Effect of topical applications of a chlorhexidine/thymol-containing varnish on fissure caries assessed by laser fluorescence

Kerstin Sköld-Larsson, Ann-Charlott Fornell, Adrian Lussi and Svante Twetman

Department of Orthodontics, Maxillofacial Unit, County Hospital, Halmstad, Sweden; Department of Restorative Dentistry, University of Bern, Bern, Switzerland; Department of Odontology, Pediatric Dentistry, Faculty of Medicine, Umeå University, Umeå, Sweden

Sköld-Larsson K, Fornell A-C, Lussi A, Twetman S. Effect of topical applications of a chlorhexidine/thymol-containing varnish on fissure caries assessed by laser fluorescence. Acta Odontol Scand 2004;62:339–342. Oslo. ISSN 0001-6357.

The aim of this study was to monitor the effect of an antibacterial varnish using the readings of a caries-detecting device (DIAGNOdent) in fissures of young permanent molars. The material consisted of 32 healthy patients with a mean age of 14.1 years undergoing orthodontic treatment with fixed appliances. The inclusion criteria were presence of one homologous pair of 2nd upper or lower molars with clinically intact occlusal surfaces. A split-mouth study design was used in which the fissures were treated with either an antibacterial chlorhexidine/thymol-containing varnish or a placebo varnish every 6th week. The follow-up period was 42 weeks and laser fluorescence (LF) readings were carried out every 12th week. The mean LF values increased significantly ($P < 0.05$) after 24, 36, and 48 weeks compared to baseline following the placebo treatments but not after treatment with the active antibacterial varnish. During the study period, micro-cavities were diagnosed in two test-treated and five placebo-treated teeth. In conclusion, the results reinforce previous findings that frequent applications of a chlorhexidine/thymol-containing dental varnish might have a protective role in fissures of young permanent molars and that this could be monitored with a chair-side caries detecting LF device. ☐ *Chlorhexidine; fissures; laser fluorescence; prevention*

Svante Twetman, Department of Odontology, Faculty of Medicine, Umeå University, SE-901 87 Umeå, Sweden. Fax. +46 90 770 330, e-mail. svante.twetman@odont.umu.se

Chlorhexidine is an antibacterial agent with a selective suppressive effect on cariogenic mutans streptococci and is therefore incorporated in dental varnishes (1). Previous studies have suggested that varnish applications of this kind may be especially effective in preventing caries in fissures of young permanent molars, i.e. explained by the retentive nature of the molar anatomy (2–6). A shortcoming, however, is that in most cases the caries diagnosis was based on clinical examination with an explorer and that only one study utilized bitewing radiographs. Diagnosis of non-cavitated fissure caries is a complex issue, and in recent years a chair-side device based on laser fluorescence (LF) has become available to provide a 'second opinion'. The accuracy has been evaluated in vitro as well as in clinical cross-sectional studies and, generally, an acceptable reproducibility and correlation to fissure caries has been demonstrated (7–9). Although in a recent publication a very weak correlation was found between LF readings, lesion depth, and bacterial counts (10), other investigators have suggested that the technique can be used to monitor progression longitudinally (11) and also regression of caries lesions (12). The background thinking for the present study was to find out whether or not LF recordings could be used as a surrogate endpoint in evaluating the caries-preventive effect of topical antibacterial treatments.

The aim of this study was therefore to monitor the effect of an antibacterial chlorhexidine/thymol-containing varnish from the readings of a caries-detecting LF device (DIAGNOdent) in fissures of young permanent molars. The null hypothesis was that the readings would not differ from baseline for either the active or the placebo varnish.

Materials and methods

The material comprised 32 healthy patients (19 F and 13 M) undergoing orthodontic treatment with fixed appliances with homologous pairs of 2nd upper or lower molars with clinically intact occlusal surfaces. Mean age was 14.1 years (range 12–18 years). With the exception of the malocclusions, the subjects had good oral health with no untreated caries lesions, and they were inhabitants of a community with low fluoride levels in the piped drinking water (<0.2 ppm F). All claimed that they brushed their teeth with fluoridated toothpaste at least once daily. During the period of orthodontic treatment, the subjects were advised to rinse daily with 10 mL of a 0.05% sodium fluoride solution. Six subjects dropped out before the final examination, four were debonded and discharged from the clinic, and two were excluded due to no-shows and failure to comply with the regime. A total of 26 patients therefore completed all registrations.

