Original article:

Efficacy of an experimental propylene glycol-based, water-free caries detecting dye in comparison with Snoop® using histological analysis

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Abstract

Background and Aim: One limitation of caries disclosing dyes is the risk of sound dentin removal. This study sought to assess the efficacy of a newly developed experimental caries disclosing dye in complete removal of infected dentin and its properties in comparison with Snoop caries detecting dye using histological analysis. ***Materials and Methods:*** A caries detecting dye with improved properties was experimentally prepared. Twenty permanent teeth with occlusal caries were selected and divided into two groups of staining with the experimental dye (group 1) and Snoop caries detecting dye (group 2). Stained carious dentin was removed and the process of staining and caries removal was repeated until no staining was observed. DIAGNOdent laser caries detection aid was used in all cavities to ensure absence of caries. After decalcification, 5 sections were made of each tooth, Gram-stained and subjected to histological analysis. Presence of bacteria in the two groups following staining with the experimental and Snoop dyes was evaluated and statistically analyzed using Fisher's exact test. ***Results:*** In the Snoop group, bacteria were present in 3 out of 50 (6.0%) specimens. In the experimental group, bacteria were found in 2 out of 60 specimens (4.0%). Fisher's exact test showed no significant difference in this respect between the two groups. ***Conclusion:*** Histological analysis revealed similar efficacy of Snoop and the experimental caries detecting dyes in terms of complete elimination of infected dentin. Considering the improved properties of the experimental caries detecting dye, it may have superior efficacy in preventing unnecessary removal of sound dentin.

Keywords: Dental caries; Caries-detectordye; Histology

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Introduction

Several methods are used for detection of carious dentin such as visual and explorer examination, caries disclosing dyes and laser fluorescence[1]. In visual and explorer examination, the clinical diagnosis of caries is made based on the color and consistency of dentin[2, 3]. This method has high specificity but low sensitivity and reproducibility[4]. In this technique, estimating the hardness of remaining dentin by the tactile sense is difficult and highly depends on the experience of the clinician. Discoloration due to caries is only visible in case of chronic caries. In acute caries, the discoloration is less noticeable and the bacterial invasion is usually more extensive than the discoloration. Therefore, a noticeable discoloration is not a suitable guide or indicator for removal of carious dentin[5]. DIAGNOdent is a recently introduced tool to overcome the shortcomings and limitations of visual examination and radiography in caries detection. DIAGNOdent operates based on a quantitative scale and has shown superior performance than the conventional caries detection techniques[6,7]. Caries detecting dyes were first introduced in 1972 for complete removal of infected dentin without removing the sound dentin[8]. These dyes are often made of 1% acid red in propylene glycol or other coloring solutions and stain the irreversibly damaged collagen fibers present in the

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infected dentin. It has been shown that not only the infected dentin, but also thes lightly demineralized dentin are stained[8]. However, some other studies have confirmed the optimal efficacy and benefits of these dyes and have reported that if not using them, the carious dentin may not be completely detected/ removed by the clinician[9, 10].

The main concern in using caries disclosing dyes is over-staining and subsequent excessive removal of dentin during cavity preparation. In other words, these dyes carry a risk of false positive results[11]. Some modifications in the chemical formulation of these dyes may improve their efficacy and overcome this shortcoming. Use of an alcohol solvent with higher molecular weight is a chemical modification suggested to be made in experimental dyes. Previous studies on dyes containing polypropylene glycol have shown that due to having a higher molecular weight, this solvent has lower permeability than monopropylene glycol and therefore, polypropylene glycol can prevent over-staining and subsequent over-preparation of dentin[5]. Considering the simple application and affordability of caries disclosing dyes, in spite of some shortcomings, they can be used as an adjunct to more complex caries detecting techniques such as laser fluorescence (FACE), DIAGNOdent and Carisolve[12]. Most commercially available caries disclosing dyes are red in color and may confuse the clinician in detecting the sound/carious state of the dentinoenamel junction, which often has a brownish-red color. Therefore, it appears that blue dyes may have greater efficacy in enhancing the clinicians' diagnosis[5]. Moreover, red color may be even mistaken for pulp bleeding in deep cavities. Another modification made in our experimental solution was addition of 2% chlorhexidine gluconate (CHX). Evidence shows that CHX has an inhibitory effect on matrix metalloproteinases (MMPs) 2,8 and 9 that play a role in collagen degradation and thus, the CHX in the caries detecting dye formulation decreases collagen degradation in dentin, disinfects the cavity and increases the bond strength of restoration[13]. This study sought to assess the efficacy and properties of a newly developed experimental caries detecting dye in comparison with Snoop caries detecting solution in terms of complete removal of infected dentin (containing bacteria) using histological analysis and laser fluorescence.

