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Original Article

Effects of chloramines and sodium hypochlorite on carious dentin

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In chemo-mechanical caries removal procedures, chloramines are typically used for chemical softening of carious dentin. However, the specific effect of chloramines to be compared to sodium hypochlorite has not been sufficiently clarified. In present study, the effect of chloramines used in the Carisolv-system on carious dentin mechanical properties and morphology were investigated, using Vickers hardness test and scanning electron microscopy (SEM).

Sections of permanent teeth with dentin caries were treated with chloramines, prepared by mixing amino acids (glutamic acid, lysine, and leucine) with sodium hypochlorite or with sodium hypochlorite alone or with purified water. There was a tendency that the application of the sodium hypochlorite solution softened the sound dentin and/or inner layer of carious dentin more than the application of the chloramines solution did. In SEM observations, the application of chloramines resulted in opening dentinal tubules in the outer layer of carious dentin: Occluded dentinal tubules were seen after sodium hypochlorite application. There is a possibility that the amino acids in the Carisolv-system decrease the aggressive effect of sodium hypochlorite on sound dentin and/or inner layer of carious dentin and also would enhance the disrupting effect on degenerated collagen in carious dentin outer layer.

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Introduction

Chemo-mechanical caries removal comprises a chemical softening of carious dentin and removal of the softened material with instruments similar to excavators[1]. The typical chemicals used for such procedures are chloramines, prepared by mixing sodium hypochlorite with amino acids. The adverse effects of sodium hypochlorite on sound dentin and soft tissue are minimized using chloramines, but the effect on carious dentin is retained.

More than 20 years ago, GK-101 (N-chloroglycine) was developed as a chemo-mechanical carious removal method[2,3]. In the procedure, carious dentin was removed by applying a solution into a caries cavity using a handpiece that was also used as an excavator. Main constituents of the liquid were sodium hypochlorite and glycine. The system was based on the non-specific proteolytic effect of sodium hypochlorite, which was reacted with glycine to reduce the aggressive effect on sound tissue. Habib et al.[2] reported that GK-101 initiated the disruption of degraded collagen in carious dentin by chlorination of hydroxyproline in the collagen molecule. GK-101 was found to affect only the outer layer of carious dentin, the portion which should be removed from a clinical standpoint, while causing almost no pain[4,5]. However, compared to traditional drilling, this method took longer time and was not effi-

cient; thus, it did not become widely used in clinical dentistry. Also, at the time of the introduction of GK-101, the use of adhesive dental materials was not common, and dentists still prepared teeth according to Black's cavity designs. Therefore, the use of a method that only removed carious dentin could not significantly reduce the need of drilling to create mechanical retentions[6].

Recently, Ericson and co-workers developed a new chemo-mechanical carious removal system, Carisolv™, based partly on the same original concept of GK-101[6,7]. In Carisolv procedure, gelified solution containing chloramines prepared by mixing sodium hypochlorite and three amino acids (lysine, leucine, and glutamic acid) is applied into the cavity and the carious dentin is removed by excavation with special hand instruments. The authors of this paper have earlier reported that Carisolv softened only the outer layer of carious dentin and the hardness of the inner layer of carious dentin and the sound dentin was not changed[8], and that Carisolv selectively dissolved the degenerated collagen in carious dentin[9]. Additionally, Ericson[6,7] explained the behavior of the amino acids in Carisolv gel from two aspects; one is the reduction of aggressive effect of the sodium hypochlorite on sound tissue and the other is that the chlorinated three amino acids of different electric charge, reacted to different moieties of carious dentin. Hanning[10] compared Carisolv with sodium hypochlorite and reported that Carisolv selectively dissolved an artificially demineralized and denatured dentin, but did not dissolve a demineralized dentin of no denaturation. Sodium hypochlorite dissolved unselectively both demineralised and denatured dentin. However, the specific effect of chloramines as compared to sodium hypochlorite alone on carious and sound dentin mechanical properties and morphology has not been sufficiently clarified. In the present study, Vickers hardness test and SEM observations were conducted and the effect of chloramines in Carisolv on the dentin mechanical property and morphology was investigated.

