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Histopathological Analysis of Sealant Infiltration in White Spot Enamel Lesions (WSEL)

Análisis Histopatológico de Infiltración de Sellante en Lesiones de Mancha Blanca en Esmalte (LMBE)

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ABSTRACT: The use of resin sealants has shown partial infiltration of White-Spot Enamel Lesions *in vitro* (WSEL). The aim of the present study was to perform a morphological evaluation of natural WSEL when infiltrated using a commercially available sealant (Concise, 3M- ESPE). 20 bicuspids extracted for orthodontic reasons from patients ranging 18 to 30 years old, which had WSEL, were used in this study. The patients agreed to donate their teeth by signing a written consent. Every WSEL was assessed microscopically (Stereo Zeiss Axiscop) and then photographed (ProScope HR microscope). Prior to applying the sealant the lesion was etched using phosphoric acid at 37 % (3M-ESPE, St Paul, MN, USA.) for 30 seconds, washed for 40 seconds and then air-dried. The sealant was marked with rhodamine B (1mg/ml) and was applied according to the manufacturer directions. A specimen of approximately 100 mm was obtained for every WSEL by cutting perpendicularly through the lesion (Isomet 1000, Buehler Co.) and grinding (600 grit). The specimens were evaluated using: clear camp, polarized light, and epifluorescence microscopy. Images were taken of each specimen for every microscopic evaluation using a slide film (Kodak Ektachrome film 400 ASA). The images were digitalized by scanning at 1200 dpi resolution (Epson Filmscan 200) and then saved as JPEG and TIFF files. The sealant infiltration into The WSEL was assessed by means of analysis, processing and digital superimposing using Adobe Photoshop 7.0 and Matrox Inspector 1.07. It was concluded that the sealant infiltrated the whole body zone of the lesion. The depth of penetration of sealants into White Spot Enamel Lesion plays an important role in the control of caries lesion progression.

KEYS WORDS: histopathological analysis, sealant, white-spot enamel lesion, microscopy

INTRODUCTION

Dental caries lesion is a continuum, from the earliest loss of ions from apatite crystals to lesion cavitation (Featherstone, 2004). Recently, attention has shifted to the early detection of dental caries to identify lesions before cavitation so that non-invasive or minimally invasive treatment approaches can be applied. White-Spot Enamel Lesions (WSEL) are a stage of the caries process, prior to cavitation, where mineral has been lost from the enamel subsurface, with an intact surface layer overlying the mineral-poor region (Arends & Christoffersen, 1986).

These lesions can be clinically assessed as either active or inactive (Nyvad *et al*., 1999) and may be treated by remineralization (Cochrane *et al*., 2010) or infiltration with low-viscosity resin materials (Paris & Meyer-Lueckel, 2010a; Ammari *et al*., 2014; Meyer-Lueckel *et al*., 2016). These treatment options require ions or infiltrant to enter the lesion body, either to repair the damaged crystals or to seal them in resin, respectively. However, removal of the WSEL surface layer by acid etching is usually required for adequate penetration of the resin into the lesion body. This has renewed interest in the structure of WSEL, particularly the surface layers and how they can be modified (Meyer-Lueckel *et al*., 2007; Cochrane *et al*.,).

The ability of resins to penetrate into the porous lesion body of enamel lesions was described almost 40 years ago (Davila *et al*., 1975; Robinson *et al*.,

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1976). Since then the penetration and arrest of artificial lesions by dental adhesives and fissure sealants have been investigated in several laboratory studies (Rodda, 1983; Donly & Ruiz, 1992; Robinson *et al*., 2001; Gray & Shellis, 2002; Schmidlin *et al*., 2004; Meyer-Lueckel *et al*., 2006; Paris *et al*., 2006). However, dental sealants and adhesives have shown only superficial penetration into artificial enamel lesions (Paris *et al*., 2007, 2014). Since natural lesions differ from artificial lesions not only with respect to the thickness and porosity of the surface layer but also regarding the histology of the lesion body, the aim of the present study was to achieve a morphological assessment of natural WSEL when treated with a commercially available sealant using microscopy.

