The effect of application time on effectiveness of two caries disclosing agents

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Introduction: The main goal of treating dental caries is complete removal of carious tissues with maximum preservation of sound dentin before restoring the cavity. The aim of this study was to evaluate the effect of application time on efficacy of an experimental caries detector dye (with polypropylene glycole as the solvent) and SNOOP (with monopropylene glycole as the solvent). **Materials and Methods:** In this experimental *in vitro* study, 100 extracted human molars and premolars with occlusal caries, were divided into five groups. In each group, the caries were removed by conventional method (optical and tactile sence) or following caries detector dye application for 10 and 20 seconds. Histological assessment was used to evaluate bacterial existance after caries excavation. Data were analyzed using Fisher's exact test. Alpha was set at 0.05. **Results:** Statistical analysis revealed that there was significant difference between clinical removal of caries and using caries disclosing dyes. However, no significant difference between removal of carious dentin after 10 or 20 seconds application of the both caries disclosing dyes was observed. **Conclusion:** Using caries disclosing dyes for 10 or 20 seconds, improves efficacy of caries removal in comparison with conventional procedure.

Keywords: Caries; Detector Dyes; Disclosing Dye; Polypropylene Glycol; Time

Introduction

The primary goal of dental caries treatment is diagnosis and complete removal of carious dentin without jeopardizing sound tooth structures (1). Although a certain way to distinguish carious tissues from sound dentin does not exist, some methods are helpful beside the visual and explorer examination, such as radiographic assessment, DIAGNO dent, fiber optic trans illumination, quantified light-induced fluorescence, and caries disclosing dyes (2, 3). Caries disclosing dyes are simple and economical to use. These dyes were first introduced by Fuzuyama in 1979 in aim of staining infected dentin (outer layer of carious dentin) and having no effect on inner layer, which can be recalcified (4, 5). Caries disclosing dyes usually composed of two main parts, a dye in a solvent (propylene glycol). Initially basic fuchsin red was used as the dye which has been replaced later by 1% acid red because of the potential carcinogenicity of the fuchsin (6). These dyes do not stain cariogenic bacteria or their products. In fact, their mechanism is staining less mineralized dentinal organic matrix (7).

Some investigations have shown that using these dyes without considering their limitations could result in over preparation of sound dental tissues. Particularly in enamel-dentin junction or deep pulpal floor of cavities, where tooth structure is more porous (8-10). Now, different caries disclosing dyes like Caries Check (Nippon Shika Yakuhin, Shimonoseki, Japan) are available, which contains polypropylene glycol with molecular weight (MW) of 300 as the solvent instead of monopropylene glycol with 76 MW (11,12). Hypothetically, higher molecular weight would restrict over penetration of the dye and consequent excessive dentin removal (13).

Iranparvar P *et al.* used polypropylene glycol as the solvent of an experimental caries disclosing dye and also made a modification by adding 2% chlorhexidine gluconate (CHX) to the solution (14). It has been shown that matrix metalloproteinases type 2, 8, 9 (MMP2, 8, 9), which are present in human dentin and degrade collagen fibers are inhibited by CHX. In addition, CHX influences degradation of the hybrid layer and increases the bond strength of resin restorative materials (15).

Another challenge after using caries detecting dyes is possibility of remained bacterial infection caused by incomplete removal of infected dentin (7, 16). Increasing the application time may reduce the risk of remnant caries and in this experimental dye may also improve bond strength of future restorations by increasing exposure time to CHX. Moreover, by using polypropylene glycol-based dyes the risk of over excavation of dentin is diminished (5, 13, 15).

Table 1. Frequency of bacterial presence after caries removal of the groups

Groups	Bacterial presence		Inter groups comparison *
	Positive	Negative	
Control	9 (45%)	11(55%)	A
SNOOP 10 seconds	2 (10%)	18 (90%)	B
SNOOP 20 seconds	1 (5%)	19 (95%)	B
Experimental 10 seconds	1 (5%)	19 (95%)	B
Experimental 20 seconds	1 (5%)	19 (95%)	B

**Groups with different letters indicate a statistically significant difference (P <0.05)*

Thus, this study aimed to compare *in vitro* efficacy of an experimental caries disclosing dye containing polypropylene glycol and one commercially available propylene glycol-based dye when applied for 10 and 20 seconds. The null Hypotheses state that there will be no difference in remnant caries after caries removal with or without using caries disclosing dyes, and application time of these dyes will make no change in their efficacy.