Materials and Methods

1. Chemicals

Solution containing three amino acid, glutamic acid, lysine, and leucine (WAKO Pure Chemical Industries, Japan) was prepared at a 0.5w/v % of each amino acid. Sodium hypochlorite solution (WAKO Pure Chemical Industries, Japan) was diluted to 0.5 w/w % before use[1]. A chloramine solution was prepared by mixing equal amounts (1 ml) of sodium hypochlorite and amino acid solution immediately before use. Purified water used in this study was supplied by Milli-Q™ system (Millipore, USA).

2. Hardness measurements

Five human permanent teeth in which a dentin carious lesion did not reach the dental pulp were stored in a freezer of −15°C immediately after extraction and used within a month. Teeth used in the hardness test were indicated in Table 1. The teeth were defrosted in distilled water before use. A scheme of the specimen production and the Vickers hardness test procedure was shown in Fig. 1. All the teeth were sectioned into two pieces through the lesion with a low speed cutting-machine (Isomet, Beular, USA). The cut surface of each piece was polished smooth with 1200 grit SiC paper, and mounted in a plastic mold with a softened black modeling compound. Half of the cut surface of the each piece was covered with adhesive tape placed along the dentinal tubules to separate a control area from a test area. Then the cut surface of the first piece of a pair was treated with chloramine solution (hereafter, CA), and the corresponding surface of the second piece of the pair was treated with a mixture of 1 ml of 0.5% sodium hypochlorite and 1ml of purified water (hereafter, NC). The solution was applied to the each cross-section for 3 minutes, and finally washed away. The tape was removed from each piece and the same carious lesion had thus been used for two test surfaces and two control surfaces.

Using a Micro-Vickers hardness tester (MVK H2, Akashi, Japan), Vickers hardness numbers (VHNs) of the both the area treated by CA or NC solution and that

Table 1. Teeth used in the Vickers hardness test and the SEM observation
All the lesions were active, soft, and stained to some degree seemed to develop rather chronically.

Teeth No.	Experiment	Age of patient	Kind of Teeth	Reason of Extraction
1	Vickers hardness test	35	18	Caries
2	Vickers hardness test	32	18	Caries
3	Vickers hardness test	29	28	Caries
4	Vickers hardness test	25	28	Caries
5	Vickers hardness test	30	48	Caries
6	SEM observation	26	28	Caries
7	SEM observation	36	28	Caries
8	SEM observation	29	38	Caries
9	SEM observation	48	42	Periodontitis
10	SEM observation	44	26	Periodontitis

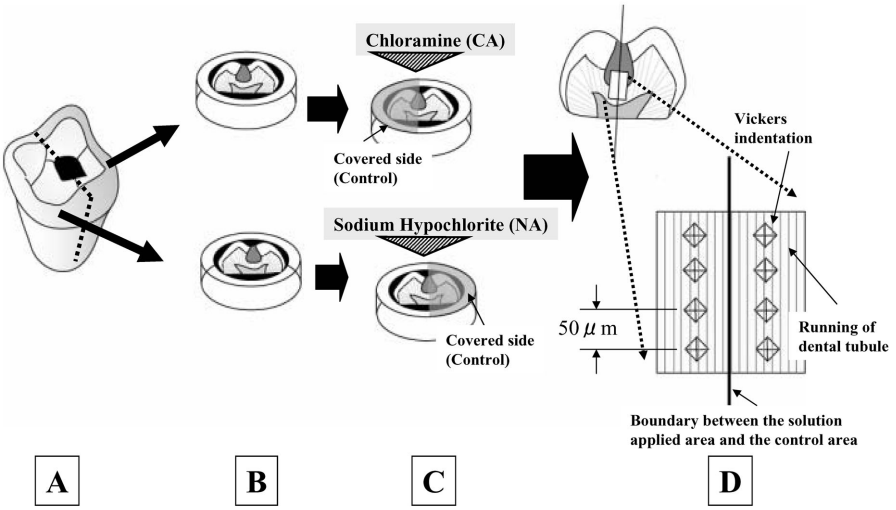

Fig. 1. Scheme of the Vickers hardness test
A: A tooth with carious lesion was sectioned into two pieces through the lesion. B: Both pieces were mounted in plastic molds with black compound after that the cut surface of each piece was polished with 1200 grit SiC paper. C: Half of the cut surface of the each piece was covered and was treated with CA or NC solution for 3 minute. D: The VHN of control area and CA or NC applied area were measured along the boundary of both areas from pulpal side to caries cavity side by 50 micrometers interval.