Materials and Methods

To prepare the experimental caries detecting dye, a search was carried out in databases to determine the basic ingredients. Next, a pilot study was performed on several samples that were made with variable percentages of basic ingredients including the polypropylene glycol with 425 Kilodalton (KD) molecular weight and brilliant blue edible dye. Physical properties based on the concentration of components were evaluated and some modifications were made. The percentage of propylene glycol was decreased in the formulation of experimental dye and a part of it was replaced with monopropylene glycol and ethanol. Pilot preparation of dye was repeated for several times until optimal concentrations of solvents were found and recorded. On the other hand, considering the unique properties of CHX, a small percentage of CHX was also added to the formulation. Ingredients were precisely measured using scaled pipettes and weighed by a digital scale. Ingredients were mixed by a mixer at room temperature and the prepared solution was kept in a screw-top container. Theproperties of the final formulation of the experimental dye were compared with those of Snoop caries detecting dye. This in-vitro, experimental study was conducted on 20 freshly extracted human permanent molars and premolars. The teeth had occlusal caries with no pulp exposure, hypoplasia or discoloration. After extraction, the teeth were stored in saline solution with no disinfecting agent. The teeth were randomly divided into two groups and exposed to the experimental dye (group 1) or the Snoop caries detecting dye (containing 47% propylene glycol) (group 2). The teeth were stained for 10 seconds and rinsed with water for another 10 seconds. Stained caries were removed by a round bur and the process of staining and caries removal was repeated until no staining was observed. DIAGNOdent was also used in all cavities to ensure complete caries removal.

After fixation, all teeth were demineralized by immersion in 10% nitric acid. The preparation steps for teeth were as follows:

1. Immersion in 10% nitric acid (15 to 20 days)
2. Rinsing under running water (15 minutes)
3. Tissue processing in the tissue processor
4. Embedding tissues into paraffin blocks
5. Sectioning with a microtome (5 sections of each tooth)
6. Dehydration of tissues (dry heat at 80-100° for 30 minutes)
7. Immersion in xylol for 15 minutes
8. Immersion in 100% alcohol for 10 minutes
9. Immersion in 96% alcohol for 10 minutes
10. Immersion in 78% alcohol for 10 minutes
11. Rinsing under running water and Gram-staining
12. A cover slip was mounted over the tissue specimen on the slide, using optical grade glue.

The processes performed in the tissue processor (step 3) included immersion in 10% formalin for 2 hours, immersion in 50% alcohol for 30 minutes, immersion in 70%, 80% and 96% alcohol and absolute alcohol for 90 minutes, respectively, immersion in pure alcohol for 2 hours, immersion in xylol for 90 minutes (twice), placement in paraffin for 2 hours, placement in paraffin for 90 minutes and placement in paraffin for the third time for 3 hours.

In step 11, slides containing dental sections were Gram-stained as follows:

1. Crystal violet dye for 30 to 45 seconds
2. Rinsing with water
3. iodine dye for 30 to 40 seconds
4. Rinsing with water
5. Decolorization: acetone alcohol for 15 to 20 seconds
6. Rinsing with water
7. Fuchsin for 30 to 45 seconds
8. Rinsing with water
9. Drying

Fixed sections were evaluated by a pathologist blinded to the group allocation of specimens under a light microscope at 100, 400 and 1000X magnifications. Dark red cylindrical structures inside the dentinal tubules (mostly close to the surface) were considered as an indicator of bacterial presence.