Materials and Methods

Sample size was calculated to be 19 in each of the five groups, using Minitab software and considering alpha and beta as 0.05 and 0.2 respectively (1). This experimental study was performed on 100 recently extracted permanent human teeth (molars and premolars) stored in normal saline solution without any disinfectant. Inclusion criteria were presence of occlusal caries which extension to the dentin were verified by radiography. The exclusion criteria were teeth with pulp exposure, discoloration, and hypoplasia.

Teeth were classified into five groups with 20 specimens in each group. After initial cavity preparation with flat end cylinder diamond bur (010, Smedent, Shanghai, China) and high-speed handpiece (Pana-Max, NSK/Nakanishi inc, Kanuma, Japan), dentinal caries was removed using round carbide bur (204RA, Dia-Tessin, Swiss) mounted in low speed handpiece (Contra-angle FX-23, NSK/Nakanishi inc, Kanuma, Japan). In control group, caries was removed using conventional method, the tactile sense and discoloration observation. In SN10 group, SNOOP caries detector dye **(**Pulpdent Corp, MA, USA) was applied for 10 seconds before removing caries. In SN20 group, SNOOP caries detector dye was applied for 20 seconds before removing caries. In EX10 and EX20 groups, before each step of caries excavation, experimental disclosing dye was applied for 10- and 20-seconds respectively. When using caries disclosing dyes, each application followed by 10 seconds water rinsing and subsequently, colored dentin was removed. Applying caries disclosing agent and caries excavation continued until no staining was observed.

Afterward, the excavated teeth were fixed for 24 hours in 10% formaldehyde, followed by demineralization in 10% nitric acid for 15 to 20 days. Following longitudinal sectioning in defect sites, six sections of each specimen were prepared for dehydration (80-100°C for 30 minutes). The slides were immersed in Xylol (15 minutes), 78% alcohol (10 minutes), 96% alcohol (10 minutes) and 100% alcohol (10 minutes) respectively and then Gram-stained (14).

The samples were examined for the presence of microorganisms in defected surface and dentinal tubules, using a light microscope (Nikon E400, Minato City, Tokyo, Japan) with 40, 100, and 200 magnification, by an oral pathologist who was not aware of identity of the samples. The cylindrical red structures inside the dentinal tubules were indicative for the presence of bacteria (Figure 1 and 2).

Data were analyzed using Fisher's exact test regarding the frequency (quantity) and percentage of bacterial presence. Alpha was set at 0.05.

Results

A total of 600 microscopical sections were evaluated and the results are presented in Table 1. The positive result implies bacterial presence at least in one of the six histological slides of each specimen.

Fisher exact test showed that results of the control group is significantly different from the other groups (control group and SN10, *P*-value=0.031/control group in comparison with group SN20, EX10, and EX20, *P*=0.008), while applying time (10 and 20 seconds) and type of the caries disclosing agent (SNOOP or experimental) had no significant effect on bacterial presence (*P*>0.05).

Discussions

The purpose of this study was to compare efficacy of a commercially available caries detecting dye (SNOOP) with an experimental disclosing dye, in different application times. The results showed that caries removal using both SNOOP and experimental caries disclosing dyes containing polypropylene glycol as the solvent, revealed the same results and increasing application time of dyes did not affect the possibility of remnant caries existence. However, applying dye solutions significantly improved caries removal efficacy in comparison with the conventional method (optical and tactile sense). Thus, the null hypothesis regarding no significant effect of using caries disclosing dyes on remnant caries was rejected while the null hypothesis regarding no significant effect of application time on efficacy of caries excavation was accepted.

Various microorganisms participate in dental caries development. But, Gram-positive bacteria such as Actinomycetes,

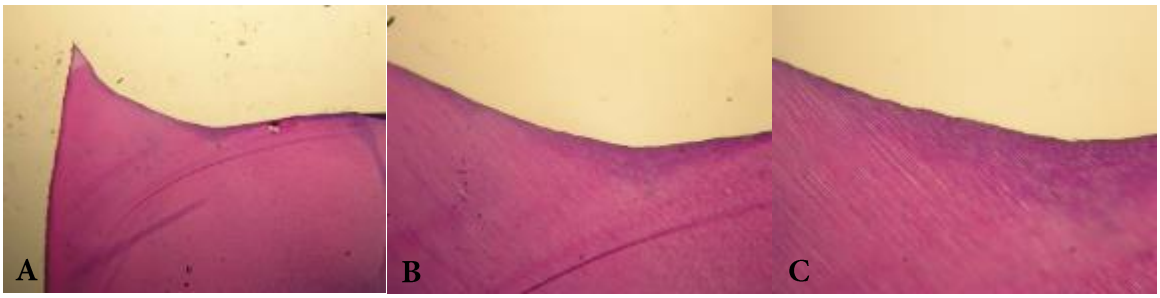

Figure 1. Absence of bacteria in dental tubules after caries removal by 10 seconds application of experimental caries disclosing dye. A. 40× magnification, B. 100× magnification, C. 200× magnification

