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Effectiveness of caries-preventing agents on initial carious lesions within the scope of orthodontic therapy

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Objective: To evaluate the effectiveness of three different caries-preventing agents on artificial caries in a *Streptococcus mutans*-based caries model. **Methods:** Sixty-five caries-free human molar enamel blocks were treated with a demineralization solution and a remineralization solution. The specimens were assigned to the following groups according to the caries-protective product applied: group A, chlorhexidine varnish; group B, fluoride-releasing chemically cured sealant; group C, fluoride-releasing lightcured sealant; group D, positive control (specimens that were subjected to de- and remineralization cycles without treatment with any caries-protective agents); and group E, negative control (specimens that were not subjected to de- and remineralization cycles). Samples in groups A–D were stored in demineralization solution with *S. mutans* and thereafter in artificial saliva. This procedure was performed for 30 days. Average fluorescence loss (ΔF) and surface size of the lesions were measured using quantitative light-induced fluorescence at baseline and on the 7th, 14th, and 30th days. **Results:** After 30 days, group A demonstrated a significant increase in ΔF and the surface size of the lesions, no significant difference in comparison with the positive control group, and a significant difference in comparison with the negative control group. Group B showed no significant changes in both parameters at any of the measurement points. While group C showed increased ΔF after 14 days, no significant fluorescence change was observed after 30 days. **Conclusions:** Both fluoride-releasing sealants (chemically or light-cured) show anti-cariogenic effects, but the use of chlorhexidine varnish for the purpose of caries protection needs to be reconsidered.
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Key words: Cervitec Plus, Maximum Cure, Pro Seal, Quantitative light-induced fluorescence

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INTRODUCTION

During orthodontic treatment, fixed appliances such as brackets and ligatures promote plaque accumulation and complicate teeth cleaning. The resulting biofilm can produce acids that can cause demineralization and the formation of visible so-called white-spot lesions.[1] These lesions are often irreversible, and the ongoing demineralization process can subsequently lead to the development of more advanced carious lesions that require invasive treatment. Thus, early detection and assessment of initial carious lesions, as well as preventive interventions, are crucial to stop lesion progression. In addition to regular follow-up, dietary recommendations, and repeated oral hygiene instructions, the use of caries-preventing agents can reduce the demineralization risk and promote the remineralization process of teeth.[2]

Clinical trials have shown the anti-caries efficacy of fluoride-releasing products.[1,3,4] Fluoride can be supplied locally in the form of mouth-rinsing solutions, gels, varnishes, sealants, and fluoride-releasing materials.[5] The use of a varnish is especially advantageous in patients with low compliance because it adheres to the tooth surface for a long duration and is independent of patient cooperation. Fluoride-releasing varnish can be used in the bracket adhesive technique to prevent demineralization of the teeth.[6] Similarly, the use of light-curing sealants with a high filler content can prevent the formation of white-spot lesions due to their increased resistance to abrasion.[6,7] Meanwhile, modern dental care products contain different antimicrobial agents for biofilm control, such as chlorhexidine, enzymes, essential oils, and phenol derivatives.

Due to the multitude of available anti-caries agents, the question arises as to which application form ensures effective protection against initial carious lesions during orthodontic treatment. To test the efficacy of different caries-preventing agents, standardized specimens and reliable diagnostic tools are desirable. The current study was therefore set up to evaluate the efficacy of two widely used fluoride-releasing sealants and a chlorhexidine/thymol-containing varnish for the prevention of initial carious lesions in a microbial caries model *in vitro* by using quantitative light-induced fluorescence (QLF). We tested the hypotheses that the application of these agents leads to lower demineralization effects and that there are no differences in effectiveness among the tested products.

