In Vitro Effect of Remineralizing Products on Changing the Enamel White Spot Lesion

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Abstract

The objectives of this research were to compare optical appearance of white spot lesion before and after intervention with two remineralizing products and to etermine which one has better ability on changing the appearance of white spot lesion by using computerized image analysis.

The researcher coated Sixty human tooth crowns with an acid resistant varnish, leaving a small window of sound enamel parallel to the horizontal plane. Artificial white spot lesion was created by thirty-minute immersion in demineralizing solution. Samples were randomly allocated into 2 groups: group A (GC tooth mousse: Casein Phosphopeptide-Amorphous Calcium Phosphate), and group B (Prevident®: 1.1% w/v Sodium Fluoride). Both groups were subjected to 60 remin/demin cycles. Photographs of each sample were taken before and after intervention at fifteen degree angle to the enamel surface and analyzed using computerized image analysis. Data were measured in term of LI% (luminance intensity proportionality) for optical appearance measurement.

Results from paired t-test showed that LI% before and after intervention of both groups were significantly different ($p<0.001$). T-test showed that changed LI% of group A was significantly different from group B ($p<0.050$).

In conclusion optical appearance of white spot lesion was decreased after applied both remineralizing products while group B (Prevident®: 1.1% w/v Sodium Fluoride) demonstrated better potency on changing the white spot lesion.

Keywords: White Spot Lesion, Optical Appearance, Remineralizing Product

1. Introduction

White spot lesion is one of undesirable complications, occurred on enamel surfaces during fixed orthodontic treatment, especially in the poor oral hygiene patients. (Chang, Walsh et al., 1997; Tufekci, Dixon et al., 2011) Orthodontic patients experience difficulty in brushing teeth from the beginning of treatment due to increasing of plaque retention site. Acidogenic bacterium in plaque cause low pH and lead to imbalance between demineralization and

remineralization. When remineralization is overcome, white spot lesion takes place. (Chang, Walsh et al., 1997; Lau and Wong, 2006; Bishara and Ostby, 2008)

Optical appearance of the lesion comes from changing of difference between refractive index of sound and abnormal enamel. When the light reflection differs, the lesion can be seen as white spot and it may compromise the esthetics of patient's smile. (Benson, 2008)

Nowadays, various products release and claim to have remineralizing potential. (Lynch, Baysan et al. 2000; Bergstrand and Twetman 2003; Azarpazhooh and Limeback 2008; Pulido, Wefel et al. 2008; Reynolds, Cai et al. 2008; Willmot 2008; Bailey, Adams et al. 2009; Karlinsey and Mackey 2009; Karlinsey, Mackey et al. 2009; Karlinsey, Mackey et al. 2009; Brochner, Christensen et al., 2010; Karlinsey, Mackey et al., 2011) Thus many evaluating processes come up to measure white spot lesion both macroscopically and microscopically depend on purpose of application.

Various investigations in vitro and in vivo have shown effect of remineralizing agents on white spot lesion measured by subsurface remineralization, lesion depth, mineral content, surface microhardness and fluoride uptake, for instance. (Mellberg, Charig et al. 1986; Arnold, Dorow et al. 2006; Azarpazhooh and Limeback 2008; Bailey, Adams et al. 2009; Brochner, Christensen et al., 2010; Karlinsey, Mackey et al., 2011) However, few studies demonstrated optical properties change, which is a factor that patient might concern about more than molecular change of the lesion.

Fortunately, another interesting method in defining enamel demineralization by using photographic technique with computerized image analysis, was proposed. Researchers have shown that under controlled circumstances and appropriate camera angle, this method is not only reproducible but also cheap and accessible. (Benson, Pender et al. 1998; Benson, Pender et al. 2000; Willmot, Benson et al. 2000; Cochran, Ketley et al. 2004; Livas, Kuijpers-Jagtman et al., 2008; Willmot, 2008)

Therefore, comparison of white spot lesion change after each remineralizing agent application would be useful for patient as another consideration in choosing appropriate product. Aim of this study is to compare white spot lesion before and after intervention with two types of remineralizing products and determines which one create higher change on the lesion using computerized image analysis.

