Ultrastructural changes of collagen fibers on cut and acid-treated dentin surfaces

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Abstract : The effects of various dentin conditioners, used to remove the smear layer, on the cut dentin surface of intact and carious human teeth were studied by transmission electron microscopy. In intact teeth, a layer showing destruction of collagen due to cavity preparation was found underneath the smear layer. The collagen in this layer was further denatured by the subsequent treatment with acids such as phosphoric acid and citric acid. In carious teeth, collagen showing morphologic changes similar to those of intact teeth was found in the superficial layer of cavity floor dentin after removal of infected dentin and treatment with acids.

抄録：スメア層を除去するために用いられている各種象牙質処理剤が，ヒト健全歯及び齲蝕歯の切削象牙質面に与える影響を透過型電子顕微鏡を用いて観察した。その結果，健全歯では窩洞形成によりスメア層直下に基質コラーゲンの構造的破壊を示す一層が存在し，この層内のコラーゲンは続くリン酸やクエン酸等の酸処理により，さらに強く変性することが明らかとなった。また齲蝕歯においても，感染象牙質除去後および酸処理後の窩底部象牙質表層に健全歯の場合と同様の形態変化を示すコラーゲンが存在することが明らかとなった。

Introduction

The cut debris layer formed on the cavity wall by cavity preparation with the cutting instrument is called "smear layer". The clinical significance of this layer with respect to pulp irritation and dentin adhesion has been discussed by a number of investigators[1)-4)]. In our previous studies, the smeared dentin resulting from routine tooth cutting was treated by HCl-collagenase digestion method[5),6)] and an undigested layer of 1.0-2.0 μm in thickness was demonstrated underneath the smear layer, indicating an effect of cutting heat on the dentin collagen[7)]. We further observed morphological changes of dentin collagen underneath the smear layer by transmission electron microscopy (TEM)[8)].

In this study, the effects of various acid conditioners, which are used to remove the smear layer in restorative treatments, on the cut dentin surface of intact and carious human teeth were evaluated ultrastructurally by TEM.

Materials and methods

In intact human premolar, class 1 cavities reaching the dentin were prepared before or immediately after extraction for an orthodontic reason, by high-speed cutting using a flat-end tapered diamond bur (# 103, Shofu, Japan) under water coolant. Then, the following treatments were applied on the surface of cavity floor dentin : a) Untreated as control, b) 40% phosphoric acid for 5 sec., c) 40% phosphoric acid for 60 sec., d) 50% citric acid for 60 sec., e) a solution of 10% citric acid and 3% ferric chloride (Super Bond C & B

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cleaning agent for dentin, SUN MEDICAL, Japan) for 30 sec., f) 10% polyacrylic acid (Dentin Conditioner, GC, Japan) for 20 sec..

In carious human teeth, the infected dentin was removed using a low-speed round steel bur with the guidance of staining with 1% acid red propylene glycol[9] (Caries Detector, Kuraray, Japan). The cavity floors were then treated with a), c), or e) of the above treatments.

The specimens were fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1M phosphate buffer (pH 7.2) 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, 11-DS, Japan).

Results

1. Intact teeth

Fig. 1 shows a transmission electron micrograph of untreated cavity floor dentin. Because of demineralization with 5% EDTA, all inorganic substances including the smear layer were completely removed. Surface collagen fibrils were broken up and raveled (Fig. 1-b). These collagen fibrils coexisted with normal fibrils over a thickness of 1.5 μm (Fig. 1-a, between arrows).

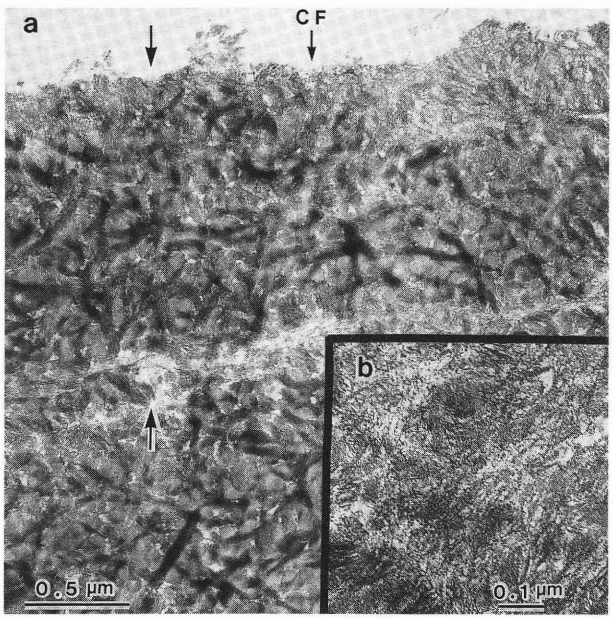

Fig. 1

a : TEM of the superficial layer of cavity floor dentin prepared with a high-speed diamond bur. Collagen fibrils were partially broken up and raveled (between arrows). CF : Cavity floor.

b : Higher magnification of the deformed collagen fibrils.

