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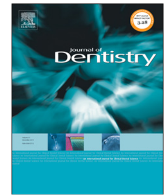

The ability of dual whitening anti-caries mouthrinses to remove extrinsic staining and enhance caries lesion remineralization – An in vitro study☆

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ABSTRACT

Objectives: This laboratory study investigated the ability of dual whitening anti-caries mouthrinses to remove extrinsic staining from artificially stained caries lesions and to enhance their remineralization and fluoridation.
Materials and Methods: Early caries lesions were created in bovine enamel specimens. The lesions were artificially stained and pH cycled for 10 days with the daily cycling regimen consisting of twice daily 60s-treatments with one of 11 mouthrinses, a 4-h demineralization period and artificial saliva treatments in between. Mouthrinses were eight commercially available products, all containing 100 ppm fluoride but utilizing hydrogen peroxide, pyro-, tri- or hexametaphosphate salts and/or sodium bicarbonate. The three control mouthrinses were 100 ppm fluoride, 30 % hydrogen peroxide and deionized water. Enamel color changes (ΔE) were determined spectrophotometrically. Vickers surface microhardness (VHN) was used to determine lesion remineralization. Enamel fluoride content (EFC) was determined using the microbiopsy technique. Data were analyzed using ANOVA.
Results: ΔE was significantly different among groups ($p = 0.0045$). Thirty percent hydrogen peroxide was superior to all other mouthrinses, while there were no differences between commercial mouthrinses and deionized water. There were small, directional but non-significant differences between commercial mouthrinses with those containing hydrogen peroxide providing better whitening. There were no significant differences between mouthrinses in their ability to remineralize caries lesions ($p = 0.2898$). EFC differed among groups ($p < 0.0001$), with the two mouthrinses containing pyrophosphate salts having lower EFC than all but the deionized water group.
Conclusions: Artificially stained caries lesions show reduced susceptibility to fluoride remineralization and whitening effects of commercial whitening and anti-caries mouthrinses.
Clinical Relevance: Artificially stained caries lesions appear to require stronger than over-the-counter interventions to successfully whiten and remineralizing them.

1. Introduction

As part of the daily oral hygiene regimen, consumers are reminded by their dental care providers to use personal care products, such as mouthwashes and toothpastes, consistently. These products provide both preventive and therapeutic benefits [1]. Therapeutic benefits include caries prevention which is usually provided through the addition of various fluoride compounds (e.g. sodium fluoride, stannous fluoride, and sodium monofluorophosphate); gingivitis prevention by utilization of antimicrobial agents (e.g. triclosan, zinc citrate, stannous fluoride, cetylpyridinium chloride or essential oils); and prevention of dentin hypersensitivity by addition of agents such as stannous fluoride, strontium chloride or potassium citrate or nitrate [1]. Preventive benefits include supragingival calculus prevention, breath freshening as well as removal and inhibition of extrinsic tooth stains (tooth whitening) [1]. Tooth whitening agents include a wide range of actives and can be divided into chemical and mechanical agents [2]. A wide range of dual whitening anti-caries mouthrinses are now commercially available. Typically, they contain sodium fluoride for caries prevention and very low concentrations of hydrogen peroxide (approx. 1.5 %) and/or pyro-, tri- or hexametaphosphate salts in addition to some also containing sodium bicarbonate [3]. The purpose of these agents is to protect the tooth surface from staining and to remove already present stains [3]. Polyphosphates are widely known for binding to proteins in the tooth pellicle. In doing so, this chemical compound helps remove stains from the teeth by absorbing chromophores [4]. Hydrogen peroxide is by far the most commonly used

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ingredient in whitening mouthrinse products. Hydrogen peroxide solutions are acidic (approx. pH 4–5). In order to be used as a bleaching agent in mouthrinses, hydrogen peroxide has to be used at a significantly lower concentration than what can be found in whitening trays and strips.