2. Materials and methods

Sixty extracted human permanent teeth which were anterior teeth, canines, premolars and molars. All teeth were macroscopically free of stains, caries, enamel defects (white spot lesions) and restorations. The patients were informed about the research information and allowed the researcher to obtain their teeth as samples. Sixty human tooth crowns were prepared. Bracket (Standard Edgewise Kit 0.018/mini dyna-lock™/3M Unitek) was adhered on each smooth surface with silicone glue. Enamel were coated with an acid resistant varnish. After bracket removal, a small window of sound enamel parallel to the horizontal plane was left as in **FIG1**. Artificial white spot lesion was created by thirty minutes immersion in demineralizing solution (pH2.2). Samples were randomly allocated into 2 groups equally for intervention: group A (GC tooth mousse: Casein Phosphopeptide-Amorphous Calcium Phosphate) and group B (Prevident®: 1.1% w/v Sodium Fluoride) are subjected to 60 remin/demin cycles. Cycle of each product was varied relating to its instruction. Group A: GC tooth mousse (CPP-ACP: Casein Phosphopeptide-Amorphous Calcium Phosphate) 35 minutes, twice per cycle. Group C: Colgate® Prevident® Gel (1.1% w/v Sodium Fluoride) 31 minutes, use once per cycle. Photographs of each sample were taken at 15 degree angle to the enamel surface. The distance between image receptor and enamel surface was kept constantly as constructed in **FIG2**. All images were converted into grayscale (8-bit range) by Photoshop Element 8.0 and analyzed using computerized image analysis (Image Pro Plus Version 3.0) before and after intervention. Gridline and ruler were set for ease of positioning and repeatability in measuring each image. Mean gray scale was measured five positions at the center of each image relating to the gridline and ruler, averaged, then calculated into

$$\text{LI\% (Luminance Intensity Proportionality)} = \left(\left(\frac{\text{mean gray level of white lesion}}{\text{mean gray level of sound}} \right) - 1 \right) \times 100$$

After 2 weeks the images were random, re-measured and calculated into LI% to test the examiner repeatability with paired t-test (at 99% CL).

The LI% of white spot lesion before and after intervention of each group was analyzed with paired t-test (at 95% CL) and the changed LI% of each group was compared with t-test statistics (at 95% CL). While examiner repeatability was tested with paired t-test (at 99% CL). All data was analyzed by SPSS, version 17.

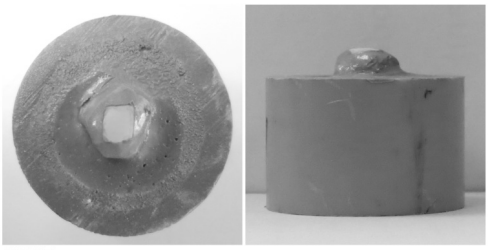

FIGURE 1

Figure 1: Photographs of a Sample: a Window of Sound Enamel Parallel to Horizontal Plane

FIGURE 2

Figure 2: Camera Setting in the Study

3. Results

Paired t-test presents no significant different of LI% (p=0.321) between repeated measurement of the same image (**table 1**). **Table 2** describes a descriptive statistics of LI% before and after intervention with each remineralizing product and their changed. Paired T-test showed that LI% decreased by an average of 2.96 $\pm$ 1.73 for group A and 4.98 $\pm$ 2.44 for group B. Value of LI% before and after intervention were significantly different for both group ($p<0.001$). In **table 3**, t-test statistics showed that changed LI% of group A and group B was significantly different ($p<0.05$).

Table 1: Repeatability test of the same image (1st and 2nd measurement): paired t-test

	1st measurement		2nd measurement		
	Mean gray scale $\bar{x} \pm$ SD.	LI%	Mean gray scale $\bar{x} \pm$ SD.	LI%	p-value*
Initial	213.883 $\pm$ 10.722		213.880 $\pm$ 10.732		
Before intervention	226.520 $\pm$ 6.443	6.083 $\pm$ 4.289	226.520 $\pm$ 6.443	6.085 $\pm$ 4.296	0.321
After intervention	218.076 $\pm$ 6.972	2.117 $\pm$ 4.134	218.075 $\pm$ 6.972	2.119 $\pm$ 4.141	0.321

*P=.01

Table 2: LI% before and after intervention with each remineralizing product: paired t-test

Remineralizing product	LI% before intervention ($\bar{x} \pm$ S.D.)	LI% after intervention ($\bar{x} \pm$ S.D.)	Changed LI% ($\bar{x} \pm$ S.D.)	p-value**
Group A	5.91 ± 4.95	2.95 ± 4.74	-2.96 ± 1.73	<0.001**
Group B	6.26 ± 3.59	1.28 ± 3.29	-4.98 ± 2.44	<0.001**

**P=.05

Table 3: Compare changed LI% of group A and group B: t-test statistics

Compare	Difference ($\bar{x} \pm$ S.D.)	p-value**
Group A / Group B	-2.02 ± 0.54	0.001**