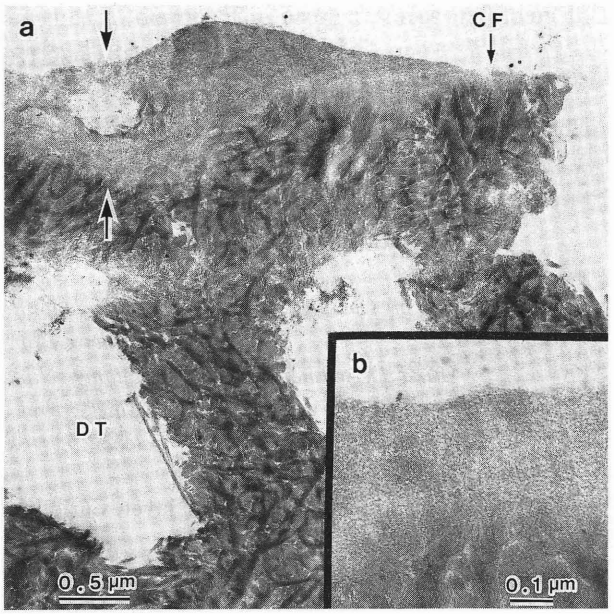

Fig. 2

a : TEM of the superficial layer of cavity floor dentin treated with 40% phosphoric acid for 5 sec.. The homogeneous layer composed of the fine filamentous or granular structures appeared (between arrows). CF : Cavity floor, DT : Dentinal tubule.

b : Higher magnification of the fine filamentous or granular structures.

When the cavity floor was treated with 40% phosphoric acid for 5 seconds, a comparatively homogeneous layer containing no collagen network was observed over a thickness of 0.5-1.0 μm (Fig. 2-a, between arrows). In a greater magnification, fine filamentous or granular structures were observed homogeneously and densely (Fig. 2-b).

The treatment with 40% phosphoric acid for 60 seconds showed a relatively homogeneous layer without normal collagen on the surface of the cavity floor, and this layer was considerably thicker (Fig. 3-a, between arrows) than that after treatment for 5 seconds. In a greater magnification, raveled collagen fibrils and fine filamentous or granular structures suggesting denaturation were observed (Fig. 3-b).

In case of treatment with 50% citric acid for 60

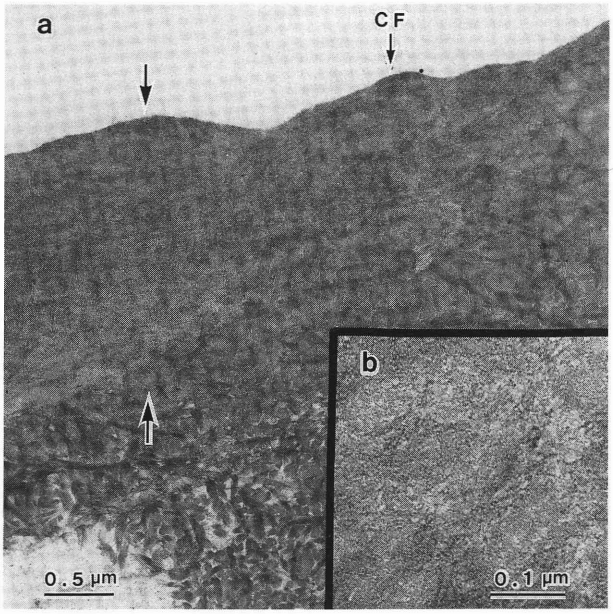

Fig. 3
a : TEM of the homogeneous layer in case of treatment with 40% phosphoric acid for 60 sec. (between arrows). CF : Cavity floor.
b : Higher magnification of the homogeneous layer.

seconds, the morphology of collagen fibrils were indistinct in a layer of 0.2-0.5 μm in thickness on the surface of the cavity floor (Fig. 4-a, between arrows). In a greater magnification, raveled collagen fibrils were observed, and fine filamentous or granular structures similar to those observed after the treatment with phosphoric acid were present underneath the top surface (Fig. 4-b).