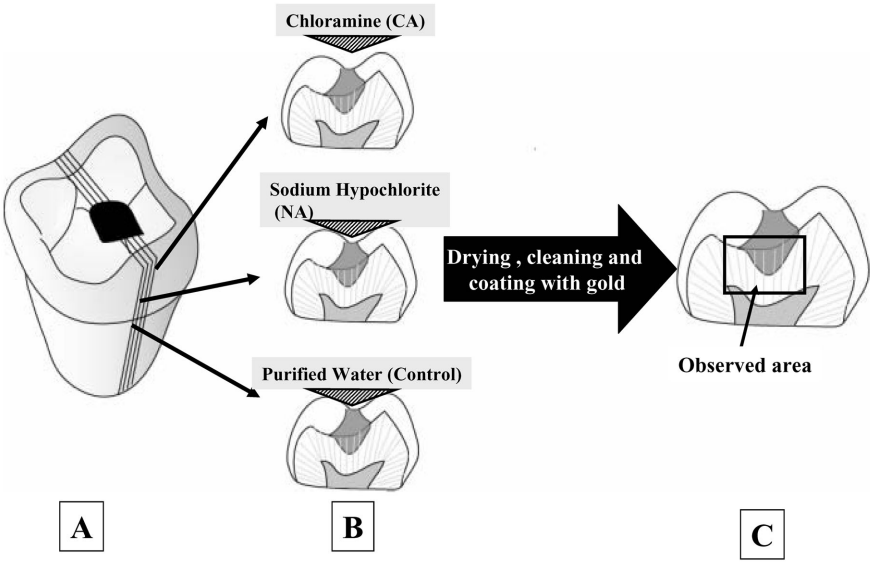

Fig. 2. Scheme of the SEM observation
A: Teeth were sliced at every 0.5 mm and three sections of 0.15 mm thickness containing both sound and carious dentin were cut out. B: The sections were polished with 1200 grit SiC paper and were treated with CA, NC and pure water (control), respectively. C: The SEM observation was done after the drying, cleaning and gold coating of the section surface; the caries cavity bottom and its subjacent area were observed.

of the control on the each cross-section were measured by application of a 10-g load for 15 seconds. Measuring points were near the boundary between the solution applied area and that of control. Measurements were taken at 50 micrometers intervals, moving parallel to the boundary line from the deep layer of the sound dentin to the surface layer of the carious dentin. After the measurements were performed, a

caries indicating solution (Caries Detector, Kuraray, Japan) was applied on the treated surfaces and then the tooth section was washed with tap water. The observed staining front line was traced with razor creating a groove that could be seen in the microscope on each test-surface. The Vickers indentations closest to the calculated center line of the groove were regarded as being on the boundary between the inner and outer layer of carious dentin, and was defined as zero point. The distance from the zero point was measured for each indentation as its own location; the caries cavity side was regarded as the carious dentin outer layer and the locations of the indentations were given a positive sign (+); the pulpal side was regarded as the carious dentin inner layer and/or sound dentin and the locations of the indentations were given a negative sign (−).

3. SEM observation

Teeth used in the SEM observation were indicated in Table 1; the condition of teeth preservation was the same as that of the Vickers hardness test. The teeth were sliced at every 0.5 mm interval and three sections of 0.15 mm thickness containing both sound and carious dentin were cut out using the previously mentioned cutting machine. The surfaces of dentin sections were polished with 1200 grit SiC paper. The sections were treated with CA, NC and purified water (control), respectively. After 3 minutes treatment, the sections were cleaned in purified water by using an ultrasonic cleaner for 30 seconds and then dried for 24 hours in a desiccator. The sections were sputter-coated with gold on one side (Ion Coater IB-3, Eiko Engineering Co., Japan) and analyzed in a SEM (DS -130c, Akashi Beam Technology Co., Japan). After the SEM observation, the back surface of each tooth sheet was treated by caries indicating solution (Caries Detector, Kuraray, Japan) and was observed with naked eyes to examine the relationships between the outer layer of carious dentin and the SEM images.