Tooth discoloration is considered one of the main reasons patients seek cosmetic dental care as it is often aesthetically displeasing and psychologically detrimental [5]. Tooth discoloration can be influenced by a combination of intrinsic and extrinsic factors. Intrinsic stains are stains within the tooth, whereas extrinsic stains are those found only on the outer enamel surface [6]. The correct diagnosis depends on the understanding of the discoloration's etiology. The understanding of the cause of discoloration will additionally assist the dental professional in explaining the precise nature of the condition to the patient. The nature of the staining may have an impact on both treatment choice and efficacy [7].

Dental caries is a chronic dynamic process involving episodes of demineralization and remineralization that occur over a period of time [8].

Fluoride compounds have been shown to inhibit tooth decay and is used commonly in the prevention and management of dental caries [8]. In addition to occurring naturally, in some areas fluoride is added to the water supply as a public health measure. Nowadays, the vast majority of toothpastes and a large proportion of mouthrinses contain cariostatic amounts of fluoride [9]. As an additional protective measure, fluoride is also available in other delivery vehicles, such as varnishes, lozenges, gels and high concentration, prescription toothpastes and mouthrinses [9].

To the authors' knowledge, no study has thus far simultaneously investigated the ability of dual anti-caries and whitening oral care products to enhance caries lesion remineralization and stain removal, in well standardized experimental conditions. Therefore, this study aimed to investigate and evaluate the response of artificially stained, partially demineralized enamel, as measured by changes in surface microhardness, enamel fluoride content and enamel color change, to dual whitening anti-caries mouthrinses, using an established pH-cycling model [10].

2. Materials and methods

2.1. Study design

Early caries lesions were created in bovine enamel specimens. The extent of lesion severity was characterized using Vickers surface microhardness (VHN). Lesions were then stained and pH cycled for 10 d with twice daily treatments with one of the mouthrinses. VHN was determined again and changes to baseline values be calculated. The color of the specimens and changes thereof were assessed at every stage during the study using a spectrophotometer. Finally, the enamel fluoride content of the specimens was determined using the microbiopsy technique. The null hypotheses were that a) dual whitening anti-caries mouthrinses have no effect on removing stains compared to deionized water, and b) dual whitening anti-caries mouthrinses reduce caries lesion remineralization and fluoridation in comparison to a non-whitening anti-caries mouthrinse.

The present primary author was solely responsible for specimen preparation, data collection and curation.

2.2. Specimen preparation

Bovine enamel specimens were used as the hard dental tissue substrate in the present laboratory study. Teeth crowns were cut into 4×4 mm^2 specimens using a slow speed saw (Buehler, Lake Bluff, IL, USA). All teeth were stored in a saturated aqueous solution of thymol (Sigma-Aldrich, St. Louis, Missouri, USA) during the sample preparation process. Then, they were ground and polished using a polishing unit (RotoPol 31/RotoForce 4, Struers, Ballerup, Denmark) to create smooth flat parallel surface of enamel and dentine to facilitate microhardness testing. The bottom side of the specimens were ground flat to a uniform thickness using 500-grit silicon carbide grinding paper. The topside of the specimen were ground using 1200-grit paper until most of the tooth surface was flattened. The specimens were then serially polished using 4000-grit paper followed by 1-μm diamond polishing suspension (Struers, USA). Specimens had at least 0.3 mm of enamel thickness. The specimens were sonicated (L&R T-21B, Fisher Scientific, Hampton, New Hampshire, USA) in de-ionized water between each grinding/polishing step. As a final cleaning step, the polished specimens were sonicated in 2 % microliquid. The specimens were assessed with a magnification of 10× for cracks and hypomineralized areas. The requirements for the specimens in this study to be acceptable were: a) not have any obvious cracks or other flaws in the enamel surface; b) to have an evenly polished, high gloss enamel surface; and c) to have no contamination on the top surface from sticky wax or any other material. Each specimen was mounted on the end of an acrylic rod (1/4″ diameter × 2″ long) using cyanoacrylate (superglue). The sides of each specimen were covered with a varnish so that only the enamel surface was exposed. A total of 250 specimens were prepared, of which 198 (11 × 18) were used in the present study.

