Effect of a Dental Cream Containing Amorphous Calcium Phosphate Complexes on White Spot Lesion Regression Assessed by Laser Fluorescence

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Purpose: To investigate and compare the effects of a dental cream containing complexes of casein phosphoprotein-amorphous calcium phosphate (CPP-ACP) and fluoride mouthwashes on the regression of white spot lesions (WSL).

Materials and Methods: The study group consisted of 26 healthy adolescents (mean age 14.6 years) exhibiting 60 teeth with 152 visible WSL sites on incisors and canines immediately after debonding of fixed orthodontic appliances. After bracket removal, professional tooth cleaning and drying, a visual scoring (0–4) and laser fluorescence (LF) readings were carried out. The patients were randomly assigned to two different treatment protocols with the aim of remineralising the lesions: A) daily topical applications of a dental cream containing CPP-ACP (Topacal) for 3 months followed by a 3-month period of daily toothbrushing with fluoridated dentifrice, or B) daily 0.05% sodium fluoride mouthwash combined with fluoridated dentifrice for 6 months. The registrations were repeated after 1, 3, 6 and 12 months and follow-up data were compared with baseline with aid of chi-square and paired t-tests.

Results: A significant improvement of the clinical WSL-scores was found over time in both groups, but there was a statistically significant difference ($p<0.01$) concerning the number of sites that totally disappeared after 12 months in favour of the CPP-ACP regime, 63% compared with 25% respectively. The clinical registrations were mirrored by a statistically significant decrease ($p<0.05$) in the LF readings at the 6- and 12-month follow-ups compared with baseline. No significant differences were displayed between the groups.

Conclusions: Clinical scoring and LF assessment suggested that both regimens could promote regression of WSL after debonding of fixed orthodontic appliances. The visual evaluation suggested an aesthetically more favourable outcome of the amorphous calcium phosphate treatments.

Key words: lesion regression, milk derivate, orthodontics, sodium fluoride mouthwash, white spot lesions

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Treatment with fixed orthodontic appliances is associated with accumulation of dental plaque with an increased risk for incipient enamel caries. The incidence of such white spot lesions (WSL) adjacent to bands and brackets has been estimated at 15–85% (Mitchell, 1992). Even in a population with low caries prevalence, as many as 61% may develop white spots during orthodontic treatment despite a comprehensive preventive program (Øgaard et al, 2001). After debonding, a saliva-mediated remineralisation normally takes place and, to facilitate this process, topical applications of fluorides are advocated (Kleber et

Table 1 Clinical index used for visual evaluation of white spot lesions (WSL) on facial sites after debonding of fixed orthodontic appliances

Score	Definition
0	No visible colour change
1	Slight white colour change, only visible after air-drying
2	Slight colour change with certain marked white areas
3	White consistent colour change
4	Distinct white colour change

al, 1999; Alexander and Ripa, 2000; Tranæus et al, 2001; Bergstrand and Twetman, 2003). In the last decade, a novel approach to enhancing remineralisation based on a milk derivate containing casein phosphopeptides (CPP) and amorphous calcium phosphate (ACP) was suggested (Reynolds, 1998). CPP are phosphorylated peptides that can stabilise calcium phosphate as nanoclusters of ACP on the tooth surface, which can act in the sub-surface part of a lesion (Reynolds et al, 2003). The CPP-ACP complexes are available in a variety of gels, creams or mousses and may also be incorporated into chewing gums. Since a number of promising *in situ* studies are available (Shen et al, 2001; Cai et al, 2003; Reynolds et al, 2003; Iijima et al, 2004; Walker et al, 2006), it was though of interest to test and compare a CPP-ACP regimen with a conventional fluoride programme for efficacy of inducing regression of early enamel lesions. One of the problems involved in such a study, however, is quantifying the enamel alterations in an objective way. The authors' group has recently described the use of a chair-side caries-detecting device based on laser fluorescence (LF; DIAGNOdent) as a surrogate endpoint in caries-preventive trials (Sköld-Larsson et al, 2004) and as a tool to monitor WSL regression in adjunct to visual inspection (Andersson et al, 2004). The aim of this study was therefore to investigate and compare the effect of topical applications of a dental cream containing CPP-ACP with conventional fluoride mouthwashes on the regression of WSL, assessed by visual inspection and LF readings after the removal of fixed orthodontic appliances.

MATERIALS AND METHODS

Patients

The study group consisted of 26 healthy adolescents of both sexes (13 boys and 13 girls) with WSL on maxillary or mandibular incisors and canines immediately after debonding of fixed orthodontic appliances. They were selected from cases that were treated with fixed orthodontic appliances during one calendar year at the Department of Orthodontics in Halmstad, Sweden. The mean age was 14.6 years (range 12–16 years) and the patients and their parents consented to participate after verbal and written information. All subjects had good oral health with no untreated caries lesions and were inhabitants in a community with low fluoride content in the piped drinking water (<0.2 ppm). They claimed regular toothbrushing habits with fluoride dentifrice at least twice a day. During the period of orthodontic treatment, the subjects had been instructed to rinse daily with 10 ml of a 0.05% sodium fluoride solution.