MATERIALS AND METHODS

Preparation of enamel blocks

Sixty-five intact, non-carious, unrestored human molars were selected out of a pool of collected teeth in accordance with a protocol approved by the Ethics Committee of the University Göttingen, Germany (No. 16/6/09). From these 65 human molars, standardized enamel blocks with a diameter of 5 mm were produced (Band System 300/310; EXAKT Advanced Technologies GmbH, Norderstedt, Germany). The surfaces of the enamel blocks were polished (Roto Pol-35; Struers GmbH, Willich, Germany) in order to obtain plano-parallel surfaces and ensure equal roughness of all specimens. Previously marked slots were drilled into the specimens in order to perform assessments at the same position during the investigation.

Demineralization solution

For the demineralization process, *Streptococcus mutans* (Clarke 1924, DSM 20523; Leibniz Institute DSMZ, Braunschweig, Germany) was used. To prepare the inocula, *S. mutans* was grown on blood agar plates (COS; bioMérieux SA, Marcy l'Etoile, France) for 48 hours. Ten colonies of *S. mutans* were inserted into 500 mL of glucose-bouillon (Merck KGaA, Darmstadt, Germany; composition in 10 L of distilled water: 50 g NaCl, 100 g peptone from meat pancreatically digested granulated, 100 g granulated meat extract dry, 100 g D(+)-glucose monohydrate and 6 mL NaOH) and incubated at 36.6°C for 23 hours under microaerophilic conditions (5% oxygen, 10% carbon dioxide, and 85% nitrogen). Contamination of cultures was verified by the Gram method.

Remineralization solution (artificial saliva)

A remineralization solution with the following composition was prepared for the experiments (materials were obtained from the pharmacies of Georg-August-University, Göttingen, Germany): 1.505 g sorbitol, 0.06 g KCl, 0.0425 g NaCl, 0.0025 g $MgCl_2$•6 H_2O, 0.0075 g $CaCl_2$•2 H_2O, 0.125 g Na_2HPO_4•12 H_2O, 0.25 g carboxymethyl cellulose sodium, and 50 g purified water.

Test material

The specimens were randomly allocated to five groups. In three groups (n = 15), different caries-protective agents were applied according to the manufacturer's instructions: group A, chlorhexidine/thymol-containing varnish (Cervitec Plus®; Ivoclar Vivadent AG, Schaan, Liechtenstein); group B, fluoride-releasing chemically cured sealant (Maximum Cure®; Reliance Orthodontic Products, Inc., Itasca, IL, USA); and group C, fluoride-releasing light-cured sealant (Pro Seal®; Reliance Orthodontic Products, Inc.). For group A, a single dose of Cervitec Plus® was applied thinly on the enamel surfaces of specimens using a micro-brush (extra fine; Kerr GmbH, Biberach, Germany). Subsequently, the varnish was dried. For groups B and C, the enamel surfaces of the specimens were etched for 30 seconds with 37%

phosphoric acid gel (Ivoclar Vivadent AG) prior to baseline varnish application, rinsed with water for 60 seconds, and dried thoroughly in oil-free air. For group B, both components of Maximum Cure® were mixed in a dappen-dish and applied in a thin uniform layer to the etched surfaces of specimens using a micro-brush. For group C, three drops of Pro Seal® were dispensed onto a mixing pad and a thin, uniform layer was applied on the etched enamel surfaces with a bristle brush. The enamel surfaces were stroked with the same brush to ensure a thin layer and good coverage. Subsequently, the layers were light-cured for 20 seconds (Ortholux™ XT Curing Light; 3M Unitek, Landsberg am Lech, Germany).

Table 1 shows the compositions of these agents. Group D (n = 15) served as a positive control (specimens only underwent the re- and demineralization cycles without application of any product) and group E (n = 5) served as a negative control (specimens were not subjected to the re- and demineralization cycles and only treated with artificial saliva).

Demineralization- and remineralization cycle

After the application of the test products (groups A–C) and rinsing, the specimens of groups A–D were stored in the demineralization solution and thereafter in artificial saliva for one hour each. These processes were repeated three times per day. Until the next cycle on the following day, all specimens (groups A–E) were stored in artificial saliva (about 15 hours). This procedure was continued for 30 days (Figure 1).