Results

Vickers hardness test

An example of VHN changes corresponding to dentin depth were shown in Fig. 3. With the control, the hardness of the caries cavity sides were constantly lower than those of the sound dentin sides; the hardness of the sound dentin varied from tooth to tooth and from location to location, though it could be said that the hardness was approximately 40 to 60 Hv. The hardness decrease was observed from around the location of the zero point to the caries cavity side. The rate of decrement also varied among teeth. The hardness finally became around 10Hv or less in the most superficial part of the carious lesion.

With CA or NC application, the hardness decrease was more prominent than with control. The hardness decrease could be detected further from the cavity surface and the hardness decrement was greater as compared to control. To evaluate the effect of the solutions, the following values were determined: The first was the location of the deepest point from which CA or NC applied dentin constantly indicated lower VHN as compared to control (hereafter α, Table 2). The second was the hardness differences between CA or NC applied dentin and control at the locations between −250 and 250 μm (hereafter β, Table 3). The α value (SD) of CA and NC applied dentin were −210 (139) and −350 (106) μm, respectively. There was a tendency that the application of NC softened the sound

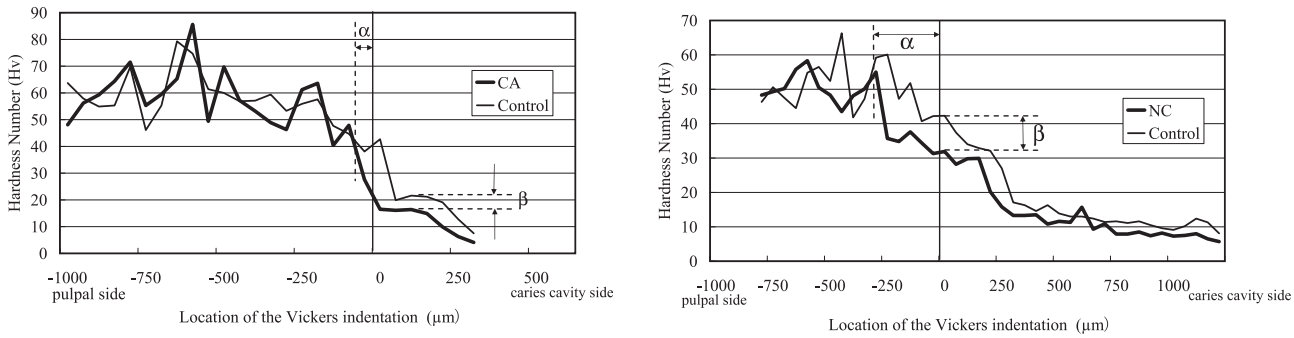

Fig. 3. VHN changes of CA or NC applied dentin.
α: The deepest location indicating the dentin softening with CA or NC application . β: The hardness differences between CA or NC applied dentin and control at the locations between −250 and 250 μm

dentin and/or inner layer of carious dentin at deeper location than the application of CA. The difference between α value of CA and NC was statistically significant by paired-t test ($p<0.05$). The β values of the carious dentin inner layer and/or sound dentin (location: -50 to -250 μm) with CA and NC application were 1.7-10.0 and 8.9-12.1Hv, respectively; there was a tendency that the hardness decrement of NC application was greater than that of CA, however, there were no significant differences between the β values of CA and NC except at the location of -200 μm by paired-t test ($p>0.05$). The β value of the carious dentin outer layer (location: 50 to 250 μm) with CA and NC application were 6.1-10.7 and 6.6-8.2 Hv, respectively; there were no significant differences between the β value of CA and NC by paired-t test ($p>0.05$).