2.3. Formation of early caries lesions

Early artificial caries lesions were formed in specimens by immersing them for 48 h into a solution of 0.1 M lactic acid and 0.2 % Carbopol which is 50 % saturated with respect to hydroxyapatite and had a pH of 5.0 (adjusted using KOH). Initial demineralization was performed at 37 °C and with 10 mL of demineralization solution per specimen [10].

2.4. Baseline hardness

After lesions were formed, initial hardness of demineralized specimens was determined by using a Vickers microhardness indenter at a load of 200 g with a dwelling time of 15 s. Four indentations on the surface of every specimen were used to determine the average lesion baseline microhardness (VHN_{base}). Indentations were placed in a central area on each specimen, approximately 100 μm apart from one another. Only specimens with a VHN_{base} that was within the range of $VHN_{base} \pm 2 \times$ standard deviation of all lesions were accepted.

2.5. Color determination

The color of the enamel specimens was analyzed throughout the study using a spectrophotometer (Minolta CM2600d, Konic Minolta, Ramsey, NJ, USA). The area of the specimens scored was a 3-mm diameter circle in the center of the enamel surface. L*, a*, and b* parameters were measured after staining and after ten days of pH cycling. A jig was used to hold the specimens in place to allow for repeat measurements. ΔE* values were calculated for each specimen using the following formula: $\Delta E^* = \sqrt{\left(L^*_{final} - L^*_{stained}\right)^2 + \left(a^*_{final} - a^*_{stained}\right)^2 + \left(b^*_{final} - b^*_{stained}\right)^2}$.

2.6. Staining procedure

The enamel staining procedure of all specimens was performed based on the staining protocol described by Stookey et al. [11]. It included the use of staining solution that contains trypticase soy broth, *Micrococcus luteus* BA13 (American Type Culture Collection, Manassas, VA, USA), tea (Nestea; Nestlé, Glendale, CA, USA), coffee (Instant Folgers Crystals; Folgers, Orrville, OH, USA), gastric porcine mucin (American Laboratories, Omaha, NE, USA), and ferric chloride (Fisher Scientific, Fair Lawn, NJ, USA). Fresh solution was prepared every day and used in a customized staining machine. Specimens were stained until they reached L* values between 10 and 50. The staining level was checked daily and the procedure continued until achieving the desired L* values.

2.7. Specimen stratification

Balanced randomization was performed to assign the specimens into the 11 treatment groups with 18 specimens per group, in order to ensure no statistically significantly differences in mean L* among groups.

2.8. Study groups

Table 1 informs about all experimental study groups. Deionized water served as a negative control, 100 ppm fluoride was the positive fluoride control, whereas 30 % hydrogen peroxide was the positive whitening control.

2.9. pH cycling model and treatment regimen

A modified version of the pH cycling model developed by White [10] was employed in this study. The daily cyclic treatment did consist of a 4 -h acid challenge in the lesion forming solution and 60s-treatments with one of the test mouthrinses two times per day with specimens stored in artificial saliva at all other times. Fresh artificial saliva (1.45 mM $CaCl_2$, 5.4 mM KH_2PO_4, 0.1 M Tris buffer, 2.2 g/L porcine gastric mucin) adjusted to pH 7.0 with KOH (all Sigma-Aldrich, St. Louis, MO, USA) was used each day and changed during the acid challenge period.

All treatments and artificial saliva were stirred at 350 rpm. After each treatment the specimen was rinsed under running deionized water and then all specimens were placed back again into saliva. The remaining time, the specimens were in saliva. The regimen was repeated for 10 days. The experiment was conducted at room temperature. The treatment schedule for this experiment is given in Table 2.

Table 2
Daily treatment schedule.