Study design, clinical examination and selection of follow-up sites

The study protocol was approved by the local ethics committee at Lund University, Sweden. After the bracket removal, all remaining composite material on the buccal surfaces was removed with a carbide bur followed by polishing with pumice paste. After thorough drying with air, each side around the former bracket base (cervical, mesial, distal, incisal) was graded with respect to WSL severity by a clinical index, as shown in Table 1 (Andersson et al, 2004). Thereafter, diagnostic LF recordings were carried out as described below. Only sites on maxillary or mandibular incisors or canines with a WSL score ≥ 2 (n=152), which meant that changes were clinically visible without air-spray drying, were included for the longitudinal follow-up. The patients were randomly assigned with a dice to one of two 6-month treatment regimes: A) a test group (n=13; 70 sites) that was asked to brush their teeth twice daily with a CPP-ACP-containing dental cream without fluoride (Topacal C-5, Nulite Systems International, Hornsby, Australia) for 3 months followed by a normal use of a standard fluoride dentifrice (1000–1100 ppm) for the next 3 months, and B) a control group (n = 13; 62 sites) that was asked to use a conventional 0.05% sodium fluoride mouthwash once daily together with a standard fluoride dentifrice for the entire 6-month period. The visual inspection and the LF readings were repeated 1, 3, 6 and 12 months after the start of the interventions. The clinical recordings were performed by one blinded examiner (AA) who was calibrated before the start of the study. The examiner could not see the previous registered clinical scores or LF values at the follow-up sessions. The intra-examiner agreement

Table 2 Relationship between visual scores and laser fluorescence readings of the selected 60 teeth (240 sites) immediately after debonding of fixed orthodontic appliances (r=0.59, p<0.01; Pearson correlation test)

Clinical score	n	Laser fluorescence readings		
		Mean	SD	range
0	64	1.8	0.9	0–3
1	44	3.2	1.7	1–11
2	58	4.5	2.1	2–12
3	34	5.9	2.4	3–13
4	40	16.0	5.2	2–70

SD, standard deviation

has previously been reported (Andersson et al, 2004). One male in group B was lost during follow-up due to relocation.

Laser fluorescence recording

The diagnostic LF recordings were performed with DIAGNOdent, a chair-side laser device (655 nm) from KaVo (Biberach, Germany) allowing reading values from 0–99. The device was calibrated using a ceramic standard provided by the manufacturer. The measurements were carried out after thorough drying with air spray and a reference value from intact buccal enamel was obtained. The flat tip was thereafter moved along the selected sites and the peak value of each site was identified. All sites were measured twice and the highest value was recorded. One single device was used for all measurements.

Statistical methods

The relationship between variables was calculated with the Pearson correlation coefficient. The follow-up LF readings were compared with baseline (debonding) values using Student's paired 2-tailed *t*-test. The categorised scores were compared using chi-square tests. Differences between the groups were assessed by the unpaired Wilcoxon test. The level of significance was set to 5% ($p < 0.05$).

RESULTS

A total of 60 teeth were followed throughout the study period, of which 57 were maxillary. The majority were lateral incisors (58%) followed by canines (24%) and central incisors (18%). There was a significant correlation between the clinical WSL scores and the LF measurements at baseline (Table 2). The distribution of the visual scores at baseline and at the designated follow-ups is presented in Table 3. A statistically significant ($p < 0.05$) improvement over time was evident in both groups, but more sites became invisible in the test group. After 3 months, the proportion of sites scored as 0 and 1 was 55% in the CPP-ACP group compared with 18% in the fluoride mouthwash group. The corresponding values after 12 months were 64% and 23% respectively, a difference that was statistically significant ($p < 0.01$). Approximately 10% of the sites remained unchanged, with the highest score (score 4) one year after debonding of the fixed appliances in both groups. The corresponding LF readings are shown in Table 4. The values decreased with time and were significantly lower ($p < 0.05$) after 6 and 12 months compared with baseline in both groups. However, no significant differences were disclosed between the two treatment regimes, either at baseline or at any of the follow-ups.