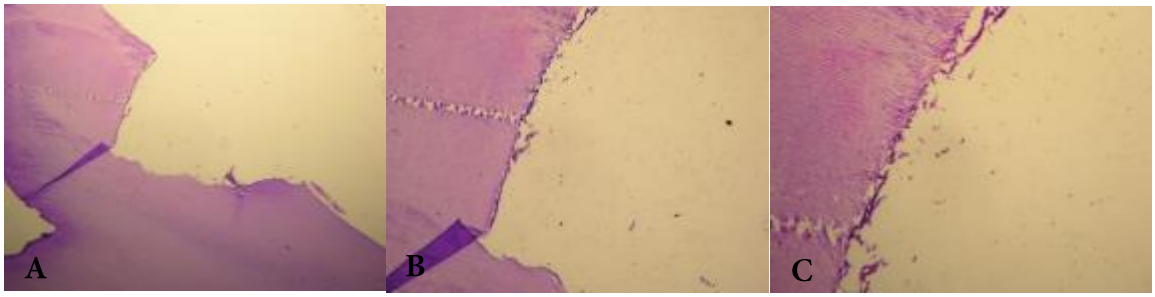

Figure 2. Infected dentin (bacterial present) after conventional caries removal. A. 40× magnification, B. 100× magnification, C. 200× magnification

Lactobacillus and predominantly Streptococcus mutans are the most important species (17). PCR is the most precise test to identify the exact microorganisms, (18) which is not applicable in all researches. Oikawa *et al.* used Vickers microhardness test to show efficacy of caries removal after applying polypropylene glycol-based caries detector dye in comparison with monopropylene glycol-based one (19). But, even sound dentin at closer areas to the pulp chamber is not as hard as superficial dentin (20). Similar to some other researches (8,14,21), histological evaluation was used in the present study. This method is simple, accessible, and affordable. However, because of two-dimensional nature of histological sections, it may cause inaccuracy due to the missing data.

Some chemical modifications have been applied in the experimental caries detector dye used in this study to decrease the probability of excessive dentin removal. Replacing conventional alcoholic solvent (monopropylene glycol) by polypropylene glycol with higher MW is one of the modifications. It has been assumed by various studies that polypropylene glycol penetrated less in dentin tissue in comparison with monopropylene glycol with lower MW (13, 22).

In addition, 2% chlorhexidine gluconate was added to the experimental dye in aim of inhibiting MMP 2,8,9,20, which can degrade dentinal collagen fibers (15). Also, Trufello AM *et al.* reported that at least 0.04% concentration of chlorhexidine is necessary to inhibit gelatinase enzyme which existed in infected dentin (23).

The results of this study showed that using any of the two caries disclosing dyes (SNOOP and experimental) improved caries removal efficacy. In contrast, Kidd *et al.* found that using caries detector dyes before caries excavation leaded to unnecessary tissue removal. They reported that caries removal using either optical and tactile sense or caries disclosing dyes had the same microbiological results (24). However, Kidd's results were due to a clinical study which did not employ histological assessment. Zacharia *et al.* and Alsehaibany *et al.* have reported results in agreement with the present study, which confirmed the superiority of caries removal efficacy after applying caries detector dyes (25, 26).

Also, in the present study no significant difference in remnant caries was observed by using any of the two caries disclosing dyes, Itoh K *et al.* and Oikawa M *et al.* reported contrary results. After using three commercially available caries disclosing dyes, they concluded that dyes containing polypropylene glycol as the solvent would improve caries removal by being more conservative (5, 27). However, their results were obtained using Vickers microhardness (5, 27) test and DIAGNOdent (27), not histological assessment.

To the best of the author's knowledge, no study has conducted to evaluate the effect of caries disclosing dye application time on remnant caries. It seems that increasing the application time makes no improvement in efficacy of caries removal and with the probability of over penetration of these dyes, reducing application time may also be effective and is recommended for future studies. Particularly when using caries disclosing dyes which contain low molecular weight solvents.

Conclusion

Within the limitation of the present study, we concluded that using caries disclosing dyes could improve efficacy of caries removal in comparison with conventional procedure (optical and explorer). It was found that the experimental caries detector dye was as effective as the commercially available one (SNOOP) and increasing application time of dyes had no significant effect on remnant caries.

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Conflict of Interest: 'None declared'.

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