Time	Procedure
8:00–8:01 a.m.	mouthrinse treatment
8:01–10:00 a.m.	artificial saliva
10:00 a.m.–2:00 p.m.	acid challenge
2:00–4:00 p.m.	artificial saliva
4:00–4:01 p.m.	mouthrinse treatment
4:01 p.m.–8:00 a.m.	artificial saliva

2.10. Post lesion surface micro-hardness

After the completion of the 10-day pH-cycling phase, the average surface microhardness (VHN_{post}) was determined from four indentations on the surface of each specimen, next to the baseline indentation, as described previously. Changes in surface microhardness were calculated as follows: $\Delta VHN = VHN_{post} - VHN_{base}$.

2.11. Enamel fluoride content (EFC)

After completion of the final color assessment, EFC of nine specimens of each group was determined using the microbiopsy technique to a depth of 100 μm [12]. Only half of the specimens from each group were analyzed as EFC was a secondary objective in the present study. The diameter of the drill holes was determined, with four holes per specimen. The enamel powder was collected from the drill hole then dissolved in (20 μL of $HClO_4$, 40 μL Citrate/EDTA Buffer and 40 μL DI water) and analyzed for fluoride by comparison to a standard curve of specimens prepared in a similar way. EFC data were calculated as μg F/cm^3: (μg F × dilution factor – volume of drilling).

2.12. Statistical analysis

Based on prior studies, the standard deviations of ΔE* and ΔVHN were estimated to be 1.5 and 7.0, respectively. With a sample size of 18 specimens per group, the study had 80 % power to detect differences of 1.5 for ΔE* and 6.75 for ΔVHN between any two groups, assuming two-sided tests each conducted at a 5% significance level.

The effects of mouthrinse on ΔE*, ΔVHN and EFC were analyzed using one-way ANOVA, with fixed effects for mouthrinse and a random specimen effect. The distributions of the measurements were examined and a transformation of the data (e.g. natural logarithm) or nonparametric tests was used if necessary. A 5% significance level was used for all tests. Analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Color change (ΔE*)

Baseline staining (L*) was comparable between groups and ranged from 25.4 ± 9.2 (H) to 26.1 ± 11.3 (C). Fig. 1 shows the ΔE* data and results of the statistical analysis for all treatment groups. ΔE* was statistically significantly different between groups ($p < 0.0001$). The whitening positive control group, H2O2, resulted in a statistically significantly greater color change than any other treatment group ($p \le 0.0090$). There were differences between the test mouthrinses as groups H and I caused more color change than groups C, E and G ($p \le 0.0352$). Groups A, B and D did not differ from all other test mouthrinses ($p \ge 0.1075$). However, none of the test mouthrinses differed from DIW ($p \ge 0.0715$), with groups C, E and G being numerically lower than DIW ($p \ge 0.6130$). The numerically lowest color change was

Table 1
Test products and their active ingredients.

Code	Test product	Whitening/stain removal agent(s)	Fluoride concentration*
A	Crest Pro-Health Advanced With Extra Whitening	Hydrogen peroxide	100 ppm
B	Listerine® Healthy White™ Restoring Fluoride Mouthwash	Hydrogen peroxide	100 ppm
C	Listerine® Healthy White™ Gentle Sodium Fluoride Anticavity Mouthrinse	Tetrapotassium pyrophosphate, pentasodium triphosphate	100 ppm
D	Listerine® Healthy White™ Vibrant Fluoride Mouthwash	Hydrogen peroxide	100 ppm
E	Crest 3D White Luxe Diamond Strong Mouthwash	Hydrogen peroxide	100 ppm
G	Arm & Hammer™ Truly Radiant™ Bright & Strong Rinse	Tetrapotassium pyrophosphate, disodium pyrophosphate, sodium bicarbonate	100 ppm
H	Reach Complete Care 8 In 1 + Whitening Rinse Mint	Hydrogen peroxide, sodium hexametaphosphate	100 ppm
I	Listerine Total Care Stain Remover Anticavity Mouthwash Fresh Mint	Hydrogen peroxide	100 ppm
100 F	100 ppm fluoride in deionized water	n/a	100 ppm
H2O2	30 % hydrogen peroxide	Hydrogen peroxide	n/a
DIW	Deionized water	n/a	n/a

* all as sodium fluoride.

