SEM and TEM Observation of Ultra Microstructure at Adhesive Interface between Resin and Caries Affected Dentin

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Abstract: To prevent over-cutting of the sclerotic dentin, we developed a new caries detector (Caries Check, Nippon Shika Yakuhin Co., Ltd., Japan) composed of polypropylene glycol containing acid red. The purpose of this study was to observe the ultra microstructure of the adhesive interface between resin composite and dentin after cutting the dentin in accordance with the conventional Caries Detector or Caries Check staining. In the Caries Check specimen, the dentin tubules were filled with debris and penetration of the bonding agent was limited in the peri-tubular dentin. In the Caries Detector specimen, the dentin tubules were open and a resin tag formed in the dentin tubules. It was possible to conclude that the sclerotic dentin was preserved under the guide of staining by Caries Check. The results suggest that dentin bonding was not obtained by monomer penetration into the superficial substrate dentin or into the dentin tubules.

Key words: caries dentin, SEM observation, TEM observation.

The idea of staining caries dentin using polyvalent alcohol containing dyestuff was initially introduced by Terashima and Fusayama in 1972. They defined that two layers could be distinguished in caries dentin and only the outer layer was selectively stained by the propylene glycol containing acid red (Caries Detector, Kuraray, Japan). It has been reported that the stained dentin should be removed because the collagen fiber in the outer layer had degenerated completely and could not re-calcify, while the inner layer of the caries dentin should be preserved because it was possible for this layer to re-calcify through dental treatment. However, treatment of the dentin that was stained pink by the Caries Detector is still the subject of discussion. In many papers concerning observation of the micro-structure of caries tooth surfaces or on the physical analysis of caries dentin using fluorescence, the dentin was likely to be over-cut if the dentist treated the caries according to the Caries Detector staining. It is widely recognized that the softening front of the caries dentin is located in the deepest part of the cavity and the surface of bacterial invasion is located in the shallowest part of the cavity. In addition, sclerotic dentin forms between caries infected dentin and sound dentin. Though the detailed mechanism of sclerotic dentin formation has not been clarified, it is understood that permeability is limited in sclerotic dentin.

The efficacy of dentin adhesives applied to the dentin is estimated by measuring the bond strength to a flat dentin surface, and the bonding mechanism has been explained by the hybrid layer formation in the superficial layer of the substrate dentin. In many papers, it was reported that bond strength to sclerotic dentin was lower than that to sound dentin because the monomer infiltration into the sclerotic dentin was interrupted. This was said to make the hybrid layer formation insufficient in sclerotic dentin. However, we have reported that sclerotic dentin is an advantageous substrate...
compared to sound dentin. This is because formation of a contraction gap was completely prevented in the sclerotic dentin cavity even when the dentin cavity wall was not primed. In addition, we suggested that the efficacy of the dentin adhesives could be consistently estimated by observing the contraction gap formation. Through contraction gap observation, the priming effect was explained by the interruption of the adhesive monomer immersion into the dentin as reported by Chigira et al. As claimed by Kusunoki, Tani, and Wu, the sclerotic dentin should be preserved not only to protect the dentin from bacterial invasion but also to improve the efficacy of the dentin adhesive. The sclerotic dentin that was located between the sound dentin and caries infected dentin should not be stained by the caries detector. We demonstrated that the hardness and the morphological characteristics of the dentin closely resembled that of sound dentin after removing the dentin that was stained pink by Caries Detector. Therefore, we developed a new caries detecting agent composed of polypropylene glycol containing acid red (Caries Check, Nishika, Japan). The Caries Check was applied in the cavity for a few seconds followed by rinse and dry. The stained dentin was removed by a round shaped steel bur mounted on a low-speed dental cutting machine with a water coolant. After removing the stained dentin, the smear layer on the cavity wall was removed by 0.5 mol/l neutralized EDTA for 60 s. Then the specimen was longitudinally sectioned through the center of the cavity and dehydrated in graduated alcohol solutions. After critical point drying and ion spattering with palladium and platinum, the ultra microstructure of the dentin was observed using a scanning electron microscope. For specimens stained using Caries Detector, the dentin stained by the Caries Detector was completely removed by a round shaped steel bur as described above. After removing the smear layer on the ground dentin cavity by EDTA, the ultra microstructure of the dentin cavity wall and sectioned dentin was observed in the same method as the Caries Check specimen. The cavity wall of the untreated moderate carious dentin was observed in the same manner after sectioning the teeth along the long axis through the center of the cavity.