The frequency and percentage of bacteria present in specimens in the two groups were calculated and the results were compared between the two groups using the Fisher's exact test.

Results

A total of 100 microscopical sections were evaluated. In 50 specimens in the Snoop group, bacteria were present in 3 cases (6.0%) and in the remaining 47 (94.0%), no sign of bacteria was noted. In 50 specimens in the experimental group, bacteria were present in 2 cases (4.0%) and the remaining 48 (96.0%) were free from bacteria (Table 1). Fisher's exact test revealed no significant difference in terms of the frequency of bacteria between the two groups (P=0.65).

Table 1. Frequency of bacteria in stained specimens in the two groups of experimental and Snoop caries detecting dyes

Caries detecting dye/ Bacteria	Absence	Presence	Total
Snoop	47 (0/94%)	3 (0/6%)	50 (100%)
Experimental	48 (0/96%)	2 (0/4%)	50 (100%)
Total	95 (0/95%)	5 (0/5%)	100 (100%)

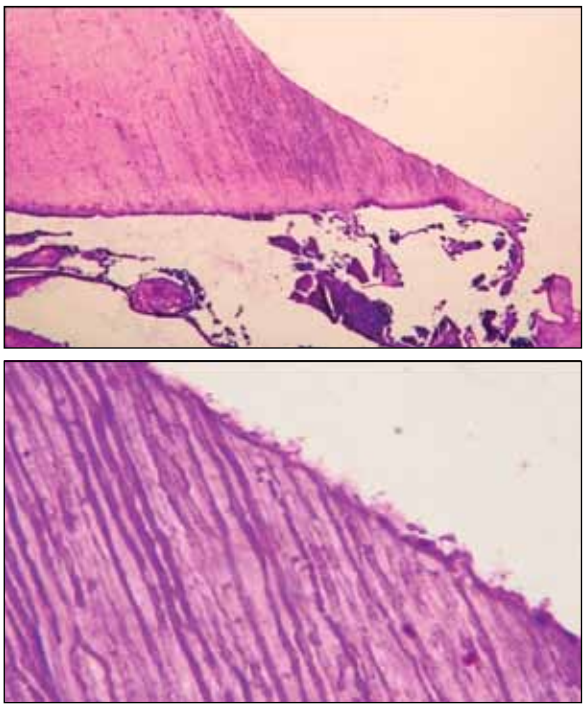

Figure 1. Histological view of a tooth section (100X and 1000X magnification)