**P=.05

4. Discussion

Various types of remineralizing products have been studied to find out if they are able to remineralize white spot lesion (Mellberg, Charig et al., 1986; Arnold, Dorow et al., 2006; Azarpazhooh and Limeback, 2008; Bailey, Adams et al. 2009; Brochner, Christensen et al., 2010; Karlinsey, Mackey et al., 2011) and enamel would be strengthen as well as before the pathology arose. However, patients that seek for orthodontic care expect mostly on esthetic result. After treatment if their teeth are function and well align but white spots are noticeable, it must not be the best result they looked forward to. Numbers of prevention were recommended for white spot lesion after orthodontic treatment. However once they occurred, better solution is to fix them. As mention above that previous studies (Lynch, Baysan et al., 2000; Bergstrand and Twetman, 2003; Reynolds, Cai et al., 2008; Bailey, Adams et al., 2009; Karlinsey, Mackey et al., 2009; Karlinsey, Mackey et al., 2009; Karlinsey, Mackey et al., 2011) concentrated on mechanical properties without mentioning the optical properties of white spot lesion which affected to patient's smile.

Consequently we focused on the luminance intensity change of white spot lesion that was observed by naked eye. Though optical property can be seen, it is hard to detect its change until the combination of photographic technique and computerized image analysis was proposed. (Benson, Pender et al., 2000; Willmot, Benson et al., 2000; Kanthathas, Willmot et al., 2005; Benson, 2008)

Photographic technique was a reproducible procedure of measuring artificial enamel demineralization while several factors were controlled such as light condition, light reflection, slanting of the camera and object's position. (Benson, Pender et al., 1998; Benson, Pender et al., 2000; Benson, 2008) Then all images were analyzed with computer.

However, because this method is sensitive, the study was designed as in vitro in order to controlled more factors. In this experiment, ring flash batteries were replaced strictly according to flash-count limit on the manual (Canon February 2010), gray card was used as a reference for image calibration and a ruler scale was used to identify the measured positions in the image in order that it can be measured repeatedly. From these additional regimens, this method is reproducible according to prior studies. (Benson, Pender et al., 1998; Benson, Pender et al., 2000; Willmot, Benson et al., 2000; Benson, Pender et al., 2003; Cochran, Ketley et al., 2004; Benson, Shah et al., 2005; Kanthathas, Willmot et al., 2005; Benson, 2008; Livas, Kuijpers-Jagtman et al., 2008) Moreover repeatability value shown in table1 is even better.

Referring to remineralizing agents, nowadays many products have been released but consumers were familiar to few names and some of them were not over the counter. The chosen products are well known and accessible. In addition, they have different active ingredients, perfect for comparative study to generate more information in making their decision.

In previous experiments (Mellberg, Charig et al., 1986; Arnold, Dorow et al., 2006; Reynolds, Cai et al., 2008; Ferreira, Aragao et al., 2009; Karlinsey, Mackey et al., 2009; Karlinsey, Mackey et al., 2009; Karlinsey, Mackey et al., 2011), intervention cycle contained same frequency and duration for each remineralizing agents application in order to control their environment. But we designed that each product's cycle conformed to its instruction to imitate the consumer's daily use. From different active ingredients, the appropriate application is also different eventually the greatest result assumed to take place when manufacturer's instruction was followed. By this method, the results represented the change of the products when using for a same period of time.

From the results, both remineralizing products decreased LI% of artificial white spot lesion significantly. According to Karlinsey et al. (Karlinsey, Mackey et al., 2011), though different variables were measured for remineralization, same results occurred. Active ingredients in each remineralizing products worked in their own way to create remineralization.

- GC tooth mousse decrease optical appearance of white spot lesion from its ability to buffer and increase the level of calcium phosphate in plaque. At the time of calcium phosphate supersaturation, GC is not only inhibiting enamel demineralization but also enhancing enamel remineralization. (Azarpazhooh and Limeback, 2008; Brochner, Christensen et al., 2010)

- Fluoride creates enamel remineralization by fluoroapatite formation and it has antibacterial effect on cariogenic bacteria. (Featherstone, 1999; Koo, 2008)

However, the difference of white spot lesion before and after intervention was obvious when measuring on the surface portion but in deeper part, no difference detected. As shown in Karlinsey et al.'s study (Karlinsey, Mackey et al., 2011) that surface microhardness after dentrifrices application was difference while cross sectional microhardness at 50 μm depth or deeper was not difference. It can be assumed that the penetrating ability of remineralizing agents was limited.

Although both groups represented improvement in white spot lesion, group B demonstrated better change on white spot lesion which same results were found for surface microhardness. (Karlinsey, Mackey et al., 2011)

Even though this study was designed as in vitro but it was a good initiation to measure white spot lesion as optical change instead of microscopic change. Suggestion for further investigation is to design appropriately for in vivo study or clinical trial.

5. Conclusion

Optical appearance of white spot lesion was decreased after applying these two remineralizing products and Prevident® demonstrated higher change on white spot lesion.

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