Changes in Demineralized Enamel around Brackets Detected by Laser Fluorescence Following Fluoride Treatment

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Abstract

Objective: To evaluate in-vitro, using red laser-light induced fluorescence (LF), the effect of three fluoride compounds on the demineralization-remineralization process around brackets bonded with glass ionomer cement (GIC) or resin-based cement.

Materials and method: The material comprised a sample of 60 premolars. 30 brackets were bonded with resin and 30 with glass ionomer cement. The enamel around the bracket was exposed to three demineralization–remineralization cycles to create a white spot lesion. After each cycle a fluorescent dye was applied to the enamel to measure laser fluorescence using a DIAGNOdent LF2190. The lesions were remineralized with three different commercial products: 12,000 ppm fluoride, 1,500 ppm fluoride, and 900 ppm fluoride. After pH cycles, the tooth received three weekly fluoride treatments and LF measurements with the dye were taken.

Results: The initial LF values of the enamel around resin-bonded (2.0 ± 0.94) or GIC-bonded brackets (1.86 ± 0.62) showed no differences (p>0.05). After pH cycling, LF values showed no difference between the groups. The LF mean was 15.15 ± 4.28. The three fluoride compounds produced enamel remineralization around the brackets. Following fluoride treatment, a score was created based on the difference between LF post fluoride treatment and the initial LF values. These LF values showed statistical differences (p<0.001). Treatment with 900 ppm fluoride produced the highest remineralization values in both the resin and the GIC group.

Conclusion: The three fluoride compounds tested had the ability to remineralize enamel lesions produced by pH cycling around brackets bonded with resin or GIC.

Keywords: Dental caries; White spot lesion; Laser fluorescence; Orthodontic brackets; Orthodontic cements

Abbreviations:

LF: Red Laser-light Induced Fluorescence; GIC: Glass Ionomer Cement; WSL: White Spot Lesion; TMPyP: Tetrakis N-methylpyridyl Porphyrin

Introduction

Dental caries is the result of an imbalance between the demineralization and remineralization of dental structures, a process that is influenced by factors that lead to demineralization, such as poor nutritional habits, and by protective factors, such as exposure to fluoride [1]. Clinically decalcified enamel appears as an opaque white spot lesion (WSL) and represents the initial stage of caries formation [2]. Over time, the WSL may be recalcified; however, the opaque color usually remains and can appear as a stain, making it even less esthetic. Enamel demineralization is a common sequela of orthodontic treatment when oral hygiene is poor. WSLs are attributed to prolonged plaque accumulation around the brackets. Incipient caries form as early as four weeks after the placement of fixed orthodontic appliances, although the formation of established caries lesions usually takes at least six months. WSLs are one of the most prevalent side effects of fixed orthodontic treatments and affect about 50% of patients [3].

It is well established that fluoride increases the initial rate of remineralization of early enamel lesions and then slows down the caries process, and also that it is the most important agent in caries prevention [4]. Self-applied methods of fluoride delivery have been recommended for WSL prevention. Regular use of fluoridated toothpastes have proved unsuccessful in preventing lesions during fixed orthodontic treatment [5]. Fluoride mouthwashes have an excellent risk-benefit ratio [6], but there is no conclusive evidence as to their benefits in preventing WSL demineralization during orthodontic treatment [7]. Some topical fluoride products in the form of varnishes, solutions or gels have been used too with limited results and there is a lack of reliable evidence as to their effectiveness for remineralizing post orthodontic WSL [8]. Demineralization and remineralization processes leading to caries activity in which the first visible sign is the white spot can be detected and quantified using various fluorescence-based optical methods for detecting the earliest signs of enamel demineralization. This therefore affords the opportunity to intervene with aggressive therapies (mainly fluorides) at an early stage, arrest the lesion, encourage remineralization, and avoid the need for restorative intervention [9]. A laser-based fluorescence device, the DIAGNOdent,
was introduced to detect and monitor the progression of caries lesions. The device generates a laser beam that penetrates the tooth surface and is absorbed by the surrounding tooth material. Carious tissue emits stronger fluorescence than sound tissue does in the red and infrared part of the spectrum (λ=655 nm) [10]; hence, fluorescence from a carious region is greater than that from sound tissue. The origin of fluorescence in carious tissue lies in oral bacterial porphyrins. Porphyrins are produced by several types of oral bacteria and can be extracted from the caries lesion. Carious material had maximum absorption in enamel treated by demineralization-remineralization pH-cycling around brackets bonded with glass ionomer cement (GIC) or resin-based cement.

