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Modification of surface pre-treatment for resin infiltration to mask natural white spot lesions

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ABSTRACT

Objectives: The aims of this study were to investigate an alternative pre-treatment method for resin infiltration using 37% H_3PO_4 with a brush applicator and to evaluate the penetration effect of the infiltrant for masking the natural white spot lesions (WSLs) in human teeth.

Methods: Seventy extracted human molars and pre-molars with non-cavitated WSLs were collected. Thirty teeth met criteria of ICDAS code 2, and were sectioned, providing a total of sixty paired halves. For the control group, 15% HCl gel was applied for 120 s, and 37% H_3PO_4, gel was gently rubbed with a brush applicator for 30 s to the experimental group. Also, to evaluate the penetration effect of the infiltrant by pre-treatment, the specimens were treated with the infiltrant (Icon®). Thicknesses of the removed surfaces and percentages of the infiltrated areas (IA%) were evaluated by CLSM, and micro-morphological changes were observed by SEM.

Results: The mean thicknesses of removed surface layers were significantly different between the control group (36 ± 7.62 μm) and the experimental group (13 ± 2.76 μm) ($p < 0.001$). But, the means of IA% were similar in both groups ($p > 0.05$). In the SEM images, the prism cores were preferentially dissolved in the control group, while the prism peripheries were preferentially dissolved in the experimental group.

Conclusions: Applying 37% H_3PO_4 gel with an applicator brush for 30 s could increase the permeability and minimize removal of the surface layer of natural WSLs. Moreover, the effect of resin infiltration was similar to the control group which was pretreated 15% HCl gel for 120 s *in vitro* study.

Clinical significance: For resin infiltration, applying 37% H_3PO_4 gel with a brush applicator can preserve the protective surface layers of the WSLs with reduced application time.

1. Introduction

During orthodontic treatment, the development of white spot lesions (WSLs) is one of the greatest challenges faced by dentists. Because fixed orthodontic appliances lead to plaque accumulation, rapidly changing the levels of acidogenic bacterial flora, the progression of WSLs is fast in orthodontic patients. Although WSLs can appear on any tooth surface, they are most frequently occurred on the facial surfaces of anterior teeth causing aesthetic problems.[1–3] Some methods such as micro-abrasion compound or restoration have been

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used to improve the appearance of facial surface of WSLs. However, unfortunately, these methods remove a large amount of dental hard tissue.[4,5]

Resin infiltration has been proposed to treat early caries lesions. In recent years, a very low-viscosity light curing resin so called 'infiltrant' is being used in WSLs to inhibit further demineralization.[6–8] In addition, several studies reported that WSLs with infiltrant showed improved aesthetic appearance. Furthermore, the treated WSLs tended to lose their whitish appearance after the treatment, appearing very similar to the surrounding sound enamel.[9–11]

Previous studies applied 15% hydrochloric acid (HCl) gel for 120 s to remove the hypermineralized superficial layer of WSLs which hampered the infiltrant penetration.[6,7,12] Moreover, some studies attempted to completely remove the surface layer by repeating the application of HCl or by using a micro-brush in order to obtain aesthetically more favourable outcome.[9,10,13] However, the surface layer is composed of high mineral contents, and acts as a protective layer against cariogenic acid attack.[14,15] Several previous studies reported that the lesions, which were pre-treated with 15% HCl for 120 s for removal of the surface layer and followed by the resin infiltration, were resistant against lesion progression. However, there was a concern about possibility of degradation of the infiltrated resin with time.[16] In addition, it was suggested that there is a need to further explore the use of phosphoric acid, which is a weak acid, to protect the surface layer, but at the same time, increase the permeability of the surface layer.[13]

Also, because the WSLs are commonly developed close to the gingival region after the orthodontic treatment,[1,3] there might be some unintended gingival damages during application of strong acid such as 15% HCl. According to an earlier study, when micro-abrasion compound containing 10% HCl was used in contact with the soft tissue for more than 30 s, it caused considerable amount of ulceration that took more than 24 h to heal.[17,18] Thus the time recommended by the commercially available resin infiltration's instructions, etching with 15% HCl for 120 s, might be relatively too long. So, it is important to explore a method to use a weaker acid with reduced contact time, so that minimizing the removed depth of the surface layer, but at the same time increasing the permeability of the surface layers of the WSLs. Therefore, we chose to use 37% phosphoric acid (H_3PO_4) gel with some mechanical technique, to enhance the effect of acid for the resin infiltration, from which WSLs can be masked.

