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Colour improvement and stability of white spot lesions following infiltration, micro-abrasion, or fluoride treatments *in vitro*

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SUMMARY

BACKGROUND/OBJECTIVES: White spot lesions (WSLs) are unwelcome side effects of fixed appliances that compromise the treatment outcome. Recently, infiltration of WSLs has been introduced as a viable treatment alternative. The objective was to evaluate the colour improvement of WSLs and their stability against discolouration following infiltration, fluoride, or micro-abrasion treatments *in vitro*.

MATERIALS/METHODS: Artificial WSLs were created in bovine enamel ($N = 96$) using acidic buffer solution (pH 5, 10 days) and were randomly allocated to four groups. Specimens were treated with infiltration (Icon, DMG), fluoride (Elmex Caries Protection, GABA), and micro-abrasion (Opalustre, Ultradent) or remained untreated (control). Groups were discoloured for 24 hours in tea or tea + citric acid. Colour components and visible colour change (L*, a*, b*, ΔE) were measured spectrophotometrically on following time points: baseline, after WSL formation, after treatment, and during discolouration (8, 16, and 24 hours). Data were analysed using Kruskal–Wallis and Mann–Whitney tests.

RESULTS: WSL formation increased (L*) in all groups. Only infiltration reduced this effect to baseline. Highest ΔE improvement was obtained by infiltration and micro-abrasion followed by fluoride. This improvement was stable only for infiltration during discolouration. L*, a*, and b* changed significantly during discolouration in all groups except infiltration. Within the same treatment group, discolouration solutions did not differ significantly.

LIMITATIONS: *In vitro* testing cannot replicate the actual mode of colour improvement or stability but can be used for ranking materials and techniques.

CONCLUSIONS/IMPLICATIONS: Infiltration and micro-abrasion treatments were capable of diminishing the whitish appearance of WSLs. Only infiltrated WSLs were stable following discolouration challenge.

Introduction

Subsurface enamel demineralizations are known as white spot lesions (WSLs), and they represent the early phase of caries formation (Derks *et al.*, 2004; Bergstrand and Twetman, 2011). Prevalence of WSLs is relatively high, affecting more than 25 per cent of the patients receiving orthodontic treatment, acquiring at least one new lesion during treatment (Hadler-Olsen *et al.*, 2012; Lucchese and Gherlone, 2013). Demineralization may take place rapidly, as fast as within 4 weeks after the placement of brackets and can stay present even years after treatment (Bergstrand and Twetman, 2011). Clinically, surfaces are intact when gently probed in early phases. However, cavitation may occur if the cariogenic challenge is ongoing, which might lead to the necessity of invasive restorative treatments (Derks *et al.*, 2004; Bergstrand and Twetman, 2011).

As light refraction through enamel is directly related to the level of mineralization, WSLs manifest themselves as white opacities visually (Derks *et al.*, 2004; Bergstrand and Twetman, 2011). The most superficial layer is richer in calcium, and the inner structure is more porous due to mineral loss. In the presence of cariogenic environment, demineralization progresses and the appearance may get more opaque (Derks *et al.*, 2004; Bergstrand and Twetman, 2011). WSLs might become even more perceptible when extrinsic staining occurs, which may compromise the aesthetic outcome of orthodontic treatment (Addy and Moran, 1995; Watts and Addy, 2001).

Although there is no golden standard for WSL treatment, three treatment modalities are more frequently preferred, depending on the degree and activity level of the lesion. Because WSL is a form of demineralization, remineralization is the most conservative method to be tried primarily