Evaluation of carious lesions

The specimens were imaged using QLF (Inspektor Research Systems BV, Amsterdam, The Netherlands) at baseline and days 7, 14, and 30. Using the QLF software package (version 2.0.0.43; Inspektor Research Systems BV), the average fluorescence loss (ΔF, %) and surface size of the lesions (mm^2) were measured. The data were statistically analyzed using Wilcoxon–Mann–Whitney test ($\alpha = 0.05$).

Statistical analysis

Statistical analysis was performed using the programs SAS (version 9.2; SAS Institute GmbH, Heidelberg, Germany) and Statistica (version 9; StatSoft [Europe] GmbH, Hamburg, Germany). The influences of test products and time on the measurements were investigated separately according to ΔF and size of lesion using two-way (non-parametric) ANOVA. In the case of a significant effect, pair comparisons were performed using the Wilcoxon–Mann–Whitney test. The level of significance was determined by $\alpha = 5\%$.

RESULTS

Average ΔF and surface size of the lesion in the specimens are presented in Table 2. Figure 2 shows the QLF images of groups A–D at all measurement points.

The specimens in group A demonstrated a significant increase in ΔF and lesion surface size after 30 days ($p = 0.014$), no significant difference in comparison with the positive control group (group D; $p = 1.000$), and a significant difference in comparison with the negative control (group E) after 30 days ($p = 0.014$).

The specimens in group B showed no changes in both parameters at all measurement points. Although the specimens in group C showed increased ΔF after 14 days, they showed no significant fluorescence change

Table 1. Compositions of the tested agents according to manufacturer specifications

Group	Product	Application	Composition (weight, %)
A	Cervitec Plus (Ivoclar Vivadent AG, Schaan, Liechtenstein)	Varnish	Ethanol, water (90) Vinyl acetate copolymer, acrylate copolymer (8) Thymol (1) Chlorhexidine diacetate (1)
B	Maximum Cure (Reliance Orthodontic Products, Inc., Itasca, IL, USA)	2-components chemically cured sealer	Component 1: Bisphenol-A-diglycidyl methacrylate (50–70) Methyl methacrylate (25–35) Amorphous silica (5–15) Hydrofluoride methacrylate (2–5) Component 2: Bisphenol-A-diglycidyl methacrylate (50–80) Benzoyl peroxide (1–5) Methyl methacrylate (20–40)
C	Pro Seal (Reliance Orthodontic Products, Inc.)	1-component light-cured sealer	Ethoxylate bisphenol-A-diglycidyl methacrylate (10–50) Urethane acrylate ester (10–40) Polyethylene glycol diacrylate (10–40) Fluoride-containing glass frit (5–40)

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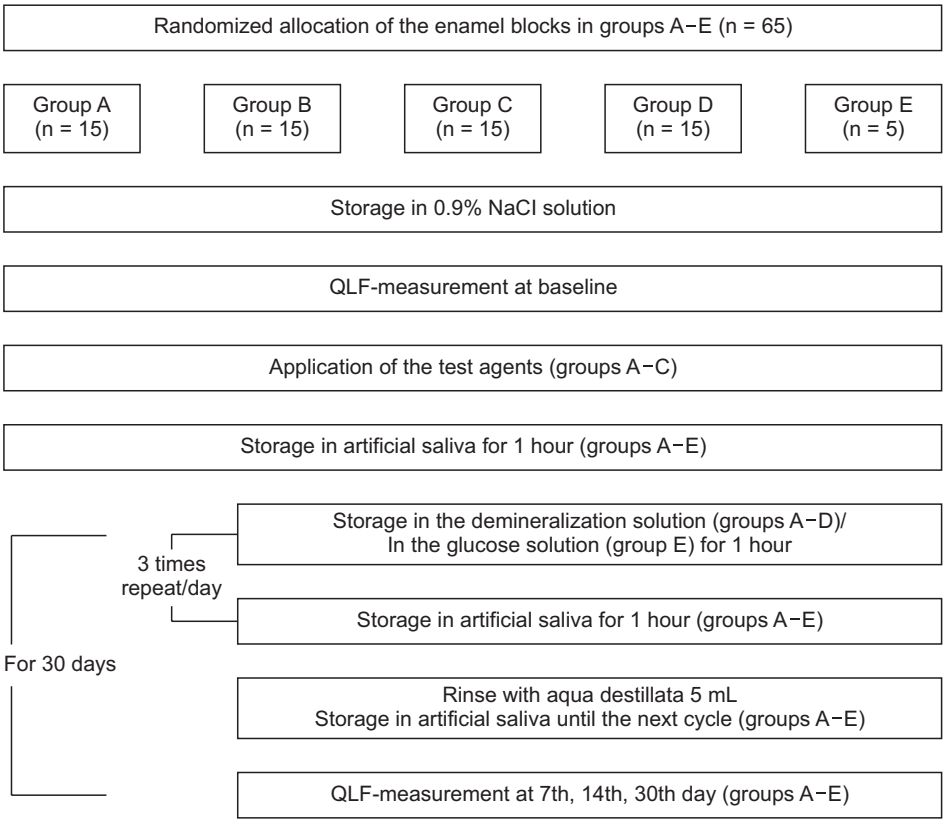

