EFFECT OF DIFFERENT WHITE SPOT LESION TREATMENT ON ORTHODONTIC SHEAR STRENGTH AND ENAMEL MORPHOLOGY

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EFFECT OF DIFFERENT WHITE SPOT LESION TREATMENT ON ORTHODONTIC SHEAR STRENGTH AND ENAMEL MORPHOLOGY: IN VITRO STUDY

Running title **Different white spot lesion treatment on orthodontics**

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Running title: Different white spot lesion treatment on orthodontics

Abstract:

Background: White spot lesion treatment is required prior to fixed appliance insertion as caries prevalence increases during orthodontic treatment. The aim of this study was to analyse the effect of fluoride varnish, CPP-ACPF varnish, and resin infiltration as white spot lesion treatments on orthodontic shear strength and enamel morphology. **Settings and Design:** An observational analysis study with post-test only control group design and blind total sampling methods. **Materials and Methods:** Sixty bovine mandible incisors were cleaned and divided into 5 groups (n=12). No treatment was applied on group 1 (Control), while 4 other groups were immersed in demineralization solution for 96 hours prior to being stored in artificial saliva

(Group 2), treated with fluoride varnish (Group 3), CPP-ACPF varnish (Group 4), and resin infiltration (Group 5). After bracket bonding with RMGIC on all samples, shear bond strength was evaluated. Adhesive Remnant Index (ARI) was observed by using digital photo. Enamel morphology was evaluated using Scanning Electron Microscope (SEM) before bracket bonding and after polishing. **Statistical analysis used:** Kruskal-Wallis test ($p>0.05$) followed by Mann-Whitney Test, was done ($p<0.05$). **Results:** Shear strength in Group 2 and 5 was slightly increased compared to Control group ($P>0.05$), while in Group 3 and 4 significantly decreased ($P<0.05$). The highest ARI score was found after treatment with resin infiltration. **Conclusion:** Pre-treatment with resin infiltration is recommended to be performed before bracket bonding on white spot lesion, however, it may need to be re-applied after debonding and polishing to conceal enamel morphology damage formed after polishing.

Keywords: white spot lesion treatment, orthodontic shear strength, enamel morphology, orthodontic treatment.

Key Messages: Pre-treatment with resin infiltration is recommended to be performed before bracket bonding on white spot lesion, however, it may need to be re-applied after debonding and polishing to conceal enamel morphology damage formed after polishing.

Introduction:

One of the side effects of fixed orthodontic treatment is the formation of white spot lesions (WSLs), defined as decalcification or demineralization of the enamel appearing as chalky-white area on tooth surface, which were considered as the early stage of dental caries caused by bacterial plaque activity. The WSLs at the end fixed orthodontic treatment prevalence has been mentioned in up to 97 of patients.[1] A meta-analysis study stated that the incidence of WSLs in patients with orthodontic appliances was 45.8%, whereas the prevalence was 68.4%.[2] The presence of WSLs is strongly influenced by the level of oral hygiene, sex, orthodontic treatment length, fluor intake, and diet.[3]

In white spot lesions, a decrease of orthodontic bracket bonding strength occurs, and which will cause the bracket to fall off more easily during the course of treatment. It may be caused by the poor quality of enamel surfaces and lack of resin tag that makes it possible for mechanical attachment between composite bonding material and enamel surface to occur. This is very detrimental to orthodontists and patients as it prolongs the overall treatment time and the duration of dental visit.[4,5]

To overcome this problem, several agents that can inhibit enamel demineralization and enhance enamel remineralization can be used, especially the ones that can be applied in one-visit to shorten the duration of dental visit and do not require patient's cooperation. Fluoride, commonly used as a remineralization agent, forms a layer of calcium fluoride on the enamel surface thus stimulating enamel remineralization.[6] Fluoride varnish application can significantly decrease the formation of the demineralization lesion around the bracket on anterior teeth.[7] The addition of fluoride to CPP-ACP (Casein Phosphopeptide-Amorphous Calcium Phosphate) strengthens the remineralization effect compared to the use of either fluoride or CPP-ACP. CPP-ACPF is able to provide calcium, phosphate and fluoride ion reservoir on tooth surfaces to form a more acid-resistant fluorapatite film.[8] In addition, white spot lesions can also be treated by the application of resin infiltration. Resin infiltration can significantly arrest the progression of early caries lesions by eroding and destroying the lesions, then coating them with TEGDMA-based resins which increase the resistance of lesions and surrounding healthy enamel against acid.[9] However, those treatments may affect the shear strength of the orthodontic bracket bonding.

