Comparison of colour improvement and stability of white spot lesions following infiltration, micro-abrasion, or CPP-ACP treatments *in vitro*

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Abstract

Objective: The objective was to compare the colour improvement of white spot lesion and the colour stability of treated enamel to after discolouration *in vitro*. **Method**: Artificial WSLs (2*2 mm) were created on the outer surface of 60 permanent premolars and randomly allocated to 4 groups. Specimens were treated with infiltration (RI), CPP-ACP (CPP), and micro-abrasion (MA) or remained untreated (UT).Groups were discoloured for 24 hours in tea or tea + citric acidVisible colour changes (ΔE) were measured spectrophotometrically on following time points: immediately after lesion formation, immediately after treatment, after 30 days of immersion in artificial saliva, anh aftere discolouration. Data were analyzed using SPSS 20 software. **Results**: WSL formation increased in all groups. ΔE1 is creater 3.7 in all groups but only infiltration reduced this effect to baseline. Highest ΔE4was obtained by CPP-ACP and resin infiltration is lowest. Between the RI and MA treatment groups, ΔE4 did not differ significantly. The improvement was more stable for infiltration and micro abrasion during discolouratio compare to the others (ΔE4 < 3.7). **Conclusions**: The method of infiltration improves the aesthetics of white spot lesions immediately after treatment and maintains treatment results during the follow-up period. Micro abrasion improved white spot aesthetics during the follow-up period. These two methods WSLs were stable following discolouration challenge.

Key words: *white spot lesion, infiltration, micro abrasion, CPP-ACP*

1. INTRODUCTION

White spot lesions are not only early sign of caries formation, but also are side-effect of orthodontic treatment because of plaque accommodation on teeth or around braces. Lesions are milky white due to demineralization resulting in a porous surface beneath the surface layer, altering the normal light reflectivity of the enamel [1]. This lesion becomes more recognizable when affected by exogenous pigments that affect the patient's aesthetics [2]. Occurently, reported rates vary from 2 to 96%, depending on methods and detection criteria as well as patient compliance with precautions. These lesions often persist long after the braces are removed and white spots can sometimes be detected even 12 years after treatment. The recent methods of white spots treatment are divided into two main groups of methods: remineralization measures and colour improvement measures. Remineralization measures include methods such as Fluoride or Casein phosphopeptide - Amorphous calcium phosphate. Other methods to improve color include micro-abrasive methods, resin penetration or bleaching [3]. Currently, there are some studies to evaluate the effectiveness of white spot lesions treatment methods such as the study of Yetkiner et al (2014), the study of Dam Minh Tuan (2016), the study of by Vo Truong Nhu Ngoc et al (2017), research by Yadav et al (2019) [2], [4], [5], [6]. However, most of these studies have only evaluated the effectiveness of a single method, while studies comparing different treatments are limited. Therefore, we compared to colour improvement and stability of white spot lesions following infiltration, micro-abrasion, or cpp-acp treatments *in vitro* in our study.

2. MATERIALS AND METHODS

2.1. Research design and subjects

The research is in vitro study, carried out at the Preclinical Department of Odonto-Stomatology, Hue University of Medicine and Pharmacy from September 2021 to April 2022 with pre-molars extracted for orthodontic reasons.

2.2. Selection criteria

The following selection criteria were used in the research:

Inclusion criteria

The tooth remains in the shape of the crown.

Exclusion criteria

- Teeth had caries, enamel hypoplasia, cracks, worn teeth.

- Teeth infected with Fluoride, Tetracycline, teeth with enamel hypoplasia.

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2.3. Research size

60 teeth were randomly divided into 4 groups including: resin infiltration (RI), micro-abrasion (MA), cpp-acp (CPP) and untreated (UT).

2.4. Research methods

Sample preparation

The tooth samples suitable to inclusion and exclusion criteria, were cleaned and preserved in saline solution and stored in a refrigerator at 5^0C. The acid resistant coating was used to cover the entire surface of the enamel crowns of each tooth, leaving only two enamel windows on the buccal surface sized 2x2mm. They applied transparent nail polish (Kim Nghia, Viet Nam) 2 times each tooth.

pH cycle

White spot lesions were generated based on the modified pH cycle of Featherstone et al (1986)[7]. The samples were soaked in a demineralized solution (consisting of calcium nitrate 2 mmol/L, potassium dihydrogen phosphate 2mmol/L and acetic acid 75mmol/L) for 3 hours with a pH of 4.3. Then the samples were washed thoroughly with distilled water for 30 min (approximately 15 s for each tooth). The teeth were expose remineralization solution (including calcium nitrate 2 mmol/L, potassium dihydrogen phosphate 2 mmol/L, potassium chloride 130 mmol/L and Trisaminomethane 100 mmol/L) for 20 hours with pH 7, then wash thoroughly with distilled water for 30 min before reintroducing it into the demineralization solution. The cycle was repeated daily for 5 days and maintained at 37°C. The samples were then immersed in the remineralization solution for the next 2 days.

Colour improvement methods

After the pH cycle finished, the acid resistant coating was removed from all teeth by aceton, and soaking them back in distilled water then randomly distributed into 4 groups that consisted of 15 teeth.