Figure 1. Workflow diagram.
Group A: Cervitec Plus®, Ivoclar Vivadent AG, Schaan, Liechtenstein; Group B: Maximum Cure®, Reliance Orthodontic Products, Inc., Itasca, IL, USA; Group C: Pro Seal®, Reliance Orthodontic Products, Inc.; Group D: positive control; Group E: negative control.
QLF, Quantitative light-induced fluorescence.

after 30 days (p = 0.392). Groups B and C showed no significant changes in the surface size of lesion compared to the negative control group (group E; p = 1.000) and a significant difference compared to the positive control (group D) after 30 days ($p \leq 0.028$).

DISCUSSION

The current study assessed the effectiveness of a chlorhexidine/thymol-containing varnish and two fluoride-releasing sealants. While the two fluoride-releasing sealants (Maximum Cure® and Pro Seal®) showed greater caries-preventing ability, carious lesion formation was observed even with the use of chlorhexidine/thymol-containing varnish (Cervitec Plus®). Therefore, our hypotheses that the fluoride-releasing sealants could prevent initial carious lesions and that the tested products did not differ in their ability to prevent the formation of carious lesions were rejected.

Fluorides play a central role in caries prevention.[4] In orthodontics, in addition to the daily supervised tooth brushing with the application of fluoride, fluoride-releasing bonding materials or fluoride-releasing sealants for brackets and bands are also used for caries prevention.[8] These products can continuously release fluoride over a long period and are therefore effective for tooth surfaces.[9] Both the fluoride-releasing sealants (Maximum Cure® and Pro Seal®) assessed in this study are used to prevent demineralization of etched areas where orthodontic brackets are affixed and to improve the adhesion of bonding materials.[6] Light-cured sealants are believed to be superior to chemically cured sealants due to their higher degree of polymerization, which can yield a more complete/stable coverage of the enamel surface.[7] In the current study, Maximum Cure® and Pro Seal® showed no significant differences in ΔF and surface size of the lesions after 30 days. Previous studies have also shown that both products influence the extent and progression of demineralization effectively.[6,7,10,11] No significant differences were observed in the effectiveness of chemically cured and light-cured sealants after 30 days. Demito et al.[12] demonstrated a reduction in demineralization depth of up to 38% after application of fluoride-releasing varnish compared to a reference group without fluoridation. The current study showed that the unprotected enamel surfaces that were exposed to demineralization- and remineralization cycles tend to develop erosive/white-spot lesions after 14 days.[13]

Chlorhexidine-containing products are used with the aim of reducing the demineralization risk by influencing the bacterial metabolism and by reducing the amount of *S. mutans*,[14] and thymol was used as a purified active compound in characterizing different microorganisms' susceptibilities. Thymol has been reported to be one of

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Table 2. Fluorescence loss and lesion size in all groups at four different measurement points