Materials and Methods

Teeth

Written informed consent was obtained from the patients to use their teeth in our study. Sixty recently extracted non-carious human premolars, for mainly orthodontics reasons, were placed in distilled water at room temperature with thymol crystals added to inhibit bacterial growth.

The teeth were visually sound; those with stains, calculus, or hypomineralization were excluded from the study. All were rinsed thoroughly under tap water and carefully cleaned with a toothbrush.

Treatment of the teeth for the demineralization-remineralization process

Each tooth was sectioned and the root discarded. The area occupied by the bracket plus a 3-mm margin were covered with adhesive tape and the remainder of the tooth was etched and covered with resin sealant (Heliosit® F, Ivoclar). 30 brackets were bonded with resin (Heliosit® Orthodontic, Ivoclar) and 30 with glass ionomer cement (GC Fuji ORTHO™ LC). The non-covered area of enamel was exposed around brackets that were bonded with resin or GIC and TMPyP.

Demineralization solution: 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 150 mM KCl, 0.1 M sodium acetate buffer, and 30 mM acetate in hydroxyethyl cellulose. The pH was adjusted to 4.7 and controlled before and after each 3-day cycle.

Remineralization solutions: 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, and 150 mM KCl at pH 7.0, again controlled before and after each 3-day cycle.

Each cycle was scheduled for 3 days and was repeated 6 times for a total experimental period of pH cycling of 18 days.

DIAGNOdent

After each cycle, 10 µl of a solution of 0.2 mM of a fluorescent dye [tetrakis(N-methylpyridyl)porphyrin (TMPyP)] (Aldrich, Milwaukee, USA) dissolved in dimethyl sulfoxide was applied to the enamel for one minute; the crown of the tooth was dipped in distilled water (two minutes, slowly stirred with a magnetic stirrer) and dried with an air syringe for 5 seconds. Laser fluorescence measurements were taken with a DIAGNOdent LF2190, (KaVo, Biberach, Germany) and using a flat-tip probe recommended for smooth surfaces. The measurement time was standardized to around 10 s. As recommended by the manufacturer, the instrument was calibrated with its own supplied ceramic standard before every measurement.

Treatment Groups

The setup of the experiment is presented in Table 1. The lesions surrounding the bracket bases were remineralized with three different commercial products: 12,000 ppm fluoride (Oral-B Minute-Gel, Procter and Gamble), 1500 ppm fluoride (Fluoroxyl gel, GlaxoSmithKline), and 900 ppm fluoride (MI Paste plus, GC America). After the pH cycles, the tooth was placed in distilled water for two minutes and the lesion dried. The fluoride compound was then applied to the lesion around the bracket for three minutes. This was subsequently washed and dried before adding the TMPyP. Laser fluorescence measurements were then taken. The tooth was placed in artificial saliva (20 mmol Na HCO₃, 3 mmol NaHPO₄, 1 mmol CaCl₂, pH 7.0) for one week and the same procedure was then repeated at 7 and 14 days.

Statistical Analysis

Since the purpose of the study was to determine the differences in remineralization in the enamel around braces bonded with resin and that around braces bonded with GIC in three different fluoride treatments using red laser-light induced fluorescence, a score was created based on the difference between LF post fluoride treatment and the initial LF values. Two-way ANOVA with two-factor variables was used to determine whether there were significant differences between the three treatments. The Tukey HSD test was used to compare the group means (p<0.05).