(Derks *et al.*, 2004; Bergstrand and Twetman, 2011; Hamdan *et al.*, 2012). Remineralization can be obtained through increasing the calcium and phosphate content in the oral environment and forming more stable compounds such as calcium fluoride (Marinho *et al.*, 2003; Beerens *et al.*, 2010; Hamdan *et al.*, 2012). Numerous studies have been performed aiming at the regression of early demineralizations by means of fluoride application. It was reported that regular topical fluoride application is an efficient method for preventing and remineralizing early enamel caries (Marinho *et al.*, 2003; Beerens *et al.*, 2010). Low concentrations of fluoride application have been advocated in order not to hyper-mineralize the outer surface, which might obstruct further remineralization of deeper enamel lesions (Marinho *et al.*, 2003; Hamdan *et al.*, 2012). Another treatment option is micro-abrasion, performed either by using hydrochloric acid (HCl) containing abrasive slurry or abrasive powders applied with high-pressurized air (Murphy *et al.*, 2007; Neuhaus *et al.*, 2010). Micro-abrasion mainly aims to remove the discoloured enamel mechanically. The prompt improvement in the appearance of the lesion and clinical results has made this technique a feasible treatment option (Murphy *et al.*, 2007; Neuhaus *et al.*, 2010; Pliska *et al.*, 2012). Because micro-abrasion is comparably more invasive in nature, delayed application was thought to be beneficial considering the spontaneous improvements of the lesion via saliva-based remineralization and spontaneous surface abrasion following debonding (Bergstrand and Twetman, 2011; Hamdan *et al.*, 2012). More recently, a minimally invasive treatment approach was introduced, in which the WSL is infiltrated using a low-viscosity resin (Paris *et al.*, 2007a,b; Kielbassa *et al.*, 2009). In this technique, the outer surface is transformed into a more permeable layer with the help of HCl etching, and the porous structure beneath is infiltrated using a triethyleneglycol dimethacrylate-based resin (Paris *et al.*, 2007a,b; Kielbassa *et al.*, 2009). It is noteworthy that this resin has a light refraction index similar to sound enamel, which improves the appearance of the lesion besides reinforcing the weakened enamel prism structure (Paris *et al.*, 2007b; Kielbassa *et al.*, 2009).

Assessment of the therapeutic effect provided by these methods has been made under various settings. Micro-abrasion (Murphy *et al.*, 2007; Neuhaus *et al.*, 2010; Pliska *et al.*, 2012) and infiltration (Kielbassa *et al.*, 2009; Torres *et al.*, 2011; Hammad *et al.*, 2012; Kim *et al.*, 2013; Paris *et al.*, 2013; Soviero *et al.*, 2013) methods were reported to produce satisfactory results compared with remineralization using fluoride or amorphous calcium phosphate derivatives. However, to the authors' best knowledge, comparison of the colour outcome achieved by micro-abrasion, infiltration, and fluoride remineralization has not been made previously. Furthermore, no information is available on how these treated surfaces will show resistance against discolouration that might also be subjected to extrinsic discolouration later.

Therefore, the aims of this study were 1. to compare the colour-masking effect of infiltration, fluoride remineralization, and micro-abrasion treatments of WSLs and 2. to compare the resistance of these treated surfaces against discolouration. The null hypotheses tested were that 1. the WSL treatment modalities would not produce differences in colour masking compared with non-treated WSLs and that 2. the resistance of treated WSLs would not be better than non-treated WSLs against discolouration.

Materials and methods

Study design

Artificial WSLs created on bovine enamel ($N = 96$, $n = 12$ per test group) were treated forming the following groups: infiltration (Icon; DMG, Hamburg, Germany), fluoride remineralization (Elmex Caries Protection; GABA, Therwil, Switzerland), micro-abrasion (Opalustre; Ultradent, Utah, USA), and control (remain untreated). All specimens were subjected to discolouration in tea or tea + citric acid solutions for 24 hours. Colour components were measured at baseline (prior to demineralization), after WSL formation, after treatment, and after 8-, 16-, and 24-hour discolouration. Chemical compositions and respective manufacturer information of the materials are summarized in Table 1.

Table 1 Composition of the low-viscosity caries infiltrant, enamel micro-abrasion slurry, and the fluoride rinse according to the manufacturers' information.

Product	Chemical composition	Manufacturer
Icon	TEGDMA-based resin matrix, Initiators—additives	DMG, Hamburg, Germany, Batch no. 634902
Opalustre	Hydrochloric acid 6%; Silicon carbide <45%	Ultradent Products, Inc., South Jordan, Utah, USA, Batch no. B6JHJ
Elmex	Aqua, PEG-40 hydrogenated castor oil, olaflur (Amine fluoride 100 ppm F-), aroma, potassium acesulfame, sodium fluoride (150 ppm F-), polyaminopropyl biguanide, hydrochloric acid	GABA International AG, Therwil, Switzerland, Batch no. 10073018

TEGDMA, triethyleneglycol dimethacrylate; PEG, polyethylene glycol.