2. SEM observation of the resin adhesive surface
For the observation of the resin adhesive surface, the exposed dentin was conditioned with 0.5 mol/l neutralized EDTA for 60 s followed by rinse and dry. The cavity was primed with 35 vol% of glyceryl mono-methacrylate (GM) solution for 60 s followed by air blasting. Then the cavity was filled with a commercial flowable resin composite (Filtek Flow, 3M ESPE, USA) modulated with a commercial dual cured dentin bonding agent (Clearfil Photo Bond, Kuraray, Okayama, Japan). The specimen was sectioned along the tooth axis through the center of the cavity and the microstructure of the adhesive interface was observed by SEM as described above. For observation of the resin adhesive surface, the tooth was dissolved in hydrogen chloride and sodium hypochlorite and the morphology of the resin surface observed.

3. TEM observation of the resin-caries affected dentin adhesive interface
The caries dentin that had extended to the middle third of the dentin was removed according to the guide of Caries Check staining as described above. The cavity wall was conditioned with 0.5 mol/l neutralized EDTA for 60 s followed by rinse and dry. Then the cavity wall was primed with GM solution for 1 s followed by gentle air blast. Finally, the cavity wall was coated with a flowable resin composite mediatesed with a commercial dual cured dentin bonding agent and irradiated for 40 s. The dentin bonding agent application and light irradiation was repeated twice. For the TEM obser-
vation, the specimens were dehydrated by ethanol and embedded in an epoxy resin for 24 h. The ultra-thin specimens 60 to 90 nm thick was prepared by using the ultra microtome. Epoxy resin embedding procedure was omitted for some specimens and TEM conservation was performed without any staining the specimen.

Results

The microstructure of caries dentin was shown from the enamel dentin junction towards the pulp (Figure 1a-h). In the superficial caries dentin, no crystals were observed, only a fibrous structure, and dentin tubule structures were mostly absent (Figure 1a, b). This layer was easily removed using a spoon excavator. In the layer beneath that, which was stained by the Caries Check, it was possible to distinguish between the dentin tubules and peritubular dentin and the course of the dentin tubules was clear. Bacterial invasion was observed in the dentin tubules in the superficial layer of the dentin that was stained by the Caries Check (Figure 1c, d). In the deepest layer of the caries dentin, the dentin tubules were completely filled with fine cubic crystals with a grain size of approximately 0.5 μm (Figure 1e-h). Bacterial invasion was not observed in this layer. This dentin was not stained by the Caries Check, however, it was stained by the Caries Detector. After removal of dentin stained by Caries Detector the dentin tubules of the cavity wall were completely open, however these were closed by debris when the dentin was cut according to the staining of Caries Check (Figure 2a, b). A similar phenomenon was seen on the sectioned surface (Figure 2c, d). When the resin adhesive surface bonded to the dentin after removal of the stained dentin by the Caries Check, it was observed that the resin tag did not form inside the dentin tubules but penetrated into the peri-tubular dentin and formed there (Figure 3a). In the specimen stained using Caries Detector, the resin tags were formed in the dentin tubules, however the morphology of the resin tag was different from that formed in sound dentin (Figure 3b). Formation of the resin tag was not seen in dentin tubules when only the area dyed by Caries Check was removed, and resin composite that filled this cavity was observed on the sectioned surface without dissolving the teeth, Crystals were also observed (Figure 3c). On the other hand, when all areas dyed by Caries Detector were removed, formation of the resin tag was observed in the dentin tubules (Figure 3d).

In the TEM observation, the dentin tubules of the cavity wall was partly or completely filled with the high density substances (Figure 4a, b). At the substrate dentin surface, the low density zone approximately 200 nm thick was observed that might be decalcified by EDTA conditioning though the adhesive interface was clearly observed (Figure 5). The high density zone at the adhesive interface was formed in the dentin beneath the adhesive interface that was produced by GM priming as reported by Chigira (Figure 6a, b).

