Comparison of microhardness of caries detector dye stained and unstained dentin

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Purpose: To evaluate microhardness of stained and unstained carious dentin which was differentiated by two caries detector dyes (Caries Detector and Sable Seek). Further, to compare the ability of the two dyes in recognizing caries-infected dentin.
Materials and Methods: Forty human molars with occlusal caries were sectioned longitudinally through the lesions in the mesio-distal plane and polished with aluminium oxide paste. Half of the specimens were stained with Caries Detector, and the remaining with Sable Seek. The specimens were microhardness tested using a Knoop microhardness indenter. The mean hardness values from each group were calculated and compared using one-way ANOVA and t-test.
Results: The mean microhardness values of dye-stained dentin from Caries Detector and Sable Seek groups were 9.99 and 7.93 KHN, respectively, which were significantly lower than those of unstained dentin (20.25 for Caries Detector group and 19.24 for Sable Seek group). There was no significant difference between microhardness values obtained from unstained carious dentin determined by the two caries detector dyes.
Conclusion: The hardness values of dye-stained dentin were lower than those of the unstained area. The ability of Caries Detector and Sable Seek in differentiation of the two layers of carious dentin was not different when dentin hardness was the only considered criterion. **(Int Chin J Dent 2006; 6: 49-52.)**
Key Words: carious dentin, caries detector dye, microhardness.

Introduction

Carious dentin consists of two layers; the outer carious layer or caries-infected dentin, which is soft, contaminated with bacteria and cannot be remineralized; and the inner carious layer or caries-affected dentin, which is hard, bacteria-free, and can be remineralized.[1] The main criteria for caries removal are color and hardness, which can be detected by visual and tactile examination. However, these may not be reliable guides. The use of caries detector dyes was therefore introduced as a guide for caries removal. Several dyes have been tested on extracted teeth to differentiate the two layers of carious dentin and it was found that 0.5% basic fuchsin in propylene-glycol was the dye that successfully served the purpose.[2] A distinct boundary separated stained and unstained zones. Bacterial invasion apparently coincided with staining and no bacteria were found when all stained dentin was removed.[3] The dye solution has therefore been recommended as a clinical guide for complete removal of bacterially infected dentin. Due to the potential carcinogenicity, basic fuchsin has later been replaced with acid red.[4] Since then, various protein dyes have been developed as caries detecting dyes.

Because an attack of dental caries gradually softens dentin, hardness is considered as one of the main criteria for caries removal. Knowledge of the dentin hardness is essential to improve caries removal and restorative procedures. The hardness values for sound dentin reported in the literatures ranged from 20 to 83 Knoop hardness number (KHN).[5-10] The microhardness testing of carious dentin by Banerjee et al.[11] demonstrated a mean value varying from 13.64 KHN in the discolored area to 48.35 KHN in the transparent dentin. However, the microhardness of stained carious dentin and that of the unstained carious dentin after two caries detector dyes used has never been reported.

Hardness testing is widely used to determine the mechanical properties of materials. The Vickers and Knoop indenters are the most commonly used instruments for microhardness testing since they employ loads of less than 1 kgf (9.8 N) and the indentations are small. Thus they are useful for providing hardness numbers of small areas.[12] The purposes of this study were to compare the microhardness values between stained and unstained

zones of carious dentin after the use of two caries detector dyes and compare the ability of the two dyes in recognizing caries-infected dentin.

Materials and Methods

Forty permanent molars, with occlusal caries to a depth of 2-2.5 mm below the central fissure as assessed with a dental probe, were used. The teeth were stored in 3% formalin solution until required for preparation. The teeth were embedded in epoxy resin, sectioned through the center of the carious lesions in the mesio-distal longitudinal plane to obtain dentin discs of 1 mm thick using low speed cutting machine (Isomet, Buehler Ltd., Lake Bluff, IL, USA). The dentin discs were polished with silicon carbide paper and finished with aluminum oxide paste up to 0.05 μm particle size. The specimens were randomly divided into two groups. One group was stained with Caries Detector (Kuraray Medical Inc., Tokyo, Japan) consist of 1% acid red dye and propylene glycol solvent, and the other with Sable seek (Ultradent Products Inc. South Jordan, UT, USA) consist of FD&C dark green dye and propylene glycol solvent. Each group was applied with the dye solution for 10 s, rinsed with water for 10 s and air-dried. The stained specimens were examined under stereomicroscope to separate and mark the boundary between stained and unstained area with sharp dental probe.