SEM observations

At 50 times magnification, the following three regions were classified in almost all the teeth; region A was at the oral surface of the carious lesion indicated by concave/convex features and partly detached material, region B was rather smooth and where no traces or scratches made by the polishing procedure, and region C had scratches which was clearly visible; the region B and C were distinguished by the existence of such scratches (Fig. 4). The regions A and B corresponded to the area being dyed red by the caries indicating solution. At 3000 times magnification, morphological differences among specimens treated with CA,

Table 2. The deepest location indicating the dentin softening with CA or NC application (α)
The difference of α values between CA and NC was statistically significant by paired-t test ($p<0.05$).

	Number of specimen	α μm
CA	5	- 210 (139)
NC	5	- 350 (106)

( ):SD

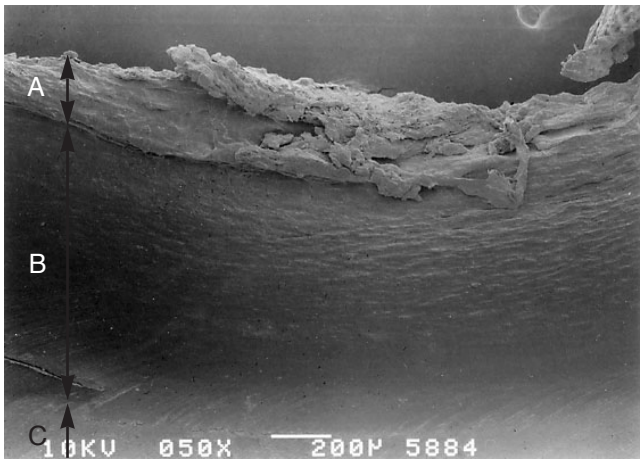

Fig. 4. SEM image of carious dentin cross-section (x50)
Three region were identified: In descending order, region A was at the surface of the caries cavity bottom and indicating concave/convex figures, region B was rather smooth and had no scratches, region C was smooth and had scratches.

Table 3. The differences of the VHNs between CA or NC applied dentin and control (β)
The values connected with a horizontal line were not significantly different by paired-t test ($p>0.05$).

Location	Number of specimen	β CA	β NC
μm		Hv	Hv
-250	5	1.7 (4.8)	9.7 (10.7)
-200	5	1.3 (7.6)	8.9 (5.1)
-150	5	7.7 (2.4)	11.1 (6.7)
-100	5	4.8 (5.7)	9.0 (3.0)
-50	5	10.0 (6.0)	12.1 (7.7)
0	5	15.5 (9.9)	9.6 (3.7)
50	5	10.7 (8.3)	6.8 (3.1)
100	5	7.5 (8.6)	8.2 (2.8)
150	5	6.9 (4.1)	6.6 (4.5)
200	5	6.3 (3.0)	6.7 (4.1)
250	5	6.1 (2.5)	6.9 (4.0)

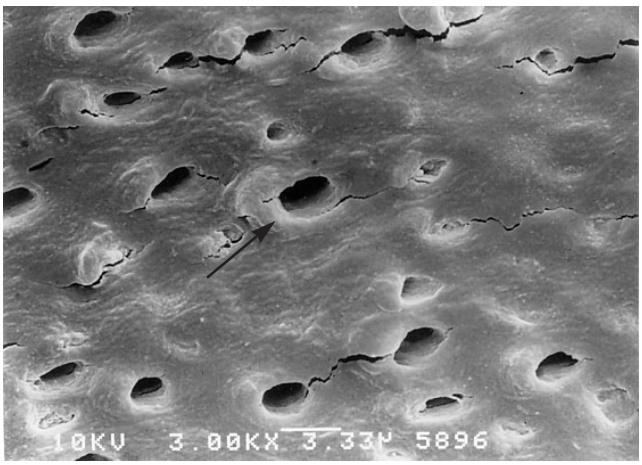

Fig. 5. SEM image of region B (x3000)
a. Image of CA-treated carious dentin. Opened dentinal tubules were observed (the arrow).