Study design

The study design was approved by the ethics committee at Lund University, and informed consent was obtained from the subjects as well as their parents. The number of

Taylor&Francis healthsciences

DOI 10.1080/00016350410010072

decayed and filled surfaces, as well as the number of proximal lesions within the enamel seen on bitewing radiographs, was collected from the latest available records (not older than 6 months) at the Public Dental Service. At baseline, the number of salivary mutans streptococci was estimated with a chair-side test (Dentocult SM-Strip mutans; Orion Diagnostica, Helsinki, Finland) in accordance with a standardized method (13, 14). The strips were categorized into low counts (score 0 + 1; <50 colony forming units, CFU) and medium or high counts (score 2 + 3; ≥50 CFU) based on the number of CFUs present on the strips after cultivation. The occlusal surfaces on the included teeth were thoroughly examined with a mirror and explorer in optimal light after drying and judged as clinically intact. Thereafter, a LF reading was taken as described below. The paired teeth were then randomly assigned to treatment with either the chlorhexidine/thymol-containing varnish (Cervitec©, Vivadent, Schaan, Liechtenstein) or the placebo varnish without the active antibacterial ingredients. After drying and cleaning, the varnishes were applied in a thin layer with a micro-brush every 6th week during the 48-week study period. The LF readings were conducted after 12, 24, 36, and 48 weeks. The patient's general dentist, who was informed of the objective of the study, maintained responsibility for deciding on and carrying out any restorative treatment during the follow-up period.

Laser-fluorescence readings

The diagnostic LF recordings were carried out with DIAGNOdent, a chair-side laser device from KaVo (Biberach, Germany) which allows reading values from 0 to 99. One device and the same tip were used throughout the study. First, the instrument was calibrated using a ceramic standard provided by the manufacturer. The measurements were performed after 5 s drying with compressed air and a reference value from intact buccal enamel was obtained. The tip was thereafter applied in the central occlusal fissure and the instrument was slightly tilted and rotated along its own axis. All sites were measured twice and the single peak value was recorded. Within each patient, all registrations were carried out by the same clinician. Intra-examiner agreement was tested by repeated measurements of 20 upper and lower molars in 10 subjects within 1 week. Prior to study, the fluorescence response for the active and the placebo varnish was evaluated in vitro on extracted teeth and no difference was explored. It should be noted that the LF measurements were performed before the applications and no remnants of the varnishes were clinically visible in the fissures at the 6-week follow-ups.

Statistical method

All data were processed with the SPSS software (version 11.5, Chicago, Ill., USA). For intra-examiner agreements, Spearman's rank correlation coefficient was calculated. The follow-up values were compared with the baseline registrations with the aid of the non-parametric Wilcoxon paired test. Odds ratio was calculated for caries development ('yes' vs. 'no') in relation to baseline mutans streptococci counts. The level of significance was set to 5% ($P < 0.05$).

Results

The mean number of decayed and filled surfaces was 1.1 DFS, ranging between 0 and 5, while the corresponding value for approximal enamel lesions was 0.3 (range 0–6). Fifty-seven percent were clinically caries-free. The majority of the participants (63%) had medium or high counts of salivary mutans streptococci at baseline (scores 2 and 3). Intra-examiner agreement of the LF measurements was statistically significant for both operators $r = 0.83$ and 0.79 ($P < 0.05$), respectively. The LF readings at baseline and at the designated follow-ups are presented in Table 1. The mean values increased significantly after 24, 36, and 48 weeks following the placebo treatments ($P < 0.05$), but not after treatment with the active antibacterial varnish. However, no statistically significant differences were obtained between the treatment groups at any follow-up, although it was close to significance ($P = 0.07$) after 48 weeks. Five occlusal surfaces were sealed or filled in the placebo group compared with two in the active varnish group during the study period. The association between salivary mutans streptococci and caries development is given in Table 2. There was a tendency for children with high bacterial counts to be more prone to develop new caries (OR = 5.4), but this relationship was not statistically significant. In the molars with caries development, the mean ΔLF values (difference between last and first recordings) were higher compared to those that remained caries-free.