Group	Measurement point	Fluorescence loss (ΔF, %)		Size of lesion (mm^2)		Fluorescence loss integrated over the lesion size (ΔQ, % × mm^2)
		Median (range)	*p*-value of comparison between baseline and 30th day	Median (range)	*p*-value of comparison between baseline and 30th day	
A	Baseline	0.00 (0.00 to 0.00)^{E,F}	0.014*	0.00 (0.00 to 0.00)^{e}	0.014*	0.00
	7th day	0.00 (−9.49 to 0.00)		0.00 (0.00 to 0.01)		0.00
	14th day	−5.94 (−7.97 to 0.00)^{E}		0.00 (0.00 to 3.48)		0.00
	30th day	−8.91 (−15.6 to −5.96)^{A,E,I}		3.49 (0.03 to 13.10)^{a,e,g,h}		−31.09
B	Baseline	0.00 (0.00 to 0.00)	0.252	0.00 (0.00 to 0.00)	0.252	0.00
	7th day	0.00 (−9.2 to 0.00)		0.00 (0.00 to 0.11)		0.00
	14th day	0.00 (−9.49 to 0.00)		0.00 (0.00 to 0.14)		0.00
	30th day	0.00 (−19.7 to 0.00)^{B}		0.00 (0.00 to 0.28)^{b,g}		0.00
C	Baseline	0.00 (0.00 to 0.00)	0.392	0.00 (0.00 to 0.00)	0.952	0.00
	7th day	0.00 (−9.9 to 0.00)		0.00 (0.00 to 0.55)		0.00
	14th day	−5.93 (−13.5 to 0.00)		0.00 (0.00 to 0.34)		0.00
	30th day	0.00 (−21.9 to 0.00)^{C,I}		0.00 (0.00 to 0.56)^{c,h}		0.00
D	Baseline	0.00 (0.00 to 0.00)^{G,H}	0.014*	0.00 (0.00 to 0.00)^{f}	0.014*	0.00
	7th day	−5.84 (−9.7 to 0.00)		0.00 (0.00 to 0.62)		0.00
	14th day	−7.51 (−9.79 to −5.90)^{G}		1.63 (0.00 to 8.77)		−12.24
	30th day	−11.80 (−20.50 to −7.06)^{B,C,D,H}		7.67 (0.65 to 15.90)^{b,c,d,f}		−90.50
E	Baseline	0.00 (0.00 to 0.00)	-	0.00 (0.00 to 0.00)	-	0.00
	7th day	0.00 (0.00 to 0.00)		0.00 (0.00 to 0.00)		0.00
	14th day	0.00 (0.00 to 0.00)		0.00 (0.00 to 0.00)		0.00
	30th day	0.00 (0.00 to 0.00)^{A,D}		0.00 (0.00 to 0.00)^{a,d}		0.00

Wilcoxon–Mann–Whitney tests were performed to compare baseline to 30th day. *$p < 0.05$.
Group A: Cervitec Plus®, Ivoclar Vivadent AG, Schaan, Liechtenstein; Group B: Maximum Cure®, Reliance Orthodontic Products, Inc., Itasca, IL, USA; Group C: Pro Seal®, Reliance Orthodontic Products, Inc.; Group D: positive control; Group E: negative control.
Groups and measurement points which showed significant differences ($p < 0.05$) during pairwise comparison using Wilcoxon–Mann–Whiteny were both marked with the same letter.