Discussion

In many papers concerning dentin bonding, it has been claimed that the strength of bonding to sclerotic dentin is lower than that to sound dentin because the immersion of the adhesive monomer into the sclerotic dentin has been interfered. Consequently it was not possible to ensure formation of a hybrid layer in the sclerotic dentin of a degree comparable to that of sound dentin. However, we verified that formation of a contraction gap of a light activated resin composite in a cylindrical dentin cavity was completely prevented when the cavity was conditioned with EDTA, primed with 35 vol% GM solution and then applied with a commercial dentin bonding agent containing phosphate ester monomer (10-methacryloxydecyl dihydrogen phosphate). In addition, we demonstrated that formation of a contraction gap in cylindrical sclerotic dentin cavities was completely prevented in half of the specimens even when the cavity was not primed. However the gap was observed in all the unprimed sound dentin cavities. Furthermore, it has been reported that dentin sensitivity is reduced by application of the dentin primer such as the aqueous solution of 2-hydroxyethyl methacrylate (2-HEMA) or 2-HEMA containing glutaraldehyde (GLUMA primer). The sedative effect of the dentin primer might be explained by the interruption of the fluid movement through the dentin tubules. Such findings suggest that the dentin primer improved the efficacy of the dentin bonding agent by decreasing monomer diffusion into the dentin structure and preventing contamination by water from the dentin structure. The sclerotic
Figure 1  a: Top layer of the caries dentin. A fibrous structure without dentin tubules was observed (*: Decay causing bacteria). b: Second layer of the caries dentin. The remainder of the dentin tubules structure was visible. c: Third layer of the caries dentin. The dentin tubules structure was clearly visible. d: Fourth layer of the caries dentin. Three dentin structures (dentin tubules, inter-tubular dentin and peri-tubular dentin) were clearly visible. In this layer, the bacteria (*) were still visible in the narrow dentin tubules. Caries Check dyed up until this layer. e: Fifth layer of the caries dentin. The crystal structure appears, and dentin tubules are closed. f: Cubic crystals observed at high magnification. g: Sixth layer of the caries dentin. Increased consistency of crystal structure. The dentin tubules gradually become filled with cubic crystals. h: Seventh layer of the carious dentin. The consistency of the crystal structure increased, and the dental tubule is now completely closed.
Figure 2  

a: Cavity wall after removal of dentin stained by Caries Check. The dentin tubules were gradually filled with cubic crystals.  
b: Cavity wall after removal of dentin stained by Caries Detector.  
c: Section of cavity wall after removal of dentin stained by Caries Check. The dentin tubules were filled with cubic crystals.  
d: Section of cavity wall after removal of dentin stained by Caries Detector. The dentin tubules did not contain any crystals.

Figure 3  

a: Adhesive surface of resin composite after the tooth stained using Caries Check was dissolved. Dentin adhesives bonded to the peri-tubular dentin(*).  
b: Adhesive surface of resin composite after the tooth stained using Caries Detector was dissolved. Dentin adhesives bonded in the dentin tubules.  
c: Resin-dentin interface after removal of dentin stained by Caries Check.  
d: Resin-dentin interface after removal of dentin stained by Caries Detector. The resin-tag formed in the dentin tubules.
Dentin should be conserved because it not only prevented the bacterial invasion but also provided an advantage as the substrate for the dentin adhesives as mentioned above. Thus dentists should remove only the carious infected dentin and preserve the caries affected dentin. Therefore, caries detector should only stain carious infected dentin and not sclerotic dentin.

As demonstrated in our previous study, there is a likelihood that sclerotic dentin is also stained by Caries Detector and is removed when the dentist removes the dentin in accordance with staining using this solution. We demonstrated that after removing the dentin stained by Caries Detector, the dentin cavity was diagnosed as sound dentin by DIAGNOdent. However, after removing the dentin stained by Caries Check, the dentin cavity was diagnosed as re-calcifiable dentin. This over-staining is caused by the over-diffusion of Caries Detector. The mechanism of the Caries Detector staining can be explained by the penetration of the propylene glycol as it stained neither the bacteria nor the collagen fiber. In addition, it is likely that the porous regions such as the dentin-enamel junction are also stained regardless of the presence of carious infection. The penetration of the experimental carious detector was limited by the higher molecular weight of the polypropylene glycol. As demonstrated in this study, after removing the dentin stained by Caries Check, the dentin tubules of the cavity wall were filled with fine cubic crystals, which could possibly be Whitrokite (β-tricalcium phosphate). Such debris in the dentin tubules was effective to prevent the bacterial invasion and the liquid flow. Thus the dentin priming effect was explained by interrupting the immersion in the dentin substances.

More effort should be required to develop the materials and methods to produce the arterially sclerotic dentin to improve the efficacy of the dentin adhesive and to sedate the dentin sensitivity.
Therefore, it was considered that Caries Check did not stain the sclerotic dentin and was able to preserve it. The dentin adhesives penetrated into the slightly decalcified peri-tubular dentin, but not into the dentin tubules.

It was possible to conclude that Caries Check stained only the carious infected dentin and not the sclerotic dentin. Therefore, when removing dentin according to the staining of Caries Check, dentists are able to avoid excessive cutting.

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References


