Assessment of the resin infiltration and CPP-ACP applications before orthodontic brackets bonding

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INTRODUCTION

The first sign of dental caries without cavitation is a white spot lesion (WSL), which can be defined as a reversible demineralization of the enamel surface. WSLs occur as a result of an imbalance between the enamel’s demineralization and remineralization processes. Although these lesions appear to be very resistant to complete remineralization, as the demineralization–remineralization cycles occur throughout a lifetime, non-invasive approaches are being preferred over preparations in the first stage of the treatment of non-cavitated WSLs. WSLs associated with fixed orthodontic appliances are a common adverse effect of orthodontic treatment. On the other hand, orthodontic treatments can be applied on individuals with existing WSLs. It has been reported that the presence of WLSs in untreated orthodontic patients varies between 11–24%, which means that most of these pre-orthodontic patients may have to undergo WSL treatment before orthodontic treatment. Fluoride treatments, topical application of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) gels, microabrasion, bleaching, and resin infiltration therapies are all referenced in the current literature as non-invasive techniques for the treatment of WSLs. Among those listed above, CPP-ACP, a remineralizing topical dental cream containing bioavailable calcium and phosphate without fluoride application, is one of the methods most preferred by doctors and patients in terms of ease of use. The manufacturer considers a daily 5-min topical application to be sufficient for remineralization. Casein is an important phosphoprotein making up 80% of the proteins in bovine milk and identified as having low cariogenicity and cariostatic activity. Casein phosphopeptides (CPP) can stabilize calcium phosphate in a state-forming CPP-ACP complex. ACP is a non-crystalline calcium phosphate that can release supersaturating levels of Ca\(^{2+}\) and PO\(_4^{3-}\) ions, which are favorable for hydroxyapatite (HAP) formation. On the other hand, as a new technique required professional practice, the usage of a low-viscosity light-curing resin infiltration is becoming widespread. The therapeutic mechanism of this technique relies upon the penetration of the low-viscosity resin into the lesion depth via capillary forces, which give rise to occluded porosities that resist the diffusion of acids and result in a slowed down or halted lesion progression.

As any bracket failure during the orthodontic treatment period can result in treatment delays, untoward tooth movement and inconvenience for patients, the effects of WSL treatment agents on the shear bond strength (SBS) of brackets has become increasingly important. In the literature, there are studies that have investigated the effect of pre-orthodontic application of CPP-ACP and resin infiltration on the SBSs of brackets, but there are no studies examining the remineralization capacities of these two agents, the effects on SBS and the correlations between these two results at the same time. The superiority of this study is to evaluate the efficiency and bonding strengths of agents at the same time and to investigate the correlation between them. Thus, the aim of this study is firstly to investigate the efficiency of CPP-ACP gel and resin infiltrant on remineralization of the decalcified enamel using the quantitative analysis technique of energy dispersive X-ray (EDX) analysis with a scanning electron microscope (SEM), which is largely used for quantification of teeth surface mineral content, and to investigate the effects of these two agents on the SBS of orthodontic brackets to explain the correlation, if any, between the surface ion release...
capacities and SBS values. It is hypothesized that the use of different agent applications will not significantly affect the amount of enamel surface ion release capacity and SBS values of orthodontic brackets.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Gaziantep University (220/29.08.2018). A power analysis of the sample size based on a 1:1 ratio among groups with a sample size of 20 teeth gave a result of 80% power to detect significant differences, with an effect size of 0.35 and alpha level of 0.05 significance.

A total of 80 freshly extracted premolar teeth were included in the study (with the patient’s informed consent) and were stored at room temperature in distilled water containing 0.1% thymol to inhibit bacterial growth until use (over a period of 2 months). Teeth showing any carries, attrition, fracture, restoration, congenital anomalies, or surface anomalies were excluded.

All teeth were cleaned and mounted vertically in chemically activated acrylic resin (Orthocryl, Dentaurum, Ispringen, Germany) until the root was embedded. The teeth were then polished with a pumice (non-fluoridated) and rubber cup and randomly divided into four groups including 20 teeth in each group. Group I was the control group and the other three groups were the experimental groups. The demineralization protocol was performed on groups II, III and IV, while groups III and IV were remineralized with Tooth Mousse® (CPP-ACP gel, GC, Tokyo, Japan) and Icon® (resin infiltrant, DMG, Hamburg, Germany), respectively. Pre-demineralization (T0) and post-demineralization (T1) EDX analyses for all experimental groups and post-application (T2) EDX analyses for groups III and IV were undertaken for the evaluation of mineral content (calcium and phosphate content in wt%). SBS tests were performed on the brackets that were directly bonded to the normal enamel in the control group, to the demineralized enamel in group II, to the enamel pre-treated with Tooth Mousse® in group III, and to the enamel pre-treated with Icon® in group IV (Table 1).