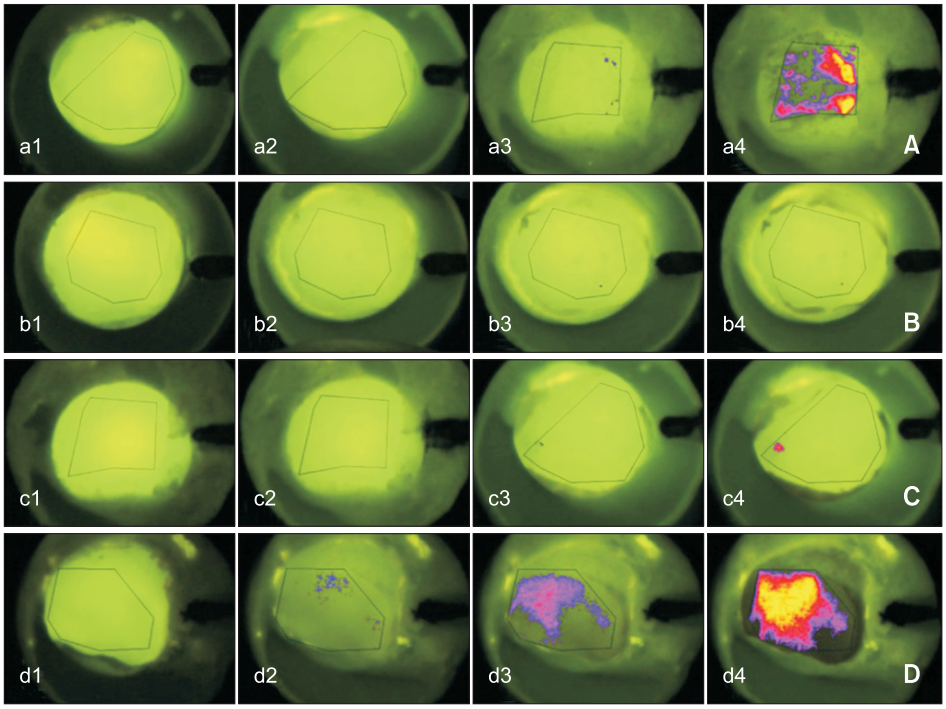

Figure 2. Quantitative light-induced fluorescence images of groups A–D at all measurement points. **A**, Cervitec Plus®; **B**, Maximum Cure®; **C**, Pro Seal®; **D**, positive control group. The images for Cervitec Plus® (**a4**) and the positive control group (**d3** and **d4**) show distinct fluorescence loss.
1, At baseline; 2, at day ; 3, at day 14; 4, at day 30.

the most active antimicrobials among the constituents of essential oils.[15] Although several studies have demonstrated that supplemental application of the chlorhexidine/thymol-containing Cervitec Plus® has a tendency to inhibit demineralization, other studies have found no evidence of caries prevention.[16–19] The current study also showed no anti-cariogenic effect of Cervitec Plus®. On the 14th day, the specimens with Cervitec Plus® showed a reduction in fluorescence, and on the 30th day, there was no significant difference between the group A and the group D. In contrast to the two fluoride-based agents investigated, Cervitec Plus® is applied to the cleaned tooth surface without any prior enamel etching process. Therefore, there may be less adhesion between the varnish and the tooth surface than between the sealant and the tooth surface. As a result, the varnish may have chipped off and the resulting discontinuities may have led to a reduced protective effect. Another possible explanation for the lower anti-cariogenic effect of Cervitec Plus® compared to the sealers is that *S. mutans* tends to recolonize over long-term application of chlorhexidine.[20] Zaura-Arite and ten Cate[21] also showed that a fluoride-releasing sealant has a greater demineralization-inhibiting effect than Cervitec Plus®. In contrast, Petersson et al.[22] found in a comparative study of Cervitec and the fluoride-releasing Fluor Protector that both products were similarly effective in controlling caries incidence. The combined use of chlorhexidine along with fluoridation could help reduce caries risk.[23,24] Nevertheless, due to the lack of evidence for chlorhexidine-containing products, fluoride-releasing products have often been considered the means of choice for preventing initial carious lesions.[4,20,25]

Compared to most other studies that used a chemical-based model of artificial caries, the current study used *S. mutans* in a microbe-based model. In comparison with natural carious lesions, artificial carious lesions allow production of a standardized specimen of any caries stage according to the need. While microbe-based caries models more closely resemble the intraoral situation and their caries development process is very similar to natural carious lesions, the existing models are costlier and require more time than chemical-based models. However, chemical-based models have disadvantages such as surface softening, implementation without intraoral conditions, and less realistic time periods of de- and remineralization.[26] This model used in the current study allowed us to 1) easily produce carious lesions under biological conditions with a high level of control, 2) show different levels of anti-cariogenic effects of different products, and 3) show the chronological progress of the anti-cariogenic effects.