MATERIAL AND METHOD

Patients ranging 18 to 30 years old, requiring tooth extraction for orthodontic reasons treated at the Dental Surgery Service at the Dental School of the Universidad de Chile were recruited for this study. Patients agreed to donate their teeth by signing informed consent. As a result, 60 human molars and premolars with WSEL were obtained.

Each tooth was carefully cleaned of soft tissues and stored in 0.1 % thymol solution until use.

In order to select only teeth with “active” (dull surface, chalky opacity) non-cavitated proximal white-spot lesion (ICDAS II, code 2), two calibrated observers (Kappa 0.8) examined the 60 teeth using ICDAS criteria (Ismail *et al*., 2007). Observations were made with a 20x Stereo Microscope Zeiss Stemi 1000 (Carl Zeiss Microscopy, LLC, USA). The final sample, after excluding cavitated as well as damaged lesions, was constituted for 20 teeth (n=20) that were photographed using a Proscope HR microscope (Bodelin Technologies, Oregon, USA) under epipolarization illumination and stored as JEPG files.

Lesions were etched with 37 % phosphoric acid gel (3M ESPE, St Paul, MN, USA) for 30 seconds, washed with water for 40 seconds and then dried with compressed air for 10 seconds. Subsequently, Concise white photopolymerizable sealant (3M ESPE, St Paul, MN, USA) marked with rhodamine B (1mg/ml) was applied following the manufacturer directions.

Each tooth was covered with a polyethylene stretch film (Euro-Lite Coreless Stretch Film) and embedded in methyl methacrylate. After resin polymerization, all specimens were sectioned mesio-distally with a water-cooled circular diamond saw in an Isomet 1000 machine (Buhler; Lake Bluff, IL, USA). Serial ground sections of 500 mm thick were cut from specimens. Thick sections were ground to approximately 100 mm, with wet 600 grit polishing paper.

Microscopy. WSEL were firstly examined by transmitted light in an Axioscop microscope (Zeiss, Jena, Germany) equipped with epi-fluorescence and epi-polarization attachments. The light source for fluorescence microscopy was a 50 W high-pressure mercury lamp. At 40x magnification, WSEL were studied using transmitted light, polarized light and epifluorescence simultaneously in the same optical plane. Images were recorded on Kodak Ektachrome film 400 ASA. The positive films were digitalized with an Epson Filmscan 200 at 1200 dpi and obtained images were stored as JPEG and TIFF file formats.

The sealant infiltration into the WSEL was assessed by means of analysis, processing and digital superimposing using Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Jose, CA, USA).

Microscopical assessment of samples:

Clear Camp microscopy. It was used to confirm histologically that clinically observed lesions were effectively enamel caries lesions by identifying each of the four areas of a WSEL.

Polarized light microscopy. It was used to observe the birefringence of the areas of the lesion.

Epifluorescence microscopy. It allowed detecting the sealant marked with rhodamine B. The infiltrated areas were seen as bright red (Fig. 1).

Images processing and digital analysis. To delimit the area of the infiltrated zone, images obtained by epifluorescence microscopy were binarized using Adobe Photoshop 7.0 software (Adobe Systems Incorporated, San Jose, CA, USA), the tools used were image/adjustments/threshold level 48. The area corresponding to the sealant marked with rhodamine B appeared white and the sound enamel black (Fig. 2).

Subsequently, in order to clearly establish the depth of sealant infiltration in the histological image, the infiltration area was delimited using the Magic Wand Tool in the binarized image. This limit was accurately superimposed to the image obtained by clear camp microscopy using the move tool to transfer the limits from the first to the second image and then the image/stroke tools to define the color and width of this limit.

This way, the depth of penetration of the sealant was judged as reaching: the translucent zone, the dark zone, the body of the lesion or the surface zone.

