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Masking white spots of enamel in caries lesions with a non-invasive infiltration technique *in vitro*

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ABSTRACT. We investigated the treatment effect of non-invasive infiltration on early caries caused by different degrees of enamel demineralization. Forty specimens of early enamel caries were prepared and divided into low and high demineralization groups. After treatment with non-invasive infiltration, the specimens were placed under cariogenic conditions. Color measurements were determined using a spectrophotometer 4 times to obtain chromatism values (ΔE1-ΔE4), including before and after production of artificial caries, and after infiltration treatment and re-demineralization. The effects of color change on early caries using non-invasive infiltration were compared between the 2 demineralization groups. Color differences before the production of artificial caries and after infiltration treatment and re-demineralization could not be distinguished by direct observation. Color differences after the production of artificial caries and after infiltration treatment and re-demineralization could be distinguished by direct observation. There were no significant differences in the 4 chromatism values (ΔE1-ΔE4) between the 2 groups. Non-invasive infiltration

showed an excellent ability to mask white spot lesions and maintained high color stability. Treatment of high and low demineralization of enamel had the same masking effect.

Key words: Non-invasive infiltration; Early caries; Demineralization; White spot lesions

INTRODUCTION

Clinical manifestations of early caries present as a dull and slightly rough enamel surface with chalky or brown caries spots that have not yet formed cavities or defects. Typically, the clinical treatment for such lesions is to promote the remineralization of early caries. However, because of the long course of treatment involved requiring high patient compliance, several limitations may occur, such as production of resistant strains (Wang and Tang, 2005). In treatments of filling restorations, even very small dental preparations may lead to the loss of healthy tooth tissue (Mueller et al., 2006). Non-invasive infiltration is a non-invasive treatment method that avoids removal of the tooth tissue. One treatment is sufficient to mask the chalky lesions, improve the appearance, and block ongoing demineralization of early caries, making treatment of early caries simple, fast, effective, and non-invasive (Meyer-Lueckel and Paris, 2008; Paris and Meyer-Lueckel, 2010; Paris et al., 2007, 2010).

Non-invasive infiltration technology was proposed and developed in Charité and Kiel University, Berlin, Germany. In 1976, infiltration treatment for natural and artificial chalk-like lesions was proposed by Davila et al. (1975). Studies of non-invasive infiltration technology *in vitro* have been conducted since 2000, and clinical studies *in vivo* are currently being developed. Previous studies have demonstrated that this technology can effectively improve the chalky white spots caused by enamel demineralization, and shows high color stability after improvement (Rocha Gomes et al., 2011). Even for old white spots caused by enamel demineralization, infiltration treatment shows some degree of improvement (Neuhaus et al., 2010). An improvement effect of infiltration treatment on chalky white spots formed during orthodontic process has also been clearly observed, but was less effective for white spots caused by insufficient enamel mineralization (Kim et al., 2011). Whether this infiltration effect is affected by the extent of demineralization is unknown.

In the present study, established specimens of early caries *in vitro* were treated with non-invasive infiltration. The efficacy of masking the chalky lesions was observed. We also investigated the effect of treating early caries caused by different degrees of enamel demineralization and determined whether this effect persisted in a cariogenic environment.

MATERIAL AND METHODS

Samples

Healthy, caries-free orthodontic subtrahend premolars were freshly extracted from children aged 12-14 years. After the occlusal surface was blow dried, 40 samples without caries, enamel hypoplasia, or other abnormalities were collected by observation under a stereomicroscope. The surfaces of the calculus, alveolar bone, and soft tissue were removed, and

the root of the tooth was amputated using a carborundum disk at 1 mm of the cemento-ename junction to prepare 5 x 5 x 2-mm enamel block. The enamel blocks obtained were immersed in a 0.1% thymol-saturated aqueous solution for further analysis. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the Affiliated Stomatological Hospital of Nanchang University (Permit Number: 20110025301). Written informed consent was obtained from the legal guardians of all participants.

Preparation of artificial cariogenic solution

Artificial cariogenic solution containing 0.2442 g NaCl, 0.2992 g KH_2PO_4, 1.56 x 10^{-3} g KCl, 3.0 g HAc, and 56.0 g KOH was prepared. The compounds above were dissolved in 1000 mL distilled water and the pH was adjusted to 4.6.