Discussion

The primary aim of this study was to evaluate the use of a caries detection device in monitoring the caries-preventive

Table 1. Mean values of occlusal DIAGNOdent readings in 2nd permanent molars treated with a chlorhexidine/thymol-containing dental varnish or placebo. Twenty-six subjects completed all registrations, but 2 and 5 teeth were either sealed or restored in the CHX/thymol and placebo groups, respectively

	CHX/thymol		Placebo	
Time	*n*	Mean ± *s*	*n*	Mean ± *s*
Baseline	32	3.5 ± 2.1	32	3.3 ± 1.7
12 weeks	32	3.4 ± 2.4	32	3.6 ± 1.7
24 weeks	30	3.8 ± 2.5	30	4.6[a] ± 3.1
36 weeks	26	3.8 ± 2.5	24	5.2[a] ± 4.2
48 weeks	24	4.0 ± 3.9	21	6.8[a] ± 6.2

[a] Statistically significant difference compared with baseline ($P < 0.05$), paired Wilcoxon test.
s = Standard error.

Table 2. Two-by-two table showing fissure caries development during the study period in relation to baseline salivary mutans streptococci (MS) scores ($n = 26$). The mean ΔLF value from baseline to latest reading is given in parentheses

	48-week caries development	
Baseline MS	Yes	No
Low (score 0 + 1)	1 (5.0)	9 (0.3)
Medium or high (score 2 + 3)	6 (6.6)	10 (2.1)

Odds ratio 5.4 (95% CI 0.5–53.9; not statistically significant).

effect of an antibacterial varnish in fissures of young permanent molars. The split-mouth technique with homologous tooth pairs used here must be considered as an adequate study design in light of the skewed distribution of caries activity, although an uncontrolled carry-over effect of active ingredients cannot be excluded. However, a possible carry-over of the active ingredient to the placebo-treated teeth would likely mask a difference rather than exaggerate one. Patients undergoing treatment with fixed orthodontic appliances were selected for practical reasons, since they had newly erupted 2nd permanent molars and were scheduled for regular appointments every 6th week. It must therefore be noted that they may not be representative of a group of adolescents with increased or high caries risk in fissures. Still, seven of the included surfaces were sealed or restored during the follow-up period.

The LF measurements were performed by two clinicians but with the one single device and one tip, which eliminated variations between different instruments. With the diagnostic device, the tooth surface was illuminated with continuous red laser light with a wavelength of 655 nm, and the fluorescence emitted was more intense for demineralized enamel compared to sound hard tissue as described by König et al. (15). Clinically, the DIAGNOdent readings were quick and easy to perform with good acceptance from the patients. The reference value of each tooth obtained from sound enamel was repeatable from time to time within each individual but varied between subjects from 1 to 4. These stable reference values indicate that the operators were successful in repositioning the tip longitudinally.

The results demonstrated significantly increased LF values with time in the placebo-treated fissures, which was in contrast to the surfaces treated with the varnish containing active components. The null hypothesis was thus rejected. It has to be pointed out, however, that the mean values in general were low, although individual values over '30' in the micro-cavitated teeth were recorded. The results supported the assumption that frequent applications of a chlorhexidine/thymol-containing dental varnish might have a protective role in fissures of young permanent molars (16). Interestingly, the present findings in favor of the antibacterial varnish were obtained in patients with regular and additional exposure of daily fluoride, while previous trials were performed in populations or study groups in which the exposure to fluoride supplements was uncertain or even lacking (2–6). Our present results were also in harmony with recent papers suggesting that LF can be used longitudinally to monitor changes of lesions (11, 12, 17), but this must be validated in further clinical studies with direct or indirect correlations to the mineral content of the lesion. Such an indirect explanation to our findings may be lower levels of microorganisms in the CHX/thymol-treated fissures since the fluorescence response may be due to bacteria or their metabolites (18). The fact that the laser-fluorescence device may offer an alternative non-invasive method for detection of caries in fissures in clinical intervention trials was highly interesting and the technology should be considered as an 'add on' to the standard visual-tactile examination in future caries trials. This may be of special importance in low-caries populations in which indications for bitewing radiographs are lacking. Moreover, the use of LF as an alternative surrogate endpoint in caries trials may enable a shorter study duration, which is an advantage in terms of human efforts and financing.