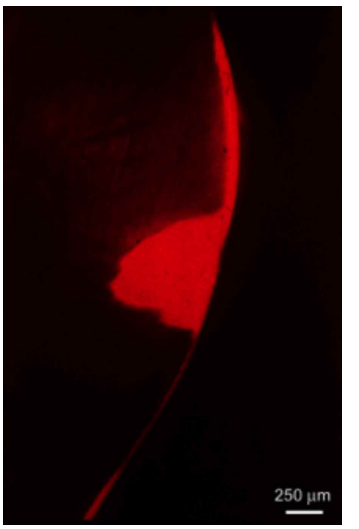

Fig. 1. In the epifluorescent microscopic images, the sealant showed a red fluorescence, whereas remaining sound enamel, was displayed black.

Fig. 2. Epifluorescent image after processing.

RESULTS

In this study, only non-cavitated proximal white-spot lesion (ICDAS II, code 2) were included (Figs. 3 and 4).

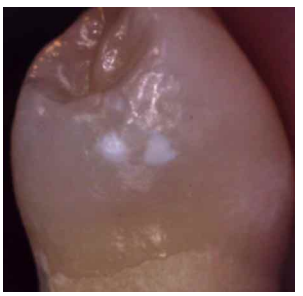

Fig. 3. WSEL in a bicuspid.

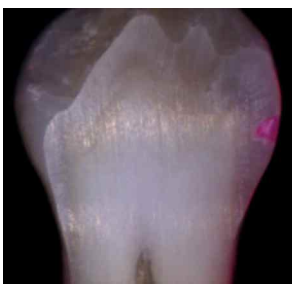

Fig. 4. WSEL after the first sectional cut.

Under clear camp microscopy, it was confirmed that all samples corresponded to caries lesions. All the WSEL were located in the enamel and no one reached the enamel-dentin junction and all samples showed histologic characteristics in agreement with rapidly progressing enamel lesions, explained by the presence of a thin dark zone (Kidd, 1983) (Fig. 5).

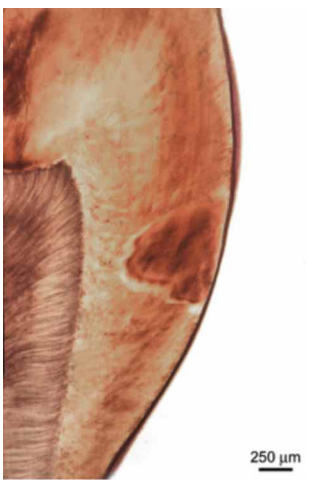

Fig. 5. The Clear Camp microscopic image shows the different zones of WSEL. It can be seen from inside to outside of the lesion: the translucent zone, the dark zone, the body of the lesion and the superficial zone.

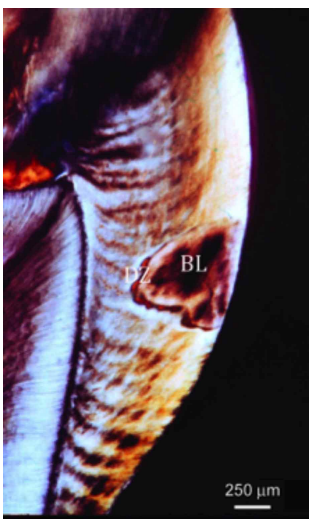

Fig. 6. The polarized light microscopic image shows the characteristics of birefringence of the different zones of the WSEL.

Observation under polarized light microscopy showed a negative birefringence in both, the superficial zone and translucent zone, whereas in the body of the lesion and dark zone a positive birefringence was observed (Fig. 6). There was a histological correlation between samples analyzed by clear camp and polarized light microscopy.

In all the specimens, it was observed that the sealant infiltrated completely the body of the lesion and reached the dark zone (Fig. 7A and B).