A digital microhardness testing machine (FM-700e Series, Future-Tech Corp., Kawasaki, Japan) with a Knoop indenter was used. Each specimen was mounted on the stage of the microhardness testing machine with double-sided adhesive tape and positioned under the microscope. The measured area of each specimen was selected to obtain the distance of approximately 1.5 mm from the boundary towards the pulp for the indentation line under low magnification. The measurements were made from carious dentin towards the pulp chamber with the load 50 g. The indentation line was selected, beginning at the stained area (carious dentin) 100 μm above the boundary and the second indentation was made at the unstained zone 100 μm below the boundary (unstained carious dentin). The next indentations were made 200 μm apart from the second indentation towards the pulp (Fig. 1). The hardness number of the normal dentin was the average number from the third measurement to the second last measurement. For the last indentation, which was considered as the pulpal area, the indentation was made at 200 μm from the pulpal wall. The total number of indentation on each specimen was eight.
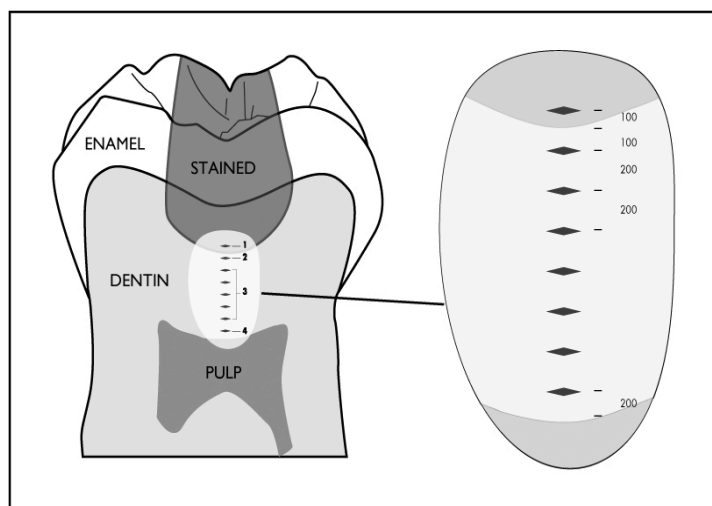
Fig. 1.
Diagrammatic representation of the relationship of the carious dentin and the indentations.
1. stained carious dentin,
2. unstained carious dentin,
3. normal dentin, and
4. pulpal area.

One way ANOVA with subsequent Scheffe multiple comparison at $p<0.05$ was used to analyze the differences of the microhardness numbers from different areas of dentin. The microhardness numbers from the two caries detector dyes were compared using t-test.

Results

The mean microhardness numbers with SDs of carious dentin and dentin from different areas after two caries detector dyes stained are shown in Table 1. The results indicated the significant differences of the microhardness numbers of different zones in the dentin. The hardness of stained area was lower than that of the unstained area. The microhardness values of pulpal area were significantly higher than those of the stained and unstained areas but lower than the normal area. There was no significant difference in hardness of stained areas between the two groups. Furthermore, a comparison of microhardness values of unstained area obtained from Caries Detector group and Sable Seek group demonstrated no significant difference.

Table 1. Knoop hardness number (KHN) of dentin after being stained with two caries detector dyes.

KHN	Stained dentin Mean	SD	Unstained dentin Mean	SD	Normal dentin Mean	SD	Pulpal dentin Mean	SD
Caries Detector	9.99	3.80	20.25	8.41	42.13	7.24	36.93	11.29
Sable Seek	7.93	2.86	19.24	5.25	46.86	5.69	32.96	6.24

Discussion

The microhardness values from various areas of dentin were significantly different. This may be due to the difference in inorganic composition in different locations.[9] The hardness of dentin also depends on the state of mineralization of tissue.[11] Meredith et al.[10] reported that the hardness of dentin decreased with distance from the dentino-enamel junction. However, there was an inverse correlation between dentin hardness and tubular density whereby tubular density increased while dentin hardness decreased as the pulp chamber was approached.[9] This is the reason for the lower hardness numbers of the pulpal area than those of normal dentin.

In carious dentin, the hardness depends on stage of caries progression, probably caused by remineralization process.[13,14] In the present study, the hardness numbers were in agreement with the previous studies.[6,7,15] The carious dentin, which was stained by Caries Detector and Sable Seek, showed the lowest microhardness numbers of all areas (9.99 KHN and 7.93 KHN, respectively). This was due to the attack of acid from caries process. The collagen fibers were irreversibly damaged by the acid and lost their function as a base for the attachment of apatite crystals.[16]

The hardness numbers of unstained dentin were higher than those of stained dentin but lower than those of normal dentin. This is because this area is also decalcified by acid. However, collagen fibers and odontoblastic process are unimpaired, which allow apatite crystals to attach and be remineralized when the caries-causing bacteria are removed. The dentin at this level of attack is uninfected by bacteria Although this dentin can be remineralized, the crystals did not possess the same hardness value as normal hydroxyapatite crystals because of the difference in crystal structure.[15]

The distance of 100 μm apart from the boundary in stained and unstained dentin was selected for the first two indentations. This is because the present study aimed to evaluate the ability of two agents in differentiation of the two layers of carious dentin. Thus, the hardness numbers most adjacent to the boundary were required. Further, with the selected distance, the two indentations were able to identified without overlapping.