The treatment with a solution of 10% citric acid and 3% ferric chloride for 30 seconds showed a layer containing collagen fibrils with indistinct morphology on the surface of the cavity floor over a thickness of 0.5 μ m (Fig. 5-a, between arrows). In a greater magnification, raveled collagen fibrils and fine filamentous or granular structures were observed (Fig. 5-b).

When the cavity floor was treated with 10% polyacrylic acid for 20 seconds, a layer containing collagen fibrils with indistinct morphology of 0.5-1.0 μm in thickness was observed on the surface of the cavity floor (Fig. 6-a, between arrows). In a greater magnification, a large number of raveled collagen fibrils were observed. But fine filamentous or granular structures were absent. This TEM profile resembled

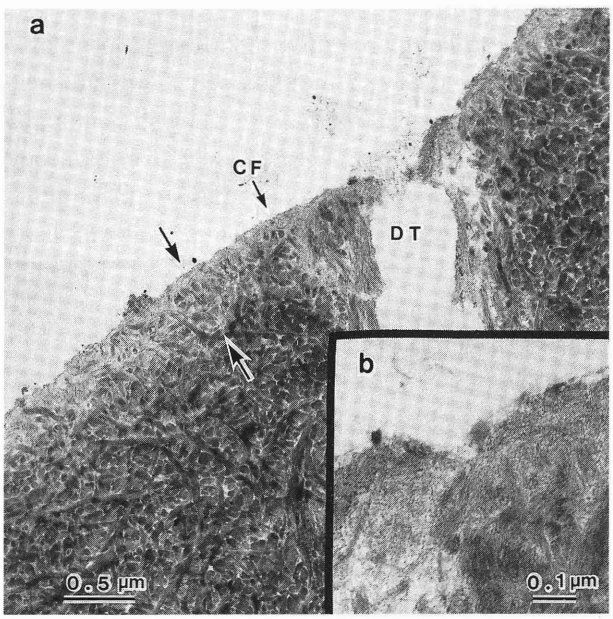

Fig. 4
a : TEM of the cavity floor dentin treated with 50% citric acid for 60 sec.. Collagen fibrils in the superficial layer were deformed into the indistinct feature (between arrows). CF : Cavity floor, DT : Dentinal tubule.
b : Higher magnification of the deformed collagen fibrils showing the fine filamentous or granular structures.

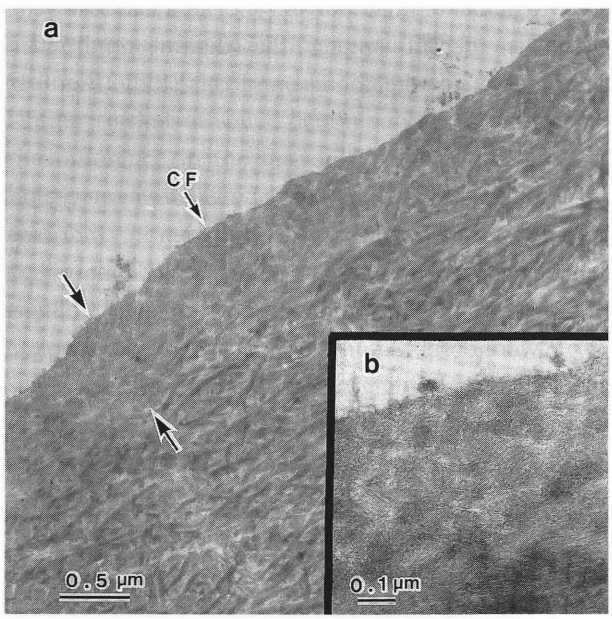

Fig. 5
a : TEM of the cavity floor dentin treated with a mixture of 10% citric acid and 3% ferric chloride for 30 sec.. Collagen fibrils in the superficial layer were deformed into the indistinct feature (between arrows). CF : Cavity floor.
b : Higher magnification of the deformed collagen fibrils showing the fine filamentous or granular structures.