The first aim of this *in vitro* study was to investigate whether the mechanical application of 37% H_3PO_4 can be an alternative pre-treatment method for resin infiltration, and the second aim was to evaluate the different penetration effects of the infiltrant according to the pre-treatment method in natural white spot lesions of the human teeth.

2. Materials and methods

2.1. Specimen preparation

Seventy extracted human molars and pre-molars with non-cavitated natural WSLs (mainly proximal lesions) were collected for this study. The protocol was approved by an Institutional Review Board for Clinical Research in Yonsei dental hospital (IRB 12-0119). After being carefully cleaned of soft tissues, the teeth were sterilized in autoclaving with distilled water, and stored at refrigeration temperature (4 °C) until used. The chosen teeth had dull and chalky opaque surface and met the criteria of ICDAS (International Caries Detection and Assessment System) code 2.

After removing the roots, the crowns were sectioned perpendicularly to the lesion surfaces with a micro-tome (TechCut 4™, Allied High Tech Products, CA, USA). The cross-sectional cut surfaces were examined using a stereomicroscope (M165FC, LEICA, Wetzlar, Germany) with magnification of 12.5. The micro-damaged enamel lesions were excluded, but the lesions that were histologically extended at least into the inner half of enamel were included in this study, of which 23.3% of the 30 lesions were on the buccal surfaces. Thirty teeth that met these criteria were sectioned, providing two halves of each lesion, making a total of sixty paired halves. The cut surfaces were covered with nail varnish. The forty paired halves were allocated for an experiment to investigate the thicknesses of the removed surface layers, and the other twenty paired halves were allocated for an experiment to evaluate the penetration effects of the resin infiltrant after different pre-treatments.

2.2. Procedure for removing surface layer

As baseline, a portion of each WSLs was covered with nail varnish, and the paired lesion halves were allocated to either one of the two groups ($n = 20$ each). The control group involved application of commercially available 15% HCl gel for 120 s, whereas the experimental group involved 30 s gentle application of 37% H_3PO_4 gel (ETCH-37™, Bisco, Schaumburg, USA) with a brush applicator that was included in the CavityShield® (3M ESPE, St. Paul, USA). The application force was less than 10 g as determined using an electronic scale. All processes were performed by one researcher to reduce the variations. After acid etching, the specimens were washed for 30 s using a dental air-water spray. From each group, twelve specimens were selected to evaluate the thicknesses of the removed surface layers, and the other eight specimens were used to evaluate the micro-morphological changes in the lesion surfaces.

First, for visualization of the WSLs, the specimens were immersed in 50% ethanol solution of 100 μM sodium fluorescein (NaFl; Sigma–Aldrich, Steinheim, Germany) for 3 min.[19] After washing with distilled water for 60 s, the depth of the WSLs and the thicknesses of the removed surface layers were evaluated using a confocal laser scanning microscope (CLSM; LSM710, Carl Zeiss, Oberkochen, Germany) equipped with an air lens (Plan-Apochromat 40×/0.95 Korr M27 or Plan-Apochromat 10×/0.45 M27) in fluorescence mode.

The images were recorded with a lateral resolution of 1024 × 1024 pixels and were acquired with pixel dwell time 1.27 μs, line average 4, 8-bit intensity resolution. Also, two dimensional images (xy-scan) were obtained from a plane directly below the cutting surfaces of specimens at plane scan mode. They were measured at six defined points using the private analysis programme of CLSM image (ZEN 2009 LE software, Carl Zeiss, Oberkochen, Germany). For the removed