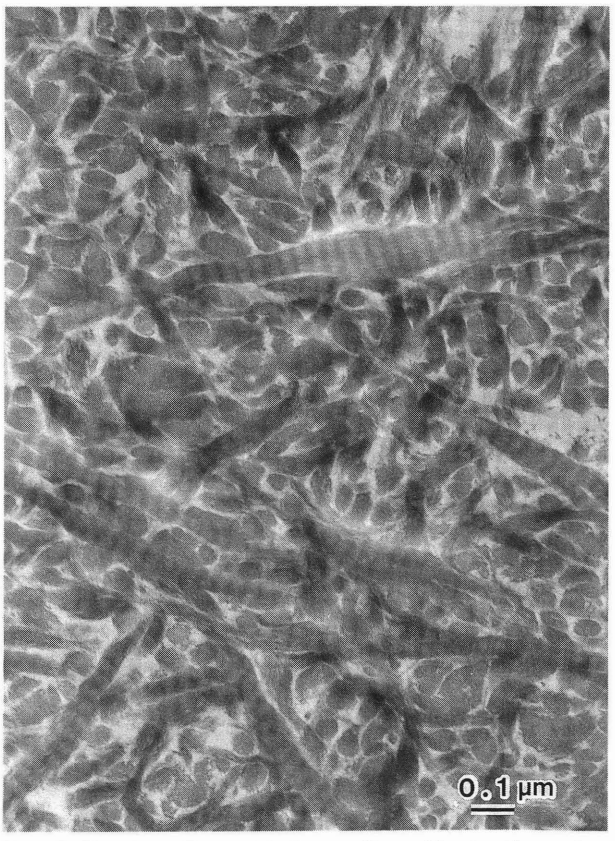

Fig. 6. Dentin collagen fibrils unaffected by tooth cutting (A demineralized section).

lagen fibrils in the most superficial region were broken up and raveled showing the denatured collagen fibrils without the cross-band structure in comparison with the morphology of collagen fibrils in deeper regions unaffected by cutting (Fig. 6).

Discussion

The smear layer formed on the dentin wall by cavity preparation is considered to be 1 to 5 μm in thickness[5]. Yamada et al.[8] treated the cavity floor dentin by the HCl-collagenase method and demonstrated the formation of a layer resistant to HCl-collagenase digestion by SEM. In this study, the smear layer on the surface of cavity floor dentin was removed by treatment with 50% citric acid in advance and an undigested layer resulting from HCl-collagenase digestion appeared underneath the smear layer removed. It is considered that this undigested layer corresponds to the layer resistant to HCl-collagenase digestion reported by Yamada et al.[8]. TEM also showed that there was a layer indicating the structural damage of collagen fibrils underneath the smear layer generated by cavity preparation. Bowen et al.[10] abraded dentin against a 320 grit abrasive cloth strip, demineralized it with EDTA, and observed it under TEM. They noted a layer which was about 1μm in thickness and less electron-stainable than deeper layers on the abraded dentin surface, and observed amorphous substances without normal collagen in this layer. Eick et al.[11] also reported similar observations by TEM. The thickness of this layer was similar to that of a layer undigested by HCl-collagenase treatment. From these findings, it is certified that a dentinal layer containing denatured collagen presents underneath the smear layer on the prepared cavity. The denaturation of collagen may be caused by the friction heat of rotary cutting instruments[12),13)]. Currently, various acidic conditioners and primers are used to remove the smear layer on cut dentin for the adhesive restorations, but the anxiety that these acidic solutions may denature the dentin collagen is suggested[14),15)]. However, the possibility that collagen fibril underneath the smear layer is already denatured by tooth cutting prior to such acid treatments should be kept in mind in further studies.

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