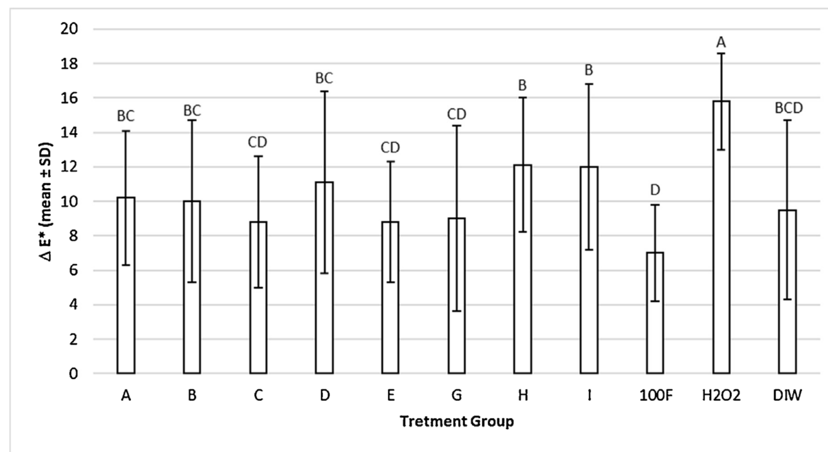

Fig. 1. Color change (ΔE*) for all treatment groups. Different letters indicate statistically significant differences between treatment groups.

achieved by the 100 F group, although it was not statistically significantly different than that of groups C, E, G and DIW ($p \geq 0.2060$).

3.2. Remineralization (ΔVHN)

Fig. 2 shows the ΔVHN data for all treatment groups. There were no statistically significant differences in ΔVHN between treatment groups ($p = 0.2898$). The numerically largest ΔVHN was achieved by group B (mean ΔVHN ± standard deviation – 6.9 ± 15.2) and the lowest by group G (-2.5 ± 10.6).

3.3. Enamel fluoride content (EFC)

Fig. 3 shows the EFC data and results of the statistical analysis for all treatment groups. EFC was significantly different between groups ($p = 0.0199$). EFC was significantly lower in groups C ($p \leq 0.0352$) and G ($p \leq 0.0304$), which were not different from one another ($p = 0.9512$), compared to all other groups apart from the negative control group, DIW, ($p \geq 0.2754$). There were no differences between DIW and all other groups ($p \geq 0.0582$) or between the other groups ($p \geq 0.4166$).

4. Discussion

In this laboratory study, we tested the ability of dual whitening anti-caries mouthrinses to remove stains from artificially stained enamel caries lesions and to enhance their remineralization. We used artificially stained caries lesions rather than stained, sound enamel (which have been studied in the past) as the test substrate as cariogenic discoloration can occur due to penetration of chromogens, from smoking or of a dietary nature, into the porous structures of caries lesions. Removal of these chromogens while promoting lesion remineralization would be beneficial for re-establishing both tooth aesthetics and mineral content.

Based on the present results, the null hypothesis a), that dual whitening anti-caries mouthrinses have no effect on removing stains compared to deionized water, was accepted. The null hypothesis b), that dual whitening anti-caries mouthrinses reduce caries lesion remineralization and fluoridation in comparison to a non-whitening anti-caries mouthrinse, was partially supported.