DISCUSSION

WSL after fixed appliances might persist as visible damage and it is therefore of great interest for both patients and orthodontists to achieve and monitor a complete remineralisation. CPP-ACP-containing products are currently marketed for white spot removal after orthodontic bracket treatment and the present study was designed to investigate whether or not this was the case. We compared the effects of a self-applied, thixotropic, tooth-surface-coating cream containing Phoscal® with a conventional fluoride mouthwash

Table 3 Distribution of clinical WSL-scores at baseline and at the designated follow-up examinations in the fluoride rinse group (n = 13) and in the CPP-ACP group (n = 13). The values denote the percentage of the sites with score ≥ 2 at baseline

Group/time	WSL-score 0	1	2	3	4
F-rinse (n = 62 sites)					
Baseline	-	-	42%	31%	27%
1 month	-	16%	53%	18%	13%
3 months	-	18%	58%	15%	9%
6 months	2%	18%	53%	16%	9%
12 months	2%	21%	53%	15%	9%
CPP-ACP (n = 70 sites)					
Baseline	-	-	46%	21%	33%
1 month	6%	39%	28%	18%	19%
3 months	11%	44%	28%	18%	9%
6 months	16%	46%	25%	4%	9%
12 months	22%	42%	23%	5%	8%

Table 4 Mean laser fluorescence values at baseline at the designated follow-up examinations in the fluoride rinse group and the CPP-ACP group.

Time	F-rinse (n = 62 sites) mean ± SD (min–max)	CPP-ACP (n = 70 sites) mean ± SD (min–max)
Baseline	9.4 ± 9.5 (2–60)	7.4 ± 10.2 (2–70)
1 month	7.6 ± 9.2 (2–60)	5.5 ± 6.7 (2–38)
3 months	6.8 ± 8.1 (2–47)	4.9 ± 5.5 (1–36)
6 months	6.4* ± 7.3 (2–47)	4.6* ± 5.1 (1–36)
12 months	6.4* ± 7.5 (2–47)	4.4* ± 5.2 (1–36)

* significantly different from baseline ($p<0.05$)

regime. It should be noted that the participants in the study had developed their WSL in spite of advocated daily rinsing with 0.05% NaF during the treatment period. The patients were highly motivated to combat their incipient lesions and there was no reason to believe that the compliance differed between the two treatment groups.

The visual recordings displayed the interesting and novel finding that the milk-derived protocol seemed to be superior to fluoride at reducing the WSL scores. This may of course be a purely optical phenomenon, but in light of previous findings (Reynolds, 1998; Reynolds et al, 2003), a beneficial sub-surface effect of the phosphoprotein–calcium-phosphate complex is plausible. The milk-protein-based formulation is designed to significantly augment the natural salivary healing process, and the nanoclusters of ACP are small enough to access demineralised areas underneath an already remineralised surface zone (Iijima et al, 2004). Therefore a 'deep' regression with a favourable aesthetic appearance may be possible to achieve. Assuming that the patient's perception of WSL is based on its visibility, almost three times as many WSL sites disappeared with the CPP-ACP therapy after 12 months. However, a marked visual difference between the treatments was seen after only 1 month and it should be stressed that a clinical improvement was also noticed among the severe sites with initial WSL scores of 3 and 4. This was especially notable for score 3, after 3 months in the CPP-ACP group when the participant changed the treatment regime to sodium fluoride dentifrice.

The LF measurements were performed by one clinician and with one single device and tip, which eliminated possible variations between instruments. The reference value of each tooth obtained from sound enamel was repeatable at different times within each individual but varied between the subjects from 1 to 4. The correlation between the LF readings and the severity of the lesions as well as the diminishing values over time was in agreement with our previous observations (Andersson et al, 2004). However, no significant difference was detected between the treatment groups and this could be explained by the fact that the LF instrument does not directly quantify the degree of mineralisation or the mineral content per se. The tooth surface is illuminated with red laser light with a wavelength of 655 nm and it has been shown that a more intense fluorescence is emitted from demineralised enamel than from a sound hard tissue, probably due to the organic content such as bacteria and their metabolites (König at al, 1998; Lussi et al, 2004). Although the LF readings should thus be regarded as an indirect measure of demineralisation, the present results were in agreement with recent studies suggesting that DIAGNOdent can be used longitudinally to monitor changes of lesions (Al-Khateeb et al, 1998; Anttonen et al, 2004; Lussi et al, 2004; Sköld-Larsson et al, 2004). The measurements were highly repeatable within the individual but varied to a great extent between the subjects. Therefore it was not possible to establish any firm cut-off points for the different WSL scores. With few exceptions, however, a DIAGNOdent value of ≥ 10 was found at the sites with the highest clinical score.

In conclusion, the present study suggests an aesthetically beneficial effect of a dental cream containing complexes of CPP-ACP on WSL regression compared with a fluoride mouthwash programme, when assessed visually. The clinical improvement was verified by chair-side LF readings showing significantly reduced values after 6 months compared with baseline in both groups. The method was not, however, able to reveal any differences between the treatment regimes. Further clinical studies are needed to establish the optimal way to prevent WSL formation during orthodontic treatment with fixed appliances and to promote its remineralisation after bracket removal.

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