Preparation of artificial saliva

Artificial saliva containing 0.33 g KH_2PO_4, 0.34 g NaH_2PO_4, 1.27 g KCl, 0.16 g CNNaS, 0.58 g NaCl, 0.17 g $CaCl_2$, 0.16 g NH_4Cl, 0.2 g urea, 0.03 g glucose, 0.002 g vitamin C, and 2.7 g mucin was prepared. The regents above were dissolved in 1000 mL distilled water and the pH was adjusted to 6.8.

Specimen preparation of early caries

Forty specimens were placed in a container to ensure that their enamels were entirely in contact with the artificial saliva; these samples were incubated for 12 h. Next, all samples were soaked alternately in artificial cariogenic solution twice per day for 1 h and in artificial saliva twice per day for 11-h incubations for 15 days. The 2 solutions were changed daily and incubated in a water bath box (Shanghai Haima Industrial Co., Ltd. Medical Equipment Factory; Shanghai, China) at 37°C.

The demineralization degree of the enamel surface of each sample was measured using DIAGNOdentPen (DDPen; Kavo, Germany) and recorded daily. The 4 corners of each sample were selected as measurement points, and the averages of the 4 measured values were recorded for sample readings. Based on specimen readings, samples were divided into 2 groups (N = 20 per group): Group I, low demineralization group (values 14-20) and Group II, high demineralization group (values 21-30).

Coating penetration of resin

According to the requirements for the product use of infiltration resin ICON (DMG; Bielefeld, Germany), after polishing using a rubber cup to clean the tooth surface, the sample was etched with 15% hydrochloric acid for 2 min and rinsed for 30 s with a water gun. An air gun was used to dry the sample for 30 s and 99% ethanol desiccant was injected into the demineralization site and allowed to remain for 30s. The sample was then completely dried using an air gun. Coat infiltration resin was applied at the demineralization site and left for 3 min, followed by irradiation for 40 s using a light-curing unit (Mini LED; Sately, France). The sample was coated a second time for 1 min and irradiated for 40 s.

Re-demineralization treatment

Forty specimens were again placed in artificial cariogenic solution or artificial saliva and treated as described above.

Color measurement

Color measurements for the 40 samples were performed 4 times. Before the production of artificial caries, the enamel blocks obtained were polished using a rubber cup at low speed and rinsed repeatedly with distilled water. After being blow dried, each window of the sample was determined using a CM-2300d spectrophotometer (Minolta; Osaka, Japan) for the first color measurement. After specimen preparation of early caries and production of artificial caries were completed, the second color measurement was performed. After infiltration treatment, the third color measurement was conducted after the infiltration resin was coated. For re-demineralization, after the samples were placed in artificial cariogenic solution for demineralization, the fourth color measurement was performed. All measurements were performed by one operator to reduce error.

White calibration was conducted. When the site of measurement on the specimen was vertically close to the window under the test, the built-in light source radiated light. A few seconds later, the results of measurement were displayed on the screen. The parameters included values of L*, a*, and b*. The data pattern automatically used the average of the 3 measurement values.

To analyze measurement results, the chromatism value, ΔE was used to quantify the overall differences of the 2 colors. The chromatism value was calculated using the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ by comparing the 4 measurement results. The USA National Bureau of Standard Unit (NBS unit) was employed as the unit of the color difference, in which ΔE = 1 indicates one NBS unit. NBS is related to the degree of the visual differences of the human eye (Table 1).

Table 1. National Bureau of Standard (NBS) unit on the degree of difference in vision.

NBS unit	Degree of visual differences of the human eye
0.0-0.5	Little
0.5-1.5	Slight
1.5-3.0	Slightly
3.0-6.0	Obvious
6.0-12.0	Notable
12.0 or more	Highly significant

The minimum chromatism value established by the NBS for which the human eye can distinguish the difference of 2 objects in color is 1.5. Therefore, ΔE = 1.5 NBS in this experiment was considered to be the critical value for visually distinguishing chromatic aberrations.