Numerous substances have been utilized to produce staining in vitro, with dietary, polyphenolic stains derived from coffee, red wine, and/or black tea being used alone or in combination with compounds known to cause tooth surface staining in their own right, such as chlorhexidine [13]. In the present study, a staining 'broth' containing a wide variety of compounds (see 2.6.) was used. The resulting stain is of clinical relevance as results of in vitro stain removal studies testing a wide range of toothpastes compared favorably to in vivo stain removal studies using the same products [11]. The eight whitening and anti-caries mouthrinses were chosen due to their wide availability in grocery stores and pharmacies in North America. While this represents a convenience sample, several of the tested mouthrinses (Table 1) are available in other countries which aids the representative value of the present study. Positive controls were included to evaluate whitening (30 % H_2O_2, H2O2) and remineralization (100 ppm F, 100 F). Likewise, a negative control

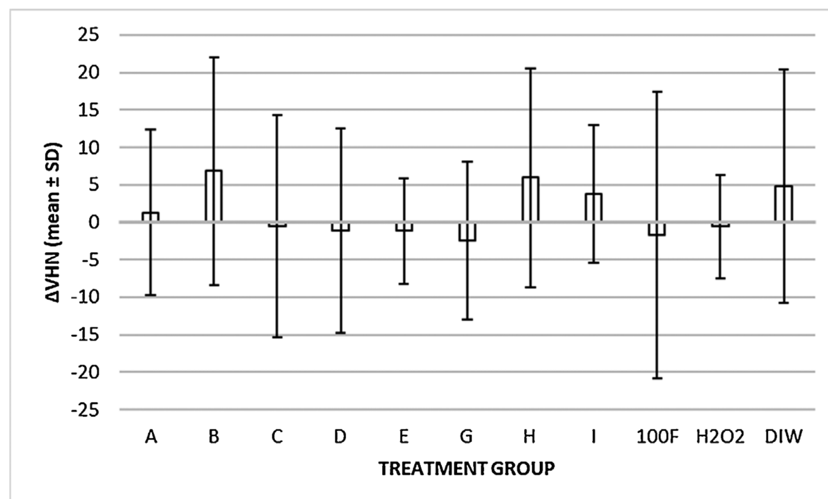

Fig. 2. Changes in Vickers surface microhardness (ΔVHN) for all treatment groups.

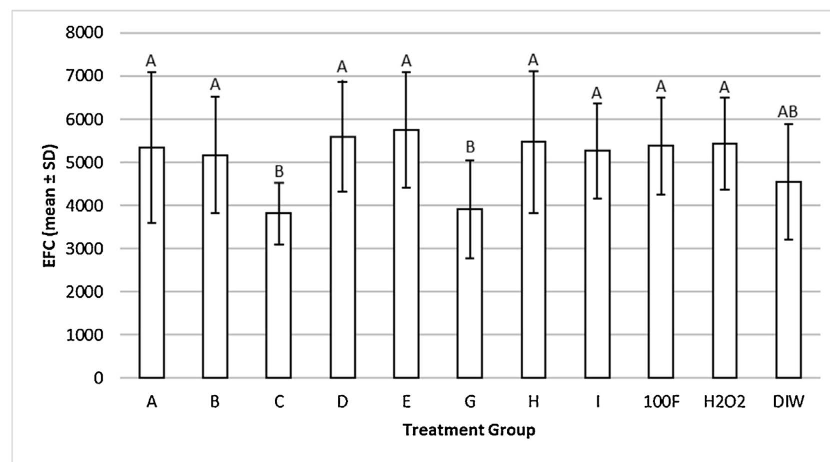

Fig. 3. Enamel fluoride content (EFC) by treatment group. Different letters indicate statistically significant differences between treatment groups.

group (deionized water, DIW) was included. The chosen pH cycling method, based on that by White [10], is one of the more established pH cycling models used to determine the remineralizing abilities of anti-caries oral care products. However, the choice of a more realistic pH cycling model incorporating multiple, short acid challenges in comparison to one long challenge per day needs to be considered in future studies. The rationale for not including toothpaste abrasion (the tested rinses are supposed to be used as an adjunct to toothbrushing) was to investigate their inherent contributions to stain removal and enamel caries lesion remineralization.