As mentioned earlier that hardness is one of the main criteria for caries removal and the results from this investigation also showed the significant difference between the microhardness numbers of the stained and

unstained areas. Thus, caries detector dyes could help the clinicians in removing carious dentin, especially those individuals who do not experience in caries removal.[17] A comparison was made between the microhardness numbers of stained and unstained dentin after using the two dyes, no significant differences were exhibited between stained areas from the two dyes although they were different in color. Caries Detector was red. Sable Seek was dark green. However, they were in the same solvent (propylene glycol). The same consequence was shown with the unstained areas, which implied that the ability in caries differentiation was not different. This suggests that the two caries detector dyes can be used in substitution of each other as an adjunctive procedure to aid the dentist in caries removal.

Caries detector dyes have been introduced to assist in diagnosis and removal of carious dentin by differentiation of the two layers of carious dentin. However, caries detector dyes should be used cautiously, as the dyes stain not only the damaged collagen fibers of the caries-infected dentin but also stain the demineralized organic material.[18,19] Hence, the dyes can stain circumpulpal dentin and dentin-enamel junction in sound teeth. This can lead to the 'false positive' with regard to detecting carious tissue and therefore increase the likelihood of excessive removal of sound tooth structure and increased likelihood of mechanical pulpal exposure.

References

1. Fusayama T. New concepts in the pathology and treatment of dental caries. St Louis: Ishiyaku EuroAmerica Inc.; 1993. p. 1-21.
2. Fusayama T, Terachima S. Differentiation of two layers of carious dentin by staining. J Dent Res 1972; 51: 866.
3. Sato Y, Fusayama T. Removal of dentin by fuchsin staining. J Dent Res 1976; 56: 678-83.
4. Fusayama T. Clinical guide for removing caries using a caries-detecting solution. Quintessence Int 1988; 19: 397-401.
5. Craig RG, Peyton FA. Elastic and mechanical properties of human dentin. J Dent Res 1958; 37: 710-8.
6. Craig RG, Gehring PE, Peyton FA. Relation of structure of the microhardness of human dentin. J Dent Res 1959; 38: 624-30.
7. Fusayama T, Okuse K, Hosoda H. Relationship between hardness, discoloration, and microbial invasion in carious dentin. J Dent Res 1966; 45: 1033-46.
8. Seaman F, Shannon IL. Fluoride treatment and microhardness of dentin. J Prosthet Dent 1979; 41: 528-30.
9. Pashley DH, Okabe A, Parham P. The relationship between dentin microhardness and tubule density. Endod Dent Traumatol 1985; 1: 176-9.
10. Meredith N, Sherriff M, Setchell DJ, Swansen SA. Measurement of the microhardness and Young's modulus of human enamel and dentine using an indentation technique. Arch Oral Biol 1996; 41: 539-45.
11. Banerjee A, Sherriff M, Kidd EA, Watson TF. A confocal microscopic study relating the autofluorescence of carious dentine to its microhardness. Br Dent J 1999; 187: 206-10.
12. Skinner EW. Physical properties of dental materials, rheology, color, thermal properties. In: Dyson J ed. Skinner's science of dental materials. Philadelphia: WB Saunders; 1991. p. 29-59.
13. Miller WA, Massler M. Permeability and staining of active and arrested lesions in dentine. Br Dent J 1962; 112: 187-97.
14. Nyvad B, Fejerskov O. Active root surface caries converted into inactive caries as a response to oral hygiene. Scand J Dent Res 1986; 94: 281-4.
15. Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent layer of human carious dentin. J Dent Res 1983; 62: 7-10.
16. Ogushi K, Fusayama T. Electron microscopic structure of the two layers of carious dentin. J Dent Res 1975; 54: 1019-26.
17. Starr CB, Langenderfer WR. Use a caries-disclosing agent to improve dental residents' ability to detect caries. Oper Dent 1993; 18: 110-4.
18. Kidd EA, Joyston-Bechal S, Beighton D. The use of a caries detector dye during cavity preparation: a microbiological assessment. Br Dent J 1993; 174: 245-8.
19. Yip HK, Stevenson AG, Beeley JA. The specificity of caries detector dyes in cavity preparation. Br Dent J 1994; 176: 417-21.

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