Specimen preparation

Bovine incisors stored in 0.5 per cent chloramine solution at 4°C no longer than 6 months were initially cut from their roots. Four discs of enamel with a diameter of 3 mm were cut from the labial aspect of each tooth using a custom-made diamond-coated trephine bur (80 µm, Intensiv SA, Lugano-Grancia, Switzerland). The discs were then flattened from the bottom to approximately 3 mm in height (Struers, Birmensdorf, Switzerland). Each piece was randomly assigned to four groups assuring equal distribution of incisal and gingival sections per group. They were embedded with their labial surfaces exposed in auto-polymerizing acrylic resin (Palapress; Heraeus Kulzer, Wehrheim, Germany) in cylindrical moulds (6 mm diameter and 3 mm thickness). Embedded specimens were ground flat and polished with water-cooled carborundum discs (1200, 2400, and 4000 grit; Struers, Erkrat, Germany) and stored in tap water until demineralization.

Demineralization procedure

Demineralization was achieved by immersing the specimens in acidic buffer solution (pH 5, 37°C, 10 days) following the formulation given by Buskes *et al.* (1985). The solution was renewed each second day to keep the pH constant.

WSL treatments

Following demineralization, the specimens were treated forming the following groups:

1. Infiltration: 15 per cent HCl (Icon etch; DMG, Hamburg, Germany) was applied for 120 seconds. Substrates were rinsed with water for 30 seconds and air-dried. They were treated with 99 per cent ethanol (Icon Dry) for 30 seconds and air-dried. Infiltrant (Icon) was applied in one coat with a micro-brush, let set for 180 seconds, light-cured for 60 seconds, a second layer was applied, let set for 60 seconds, and light-cured for 40 seconds. The surfaces were polished with fine and superfine aluminium oxide discs for 20 seconds each (Sof-Lex; 3M, Neuss, Germany).
2. Fluoride: Specimens were immersed in 2 ml of fluoride solution (Elmex Caries Protection) for 1 minute daily for 30 days. In between, they were stored in artificial saliva as described below.
3. Micro-abrasion: Specimens were treated with rubber polishing cups (Produits Dentaires; Vevey, Switzerland) using HCl containing abrasive slurry (Opalustre) at 300 rpm for 1 minute. The surfaces were polished with rubber cups afterwards for 20 seconds.
4. Control: Specimens remained untreated.

All specimens were stored in artificial saliva (3 specimen/25 ml, 37°C) under dark conditions for 30 days prior to discolouration. Artificial saliva was prepared according to the formula given by Klimek *et al.* (1982) and was renewed every 2 days. Measured pH ranged between 6.5 and 6.8.

Discolouration procedure

Black tea and black tea + citric acid were used as discolouration solutions. Black tea was prepared by steeping of 5 teabags (3.125 g/bag, extra strong, Marks and Spencer, Chester, UK) in 1 l of boiling distilled water for 10 minutes. Following this, the tea bags were removed, and the solution was left for cooling. Finally, the pH was measured, which ranged between 4.2 and 4.4. For the preparation of tea + citric acid, the same procedure was repeated, and 0.1 M citric acid was added until pH 4.0 was obtained.

During discolouration, specimens were fixed to the base of boxes containing the discolouration solution using moulding dough (Plastilin; Pelikan, Hannover, Germany). The boxes were then placed in a water bath (3 specimens/25 ml, 37°C, under constant motion). The solution was renewed every 8 hours.

Colour measurements

Colour measurements were assessed at standardized ambient conditions using a spectrophotometer (CM-2600d, Konica Minolta; Osaka, Japan), which was set to standard illuminant D65, 3 mm reading area and 6 mm lighting area. Observer angle was set to 2 degree, and specular component was included. Colour and spectral distributions were measured according to Commission International de l'Eclariage (CIE) L*a*b* system (CIE Colorimetry Publication, 1986), using Spectra Magic NX Version 1.9 colour data software (Konica Minolta, Osaka, Japan). The L* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a* value is the red/green axis where an increase indicates a higher red colour component. The b* value is the yellow/blue axis where an increase indicates higher yellow colour. The visible colour change (ΔE) was calculated as follows (CIE Colorimetry Publication, 1986):

$$\Delta E = [(L_1^* - L_2^*)^2 + (a_1^* - a_2^*)^2 + (b_1^* - b_2^*)^2]^{1/2}$$

ΔE was accepted clinically detectable when it exceeded 3.7 units (Johnston and Kao, 1989). The spectrophotometer was calibrated before each measurement. Prior to each colour measurement, the specimens were taken out from the relevant storage solution regarding the time point, and 300 brushing strokes (Paro M43; medium bristle stiffness, Esro, Kilchberg, Switzerland) were administered in order to eliminate the superficial staining (Attin *et al.*, 2003) using a two-axis brushing machine (Willytec, Feldkirchen-Westerham, Germany) with 2.5 N force (Wiegand and Attin, 2011). The toothpaste slurry consisted of 85 per cent glycerine (10 per cent), 1.62 per cent sodium bicarbonate (10.3 per cent), and carboxymethylcellulose (Göhring *et al.*, 2004).