The present study has shown that dual-action whitening and anti-caries mouthrinses do not appear to provide clinically meaningful tooth whitening benefits in their own right (Fig. 1). H2O2 was the only intervention that was able to lead to significant stain removal compared to DIW. The DIW group exhibited color improvement which would suggest that the daily acid challenge lead to some stain removal. Hydrogen peroxide in high concentrations (25–40 %) is used for in-office tooth whitening in the form of gels with an often immediate tooth whitening outcome [14,15]. Here, the twice-daily 1-min application of H2O2 over a period of 10 days (i.e., 20 min in total) resulted in measurable stain removal. However, it must be noted that the chosen positive whitening control H2O2 (i.e., 30 % hydrogen peroxide) is not suitable for application in a rinse format. It was chosen to mimic in-office whitening products that lead to significant whitening as there are presently no clinically proven whitening rinses that could have been used as a positive whitening control. It could be argued that the hydrogen peroxide concentration in the test mouthrinses was insufficient. In this context, it must be noted that none of the test mouthrinses displayed the concentration of hydrogen peroxide on the bottle label. Likewise, the stain removal action of the other chemical whitening agents was too low to have an effect under the present conditions. While none of the test mouthrinses offered any benefits, it cannot be excluded that they may provide some benefits in combination with toothbrushing abrasion, which was not the purpose of the present study. Although none of the rinses are indicated for use prior to toothbrushing, where loosening of stain by chemical means could ease its removal through subsequent abrasion, the reverse action caused by chemical whitening agents in toothpastes can be considered and is worthy of further investigation.

The present results are partially in agreement with previous studies, which showed that whitening rinses do not have the whitening potential of a professional whitening agent [16] and that they are incapable of providing significant whitening compared to a no-treatment control [17]. However, a differential whitening between mouthrinses and compared to DIW was observed previously [16]. Furthermore, an in vivo study [18] indicated that the use of whitening mouthrinses containing 1.5 % hydrogen peroxide for 6 months was effective in tooth whitening and decreasing the severity of gingivitis. This study included twice-daily toothbrushing which may explain the positive whitening result. Another study demonstrated similar whitening benefits for some mouthrinses compared to a 10 % carbamide peroxide whitening gel [19]. The reasons for the discrepancy between studies are manifold and include differences in the test substrate (e.g. caries lesion presently vs. sound enamel in other studies), staining procedures (e.g. complex stain vs. coffee), inherent in vitro model differences and study duration. It must be noted, however, that a significant whitening effect was noted for all treatment groups in the present study. ΔE^* values greater than 3.3 have been associated with clinically visible changes in whitening previously [20], with the minimum observed presently being 7.0 for 100 F. This whitening effect may be due to the daily acid challenge during the pH cycling phase, a model parameter not included in previous investigations.

Topical fluorides play an important role in the prevention of dental caries. Multiple clinical trials have demonstrated a positive relationship between fluoride dose and reduction in caries incidence [21–23]. For fluoride to be effective in any toothpaste it should contain at least 1000–1100 ppm fluoride and any dose less than 600 ppm was considered to be less beneficial [24]. However, there is no guidance on a minimum fluoride concentration for mouthrinses.

In the present study, all test mouthrinses contained 100 ppm F (Table 1). A previous study investigated salivary fluoride concentrations after brushing with a 1450 ppm F toothpaste followed by rinsing with a 100 ppm F rinse or water [25]. The authors found that the 100 ppm F rinse did not provide enhanced intra-oral fluoride retention compared to brushing with toothpaste alone. Therefore, the anti-caries benefits of the tested rinses can be questioned and given the widespread use of fluoride toothpastes. In hindsight, however, the use of an additional, higher fluoride concentration to determine a dose-response and demonstrate model sensitivity would have been advantageous. However, the lack of remineralization in the present study was most likely in that staining of the lesions caused them to at least partially arrest and/or that considerably higher fluoride concentration would be needed to remineralize such lesions. Hardness changes were minimal after completion of the pH cycling phase (Fig. 2), which would not necessarily support the ‘arrest hypothesis’ as arrested lesions would have been expected to exhibit higher hardness values. However, it could have been likely that the staining procedure caused the demineralized enamel structure to become almost inert due to the deposition of chromophores onto enamel crystallites, thereby lowering their ability to remineralize or demineralize further. Nonetheless, a recent study [26] demonstrated the ability of 38 % silver diamine fluoride (SDF) to remineralize stained caries lesions while using the same staining protocol as employed presently. However, the fluoride concentration of SDF is approx. 44,800 ppm compared to 100 ppm F used presently. Furthermore, application mode and frequency differ significantly between these two interventions, making a comparison not straightforward. It appears further studies using a wider range of