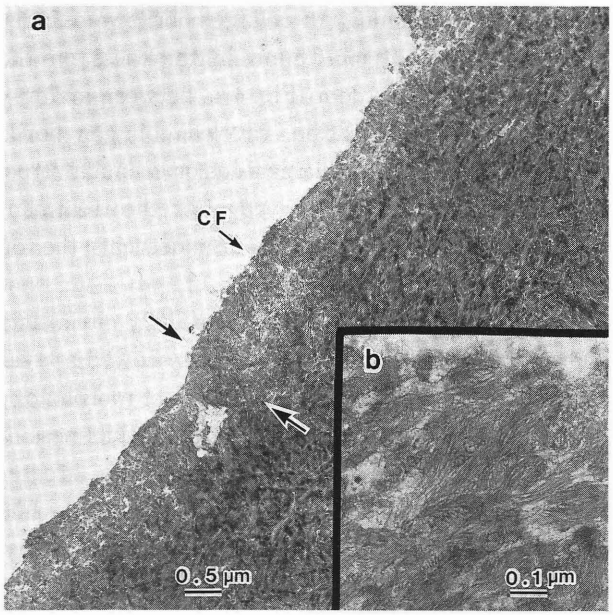

Fig. 6

a : TEM of the cavity floor dentin treated with 10% polyacrylic acid for 20 sec.. Collagen fibrils in the superficial layer were deformed into the raveled feature (between arrows). CF : Cavity floor.

b : Higher magnification of the raveled collagen fibrils.

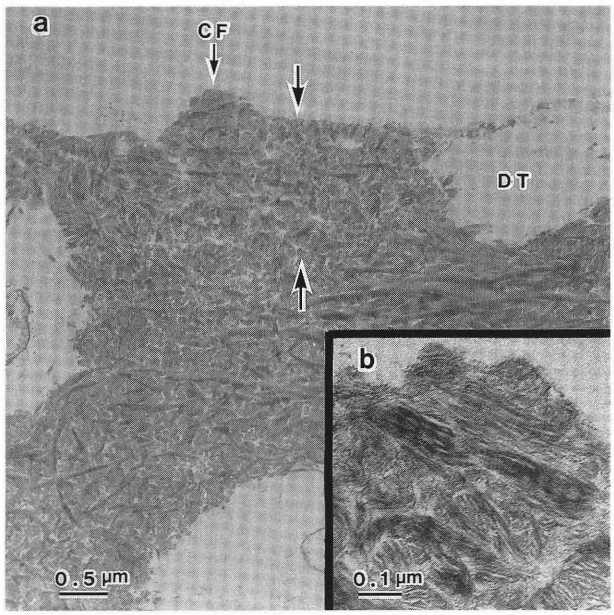

Fig. 7

a : TEM of the cavity floor dentin after the removal of carious dentin guided by Caries Detector. Collagen fibrils in the superficial layer were partially broken up and raveled (between arrows). CF : Cavity floor, DT : Dentinal tubule.

b : Higher magnification of the deformed collagen fibrils.

that of control without acid treatment and was considered to indicate that polyacrylic acid has the least effect on dentin among the dentin conditioners examined under the condition of this study (Fig. 6-b).

2. Carious teeth

Fig. 7 shows a TEM image of untreated cavity floor dentin after removal of infected dentin. Inorganic substances were completely removed by demineralization with 5% EDTA, and a large number of collagen fibrils were observed. In the superficial region, broken-up and raveled collagen fibrils were observed (Fig. 7-a, between arrows) as well as in intact teeth, but the degree of their morphologic changes was smaller than in intact teeth, with some collagen fibrils retaining their normal morphology (Fig. 7-b).

After the treatment of the cavity floor with 40% phosphoric acid for 60 seconds, a relatively homogeneous layer without normal collagen fibers was observed (Fig. 8-a, between arrows) as in intact teeth. In a greater magnification, this layer contained fine filamentous or granular structures, but more fibrils retained the fibrous structure than in intact teeth (Fig. 8-b).

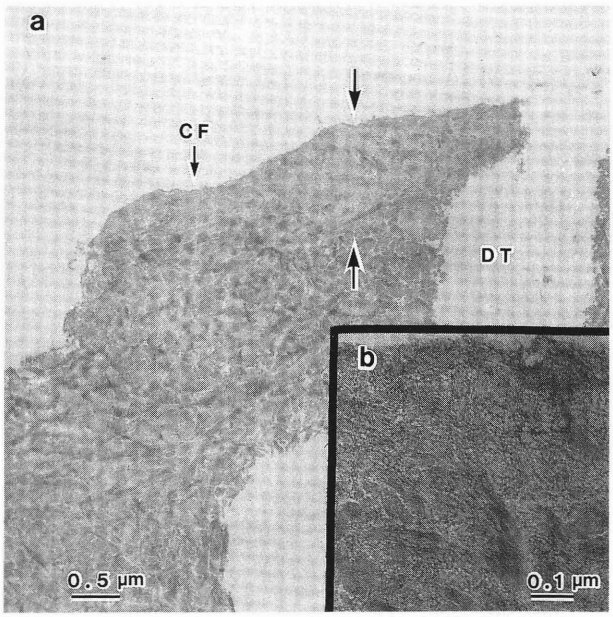

Fig. 8

a : TEM of the superficial layer of cavity floor dentin treated with 40% phosphoric acid for 60 sec. after the removal of carious dentin. The comparatively homogeneous layer composed of the fine filamentous or granular structures appeared (between arrows). CF : Cavity floor, DT : Dentinal tubule.

b : Higher magnification of the fine filamentous or granular structures.