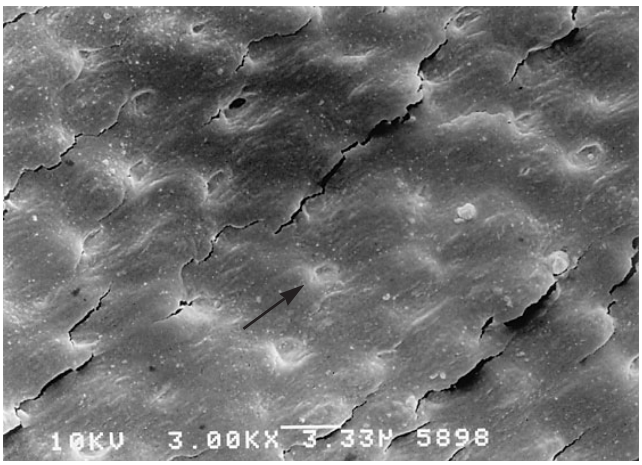

Fig. 5. SEM image of region B (x3000)
b. Image of NC-treated carious dentin. Occluded dentinal tubules were observed (the arrow).

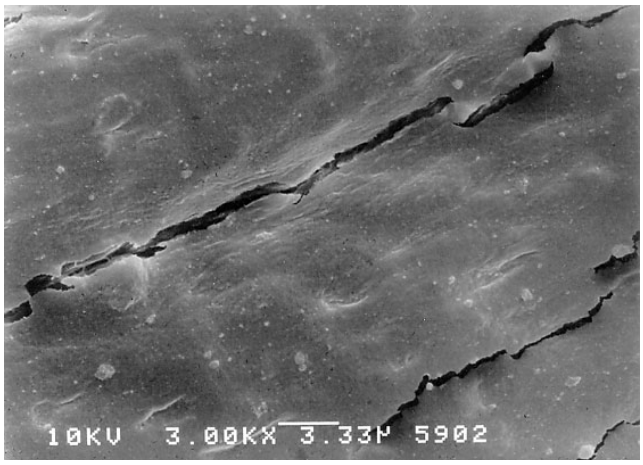

Fig. 5. SEM image of region B (x3000)
c. Image of carious dentin treated by purified water (control). No dentinal tubules were observed.

NC and control were hardly identified in regions A and C. On the other hand, in region B, differences were observed as regards the morphology of dentinal tubules. An example of a tooth was shown in Fig 5 a-c. The opening dentinal tubules were observed in CA and occluded dentinal tubules were observed in NC. No dentinal tubules were observed in control.

Discussion

All the teeth in this study were used after obtaining consent of the donating patients. Even though the carious lesions of all teeth were active status judging from color, tactile examination by explorer and history, they were not identical in all respects. Therefore, the effects of the solutions were studied within a small area using a split tooth design.

Carisolv gel contains equal amounts of three amino acids, glutamic acid, lysine, and leucine: the total amount of the amino acids is 0.1M before mixture[1]. In present study, the concentrations of each glutamic acid, lysine, and leucine in prepared solution before mixture were all 0.5w/v %, that is 0.034, 0.038 and 0.034 M, respectively; the amounts were almost the same as Carisolv gel.

It was difficult to see the staining front in the optical microscope used for the Vickers hardness test. Therefore, the staining front line was traced with a razor and a groove was created in the dentin according to Igarashi et al.[8] The indentations closest to the groove center line were regarded as being on the boundary between the inner and outer layer of carious dentin. The width of the groove was approximately 30-50 μm, therefore the measuring error was considered to be within 15-25 μm.

In this investigation, the solutions were applied to the cross-section surface of the carious dentin and VHN changes corresponding to caries depth could be examined. For evaluation of the influenced ranges by the solutions and VHN changes of each location, α and β value were defined and used. From α value, it was revealed that the dentin specimens treated with NC were softened at deeper point than that of CA in the area of the inner layer of carious dentin and/or sound dentin and the difference was statistically significant.

On the other hand, β value indicated that VHN changes of the inner layer of carious dentin and/or sound dentin of CA application had a tendency to be lower than that of NC, however, there was no significant difference of the VHN changes between CA and NC application from sound dentin to carious dentin except at the location of -200 μm. It was suggested that the amino acids uses to generate chloramines had a conservative effect on the inner layer of carious dentin and/or sound dentin.