Two millilitres of slurry for each brushing session was used assuring that the specimens were sufficiently covered. Following brushing, the specimens were rinsed under running water to remove toothpaste remnants and finally rinsed with distilled water. Each specimen was dried using drying paper and immediately placed into the 2 mm diameter frame for colour reading. The reading frame allowed precise repositioning of each specimen at each time point.

Statistical analysis

A sample size of 12 in each group was calculated to have 90 per cent power to detect a difference in means of 3.7 ΔE. This assumes that in one group (control) the standard deviation is 1.4, and in the other group (infiltration) the standard deviation is 3.5 using a two-group Satterthwaite *t*-test with a 0.05 two-sided significance level. Kolmogorov–Smirnov and Shapiro–Wilk tests were used to test normal distribution of the data. As the data were not normally distributed, Kruskal–Wallis test was applied to analyse possible differences between the groups at the same time points and the differences between the time points within each group. This was followed by Mann–Whitney test, separately for all combinations of two group comparisons. Level for significance was set at $P < 0.0083$ for comparisons at the same time points between groups and at $P < 0.0033$ for comparisons between time points within each group according to Bonferroni correction.

Results

Median values, confidence intervals, and groups presenting significant colour changes at baseline, after WSL formation, after treatment, and after discolouration cycles of 8, 16, and 24 hours are presented in Tables 2 and 3.

Lightness (L value)*

Formation of WSL increased the lightness (L* value) significantly in all groups. Only the infiltration treatment diminished the whitish appearance back to the baseline level. Micro-abrasion reduced L* value significantly better than the control and fluoride rinse, but this value was still significantly higher than the baseline measurement. L* value presented a decreasing trend throughout the whole discolouration procedure for all groups except infiltration.

Red–green chromaticity (a value)*

The a* value presented a decrease in all groups after the formation of WSL, indicating a shift to the green component. Following WSL treatment, a* value increased significantly in infiltration and micro-abrasion groups. Starting with the discolouration procedure, a* value increased in all groups, indicating a shift to the red component except for infiltration.

Table 2 Median values and confidence intervals of colour measurements at baseline, after WSL formation, after treatment, and after discolouration cycles (black tea) of 8, 16, and 24h.