Based on those facts, this research is conducted to determine the effect of several one-visit treatments of white spot lesions prior to orthodontic fixed treatment on shear strength of RMGIC orthodontic bonding agent and their effect on enamel morphology.

Materials and Methods:

This in-vitro study was using post-test only control group design, done in Faculty of Dentistry Airlangga University Dental Hospital (Number: HRECC.FODM/XII/2018). Sixty samples of bovine (*Bos indicus*) permanent mandibular incisors were cleaned using pumice on a low-speed handpiece brush to remove debris, then cut by separating disc (7/8 SS Flat Baker) mounted on a straight low-speed handpiece. The teeth were cut on the cervical section to separate the crown and the root. The cut tooth was then placed inside a pre-formed plastic tube with its labial surface facing upwards and at an equal height to the top of the plastic tube, using self-cured acrylic (Vertex) as its stabilizer. The plastic tube was made from 5 cc syringe tube, with a screw half-inserted into it as it acted as handle during shear strength test procedure.

The samples were then divided into 5 groups. No treatment was applied on Group 1 (control). Each sample in other 4 groups was immersed in 10 mL of demineralization solution (HCl 33%, NaOH 40%, dan H_2SO_4 27%) for 96 hours in incubator at 37 ° C. This immersion was performed to produce artificial caries lesion as deep as 120-200 μm. Samples on Group 2

were then stored in artificial saliva. White spot lesion treatments were performed on the other 3 groups: fluoride varnish (*Fluor Protector,* Ivoclar Vivadent) in Group 3, CPP-ACPF varnish (*MI Varnish*, GC) in Group 4, and resin infiltration (*ICON Infiltrant*, DMG) in Group 5. All treatments were performed according to the manufacturer's instruction. After treatments, all samples were put in artificial saliva until bracket placement.

RMGIC bonding agent was applied on maxillary central incisor bracket mesh (*Integra*, RMO) using an applicator stick. Brackets were then attached onto the teeth (Figure 6). Excessive adhesive agent was removed using a probe. The brackets were then cured using a light curing unit from four directions, each for 10 seconds. All samples were stored in artificial saliva for 1 day before shear strength test to make complete polymerization of RMGIC bonding agent possible.

Each sample was then placed on shear strength measurement tool (Autograph Shimatzu, Japan). After that, obtained data were analyzed using Kolmogorov-Smirnov One-Sample normality test ($p>0.05$), one-way ANOVA and Tukey HSD statistical tests ($p<0.05$). A visual scoring of ARI using digital camera was performed by 3 different observers using 4-point scale of Artun and Bergland (1984), where 0: no adhesive left on tooth surface; 1: less than 50% of the adhesive is left on tooth surface; 2: more than 50% of the adhesive is left on tooth surface; 3: all adhesive is left on tooth surface with a distict impression of the bracket base.[11] ARI evaluation scores obtained were analyzed by Kruskal-Wallis Test and Mann-Whitney Test ($p<0.05$).

Further visual observation on enamel surface by Scanning Electron Microscopy (SEM) was made before the application of white spot treatments and after debonding and polishing. Crack length and porosity diameter were analyzed using the Kolmogorov-Smirnov One-Sample normality test, one-way ANOVA and Tukey HSD statistical tests. T-test was used to analyze the difference of crack length before and after debonding and polishing.

Statistical Analysis:

All statistical analyses were performed using software 20.0 version of Statistical Package for Social Science (SPSS, IBM Corporation, Chicago, Illinois, US) Descriptive statistics were performed for the calculation of mean, standard deviation, maximum, and minimum. Kruskal-Wallis test ($p>0.05$) followed by Mann-Whitney Test, was done ($p<0.05$).