CPP group: the teeth were bloted and applied CPP-ACP gel to study windows 2 times a day, 4 minutes each time. Then they are placed back in artificial saliva solution without rinsing and maintained within 2 months.

MA group: White spot was treated with micro abrasion by placing a layer of the mixture (6.6% HCl and silicon carbide) approximately 1mm over the white spot lesion and using the same rubber pad and slow handpiece with medium pressure at about 300 rpm for 60 seconds polishing for 20 seconds.

RI group: the teeth were gently applied an appropriate amount of Icon etch (15% hydrochloric acid) to the affected area for 120 seconds. Then rinsing off the residue with water for 30 seconds and blowing dry. They were pumped onto the surface an amount of ICON Dry (ethanol 99%) and wait for 30 seconds before blowing dry. The resin (ICON) was then injected onto the lesion, left for 180 seconds, and illuminated for 60 seconds. Continue injecting the second layer of ICON, leave for 60 seconds and light up for 40 seconds then polising with the rubber band for 20 seconds.

UT group: un treated

The samples were soaked in artificial saliva (Salisol, Hago, Vietnam) for 30 days to evaluate the post-treatment color stability. The artificial saliva solution is renewed daily.

Dicolouration process

The teeth are soaked in a mixed solution of tea and coffee for 5 minutes a day. As the rest time, the teeth are soaked in artificial saliva. This staining process is done in 5 days. The coloring solution is prepared by brewing 20 grams of tea and 20 grams of coffee in 2 liters of boiled water and then filtering. After five days, the samples were washed and gently brushed with a soft-bristled brush to remove the outer yellow layer, then rinsed with water and dried.

Image analysis

The teeth were colored using a Crystaleye spectrophotometer (Olympus, Tokyo, Japan). Captured images are transferred via a cable that connects the stand and a computer with the dedicated Crytaleye Application Master software (Version 1.4). Colors are displayed in three values L*, a*, b* in the CIELAB color space defined by the International Commission on Illumination (1976) [8]. L* valuerepresents the luminance value from 0 (complete black) to 100 (complete white). a and b value represent saturation, relative to the red-green and yellow-blue axes, with a positive a indicating red tones, and positive b indicating yellow tones [9].

Parameters of color and brightness (L*, a*, b*) were analyzed and recorded by the software at the time before treatment (T0), when white spot lesions were formed (T1). , immediately after treatment (T2), 30 days after treatment (T3), after soaking in colored solution (T4). The color change is calculated using the formula:

$$\Delta = \sqrt{(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2}$$

If ΔE value is greater than 3.7, the color is clinically recognizable to the naked eye [13]. The color change is calculated between time points:

ΔE1 (T1-T2); ΔE2 (T2-T3); ΔE3 (T3-T4), ΔE4 (T0-T3).

Data analysis

Data was statistically analyzed using SPSS software ver 20.0. Calculate mean and standard deviation of measured value was analyzed by Paired samples test. Comparing 4 independent groups by ANOVA, Kruskall-Wallis test and using Mann-Whitney's U test to compare 2 independent values in the same group on different time.

3. RESULTS

3.1. Comparison of colour improvementof white spot leisons

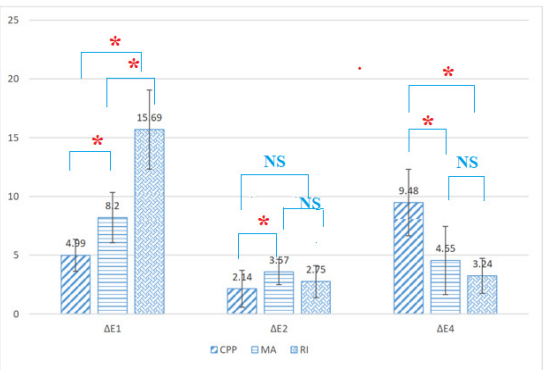

Chart 1. Mean and standard deviation of the values E1, ΔE2, E4 of the three treatment groups CPP, MA, and RI

*: The difference is statistically significant

NS: The difference is statistically non significant

When making comparison, the ΔE1 color change is highest in the RI group. The MA group has a lower ΔE1 value than RI group and the lowest is the CPP group. The ΔE1 color change of 3 groups CPP, MA, RI show $p<0.05$ and the difference between groups is statistically significant. After soaking teeth for 30 days in artificial saliva, the MA group has the largest ΔE2 color change. The result of comparing the ΔE2color change between CPP and MA has $p<0.05$ so the difference is statistically significant, while others (MA and RI), (CPP and RI) show $p>0.05$. The color change between time points (T0-T3) shows that the CPP group has the greatest difference in ΔE4. The result of comparing the ΔE4color change between the MA and RI groups has $p>0.05$ while remain groups: (MA, CPP) and (RI, CPP) have $p<0.05$ and show the statistically significant difference.