Statistical analysis

In this study, SPSS 12.0 was used for data collection and analysis (SPSS, Inc.; Chicago, IL, USA). The results of color measurement were analyzed using the Student *t* test; statistical significance was set at $P < 0.05$.

RESULTS

For the statistical analysis of the 4 color measurement results, a single sample-sided Student *t* test was carried out using ΔE = 1.5 as the overall average, assuming that ΔE is not less than 1.5 (Tables 2 and 3).

Table 2. Analysis results of ΔE1 (the chromatic difference value between after non-invasive infiltration and before the production of artificial caries) and ΔE2 (the chromatic difference value between after a new acid challenge and before the production of artificial caries).

Chromatic difference value	Cases		Composition ratio for ΔE ≤ 1.5	Mean and standard deviation for ΔE	*t**	P
	ΔE ≤ 1.5	ΔE > 1.5				
ΔE1	37	3	92.5%	0. 848 ± 0. 292	-14.113	<0.001
ΔE2	36	4	90.0%	0. 954 ± 0. 245	-14.120	<0.001

*Single sample sided *t* test was carried out using ΔE = 1.5 as the overall average, assuming ΔE is not less than 1.5.

Table 3. Analysis results of ΔE3 (the chromatic difference value between after non-invasive infiltration and after the production of artificial caries) and ΔE4 (the chromatic difference value between after a new acid challenge and after the production of artificial caries).

Chromatic difference value	Cases		Composition ratio for ΔE ≥ 1.5	Mean and standard deviation for ΔE	*t**	P
	ΔE ≥ 1.5	ΔE < 1.5				
ΔE3	40	0	100.0%	2.319 ± 0. 912	5.684	<0.001
ΔE4	38	2	95.0%	2.505 ± 0. 940	6.767	<0.001

*Single sample sided *t* test was carried out using ΔE = 1.5 as the overall average, assuming ΔE is not less than 1.5.

Statistical analysis showed that ΔE1 (difference in chromatic values obtained after non-invasive infiltration and before production of artificial caries) and ΔE2 (difference in chromatic values obtained after a new acid challenge and before production of artificial caries) were less than 1.5 NBS, suggesting that the color difference could not be distinguished by direct observation. ΔE3 (difference in chromatic values obtained after non-invasive infiltration and after production of artificial caries) and ΔE4 (difference in chromatic values obtained after a new acid challenge and after production of artificial caries) was higher than 1.5 NBS, suggesting that the color difference could be distinguished by direct observation.

As shown in Table 4 and Figure 1, there were no significant differences in ΔE1, ΔE2, ΔE3, and ΔE4 between the low and high demineralization groups (P > 0.05).

Table 4. Statistical results of four group comparisons of the chromatic difference value between groups I and II.

Chromatic difference value	*t*	P
ΔE1	1.373	0.178
ΔE2	0.473	0.639
ΔE3	-0.964	0.341
ΔE4	-0.449	0.656

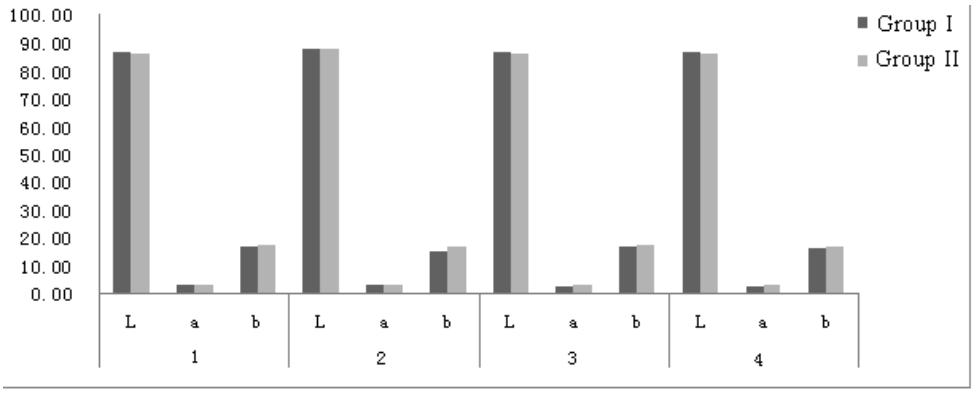

Figure 1. Histogram of L*, a*, b* value with color measurement in four times between groups I and II. 1 = before the production of artificial caries; 2 = after the production of artificial caries; 3 = after the infiltration treatment; 4 = re-demineralization.