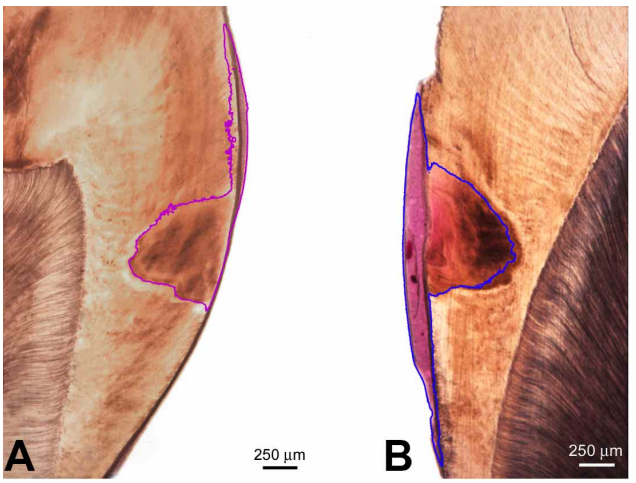

Fig. 7A-B. The pattern of penetration was similar in all samples. The image processing confirmed that the sealant penetrated completely the body of the lesion zone in all samples.

DISCUSSION

Different techniques aiming either to arrest or to reduce the progression of enamel caries have been proposed as treatment options of proximal white-spot lesions.

Martignon *et al*. (2006) have used phosphoric acid (37 %) and a dental adhesive. Gomez *et al*. (2007) replaced the adhesive with a fissure sealant; Meyer-Lueckel *et al*. (2007) have suggested replacing the use of phosphoric with hydrochloric acid, since it has been seen that an important factor that allows the penetration of resins inside the zones of a WSEL is the etching treatment of the low porous surface of enamel caries and an effective reduction in this surface can be achieved by etching with 15 % hydrochloric acid gel for 90–120 seconds. Then, in 2007 infiltrants were introduced. The main characteristics of these products are low viscosity and relatively high penetration coefficient and they have demonstrated to be capable of almost completely inhibiting the progression of natural enamel caries lesions in a low-demineralizing environment (Paris *et al*., 2007; Meyer-Lueckel & Paris, 2010).

Nevertheless, despite the results of recent studies that orient to the use of resins with a high penetration coefficient (Meyer-Lueckel & Paris, 2010; Paris & Meyer-Lueckel, 2010b) and that, according to Paris *et al*. (2007), only superficial penetration can be expected with the use of fissure sealants, they have demonstrated to be effective for arresting non–cavitated enamel and dentine lesions in the short and medium term, and an effective method for caries control in primary and permanent teeth (Gomez *et al*., 2005, 2007; Martignon *et al*., 2010, 2012; Ammari *et al*.,).

Moreover, the current *in vitro* study provides evidence of efficacy of a commercially available fissure sealant (Concise) to infiltrate proximal WSEL after the surface of lesions was etched with a conventional phosphoric acid gel during 30 seconds. Also, as seen in the histopathological analysis the sealant penetrated as deep as the dark zone in all the samples studied.

Even though the surface of a WSEL is much more acid-resistant than areas of sound enamel (Lee *et al*., 1995; Iijima & Takagi, 2000), under the conditions of this study the use of phosphoric acid over a cleaned surface appeared to be enough to provide access for the sealant to infiltrate the natural enamel lesion.

The results of this study note that depth of penetration could also be influenced by other factors besides pretreatment, etching product, and the way of its application as well as the resins used to infiltrate or seal the lesion. In that sense, the authors consider that histopathology of the lesion plays an important role in the permeability of WSEL influencing the penetration of the sealant. As known, every zone of an enamel caries lesion has a different porosity. In this sense, an enamel caries lesion that has a comparatively small dark zone offers more permeability than an enamel caries lesion with a bigger one, since the dark zone has a lesser porosity (5-10 %) than the body of the lesion (25-25 %) (Robinson *et al*., 2000). This could help to explain the diversity of results obtained by Lausch *et al*. (2015, 2017) and Paris *et al*. (2013, 2014).