3.2. Comparison of stability of white spot leisons

Table 2. Mean and standard deviation of values of 4 groups at time points T3, T4

Method			CPP	MA	RI	UT
Values at time points T3, T4	L	T3	77.83 ± 3.09	72.03 ± 1.83	69.57 ± 2.11	79.90 ± 2.55
		T4	70.74 ± 2.74*	69.14 ± 2.47*	68.18 ± 2.56^{NS}	72.02 ± 1.78*
	a	T3	2.18 ± 1.38	0.60 ± 0.89	1.40 ± 0.82	0.97 ± 0.99
		T4	1.96 ± 1.36^{NS}	0.92 ± 1.03*	1.91 ± 0.80*	1.27 ± 0.86*
	b	T3	10.64 ± 2.17	15.99 ± 2.69	17.39 ± 2.35	10.51 ± 1.90
		T4	13.56 ± 1.87*	16.27 ± 2.07^{NS}	18.71 ± 1.63*	13.89 ± 1.82*
	ΔE3		8.82 ± 2.37	8.06 ± 1.35	3.46 ± 2.35	2.54 ± 1.15

*Use Paired samples test with *: p<0.05 and NS: p>0.05 when comparing L, a, b values at two consecutive time points.*

After soaking in the colored solution, L* values of all groups UT, CPP, MA, RI decrease, a values of the UT, MA, and RI groups increase while the CPP group remains unchanged. At the same time, the UT, CPP, and RI group show increase in b valuewhile the MA group do not change. The ΔE3 mean of the groups has p<0.05, the difference is statistically significant.

4. DISCUSSION

4.1. Comparison of colour improvementof white spot leisons

The ΔE1 value represents the color change before and after treatment. Immediately after treatment, ΔE1 values of three groups are higher than 3.7. It means that all three methodsare effective in improving white spot color. The ΔE1 values comparisonof three groups shows a statistically significant difference (p<0.05). And when comparing in pairs (CPP and MA), (MA and RI), (CPP and RI), the results points out statistically significant differences (p<0.05).These demonstrate the effectiveness of color improvement methods in varying levels. The highestΔE1 mean in the RI group shows that the resin infiltration is most effective. It fits into the study of Yetkiner et al (2014) and the study of Yadav et al (2019) [2], [6].

The E2 value represents the color change between two time points: immediately after treatment and after 30 days of soaking in the artificial saliva. The ΔE2 mean of the MA group is highest demonstrating effectiveness of micro-abrasion method in white spot lesions treatment. This can be explained that the micro-abrasion method removes the surface layer of the lesion and allows the remineralization agent in the artificial saliva to contact the central area of the lesion [11]. The surface layer of the lesion, which is 10-30 µm in dense has a mineral content to be similar to intact enamel. Meanwhile, micro-abrasion method can remove 25-200 µm of enamel layer after 5-10 times [10]. This result is consistent with the study of Xi Gu et al (2019) [11].

In conclusion, resin infiltration method improved the colour of white spot lesions immediately after treatment and maintained stability during the follow-up period. Whereas, micro-abrasion method continuously enhancedaesthetics during the follow-up period. This was similar to the study of Xi Gu et al (2019) and the study of Di Shan et al (2020) [11], [12].

4.2. Evaluation of stabilityof white spot leisons

After soaking in the stained solutions, all groups had a decrease in L* values indicating that these solutions made the teeth tend to be darker. Colored solutions increase a and b values which means that the color shifts towards red and yellow. The b value increased because of tea and coffee in the stained solution containing yellow pigments called Tannins with different polarities [14]. The colorants in tea are highly polar attraction and are easier to be absorbed on the tooth surface while coffee can infiltrate inside due to compatibleability with the polymer phase of composite materials. When assessing the ΔE3 value, the CPP and the UT group changed more than others. The ΔE3 color change of MA and the RI group changed less and the mean of these two methods < 3.7, indicating that the white spot lesions treated with micro-abrasion and resins infiltration can resist staining after soaking in coloured solution. Penetration of infiltrated resin filling the pores may be a factor to enhance staining agent resistance. This is consistent with the study of Paris et al (2013), Yetkiner et al (2014) [2], [15],. The micro-abrasion can resist staining, which is different from the study of Yetkiner et al (2014) [2]. This can be explained by the fact that in our study, the extra time of soaking in artificial saliva enhanced remineralization after the superficial layer of the lesion had been removed, the polished residues beccame smooth and mineralized so they could resist staining.

White spot lesions treatment with CPP-ACP is a time-requiring method because remineralization is a slow process and depends on calcium ion deposition [16]. Although color changed after treatment, the lesion is visible. The reason was incomplete remineralization of the lesion sothe color change of the CPP group was similar to untreated groups when it was added to the stained solution [2].

5. CONCLUSION

All of treating white spot lesions methods including: micro-abrasive, infiltration resin, casein phosphopeptide- amorphours calcium phosphate both provide color improvement effect. When comparing the three methods, the color change ΔE decreases with the order: infiltration resin, micro-abrasive, casein phosphopeptide- amorphours calcium phosphate.

After soaking in colored solution, white spots treated with casein method phosphopeptide-amorphours calcium phosphate has more color change and similar to the untreated group, while the change color of micro-abrasive and infiltration resin groups is lower and equal.

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