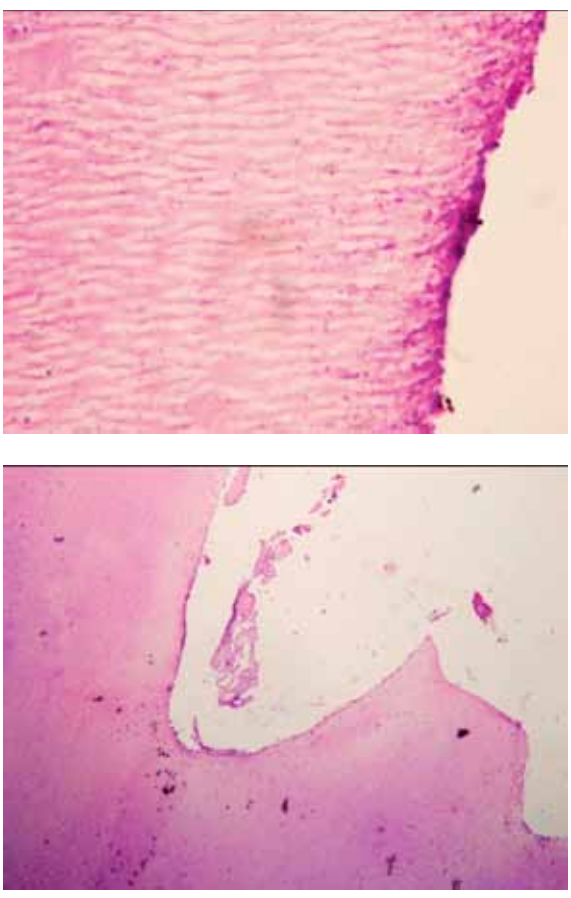

Figure 2. Histological view of another tooth section (100X and 1000X magnification)

Discussion

Based on the results, in the Snoop group, bacteria were present in 3 cases (6.0%) and in the remaining 47 (94.0%), no sign of bacteria was noted. In the experimental group, bacteria were present in 2 cases (4.0%) and the remaining 48 (96.0%) were free from bacteria (Table 1). Fisher's exact test revealed no significant difference in terms of the frequency of bacteria between the two groups. Thus, the experimental caries detecting dye can be used for detection of carious dentin during cavity preparation. The mechanism of action of caries disclosing dyes used for detection of carious dentin during cavity preparation was believed to be based on selective staining of demineralized dentin collagen matrix. The collagen fibers in carious dentin have been irreversibly damaged and the inter-molecular cross-links have been degraded[14]. However, this mechanism is now refuted and it has been discussed that the caries disclosing dyes penetrate into porous carious dentin and the acid red easily stains the organic matrix or collagen fibrils irrespective of the changes in the inter-molecular cross-links[15,16]. Considering the higher porosity of dentin substrate [15], an explanation has also been given regarding the mechanism of staining of dentinoenamel junction, sound dentin and deep dentin even after complete caries removal. Thus, application of conventional caries disclosing dyes may result in unintentional and unnecessary removal of affected dentin, which has been demineralized to some extent but is free from bacteria. Thus, it has been suggested that the less the mineralization and the higher the porosity of dentin beneath the dentinoenamel junction, the more susceptible are the areas to staining[16]. The experimental solution prepared in the current study was based on polypropylene glycol solvent and aimed to decrease the risk of staining of affected porous dentin via limiting the diffusion of dye into dentinal porosities. Propylene glycol is a dihydric alcohol and allows the staining of safranin O. Safranin O is an aniline dye and not an acid red dye. In comparison with distilled water (with a molecular weight of 18KD), it penetrates into the root dentin more rapidly and to a higher depth[17]. Low surface tension and high wettability and infiltration of propylene glycol may result in over-staining of carious dentin or the affected dentin beneath the soft carious dentin. On the other hand, evidence shows that use of caries disclosing dyes decreases the ability of microorganisms to penetrate into tooth structure and porosities but cannot completely eliminate them[18-20]. Moreover, occlusal caries may be ceased for up to 10 years by the application of resin sealant[21]. However, it is not known whether the entrapment of bacteria below the restorations affects the prognosis of restored teeth or not. However, it appears that caries progression is actually preventedby the application of resin sealant; because there is a possibility that the bacteria present in dentinal tubules no longer show cariogenic activity after the dentinal tubules are sealed with restorative materials. The main concern in using caries disclosing dyes is over-staining and subsequent excessive removal of dentin during cavity preparation. In other words, these dyes carry a risk of false positive results[11]. Kidd et al, in 1993 evaluated the use of caries disclosing dye during cavity preparation and showed that repeated use of caries detecting dye resulted in unnecessary removal of hard dentin[22]. According to Banerjee et al, in 2003, use of caries disclosing dyes is not indicated for carious lesions extending to the middle third of dentin or for deeper caries due to the risk of pulp exposure during cavity preparation[15]. In the current study, we tried to decrease unnecessary dentin removal by making some modifications in the chemical formulation of caries disclosing dyes. One modification made in the formulation of caries detecting dye was changing the solvent. This was done based on two previous studies demonstrating decreased diffusion of dyes containing solvents with high molecular weight into the porous dentin[22, 25]. Accordingly, instead of propylene glycol (with a molecular weight of 76 KD) or combination of propylene glycol and water used in most commercially available disclosing agents, polypropylene glycol with larger molecules (molecular weight of 300KD) has been used in new formulations of these agents[26]. Two recently introduced caries detecting dyes namely Caries Check Red ™ and Caries Check Blue ™are manufactured (Nishika Company) and marketed in Japan with the formulation of 1% acid red (or brilliant blue) in polypropylene glycol solvent with a molecular weight of 300 KD[27]. Evidence shows that solutions containing polypropylene glycol may be more conservative in removal of tooth structure. Comparison of tooth surfaces by Vickers microhardness test after complete removal of caries stained with polypropylene glycol or propylene glycol based dyes revealed that the polypropylene glycol group had a significantly smoother surface than the propylene glycol group (MVH of 32.7 versus 44.7)[28]. Since carious dentin in deeper areas is often harder, these results suggest more conservative