Results:

Effect of treatments on enamel surface

On 50x magnification of SEM observation (Figure 1), it was seen that enamel surface in the control group showed slight depressions with defined margin. In demineralization group, the enamel surface looked chalky with more clearly seen vertical depressions, some with defined margin. After fluoride varnish was applied, enamel depressions appeared to be filled with the material so that they were not as deep as those in demineralization group. In contrast, there were the depressions found in CPP-ACPF varnish treatment group because it appeared to be concealed by a homogeneous layer. Treatment with resin infiltration caused the enamel surface to look smoother than that of control group.

It appeared that most of the direction of enamel cracks in the all groups were vertical and diagonal, but there were also horizontal cracks in the demineralization group (Figure 2). The black dots, which were enamel porosities, were more clearly seen in the larger area on the demineralization group than the control group. In contrast, there were less porosities in the treatment group with fluoride varnish, CPP-ACPF varnish, and resin infiltration.

The porosity in both control and demineralization group had honeycomb structure, but it was deeper in demineralization group compared to that in control group as it had more contrast color. However, fluoride varnish application altered the porosity to look like layered-honeycomb, in which the porosity was covered by varnish material, and the varnish itself also forms a honeycomb coating layer. The enamel surface appeared to be covered by a layer of varnish in CPP-ACPF varnish treatment group, with no porosity found on some parts of the image. In resin infiltration treatment group, the porosity appeared to be defective, as characterized by the irregular porosity margin and the presence of interconnected porosity or damaged honeycomb. However, the size of each porosity appears to be smaller than that of the control group and seemed covered in some places.

Shear strength test

It was indicated that demineralization and resin infiltration groups had slightly higher mean of shear strength than control group, whereas treatment with fluoride varnish and CPP-ACPF varnish caused lower shear strength (Table 1). However, there was no significant difference in shear strength among demineralization, control, and resin infiltration group although the mean of demineralization group was higher than that in other groups (Table 2). Shear strength in the CPP-ACPF varnish treatment group was significantly lower than in the other 4 groups. Treatment with fluoride varnish also resulted in lower shear strength than in all groups but in CPP-ACPF varnish group.

Adhesive Remnant Index (ARI)

The scores of Adhesive Remnant Index (ARI) obtained were ordinal data so, non-parametric statistics were used in the calculation (Table 3). The ARI score mode in resin infiltration treatment group was the highest of all the other 4 groups.

Based on Kruskal-Wallis test followed by Mann-Whitney Test (Table 4), it can be concluded that the ARI score mode in resin infiltration treatment group was the highest of all the other 4 groups. Besides, the ARI score was significantly higher in demineralization group than those of CPP-ACPF varnish group.

Effects on enamel surface after debonding and polishing

After debonding and polishing process, observation was made using SEM to see enamel surface alteration further. Figure 3 showed that there was some adhesive material left in control and demineralization group, even after visually-checked polishing had been performed. In contrast, there was no visible residual adhesive in other 3 treatment groups. However, a layer of varnish material disappeared. Treatment with resin infiltration enabled the enamel surface to appear as smooth as before bracket placement.

On higher magnification, it was revealed that there was residual adhesive on the enamel surface of all samples (Figure 4). Similar to the observations before bracket placement, vertical depressions were clearly visible, with deeper depressions in the in the demineralization group. In contrast, enamel damage was found on resin infiltration treatment group, as marked by the presence of black areas on several spots.

A greater amount of adhesive remnant was found in the demineralization than in control group, whereas that in resin infiltration group was higher than in the demineralization group (Figure 5). They appeared only as white dots in fluoride varnish treatment group. No residual adhesive was seen in CPP-ACPF varnish treatment group.