SEM observations were conducted with the cross-section of the carious dentin, which was the same as the hardness test. As a result, three regions A, B and C were detected. With observation with naked eyes, the regions A and B corresponded to the area dyed red, that is, the carious dentin outer layer. The region C corresponded to not-dyed area. The region B and C were distinguished by the presence of scratches in the SEM images made by the polishing procedure. Scratches could be created in dentin which possessed a certain degree of hardness. Therefore, it can be considered that the region B and C had different hardness. Consequently, the boundary of the region B and C would not correspond exactly to the zero point of the hardness test because the hardness change compared with the zero points were different among CA, NC and control.

The region A had complicated morphology and varied from tooth to tooth; it was difficult to describe the common features as the results of the solution effect among the different teeth. The morphology of the region B was less complicated than that of the region A. Such a condition might make the difference of solution effect more prominent. CA application resulted in the opened dentinal tubules in the region B, while the dentinal tubules in the region B could be observed but were occluded after NC application. Shimizu et al.[11] reported that the arrangement of collagen matrix fibers and periodic crystal attachment was completely obscure in intertubular dentin with a TEM observation; though proper thickness of the peritubular dentin was maintained in empty tubules, partial or complete invasion of bacteria enlarged the lumens in the outer layer of carious dentin. The region B was softer because of demineralization, however, would maintain its original structure such as dentinal tubules. By the machining procedure, the dentinal tubules of the region B were plugged by smear that might consist of the small particles of carious dentin tissue; there was a possibility that generated chloramines in CA would chlorinate and disrupt the denatured collagen to remove such small particles.

There were some reports which investigated the SEM image of dentin surfaces after caries removal with Carisolv[12-14]; one common finding was that the dentinal tubules on the dentin surface after Carisolv treatment were opened. In these reports, the observed dentin surface was corresponded to the region C in present study, though direction of observation was not identical. The dentinal tubules in the region C were observed but occluded, and no difference was found among CA, NC and the control samples in the present study. The state of dentinal tubules after caries removal is claimed to be influenced by the speed of the caries process. Most of the tubules were occluded in arrested caries while most of them were open in active caries[15]. Hashimoto et al.[12] reported a difference in opening of dentinal tubules after caries removal by using Carisolv; there was a tendency that the dentinal tubules opened more with acute caries than with chronic caries. All the teeth used in the present study were considered to be active however seemed to have rather chronic development; there was a possibility that such chronic development of the lesions influenced the morphology of dentinal tubules of the region C.

Sodium hypochlorite has two main characteristics depending on solution pH; one is a sterilizing effect at around pH 7 and the other is solvent effect on organic material at higher pH[16]. Chloramines are generally produced by combination of sodium hypochlorite and amino nitrogen, which makes the effect of sodium hypochlorite less aggressive and prolonged[16]. Chloramines are commonly used as disinfectant; however, the solvent effect is expected for application of chemo-mechanical caries removal. As previously mentioned, Ericson[6,7] presented a hypothesis on the behavior of the amino acids and chloramines used in the Carisolv system from two aspects; the reduction of aggressive effect of the sodium hypochlorite on sound tissue, and the promotion of the chlorinating effect on carious dentin collagen. Hanning's[10] study, reported less dissolving effect of Carisolv than pure sodium hypochlorite, was concerning with the former aspect of amino acids behavior. In the present study, the result of Vickers hardness test showed such conservative effect. On the other hand, the second chlorination promoting behavior was observed as the change in morphology of dentinal tubules in the carious dentin outer layer in this study; the presence of chloramines and amino acids seemingly had some effect on the carious dentin outer layer. However, chloramines activity was supposed to be influenced by pH of the solution,

kinds of amino acids and proportion of amino acids and sodium hypochlorite; therefore, further investigations are needed to clarify the effect of chloramines on carious dentin.

In conclusion, there is a possibility that the amino acids used in the Carisolv system would decrease aggressive effect of sodium hypochlorite on sound dentin and inner layer of carious dentin and also would enhance the disrupting effect on degenerated collagen of carious dentin.

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