dentin removal by the application of polypropylene glycol based dye[29]. Since in histological analysis, both groups were found to be free from bacteria, it was concluded that application of polypropylene glycol may yield superior results. However, since by increased depth the hardness of sound dentin decreases, clinical interpretation of the results of hardness test as such may not be completely accurate[27]. Following the above-mentioned preliminary study, other studies using laser fluorescence found similar results at least for permanent teeth. A previous study evaluated the auto-fluorescence properties of carious dentin[30]. It was demonstrated that the DIAGNOdent values were higher for specimens in which, caries had been removed according to the results of application of polypropylene glycol based dye compared to the specimens in which, caries had been removed according to the results of application of propylene glycol based dye (at least for permanent teeth)[22,31]. Also, one study showed that caries stained with polypropylene glycol and removed were stained again with propylene glycol; this finding confirmed the hypothesis that use of polypropylene glycol based solutions results in more conservative caries removal and prevents unnecessary removal of sound/affected dentin. One chemical modification made in the experimental caries detecting dye was the use of alcohol solvent with higher molecular weight. Incorporation of brilliant blue dye into the formulation of experimental caries detecting dye can help the clinician better detect caries[5]. Similar to our findings, Hosoya et al, in 2008 showed that caries detecting dyes with blue color had higher efficacy in removal of infected, carious dentin and prevented unnecessary removal of affected or sound dentin[5]. Another modification made in the experimental dye was incorporation of 2% CHX into the caries detecting dye formulation. Degradation of mineral matrix in mammals primarily occurs by the activity of MMPs. MMPs 2,8, 9 and 20 have been detected in sound dentin matrix and these enzymes are believed to be responsible for dentin destruction during or after demineralization by the activity of acidogenic bacteria[32]. CHX at a concentration of 0.04% or higher can inhibit the gelatinases present in carious dentin. Thus, CHX can be used in formulation of materials that come into contact with dentin in the process of restoration or prevention[35]. Mutans streptococci are the dominant cariogenic bacteria and play a role in the etiology of human dental caries. Streptococcus mutans has been isolated from the dental plaque, which is known to be the primary cause of caries. High number of other microorganisms have also been detected in deep carious lesions such as the facultative anaerobes and obligate anaerobes. Polymerase chain reaction is an accurate technique for detection of microorganisms[3]. Similar to some previous studies[9,16], histological analysis was used in the current study for detection of bacteria. This method is simple, easily accessible and practical. However, the main limitation of microscopical observations is their two dimensional nature and that a two-dimensional image is obtained of a three dimensional structure; thus, error are likely[2]. Not using the polymerase chain reaction for detection of microorganisms in dentin following the use of caries detecting dyes was a limitation of the current study.

Conclusion

Considering the modifications and improvements made in the composition of our experimental caries detecting dye, it may have superior efficacy in preventing unnecessary removal of sound dentin.

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