**Demineralization protocol**

The compositions of the demineralization and remineralization solutions are shown in Table 2. Samples were kept in a demineralizing solution for 22 consecutive hours and after being washed with deionized water, the samples were kept in a remineralizing solution for 2 h to complete a 24-h cycle. These dynamics were reproduced 855

| Table 1 | Description of groups, agent application procedures, timing of EDX analysis and SBS |
|---------|--------------------------------------------------------------------------------|
| Groups  | Pretreatment procedures | EDX analysis | SBS | Agent application procedure |
| Group I | Control                   | —            | Directly | —                          |
| Group II| Demineralization only     | T0 and T1    | After demineralization | —                          |
| Group III| Demineralization +Tooth Mousse® | T0, T1 and T2 | After Tooth Mousse® application | According to the manufacturer’s recommendations, CPP-ACP paste was applied to the demineralized enamel surface for 5 min and then rinsed with deionized water and kept in artificial saliva. This procedure was repeated once a day for 28 days. |
| Group IV| Demineralization +Icon®   | T0, T1 and T2 | After Icon® application | According to the manufacturer’s recommendations, a resin infiltrant was applied to the demineralized enamel surface before bonding procedure. Preconditioning with the resin infiltrant included 15% hydrochloric acid etching for 2 min (Icon-Etch®), water rinsing for 30 s, surface drying by ethanol for 30 s (Icon-Dry®), application of a low-viscosity resin infiltrant for 3 min, (Icon-Infiltrant®), and lightcuring (LED) for 40 s., a final application of Icon-infiltrant® for 1 min. and lightcuring for 40 s. |

| Table 2 | Composition of demineralization and remineralization solutions |
|---------|----------------------------------------------------------------|
| Demineralization solution | 3 mmol/L calcium, 3 mmol/L phosphate, 50 mL/L acetic acid, and 0.308 g ammonium acetate with the pH adjusted to 4.5 with sodium hydroxide |
| Remineralization solution | 1.54 mmol/L calcium, 1.54 mmol/L phosphate, 20 mmol/L acetic acid, and 0.308 g ammonium acetate, adjusted to pH 7.0 with potassium hydroxide |
over a period of 14 days, during which the solutions (neutral and acidic) were changed every day\textsuperscript{20}.

**Application of Tooth Mousse\textsuperscript{*}**
After demineralization, Tooth Mousse\textsuperscript{*} was applied for 5 min to the enamel surface, rinsed with deionized water, and the tooth was then kept in artificial saliva. The protocol was repeated over 28 days and the artificial saliva was changed daily\textsuperscript{21}.

**Application of Icon\textsuperscript{*}**
After demineralization, Icon\textsuperscript{*} was applied in accordance with the manufacturer’s instructions. Icon-Etch\textsuperscript{*} was applied and left to sit for 2 min, rinsed off with water for 30 s, and then dried with oil and water-free air. Icon-Dry\textsuperscript{*} was then applied and left to sit for 3 min, and then light-cured for 40 s. Finally, Icon-Infiltrant\textsuperscript{*} was applied again, left to sit for 1 min and light-cured for 40 s. At the same time as the Tooth Mousse\textsuperscript{*} was tested (over 28 days), the group applied with Icon\textsuperscript{*} also rested in artificial saliva that was changed daily.

**EDX analysis**
The samples were analyzed with a SEM (JEOL, Tokyo, Japan), which was connected to an energy-dispersive X-ray spectrometer (EDS; IXRF Systems, Austin, TX, USA) with a standardized integrated reading time of 100 s per surface area of 1 \( \mu \text{m}^2 \).\textsuperscript{23}

**Bonding procedure**
Samples were initially rinsed with an air-water syringe for 5 s. The enamel was then treated with 37% orthophosphoric acid (Gel Etch, 3M Unitek, Monrovia, CA, USA) for 20 s, rinsed, and air-dried for 15 s. The Mini Master (American Orthodontics, Sheboygan, WI, USA) stainless steel premolar brackets were then bonded using Transbond XT primer and Transbond XT adhesive (3M Unitek) according to the manufacturer’s instructions. The brackets were bonded to the middle of the buccal surface of the tooth crown, excessive primer and adhesive remnants were removed, and each tooth was cured for 20 s using LED (Valo, Ultradent, South Jordan, UT, USA).