Discussion:

In this study, the SEM analysis revealed that demineralization process resulted in more enamel cracks than in the control group. In addition, enamel surface got rougher, whitish, with deeper depression than that of control group. Enamel porosities were deeper as observed with greater magnification. This result was consistent with the study of human premolars by Nhu et al. in which honeycomb appearance was seen after immersion in demineralization solution, confirmed the occurrence of enamel's mineral solubility.[12] Other studies by Worawongvasu and Hicks et al also stated that demineralization process led to partial disappearance of central

prisms nucleus, while the peripheral prims remained. The open structure of the prisms produced a honeycomb appearance.[13,14]

Smoother enamel topography was obtained after applying white spot treatment materials. Varnish material could fill the depression caused by demineralization process. CPP-ACPF varnish layered enamel surface with a homogenous substance, and thus it looked smooth. This result was consistent with experiments by Poggio et al. and Kucuk at al. using CPP-ACP in the form of paste. SEM analysis revealed that the porosities were perfectly sealed in some parts of the tooth by CPP-ACP material.[15,16] Application of resin infiltration made enamel surface looked smoother than normal tooth.[17] The appearance of destructed pores might be due to etching process in resin infiltration, but some pores seemed to be sealed by resin material which filled the pores.

There was no significant shear strength difference in control and demineralized samples. This was contradictory to studies by Baka et al. and Baysal and Uysal which stated that early caries (demineralized) tooth had lower shear strength than normal tooth.[4,18] The difference could be caused by different bonding agent used. In those studies, composite resin was used, but in this study, RMGIC was used. In addition, exposed porosity resulting from demineralization process was also required for bonding agent's resin tag penetration.[19] Treatment with resin infiltration produced greater shear strength than that in demineralization group, although not significant. Penetration of resin tag into enamel pores, followed by chemical bonding of RMGIC bonding agent with resin infiltration made brackets adhere stronger to enamel. On the contrary, the shear strength of varnish group was significantly lower than that of control group. It may be caused by the direct bracket placement to varnish layer without being polished with pumice, so that it did not bond with remineralized enamel surface.

This result was consistent with experiment by Bayrak et al. and Endo et al., in which fluoride varnish was applied before bracket attachment to human tooth samples using RMGIC bonding agent.[20,21] Nonetheless, this result was inconsistent with a study by Nhan et al. in which fluoride varnish application did not contribute to the difference of shear strength of brackets bonded with composite resin bonding agent and phosphoric acid etch.[22] However, study by Viana et al. concluded that low fluoride level of enamel did not significantly decrease the bond strength, e.g. the use of fluoride-containing pumice.[23] On the other hand, the use of fluoride-containing self-etch decreased the bond strength. It could be caused by the decrease of resin tag penetration depth. Nonetheless, in an experiment using acidulated phosphate fluoride (APF)-containing etch, there was no change in the bonding strength.[24]

In this study, application of CPP-ACPF prior to bonding process resulted in a low shear strength. It was contradictory to a study by Cehreli et a. in which stated that application of CPP-ACPF paste to human teeth before bonding process with composite resin and etch-and-rinse system and self-etch only insignificantly reduced the shear strength.[25] Experiment by Karabekiroglu et al. also found that application of CPP-ACPF before or after etching process did not affect the shear strength.[26] This difference might be because the CPP-ACPF preparation used in that study was in paste form, while in this study, varnish form was used. In addition, the bonding agent used in those studies was composite resin. In this experiment, the average shear strength of control, of demineralization, and of resin infiltration treatment group was in the suggested range, which was 6-8 MPa.[27,28] The shear strength after varnish treatment was lower than the suggested strength, thus varnish application should be avoided prior to orthodontic treatment.

Lower ARI scores allowed orthodontists to clean residual adhesive material faster with minimum enamel destruction.[29] In this study, ARI scores were only observed on enamel surface to determine post-debonding enamel quality. Basically, ARI scores in RMGIC bonding agent revealed that bonding failure occurred on the interface of enamel surface - bonding agent, while the bonding failure of composite resin bonding agent was on the interface of adhesive and brackets.[27]

In this experiment, the ARI scores were found significantly high in resin infiltration treatment group. It supported a theory that chemical bond between resin infiltration and resin in bonding agent occurred, thus enabling resin tag to penetrate deeper into the porosity after hydrochloric acid etching process while applying resin infiltration. In other 4 groups, there was no significant difference of ARI scores, probably due to manual score observation (with eyes). ARI observation method performed with different magnifications had different reliability results. In greater magnification (above 20 times), lower ARI scores tended to decrease and higher scores increase compared to manual observation.[25] Nonetheless, in reality, orthodontists manually observed residual adhesive material after debonding.