Factor	Test group	Baseline	WSL	Treatment	8-h discolouration	16-h discolouration	24-h discolouration
L*	Control	58.10 A-a, (57.03; 60.91)	79.23 A-b, (75.49; 80.48)	76.74 A-b, (73.62; 78.74)	60.54 A-a, (59.03; 63.86)	59.16 A-a, (57.42; 62.51)	53.82 A-c, (51.82; 56.46)
	Icon	54.09 B-a, (52.17; 55.49)	76.18 A-b, (72.29; 78.73)	58.57 B-a, (56.04; 59.45)	57.14 B-a, (55.89; 58.24)	57.45 A-a, (54.99; 57.86)	55.3 A-a, (53.26; 56.00)
	Fluoride	55.34 AB-a, (53.43; 58.31)	77.60 A-b, (74.42; 79.84)	72.10 A-b, (68.63; 74.97)	53.22 C-ac, (51.86; 55.46)	48.43 B-cd, (47.41; 51.53)	47.36 B-d, (45.88; 48.47)
	Abrasion	55.15 AB-a, (53.25; 57.56)	78.87 A-b, (76.02; 80.53)	59.72 B-c, (58.45; 62.52)	54.99 BC-ad, (52.97; 56.04)	52.45 B-de, (49.25; 54.21)	48.90 B-e, (46.25; 50.05)
a*	Control	−1.27 A-a, (−1.45; −0.82)	−1.50 A-a, (−1.64; −1.36)	−1.44 A-a, (−1.56; −1.27)	1.27 A-b, (0.11; 1.58)	2.32 A-bc, (1.79; 4.84)	5.64 A-c, (4.48; 7.07)
	Icon	−0.54 B-a, (−0.68; −0.18)	−1.68 A-b, (−1.79; −1.45)	−0.28 B-a, (−0.33; −0.05)	−0.40 A-a, (−0.45; −0.17)	−0.31 B-a, (−0.47; −0.3)	−0.27 B-a, (−0.55; 0.18)
	Fluoride	−0.72 AB-a, (−0.93; −0.53)	−1.56 A-b, (−1.69; −1.43)	−1.41 A-b, (−1.60; −1.32)	6.00 B-c, (3.00; 6.70)	4.54 A-c, (2.80; 5.19)	4.48 AC-c, (3.53; 4.92)
	Abrasion	−0.64 AB-a, (−0.82; −0.33)	−1.52 A-b, (−1.63; −1.42)	−0.65 C-ac, (−0.81; −0.40)	0.35 A-c, (−0.39; 1.66)	1.27 B-d, (0.54; 2.91)	2.73 C-d, (2.23; 4.40)
b*	Control	−3.42 A-a, (−4.60; −2.81)	−4.08 A-a, (−5.05; −3.47)	−1.86 A-b, (−2.93; −1.33)	10.17 A-c, (6.52; 10.71)	10.19 A-cd, (9.06; 12.65)	13.24 A-d, (11.03; 14.41)
	Icon	−4.07 B-a, (−5.47; −2.43)	−5.89 A-b, (−6.32; −4.63)	−3.12 A-a, (−4.09; −2.24)	−2.29 B-a, (−3.30; −1.11)	−2.21 B-a, (−3.46; −0.69)	−1.33 B-a, (−2.84; 0.45)
	Fluoride	−4.17 A-a, (−5.54; −3.13)	−5.26 A-a, (−5.81; −4.44)	−4.73 B-a, (−5.61; −4.11)	10.28 A-b, (7.91; 11.63)	5.61 C-c, (4.94; 6.88)	5.97 C-bc, (4.80; 7.94)
	Abrasion	−4.03 A-ab, (−4.82; −2.94)	−4.35 A-a, (−4.92; −3.45)	−2.92 A-b, (−3.65; −1.88)	3.85 C-c, (3.21; 7.18)	5.74 C-c, (3.82; 7.00)	7.90 C-c, (6.62; 8.89)

WSL, white spot lesion. Comparisons of the treatments at each time point of measurement that are not significantly different are marked with the same capital letters within L, a, or b values (read vertically). Comparisons of the measurements for each treatment at different time points that are not significantly different are marked with the same lower-case letters (read horizontally).

Table 3 Median values and confidence intervals of colour measurements at baseline, after WSL formation, after treatment, and after discolouration cycles (black tea + citric acid) of 8, 16, and 24h.

Factor	Test group	Baseline	WSL	Treatment	8-h discolouration	16-h discolouration	24-h discolouration
L*	Control	59.27 A-a, (57.54; 61.01)	77.29 A-b, (75.46; 79.11)	75.22 A-b, (73.29; 77.16)	67.86 A-c, (64.86; 70.86)	61.28 A-ac, (57.57; 64.98)	51.58 A-d, (49.70; 53.46)
	Icon	57.62 AB-a, (56.18; 59.05)	78.07 A-b, (76.67; 79.48)	58.09 B-a, (56.59; 59.59)	56.81 B-a, (55.87; 57.74)	56.07 B-a, (54.97; 57.18)	55.94 B-a, (54.50; 57.38)
	Fluoride	56.00 B-a, (54.74; 57.26)	78.59 A-b, (76.91; 80.26)	71.75 A-c, (69.05; 74.45)	55.89 B-a, (54.87; 56.90)	52.58 C-d, (51.74; 53.42)	50.82 A-d, (49.98; 51.66)
	Abrasion	55.14 B-a, (53.96; 56.31)	78.37 A-b, (76.32; 80.41)	62.57 C-c, (60.60; 64.53)	55.35 B-a, (53.73; 56.96)	50.55 C-d, (49.33; 51.76)	46.70 C-e, (44.87; 48.52)
a*	Control	−1.02 A-a, (−1.26; −0.78)	−1.56 A-b, (−1.67; −1.44)	−1.38 A-ab, (−1.50; −1.26)	−0.80 A-a, (−1.33; −0.27)	1.42 A-c, (0.10; 2.18)	4.88 A-d, (3.83; 5.89)
	Icon	−0.72 AB-a, (−0.95; −0.48)	−1.60 A-b, (−1.72; −1.49)	−0.25 B-a, (−0.47; −0.02)	−0.42 A-a, (−0.63; −0.21)	−0.40 B-a, (−0.63; −0.1)7	−0.43 B-a, (−0.61; −0.24)
	Fluoride	−0.45 B-a, (−0.75; −0.16)	−1.52 A-b, (−1.61; −1.43)	−1.42 A-b, (−1.56; −1.29)	3.38 B-c, (2.56; 4.20)	4.15 C-c, (3.64; 4.65)	4.38 A-c, (3.91; 4.86)
	Abrasion	−0.45 B-a, (−0.69; −0.21)	−1.57 A-b, (−1.69; −1.46)	−0.68 C-a, (−0.88; −0.49)	1.25 C-c, (0.33; 2.46)	3.93 C-d, (2.98; 4.88)	3.90 A-d, (3.10; 4.70)
b*	Control	−2.93 A-a, (−4.10; −1.75)	−4.65 A-a, (−5.33; −3.97)	−3.92 A-a, (−4.53; −3.31)	3.87 A-b, (1.68; 6.06)	7.21 A-b, (4.51; 9.92)	10.88 A-b, (8.71; 13.04)
	Icon	−4.95 B-a, (−5.66; −4.24)	−2.74 B-b, (−3.81; −1.68)	−2.39 B-bc, (−3.15; −1.63)	−2.79 B-c, (−3.41; −2.15)	−3.03 B-c, (−3.59; −2.46)	−2.04 B-c, (−2.83; −1.25)
	Fluoride	−3.10 A-a, (−4.03; −2.18)	−3.96 A-b, (−4.63; −3.29)	−4.80 A-ab, (−5.30; −4.31)	7.05 A-c, (6.08; 8.01)	7.65 A-c, (6.59; 8.71)	7.98 A-c, (7.04; 8.92)
	Abrasion	−4.84 A-a, (−5.76; −3.93)	−3.80 A-ab, (−4.56; −3.05)	−2.20 B-b, (−3.09; −1.32)	7.14 A-c, (4.92; 9.36)	8.68 A-c, (7.16; 10.20)	6.96 A-c, (5.82; 8.09)