The results of the current study, in which all the samples showed histologic characteristics in agreement with rapidly progressing enamel lesions, explained by the presence of a thin dark zone, which, either in slowly progressing or arrested lesions is frequently very wide (Kidd), show that penetration depth of ConciseTM is interestingly deep enough, to be considered as a viable preventive measure, especially considering that penetration depth has been strongly correlated with the ability of materials to hinder lesion progression of artificial lesions *in vitro* (Meyer-Lueckel & Paris, 2008).

However, in specific clinical situations, it is hard to decide whether a WSEL is a rapidly or slowly

progressing lesion. Some studies performed in developed countries, state that occlusal and proximal caries progression has slowed, so the nature of primary caries appears to have changed from a rapidly progressing disease of childhood to a slowly progressing disease, which commences in childhood but progresses steadily in adulthood (Mejàre *et al*., 1998, 1999; Gustafsson *et al*., 2000; Isaksson *et al*., 2013). The authors estimate that in those clinical situations in which there is a high cariogenic activity the proportion of rapidly progressing lesions should be higher. Therefore, this non-invasive procedure could be a viable approach to hinder lesion progression in such clinical situations, replicating the results obtained with infiltrates (Meyer-Lueckel *et al*., 2016; Meyer-Lueckel & Paris, 2016).

The results of the present study could allow explaining from the point of view of histopathology the success rates in clinical studies of the therapeutic seal of proximal carious lesions (Gomez *et al*., 2005, 2007; Martignon *et al*., 2006, 2010, 2012; Ammari *et al*.).

CONCLUSIONS

According to the results of the present study, it can be concluded that the use of a conventional fissure sealant, on the surface of rapidly progressing white spot enamel lesions, etched with phosphoric acid is a procedure that achieves deep penetration of the sealant, which constitutes an important foundation for its use as a micro invasive treatment to hinder caries progression

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RESUMEN: El uso de sellantes de resina ha demostrado lograr la infiltración parcial de lesiones de mancha blanca en esmalte *in vitro* (LMBE). El objetivo del presente estudio fue realizar una evaluación morfológica de la infiltración a LMBE naturales utilizando un sellante disponible comercialmente (Concise, 3M-ESPE). Se utilizaron en este estudio, 30 bicúspides extraídos por razones de ortodoncia en pacientes que tenían entre 18 a 30 años de edad, y presentaban LMBE. Los pacientes aceptaron donar sus dientes firmando un consentimiento informado. Cada LMBE se evaluó microscópicamente (Stereo Zeiss Axiscop) y luego se fotografió (microscopio ProScope HR). Antes de aplicar el sellante, la lesión se grabó usando ácido fosfórico al 37 % (3M-ESPE, St Paul, MN, EE. UU.) durante 20 segundos, luego se lavó durante 40 segundos y finalmente se secó con aire de jeringa triple. El sellante se marcó con rodamina B (1 mg / ml) y se aplicó siguiendo las instrucciones del fabricante. Se obtuvo una muestra de aproximadamente 100 mm para cada LMBE cortando perpendicularmente a través de la lesión (Isomet 1000, Buehler Co.) y trituración (grano 600). Las muestras se evaluaron usando: campo claro, luz polarizada y microscopía de epifluorescencia. Se tomaron imágenes de cada espécimen para la evaluación microscópica usando una película deslizante (Kodak Ektachrome film 400 ASA). Las imágenes se digitalizaron escaneando a una resolución de 1200 ppp (Epson Filmscan 200) y luego se guardaron como archivos JPEG y TIFF. La infiltración del sellante en las LMBE se evaluaron mediante análisis, procesamiento y superposición digital utilizando Adobe Photoshop 7.0 y Matrox Inspector 1.07. Se concluyó que el sellante infiltró toda la zona del cuerpo de la lesión. La capacidad de penetración en profundidad de los sellantes en Lesiones de Mancha Blanca del Esmalte desempeñan un rol importante en el control de la progresión de las lesiones de caries.

PALABRAS CLAVE: análisis histopatológico, sellante, lesión de mancha blanca del esmalte, microscopía.

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