In conclusion, this study displayed significantly different LF readings over time in molars treated with antibacterial and placebo varnishes, suggesting that this method may offer an alternative way of monitoring the outcome of caries preventive measures in fissures of young permanent teeth. Further clinical validation studies and extended prospective trials are needed, however, in which the LF readings are linked to the progression, or regression, of caries lesions.

Acknowledgements.—This study was supported by a grant from the Swedish Patent Revenue Fund and the County Council of Halland. The varnishes were generously provided by Vivadent, Schaan, Liechtenstein.

References

1. Emilson CG. Potential efficacy of chlorhexidine against mutans streptococci and human dental caries. J Dent Res 1994;73:682–91.
2. Bratthall D, Serinirach R, Rapisuwon S, Kuratana M, Luangjarmekorn, Luksila K, et al. A study into the prevention of fissure caries using antimicrobial varnishes. Int Dent J 1995;45: 245–54.
3. Fennis-Ie YL, Verdonschot EH, Burgersdijk RCW, König KG, van't Hof MA. Effect of 6-monthly applications of chlorhexidine varnish on incidence of occlusal caries in permanent molars. J Dent 1998;26:233–8.
4. Joharji RM, Adenubi JO. Prevention of pit and fissure caries using antimicrobial varnish: 9-month clinical evaluation. J Dent 2001;29:247–54.
5. Araujo AMPG, Naspitz GMCC, Chelotti A, Cai S. Effect of Cervitec® on mutans streptococci in plaque and on caries formation on occlusal fissures of erupting permanent molars. Caries Res 2002;36:373–6.
6. Baca P, Munoz MJ, Bravo M, Junco P, Baca AP. Effectiveness of chlorhexidine-thymol varnish for caries reduction in permanent first molars of 6–7-year-old children: 24-month clinical trial. Community Dent Oral Epidemiol 2002;30:363–8.

7. Lussi A, Imwinkelried S, Pitts NB, Longbottom C, Reich E. Performance and reproducibility of a laser fluorescent system for detection of occlusal caries in vitro. Caries Res 1999;33:261–6.
8. Lussi A, Megert B, Longbottom C, Reich E, Francescut P. Clinical performance of a laser fluorescence device for detection of occlusal caries lesions. Eur J Oral Sci 2001;109:14–19.
9. Lussi A, Francescut P. Performance of conventional and new methods for the detection of occlusal caries in deciduous teeth. Caries Res 2003;37:2–7.
10. Ástvaldsdóttir A, Holbrook WP, Tranæus S. Consistency of DIAGNOdent instruments for clinical assessment of fissure caries. Acta Odontol Scand 2004;62:193–8.
11. Anttonen V, Seppä L, Hausen H. A follow-up study of the use of DIAGNOdent for monitoring fissure caries in children. Community Dent Oral Epidemiol 2004;32:312–8.
12. Andersson A, Sköld-Larsson K, Hallgren A, Petersson LG, Twetman S. Measuring regression of enamel lesions with a laser fluorescence device (DIAGNOdent): a pilot study. Orthodontics 2004;1:201–5.
13. Jensen B, Bratthall D. A new method for the estimation of mutans streptococci in human saliva. J Dent Res 1989;68:468–71.
14. Twetman S, Frostner N. Salivary mutans streptococci and caries prevalence in 8-year-old Swedish schoolchildren. Swed Dent J 1991;15:145–51.
15. König K, Flemming G, Hibst R. Laser induced autofluorescence spectroscopy of dental caries. Cell Mol Biol 1998;44:1293–2000.
16. Twetman S. Antibacterials in future caries control? A review with special references to chlorhexidine treatment. Caries Res 2004;38:223–9.
17. Lussi A, Hibst R, Paulus R. DIAGNOdent: an optical method for caries detection. J Dent Res 2004;83:C80–3.
18. Hibst R, Paulus R, Lussi A. Detection of occlusal caries by laser fluorescence: basic and clinical investigations. Med Laser Appl 2001;16:205–13.

Received for publication 15 October 2004
Accepted 29 November 2004