After polishing, the enamel surface was re-observed by SEM to determine any enamel destruction. In greater magnification of SEM observation, there was still residual adhesive material left in all samples. Consistent with the increase of shear strength mean, although statistically insignificant, the residual adhesive material after resin infiltration application was seen thicker than that of other groups. However, enamel destruction also occurs worse in resin infiltration treatment group. New horizontal cracks were found in demineralization group. On the contrary, in the varnish treatment group, there was little residual adhesive material, and

varnish layer disappeared as wide as the brackets, exposing relatively smooth enamel contour underneath. It might be caused by the bracket attachment on varnish layer, not directly to remineralized enamel surface, which resulted in low shear strength and ARI scores.

Based on the result of this study, the shear strength of RMGIC bonding agent on demineralized enamel was not significantly different from that in normal enamel. It indicated that RMGIC could be used as orthodontic bonding agent on demineralized enamel without reducing the bond strength. Therefore, treatment on white spot lesion before short-term fixed orthodontic treatment might not be required. In addition, RMGIC could also reduce the risk of advancing demineralization lesion due to its fluoride content.

The use of varnish could repair enamel surface destruction caused by demineralization process, but it caused the shear strength to be very low since the brackets bonded with varnish layer. Further studies using XPS (X-ray Photoelectron Spectroscopy) were needed to determine the extent of remineralization in underlying enamel. XPS observation had some benefits: pre-treatment on samples was not needed (unlike SEM analysis) so that it would not cause further alteration to enamel surface and atomic content in the form of chemical elements can be measured (Viana et al., 2016; Santak et al., 2017).[23,30] However, if the underlying enamel had been remineralized, polishing using pumice was suggested to be performed prior to bracket placement to remove residual varnish layer, and thus the bonding material could attach directly to the enamel surface. Varnish application could also be performed for remineralising tooth surface after debonding due to demineralization and enamel damage occurred during the course of orthodontic treatment and polishing process.[31,32]

The limitation of this study was there was no measurement was done on: demineralization depth in bovine enamel samples, layer thickness of applied fluoride varnish, CPP-ACPF varnish, and resin infiltration, and the depth of enamel lost during polishing. These made the degree of real enamel destruction could not be measured in 3-dimensional way.

Conclusion

Pre-treatment with resin infiltration is recommended to be performed before bracket bonding on white spot lesion; however, it may need to be re-applied after debonding and polishing to conceal enamel morphology damage formed after polishing. Besides, RMGIC bonding agent can also be used on white spot lesion without any pre-treatment.

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Figure 1. Enamel topography (50x SEM magnification) on group: a) 1 (control); b) 2 (demineralization); c) 3 (fluoride varnish); d) 4 (CPP-ACPF varnish); e) 5 (resin infiltration).

Figure 2. Enamel topography (1000x SEM magnification) on group: a) 1 (control); b) 2 (demineralization); c) 3 (fluoride varnish); d) 4 (CPP-ACPF varnish); e) 5 (resin infiltration).

Figure 3. Adhesive remnant and the missing of varnish layer after debonding and polishing (30x SEM magnification) on group: a) 1 (control); b) 2 (demineralization); c) 3 (fluoride varnish); d) 4 (CPP-ACPF varnish); e) 5 (resin infiltration). Note: purple arrow showed adhesive remnant; blue arrow showed the disappearance of varnish layer.

Figure 4. Enamel topography after debonding dan polishing (50x SEM magnification) on group: a) 1 (control); b) 2 (demineralization); c) 3 (fluoride varnish); d) 4 (CPP-ACPF varnish); e) 5 (resin infiltration).

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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13