fluoride concentrations, fluoride compounds and non-fluoride remineralizing agents is necessary to provide more conclusive evidence on the matter.

The EFC data (Fig. 3) not necessarily mirrored the hardness data as differences were observed between treatment groups. These data were, however, somewhat improbable as the fluoride-free H2O2 group had statistically significantly higher EFC values than two groups of specimens that were treated with 100 ppm F (C, G). These two mouthrinses were the only products that did not contain hydrogen peroxide but pyro- and triphosphate salts as whitening agents instead (Table 1). Previous research has shown that these compounds do not impede the interaction of fluoride with enamel under in situ conditions [27], contradicting present observations. The inherent fluoride content of the enamel specimens after staining was not determined presently due to the destructive nature of the microbiopsy technique (which in turn would have impeded the more relevant color measurements). Hence, it can only be speculated that the measured EFC values were not a true representation of the fluoridation abilities of the test mouthrinses, but instead represent the sum of the inherent enamel fluoride content after staining and the fluoride acquired during the pH cycling phase. While the staining procedure is unlikely to differentially alter the fluoride content of the enamel lesions, structural changes in the enamel may occur that may make it more difficult for fluoride to be retained. Likewise, the daily acid challenge may have removed applied, loosely-bound fluoride before it had a chance to be permanently retained in the enamel structure. This will be the subject of further investigations.

In addition to the limitations mentioned earlier, the present study has several other limitations that need to be taken into consideration in the interpretation of the findings. In vitro pH-cycling models have some limitations in that they do not mimic the fluoride retention phase, which was shown to be important in the protection against dental caries [28]. Bovine teeth have some limitations compared to human teeth, as there are structural and compositional differences [29]. In bovine teeth the de- and remineralization processes occur faster than in human teeth resulting in potentially misleading findings [30,31]. However, a recent pH-cycling study [31] highlighted that these variances in response to de- and remineralization challenges and fluoride are overcome by differences in lesion mineral distribution. Hence, the utilization of bovine teeth in studies like the present one can be justified. Furthermore, a longer pH cycling phase or the utilization of a more aggressive model (i.e., more net de- or remineralization) could have potentially allowed for a fluoride effect to be observed. Lastly, dilution of mouthrinses by saliva as it would occur in vivo may alter their whitening effect through chemical interaction between saliva proteins and minerals and chemical whitening agents.

5. Conclusions

Within the limitations of this in vitro study, it can be concluded that artificially stained enamel caries lesions shows reduced susceptibility to fluoride remineralization and whitening effects of commercial whitening and anti-caries mouthrinses. Further clinical studies utilizing typical oral care regimens with twice-daily toothbrushing will be needed to provide conclusive evidence to the usefulness of the presently tested products in comparison to mouthrinses providing clinically proven therapeutic benefits, such as reductions in the severity of gingivitis.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Ahid A. Al-Shahrani: Conceptualization, Data curation, Investigation, Methodology, Project administration, Writing - original draft. **John A. Levon:** Methodology, Writing - review & editing. **Anderson T. Hara:** Conceptualization, Methodology, Writing - review & editing. **Qing Tang:** Formal analysis, Writing - review & editing. **Frank Lippert:** Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

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