WSL, white spot lesion. Comparisons of the treatments at each time point of measurement that are not significantly different are marked with the same capital letters within L, a, or b values (read vertically). Comparisons of the measurements for each treatment at different time points that are not significantly different are marked with the same lower-case letters (read horizontally).

Yellow–blue chromaticity (b value)*

Formation of WSL created a slight decrease in the b* component for infiltration groups in both discolouration specimens and the fluoride group in the tea + citric acid specimens. Infiltration produced an increase causing the b* value to return to baseline, whereas micro-abrasion and artificial saliva treatments (control) caused a decrease for the tea discolouration specimens. None of the treatments induced a change in the b* component in the tea+ citric acid discolouration specimens. A significant shift to the yellow component (increase in b* value) was seen immediately after the first 8-hour discolouration in all groups except infiltration, and this increase was sustained throughout the whole discolouration process for all groups except infiltration.

Delta E

Highest colour change obtained by the treatment of WSL was in infiltration and micro-abrasion groups followed by fluoride. The least affected group by the discolouration process was infiltration. Within each treatment group, the two used discolouration solutions did not differ significantly. Colour change obtained between time points and significant differences between groups at each time point are shown in Figures 1 and 2.

Discussion

The colour improvement of WSLs following infiltration, micro-abrasion, or fluoride treatments and their resistance against two discolouration solutions were investigated in this study. Based on the obtained results, infiltration and micro-abrasion treatments performed better in diminishing the opaque WSL appearance compared with the fluoride treatment and control. This effect was stable only for the infiltration treatment under discolouring effects. Both null hypotheses are rejected.

In this study, bovine enamel was chosen as the test substrate in order to facilitate homogenous allocation of specimens from the same crown to the four test groups. Despite the fact that use of human enamel would be more preferable in dental material testing, it has been stated that bovine enamel could be safely used as a substitute for human enamel, particularly when a large crown size for preparing samples from the same crown was necessary (Wiegand and Attin, 2011).

Effects of different treatments in terms of improving the appearance of WSL and the resistance of the treated surface against discolouration were evaluated by the change in colour components (L*, a*, and b*). The standard quantification of colour change was performed using a spectrophotometer in the present set-up. The reproducibility of the measurements and the reliability of this method have led to its frequent use in such studies (Torres *et al.*, 2011, Kim *et al.*, 2013).