**SBS test**
A universal testing machine (AGS-X, Shimadzu, Kyoto, Japan) at a constant crosshead speed of 1 mm/min was used to record the SBS of brackets bonded onto the differently conditioned enamel surfaces, until failure occurred. The force needed to remove the brackets was measured in newtons (N), and the SBS was then calculated by dividing the force values by the bracket base area (10.27 mm\textsuperscript{2}).

**Adhesive remnant index (ARI)**
The composite resin remnants on the enamel surface after shear testing were examined with a light stereomicroscope (M165C, Leica Microsystems, Wetzler, Germany) at a magnification of 10\times to determine the fracture pattern in each group. The patterns were then classified into four types according to the ARI\textsuperscript{15}.

**Statistical analysis**
SPSS software (v. 21.0 for Mac, SPSS, Chicago, IL, USA) was used for all statistical analyses. The Kolmogorov-Smirnov test was used for evaluating normality. Levene’s test of error variance equality was applied for variances. The Friedman test was used for statistical analysis of non-normally distributed variables, and the Wilcoxon test was used for dependent measurement comparisons. One-way ANOVA LSD multiple comparison tests, and the Student’s \( t \)-test were used to compare variables in independent groups, and a paired samples \( t \)-test was used for dependent variables with normal distribution. The chi-square test was used to test the relationship between categorical variables. The Spearman correlation test was used to determine the correlation between changes in SBS and calcium phosphate ratios. For the descriptive statistics, mean ± standard deviation values were given for the numerical variables. \( p<0.05 \) was considered statistically significant.

**RESULTS**
Descriptive statistics and comparisons of mean Ca/P ratios at T0 and T1 between and within experimental groups are shown in Table 3. Statistical analysis of the EDX data revealed that there were no statistically significant differences before or after the demineralization procedure between the experimental groups (\( p>0.05 \)). Although there was a decrease in the Ca/P ratios within

| Ca/P Ratio | Time  | Demineralization only (\( n=20 \)) Mean±SD | Tooth Mousse\textsuperscript{*} (\( n=20 \)) Mean±SD | Icon\textsuperscript{*} (\( n=20 \)) Mean±SD | \( p \) |
|------------|-------|------------------------------------------|-------------------------------------------|-----------------------------------|------|
|            |       | Mean±SD                                  | Mean±SD                                   | Mean±SD                           |      |
| T0         | 1.16±0.55 | 1.25±0.53                                | 1.42±0.17                                 | 0.177                             |      |
| T1         | 1.14±0.03 | 1.21±0.04                                | 1.31±0.34                                 | 0.1                               |      |
| \( p \)    | 0.909  | 0.455                                    | 0.387                                     | —                                 |      |
the experimental groups, this was also found not to be statistically significant.

Descriptive statistics and a comparison of mean Ca/P ratios at T0, T1, and T2 between and within the agent application groups are shown in Table 4. In the Tooth Mousse® group, there were statistically significant differences between the T0, T1, and T2 Ca/P ratios ($p=0.001$). Ca/P ratios significantly increased after the

| Group                  | n  | Mean Difference (T2-T0) | p    |
|------------------------|----|------------------------|------|
| Tooth Mousse® (n=20)   | 20 | 0.29±0.39              | 0.029|
| Icon® (n=20)           | 20 | 0.13±0.23              |      |

The descriptive statistics and comparison of ion release of agent applications are shown in Table 5.

Table 5  Descriptive statistics and comparison of ion release of agent applications

| Group     | n  | Mean Difference (T2-T0) | p    |
|-----------|----|------------------------|------|
| Tooth Mousse® | 20 | 0.29±0.39              | 0.029|
| Icon®     | 20 | 0.13±0.23              |      |

The descriptive statistics and results of analysis of variance (ANOVA) comparing shear bond strengths are given in Table 6.

Table 6  Descriptive statistics and results of analysis of variance (ANOVA) comparing shear bond strengths

| Groups                      | n  | SBS Mean±SD | Multiple comparisons | p    |
|-----------------------------|----|-------------|----------------------|------|
| Control                     | 20 | 16.83±4.75  | Demineralization only | 0.001|
| Demineralization only       | 20 | 13.07±3.73  | Tooth Mousse®        | 0.000|
| Tooth Mousse® (n=20)        | 20 | 4.8±1.97    | Icon®                | 0.000|
| Icon®                       | 20 | 4.36±2.24   |                      |      |

The frequency distribution of adhesive remnant index (ARI) scores of the groups is shown in Table 7.