QLF is considered to be a validated caries diagnostic tool, especially for detection of initial caries.[27] Transverse microradiography is known as the gold standard method for determination of mineral loss, but it is destructive and invasive. In contrast, QLF provides non-invasive multiple measurements and the evaluated images can be archived, enabling longitudinal monitoring of caries development or progression.[28] Previous studies presented

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a high correlation between ΔF and mineral loss and confirmed QLF as a suitable diagnostic tool.[28-30] Ando et al.[29] reported that there is a non-linear correlation or even a non-correlation between ΔF and the size of the lesions. In contrast, an imperfect linear correlation between both parameters was observed in the current study regardless of groups and times of measurement.

The limitations of the current study are related to its experimental setup. No histological analysis of carious lesions was conducted after the QLF assessment to validate lesion formation. This analysis could have been performed after 30 days, e.g., using scanning electron microscopy or transverse microradiography. Additionally, the current study was performed *in vitro*, in which the entirety of clinical conditions and physiological processes could not be fully reproduced. Furthermore, this study lacks a simulation of orthodontic treatment procedures such as fixing of the brackets on the specimens, and it compared test products with different types of application (varnish and sealant), which may impair comparability. These shortcomings should be considered in follow-up studies.

CONCLUSION

Demineralization performed without enamel protection in the caries model resulted in the formation of white-spot lesions within 2 weeks. Although both fluoride-releasing sealants provided anti-cariogenic effects, the application of chlorhexidine-containing varnish for caries protection should be reconsidered.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Gorton J, Featherstone JD. In vivo inhibition of demineralization around orthodontic brackets. Am J Orthod Dentofacial Orthop 2003;123:10-4.
2. Lopatiene K, Borisovaite M, Lapenaite E. Prevention and treatment of white spot lesions during and after treatment with fixed orthodontic appliances: a systematic literature review. J Oral Maxillofac Res 2016;7:e1.
3. Lovrov S, Hertrich K, Hirschfelder U. Enamel demineralization during fixed orthodontic treatment-incidence and correlation to various oral-hygiene parameters. J Orofac Orthop 2007;68:353-63.
4. Bergstrand F, Twetman S. A review on prevention and treatment of post-orthodontic white spot lesions-evidence-based methods and emerging technologies. Open Dent J 2011;5:158-62.
5. Marinho VC, Higgins JP, Logan S, Sheiham A. Topical fluoride (toothpastes, mouthrinses, gels or varnishes) for preventing dental caries in children and adolescents. Cochrane Database Syst Rev 2003;(4):CD002782.
6. Hu W, Featherstone JD. Prevention of enamel demineralization: an in-vitro study using light-cured filled sealant. Am J Orthod Dentofacial Orthop 2005;128:592-600; quiz 670.
7. Buren JL, Staley RN, Wefel J, Qian F. Inhibition of enamel demineralization by an enamel sealant, Pro Seal: an in-vitro study. Am J Orthod Dentofacial Orthop 2008;133:S88-94.
8. Benson PE, Parkin N, Dyer F, Millett DT, Furness S, Germain P. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. Cochrane Database Syst Rev 2013;(12):CD003809.
9. Cohen WJ, Wiltshire WA, Dawes C, Lavelle CL. Long-term in vitro fluoride release and rerelease from orthodontic bonding materials containing fluoride. Am J Orthod Dentofacial Orthop 2003;124:571-6.
10. Banks PA, Richmond S. Enamel sealants: a clinical evaluation of their value during fixed appliance therapy. Eur J Orthod 1994;16:19-25.
11. Cain K, Hicks J, English J, Flaitz C, Powers JM, Rives T. In vitro enamel caries formation and orthodontic bonding agents. Am J Dent 2006;19:187-92.
12. Demito CF, Vivaldi-Rodrigues G, Ramos AL, Bowman SJ. The efficacy of a fluoride varnish in reducing enamel demineralization adjacent to orthodontic brackets: an in vitro study. Orthod Craniofac Res 2004;7:205-10.
13. Melrose CA, Appleton J, Lovius BB. A scanning electron microscopic study of early enamel caries formed in vivo beneath orthodontic bands. Br J Orthod 1996;23:43-7.
14. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 1994;73:682-91.
15. Marchese A, Orhan IE, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF, et al. Antibacterial and antifungal activities of thymol: a brief review of the literature. Food Chem 2016;210:402-14.
16. Twetman S, Hallgren A, Petersson LG. Effect of an antibacterial varnish on mutans streptococci in plaque from enamel adjacent to orthodontic appliances. Caries Res 1995;29:188-91.
17. Ogaard B, Larsson E, Glans R, Henriksson T, Birkhed D. Antimicrobial effect of a chlorhexidine-thymol varnish (Cervitec) in orthodontic patients. A prospective, randomized clinical trial. J Orofac Orthop 1997;58:206-13.