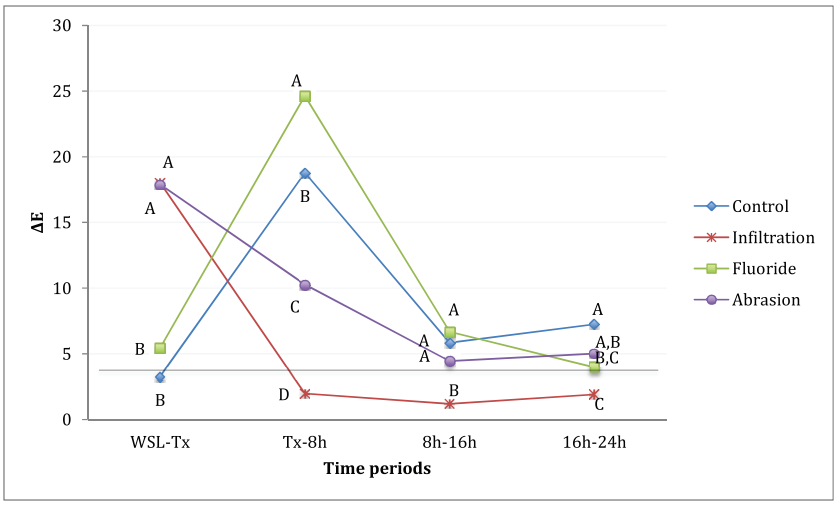

Figure 1 Colour change (ΔE) between each time period (WSL-Tx: WSL formation to treatment; Tx-8h: treatment to 8-h discolouration) for the black tea specimens. The horizontal line represents ΔE 3.7, which is the clinical detection limit (minimum) by naked eye. Comparisons of measurements that are not significantly different at each time period are marked with same capital letters (read vertically).

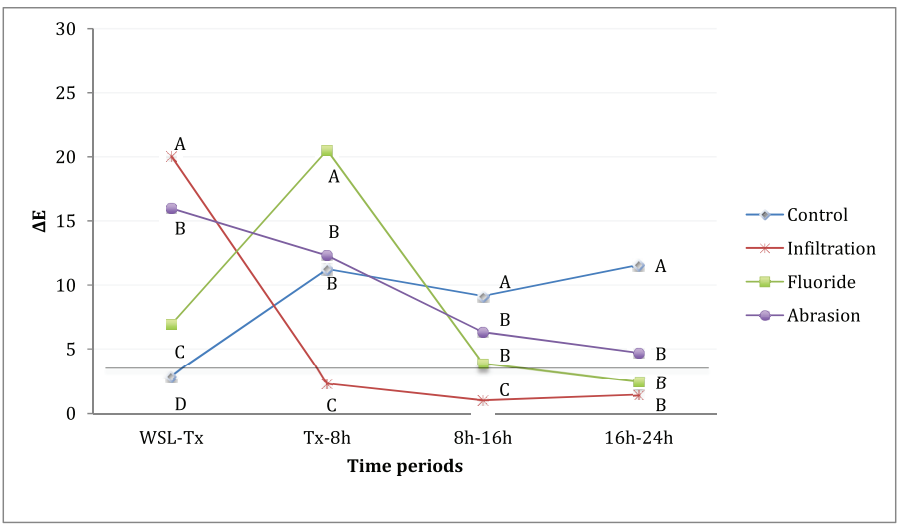

Figure 2 Colour change (ΔE) between each time period (WSL-Tx: WSL formation to treatment; Tx-8h: treatment to 8-h discolouration) for the black tea + citric acid specimens. The horizontal line represents ΔE 3.7, which is the clinical detection limit (minimum) by naked eye. Comparisons of measurements that are not significantly different at each time period are marked with same capital letters (read vertically).

Extrinsic enamel discolouration is frequently seen due to effects such as smoking, consumption of tannin-rich foods, and long-term use of cationic agents (Addy and Moran, 1995; Watts and Addy, 2001). The mechanism of these negative side effects is associated to nonenzymatic browning, protein denaturation, formation of pigmented metal sulphides, and precipitation of dietary chromogens (Addy and Moran, 1995; Watts and Addy, 2001). In this study, regular and citric acid added black tea was chosen as discolouration solutions. The rationale behind this approach was twofold. Black tea had been reported as one of the most powerful discolouring agents previously (Addy and Moran, 1995; Watts and Addy, 2001). However, because it is a natural product and the final solution may vary in terms of pH depending on the processing of the tea plant (Kumar *et al.*, 2013), citric acid was used to obtain a second discolouration solution with standard pH. Enhancement of discolouration by increasing the permeability of the superficial WSL surface as well as imitating the consumption of acidic soft drinks was also aimed secondarily (Paris *et al.*, 2007a; Neuhaus *et al.*, 2013). Nevertheless, the discolouration results showed no difference between the two solutions in this study. The lack of this effect was attributed to the slight difference between the pH values. The black tea solution already had a pH of 4.2–4.4, and this value was adjusted to pH 4 in the second group. A more acidic pH was avoided with respect to possible erosive effects and considering the buffering effect

of saliva avoiding lower pH levels *in vivo* (Simpson *et al.*, 2001; Wiegand and Attin, 2011).