Table 7  Frequency distribution of adhesive remnant index (ARI) scores of the groups

| Groups                      | Score 0 | Score 1 | Score 2 | Score 3 | Total | p    |
|-----------------------------|---------|---------|---------|---------|-------|------|
| Control                     | 1       | 6       | 7       | 6       | 20    |      |
| Demineralization only       | 0       | 8       | 9       | 3       | 20    | 0.003|
| Tooth Mousse® (n=20)        | 6       | 11      | 3       | 0       | 20    |      |
| Icon®                       | 3       | 13      | 3       | 1       | 20    |      |

ARI scores: 0=No adhesive remaining on the enamel surface; 1=Less than 50% adhesive remaining on tooth; 2=More than 50% adhesive remaining on tooth; 3=All adhesive remaining on tooth surface.

The results of correlation between SBS values and ion release of agent applications are presented in Table 8.

Table 8  Results of correlation between SBS values and ion release of agent applications

| Groups                      | Mean difference (T2-T0) | Spearman correlation coefficient | p    | n  |
|-----------------------------|-------------------------|----------------------------------|------|----|
|                            |                         |                                  | 0.612| 40 |
application of Tooth Mousse®, compared with the Ca/P ratios at the beginning and after demineralization ($p=0.007$ and $p=0.001$, respectively). In the Icon® group there were also statistically significant differences between the T0, T1, and T2 Ca/P ratios ($p=0.031$). Ca/P ratios significantly increased after Icon® was applied, compared with the Ca/P ratios at the beginning and after demineralization ($p=0.008$ and $p=0.017$, respectively).

To compare the ion release potentials of the agents, the mean differences of the Ca/P ratios between T0 and T2 were analyzed. A statistically significant difference was found between the groups ($p=0.029$) (Table 5).

Descriptive statistics and a comparison of the mean SBS values of all groups are shown in Table 6. The statistical analysis revealed statistically significant differences between the SBS values of the groups ($p<0.001$). SBS values reduced significantly after demineralization compared with the control group ($p=0.001$). Both the Tooth Mousse® and Icon® groups showed a statistically significant decrease in SBS values compared to the control and demineralization only groups ($p<0.001$).

The frequency distribution of the groups’ ARI scores is shown in Table 7. The residual amount on the enamel surface was observed to be minimal in both the Tooth Mousse® and Icon® groups. As a result of the statistical assessment, a significant difference was seen between the groups in terms of ARI index ($p=0.003$).

The correlation results between the SBS values and the post-agent application Ca/P ratios for Tooth Mousse® and Icon® are shown in Table 8. There was no correlation found between the values ($p>0.05$).

**DISCUSSION**

Fixed appliance therapy in orthodontics involves bonding brackets to teeth for a period of nearly 2 years. Having healthy enamel surfaces at the beginning of treatment is essential. The treatment of WSLs at the beginning of orthodontic treatment without compromising the SBS of brackets has become an area of interest in orthodontics due to the undesirable prolongation of treatment time resulting from bracket failure. This current *in vitro* study aimed to compare the efficiency of two popular applications (Tooth Mousse® and Icon® resin infiltration) using EDX, a quantitative analysis method, to compare the effects of the applied techniques on the SBS of brackets and to establish a correlation between the treatment outcomes and SBS values before treatment, in order to meet the orthodontic treatment needs of demineralized enamel. The originality of this study comes from the fact that the effects of these two agents and the effects on SBS were evaluated within the same protocol, which in fact allowed the two results to be associated with each other. According to the results of the present study, the null hypothesis was partially rejected. The different agents’ effects on SBS were similar. However, a difference was found between the surface ion release capacities of the two agents.

To validate mineral loss from the enamel, quantitative light-induced fluorescence, micro computed tomography scans, laser scanning microscopy, polarized light microscopy, and SEM are microscopic methods available that are often used in studies. The evaluation method should be accurate, reproducible, and easy to conduct use. An improvement on the Electron microscope has been the EDX analysis with SEM which is a micro-analytical technique employed to quantitatively estimate the amount of minerals in a given sample. The technique sensitivity of this method depends upon the atomic number of the element to be categorized, the atomic number of all elements present in the sample, and the technique used for sample preparation during microanalysis. The present study analyzed only calcium and phosphate, the HAP crystal compounds that form the main structure of enamel EDX can quantitatively analyze calcium and phosphate, which have high atomic numbers (20 and 15, respectively). A reduction in the Ca/P mineral ratio on the enamel surface represents the demineralization of the tooth, while an increase represents remineralization. Rather than measuring the depth of mineral loss from the tooth structure, measuring the quantity of mineral loss provides an opportunity to understand the biological events during the demineralization/remineralization process.