18. Madléna M, Vitalyos G, Márton S, Nagy G. Effect of chlorhexidine varnish on bacterial levels in plaque and saliva during orthodontic treatment. J Clin Dent 2000;11:42-6.
19. George AM, Kalangi SK, Vasudevan M, Krishnaswamy NR. Chlorhexidine varnishes effectively inhibit Porphyromonas gingivalis and Streptococcus mutans-an in vivo study. J Indian Soc Periodontol 2010;14:178-80.
20. Autio-Gold J. The role of chlorhexidine in caries prevention. Oper Dent 2008;33:710-6.
21. Zaura-Arite E, ten Cate JM. Effects of fluoride- and chlorhexidine-containing varnishes on plaque composition and on demineralization of dentinal grooves in situ. Eur J Oral Sci 2000;108:154-61.
22. Petersson LG, Magnusson K, Andersson H, Almquist B, Twetman S. Effect of quarterly treatments with a chlorhexidine and a fluoride varnish on approximal caries in caries-susceptible teenagers: a 3-year clinical study. Caries Res 2000;34:140-3.
23. Øgaard B, Larsson E, Henriksson T, Birkhed D, Bishara SE. Effects of combined application of antimicrobial and fluoride varnishes in orthodontic patients. Am J Orthod Dentofacial Orthop 2001;120:28-35.
24. Pinar Erdem A, Sepet E, Kulekci G, Trosola SC, Guven Y. Effects of two fluoride varnishes and one fluoride/chlorhexidine varnish on Streptococcus mutans and Streptococcus sobrinus biofilm formation in vitro. Int J Med Sci 2012;9:129-36.
25. James P, Parnell C, Whelton H. The caries-preventive effect of chlorhexidine varnish in children and adolescents: a systematic review. Caries Res 2010;44:333-40.
26. Buzalaf MA, Hannas AR, Magalhães AC, Rios D, Honório HM, Delbem AC. pH-cycling models for in vitro evaluation of the efficacy of fluoridated dentifrices for caries control: strengths and limitations. J Appl Oral Sci 2010;18:316-34.
27. Gomez J, Tellez M, Pretty IA, Ellwood RP, Ismail AI. Non-cavitated carious lesions detection methods: a systematic review. Community Dent Oral Epidemiol 2013;41:54-66.
28. Kühnisch J, Heinrich-Weltzien R. Quantitative light-induced fluorescence (QLF)--a literature review. Int J Comput Dent 2004;7:325-38.
29. Ando M, van Der Veen MH, Schemehorn BR, Stookey GK. Comparative study to quantify demineralized enamel in deciduous and permanent teeth using laser- and light-induced fluorescence techniques. Caries Res 2001;35:464-70.
30. Kim HE, Kim BI. An in vitro comparison of quantitative light-induced fluorescence-digital and spectrophotometer on monitoring artificial white spot lesions. Photodiagnosis Photodyn Ther 2015;12:378-84.