DISCUSSION

The most common method of treating early caries is to use fluoride and remineralization. Fluoride is simply used for remineralization without considering the masking effect. A previous study showed that topical application of fluoride for the treatment of mouth early caries can promote remineralization and effectively prevent caries development (Yamazaki et al., 2007). If early lesions of dental caries are completely mineralized, a color change would be observed, resulting from color changes of the translucent enamel due to mineral content changes (Houwink, 1974; Brodbelt et al., 1981). However, this remineralization is superficial (Theuns et al., 1986; Anderson and Elliott, 2000). Although the enamel surface was mineralized, its body was more likely to decalcify; after remineralization, the optical reflectance in the body portion with caries lesions did not significantly decrease. This indicates that the body of carious lesions did not reach the same extent of remineralization as that of the surface. Therefore, after remineralization treatment, early caries lesions improved to some extent and the development of dental caries was terminated, but white spots were not improved.

Treatment of both non-invasive infiltration and remineralization can block the development of early caries. Chalky spots of early caries can also be improved by non-invasive infiltration. The improvement effect on the chalky spots formed in the orthodontic process is clear and better than those formed by poor enamel mineralization (Kim et al., 2011). This suggests that infiltration treatment does not have the same effect on all early caries. We examined whether different degrees of demineralization had the same effect on chalky lesions.

The results of the present study indicated that non-invasive infiltration technology can change the color of early caries lesions and achieve a good aesthetic effect by masking the chalky spots of early caries so that it appears close to its normal color. Previous studies also demonstrated that infiltration treatment could improve chalky spots caused by enamel demineralization, and that the color stability after improvement was very high (Rocha Gomes et al., 2011). Chalky spots caused by prior enamel demineralization could be improved by infiltration treatment (Neuhaus et al., 2010).

However, the masking effect is not affected by the extent of enamel demineralization;

treatment of enamel with high and low levels of demineralization showed the same effect. Although the depth of infiltration can be affected by the degree of demineralization, the effects on color change were very similar. The reason may be that regardless of infiltration depth, the enamel surface and most of the carious lesion bodies were filled with infiltration resin matrix, resulting in nearly the same light reflectivity and performance for color measurement.

Some data in this experiment showed relatively large deviations, which may have been caused by fractionated determinations and measurement errors. Under the same environment and operator, it is difficult to control the measurement position, air humidity, and other factors that may have influenced light refraction. In addition, due to the radian of the sample surface, loss of a small amount of reflected light may have affected the measurements. Taking into account the problem of position deviations at different time points, continuous color measurement may be used to overcome this limitation.

Regarding the efficacy stability of non-invasive infiltration technology, in addition to maintenance in the short-term cariogenic environment, this technique should withstand the challenges of the complex oral environment for clinical application. More objective clinical observational studies are required to determine whether this method can maintain a good masking effect on the chalky spots.

Mueller et al. (2011) and Belli et al. (2011) observed the morphology after resinous infiltration of subsurface bovine enamel lesions and found that a large difference in surface roughness and morphology remained compared with normal enamel. Gloss on the object surface with the same chrominance will have a significant impact on the appearance. A smooth surface with high gloss has strong light with specular reflection and weak light of diffused reflection. The rough surface, or that with low gloss, shows weak light of specular reflection and strong light of diffused reflection. Typically, when observing the color of an object, one should avoid the angle of specular reflections, and diffused reflection light should be used as a reference to determine the object's color. Dietschi et al. (1994) pointed out that the average value of the resin color difference was related to the surface roughness, which could be decreased by 26-74% in the polishing process; a smooth surface is more resistant to colorant dyeing. Thus, further study should be conducted to ensure that surface roughness and morphology after infiltration treatment are more similar to those of normal enamel.

The results of the present study indicate that for early caries measured using a laser diagnosis pen with readings ranging from 14-30, white spots could be masked to reach a favorable aesthetic effect after treatment with non-invasive infiltration. Furthermore, color stability was maintained under cariogenic conditions.

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