The results of the EDX analysis show that in all experimental groups, the before and after demineralization Ca/P ratios between groups did not differ from each other, which means that similar tooth groups existed before agent application. At the same time, when the intra-group differences are checked both before and after demineralization, although there was a noticeable decrease in Ca/P ratios within the groups, this was not statistically significant. Klimuszko et al. reported that the results of acid application impacted the entire mineral content of the enamel and that the structure’s Ca content was affected by other minerals on the surface moving away at different rates in the presence of the acid. In this present study, it may not be possible to determine the exact amount of demineralization for the reasons mentioned above. This may show that in order to evaluate demineralization, not only Ca and P ions, but every ion present on the enamel surface should be included in the EDX analysis, to be considered together.

The EDX values after application of Tooth Mousse® and Icon® indicated that usage of both methods increased the Ca/P ratios on demineralized teeth, with treated enamels even showing higher Ca/P ratios than the initial measurements. In accordance with this study’s results, Gjorgievksa and Nicholson, who also examined the enamel remineralization potential of applying Tooth Mousse® and Novamin using EDX analysis in their study, stated that Tooth Mousse® significantly increased the level of Ca and P compared to the control, resulting in enhancement of the remineralization potential of tooth enamel. In another study aiming to compare the quantitative evaluation of mineral gain of Tooth Mousse®, Vantej® and Icon® on enamel surface, Shaik et
as a result of low viscous resin, which possibly occluded the underlying pores in the carious lesion. The results of some other studies evaluating the application of Icon® conform with those of the present study. However, other authors’ results conflict with the current results. Costenoble et al.19 investigated the effects of calcium silicate-sodium phosphate salts, or Icon®, on SBS values with immediate and 1-month delayed bracketing subgroups. They reported that followed by a delayed bonding, Icon® produced lower SBS values equivalent to those in this current study, and advised that orthodontic bonding should be performed shortly after resin infiltration. On the other hand, Mews et al.17 reported that the application of Icon® in addition to a primer-adhesive system did not affect bond strengths to enamel. Vell et al.16 and Baka et al.18 compared the SBS values of CPP-ACP application, microabrasion, fluoride varnish, and Icon® application, in addition to a primer-adhesive system and self-etching protocols, respectively, and both concluded that the application of Icon® after demineralization led to significantly better adhesion of brackets compared to the other methods described above. Although the SBS value of 1 min at 4 MPa has previously been reported as adequate for the clinical application of brackets20, the results of this study show that further studies are needed on this subject, especially with regards to determining bracketing times after agent application.

The ARI score comparisons in this study, supporting the SBS findings, show that both the Tooth Mousse® and Icon® groups had higher amounts of composites on the bracket surface, meaning that the bond strength was higher between the bracket and composite, compared to the bond strength between the composite and the tooth. Finally, the results of this study do not reveal a correlation between the agents’ application effects on surface ion release capacity and SBS values, meaning that there may be different parameters that influence SBS values more prominently than enamel surface mineral contents. Based on the results of the present study, it is possible to conclude that although the application of Icon® and CPP-ACP before orthodontic treatment significantly reduces the SBS values, it does not result in an enamel surface that is unable to withstand the mechanical orthodontic forces. However, it should be kept in mind that bracket failure may occur more easily due to factors reliant upon a patient’s unique masticatory performance or trauma that cannot be imitated in vitro. As Icon® is only applied to teeth with WSLs, while CPP-ACP is applied to all tooth surfaces by the patient, it may be said that CPP-ACP should be used more carefully and should be recommended to be applied only to teeth with lesions.
CONCLUSIONS

Within the limitations of this in vitro study, the following clinical conclusions can be drawn.

Firstly, CPP-ACP and resin infiltrant therapies enhanced the tooth surface mineral content.

Secondly, CPP-ACP and resin infiltrant therapies resulted in lower SBS values than the control and demineralization only groups.

Finally, there was no correlation found between the SBS values and ion release capacities of CPP-ACP and resin infiltrant therapies.

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