It is generally accepted that WSLs tend to regress in the presence of remineralizing agents, when the cariogenic attack is avoided (Marinho *et al.*, 2003; Derks *et al.*, 2004; Beerens *et al.*, 2010; Bergstrand and Twetman, 2011). Treatment of these surfaces with fluoride was shown to enhance subsurface remineralization (Marinho *et al.*, 2003; Derks *et al.*, 2004; Beerens *et al.*, 2010). In this study, a low-concentration fluoride rinse (250 ppm) containing sodium and amine fluoride was used for this purpose (Marinho *et al.*, 2003; Hamdan *et al.*, 2012). The rationale for applying low doses of fluoride was to avoid hypermineralization of the lesion surface, which might obstruct further regression (Marinho *et al.*, 2003; Hamdan *et al.*, 2012). Colour improvement of the WSLs was achieved with the effect of fluoride to some extent, which was superior compared with the control. However, this effect did not bring the colour components back to the initial levels. Previously, it was reported that a similar demineralization solution used on bovine enamel (pH 4.55) presented a lesion depth of 95 ± 32 µm even after the application of low-dose fluoride (250 ppm) for 28 days (Chin *et al.*, 2009). This implied an incomplete remineralization of the porous enamel; thus, the susceptibility to discolouration was still increased. Similarly, this might be the reason for the limited improvement and instability of the WSL colour in this study. However, verifying this claim microscopically was not performed in this study.

Infiltration and micro-abrasion were the two effective treatment modalities significantly improving the whitish appearance of WSLs as well as creating significant visible colour improvement. Infiltration resulted in regression of all colour components back to the baseline values except b* value in tea + citric acid specimens, whereas micro-abrasion could not revert lightness to the initial levels. These two findings were in accordance with previous *in vitro* and *in vivo* results in which the treatment efficacy of these two methods was compared with fluoride therapy or saliva remineralization (Torres *et al.*, 2011; Kim *et al.*, 2013; Neuhaus *et al.*, 2013; Paris *et al.*, 2013). However, effects of infiltration and micro-abrasion treatments on improving WSL appearance were not compared previously. The main difference between these two treatment methods is that micro-abrasion removes the demineralized enamel, whereas infiltration stabilizes the lesion and reinforces the weakened prism structure within the lesion (Paris *et al.*, 2007b; Kielbassa *et al.*, 2009). Previously, the infiltrant was shown to penetrate subsurface demineralized areas up to 400 µm (Paris *et al.*, 2007a; Neuhaus *et al.*, 2013). The deep penetration of the resin infiltrating leading to the plugging of porosities within the WSLs might be the factor increasing the resistance against discolouration and improving the colour by having a similar light refraction index as shown in the present results (Paris *et al.*, 2013).

Previously, HCl micro-abrasion was shown to remove demineralized enamel up to 134 ± 35 microns (Schmidlin *et al.*, 2003). In this study set-up, micro-abrasion was applied for 1 minute, but the amount of enamel loss was not measured. The treatment was successful in improving the whitish appearance but was more prone to discolouration compared with infiltrated specimens. This can be explained in two ways; micro-abrasion with 6.6 per cent HCl slurry for 60 seconds might not have been enough to remove all the porous structure, which might have caused these specimens to be more prone to discolouration. Second, although the surface was polished following micro-abrasion, the surface might have been rough still (Paic *et al.*, 2008), thus presenting an increased susceptibility to discolouration.

Conclusion

Within the limitations of this study, the following could be concluded:

- Infiltration of WSLs can treat the white opaque appearance, and this outcome is stable under discolouring effects.
- Micro-abrasion reduces the white opaque appearance of WSL considerably; however, this outcome is not resistant to discolouration.
- Low-concentration fluoride treatment improves the WSL appearance more than the clinical detectable limit, but the stability is not different than the effect of saliva remineralization.

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