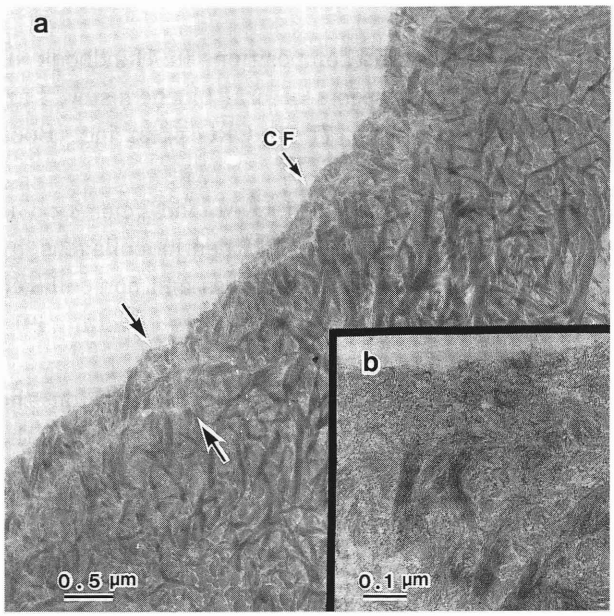

Fig. 9

a : TEM of the cavity floor dentin treated with a mixture of 10% citric acid and 3% ferric chloride for 30 sec. after the removal carious dentin. Collagen fibrils in the superficial layer were deformed into the indistinct feature (between arrows). CF : Cavity floor.

b : Higher magnification of the deformed collagen fibrils showing the fine filamentous or granular structures.

In case of treatment with a solution of 10% citric acid and 3% ferric chloride for 30 seconds, a layer containing collagen fibrils with indistinct morphology appeared (Fig. 9-a, between arrows) as did in intact teeth. In a greater magnification, raveled collagen fibrils and fine filamentous or granular structures were observed, but the fibrous structures were retained more frequently than in intact teeth (Fig. 9-b).

Discussion

In this study, intact human teeth were demineralized after cavity preparation and examined under TEM. A layer containing deformed collagen fibrils was observed underneath the smear layer in the superficial layer of cavity floor dentin. We previously identified this layer[8] with the layer resistant to HCl -collagenase digestion reported by Yamada et al.[6].

Collagen accounts for about 90% of organic components of dentin, and most of it is reported to be insoluble, very stable, and resistant to acids[10]. It was also shown to be stable against treatment with pepsin[11].

Takahashi et al.[12] examined the cut dentin surface after treatment with various cavity conditioners under the scanning electron microscope and observed a layer presumably of denatured collagen below the smear layer. Mizunuma[13] and Okamoto et al.[14] biochemically demonstrated that the solubility of bovine dentin collagen by pepsin and trypsin digestion increased when it had been treated with phosphoric acid.

In this study, collagen fibrils in the superficial layer of cavity floor dentin changed into fine filamentous or granular structures after treatment with phosphoric or citric acid, indicating that denaturation of dentin collagen caused by tooth cutting underneath the smear layer was enhanced by acid treatment.

Clinically, adhesive resin restoration is more often performed in carious teeth. Therefore, we carried out similar evaluations using carious teeth. A layer of denatured collagen fibrils similar to that observed in intact teeth was noted also in the superficial layer of cavity floor dentin in carious teeth after removal of infected dentin and acid treatment. The degree of denaturation, however, was less in carious teeth than in intact teeth. In this study, the cavity floor dentin after removal of infected dentin according to the staining with Caries Detector corresponded with the inner carious dentin of Fusayama[15]. Ultrastructural features of collagen in this layer were shown to be similar to that of sound dentin collagen[16]. Therefore, the difference in the degree of denaturation of collagen observed between the intact teeth and carious teeth may be ascribed to the difference in the cutting condition, i.e. the difference between high-speed cutting and low-speed cutting. Usami et al.[7] investigated the effects of various cutting conditions on the superficial layer of cavity floor dentin using the HCl-collagenase method and reported that the effect of low-speed cutting was slightly less than that of high-speed cutting.

Further studies are needed to clarify the effects of dentin conditioners according their concentration, pH, and duration of treatment.

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