Results

The LF values of the enamel around resin-bonded (2.0 ± 0.94) or GIC-bonded brackets (1.86 ± 0.62) showed no initial differences (p>0.05) (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin-bonded brackets</td>
<td>30</td>
<td>2</td>
<td>0.95</td>
</tr>
<tr>
<td>GIC-bonded brackets</td>
<td>30</td>
<td>1.87</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Table 1: Initial laser fluorescence values of enamel around brackets bonded with resin or GIC and TMPyP

The demineralization-remineralization process is not affected by the bonding material. There are no differences between the LF values of the enamel surface around resin-bonded brackets after pH cycling (16.43 ± 3.6) and those around GIC-bonded brackets (13.86 ± 4.58) (p>0.05) (Table 2).
Table 2: Laser fluorescence values of enamel around brackets bonded with resin or GIC after three demineralization-remineralization cycles and TMPyP (p>0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin-bonded brackets</td>
<td>10</td>
<td>5.9</td>
<td>1.10</td>
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<tr>
<td>Resin-bonded bracket (900 ppm fluoride)</td>
<td>10</td>
<td>3.5</td>
<td>1.18</td>
</tr>
<tr>
<td>GIC-bonded bracket (900 ppm fluoride)</td>
<td>10</td>
<td>4.9</td>
<td>1.72</td>
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<tr>
<td>Total</td>
<td>60</td>
<td>5.5167</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Table 3: Laser fluorescence values of enamel around brackets bonded with resin or GIC after treatment with three weekly fluoride treatments and TMPyP

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin-bonded bracket (12,000 ppm fluoride)</td>
<td>10</td>
<td>3.4</td>
<td>1.17</td>
</tr>
<tr>
<td>Resin-bonded bracket (1,500 ppm fluoride)</td>
<td>10</td>
<td>5.9</td>
<td>2.08</td>
</tr>
<tr>
<td>Resin-bonded bracket (900 ppm fluoride)</td>
<td>10</td>
<td>2.6</td>
<td>1.05</td>
</tr>
<tr>
<td>GIC-bonded bracket (12,000 ppm fluoride)</td>
<td>10</td>
<td>3.4</td>
<td>2.01</td>
</tr>
<tr>
<td>GIC-bonded bracket (1,500 ppm fluoride)</td>
<td>10</td>
<td>3.9</td>
<td>1.45</td>
</tr>
<tr>
<td>GIC-bonded bracket (900 ppm fluoride)</td>
<td>10</td>
<td>2.6</td>
<td>0.97</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>3.4</td>
<td>1.455</td>
</tr>
</tbody>
</table>

Table 4: Difference between the LF values of enamel around brackets after three weekly fluoride treatments and TMPyP and the initial LF values of the enamel

The score indicates that the best mineralization rates were produced by 900 ppm fluoride (mean 2,3), average rates by 12,00 ppm fluoride (3,3), and the greatest difference by 1,500 ppm fluoride (5,5).

The Tukey HSD test showed statistically significant differences in the remineralization rate of the groups treated with 900 ppm fluoride gel (p<0.001) (Table 4).

Treatment with 900 ppm fluoride produces the highest remineralization values in both the resin and the GIC group.

Discussion

White spot lesions around the fixed appliance are an undesirable consequence of orthodontic treatment and can be attributed to the patient’s lack of commitment to achieving proper plaque removal. Dentists face this problem during and after treatment and therefore should use all available strategies to intervene both at the beginning of and during the treatment, and after debonding.

Demineralization caused by bacterial metabolism removes Ca ions and PO4 ions from the hydroxyapatite crystals that make up the enamel rods, thereby creating a porous structure that allows the entry of water and, if desired, air, when the enamel surface is dried off with a triple syringe [15]. The demineralization is repaired by the precipitation of Ca and PO4 ions dissolved in saliva, which can be enhanced by the use of fluorides [2].

This study was performed for the purpose of determining which treatment method is most successful in treating in-vitro the demineralized white spot lesions that occur in the area adjacent to orthodontic brackets, which was done by assessing the laser fluorescence of enamel lesions with protoporphyrins.

Laser fluorescence (measured using a DIAGNOdent) has been used to determine the depth of caries lesions and, in conjunction with the caries risk assessment, define the type of treatment that these lesions require [16].

The laser fluorescence device in question allows for a quantitative method based on the emission of light from a diode laser and the measurement of the fluorescence emitted primarily from the carious tissues [10]. In clinical practice, the DIAGNOdent measures bacterial presence in the porous structure of demineralized enamel; therefore, in an in-vitro study such as ours, we need to incorporate the molecules present in bacteria before we can measure the laser fluorescence of enamel that has suffered acid attack in-vitro.

The initial LF values of the enamel in the resin-bonded group were 2.0 ± 0.94 and in the group bonded with GIC 1.86 ± 0.62, which correspond to the values for sound enamel reported [17,18].

In an in-vitro study [13], laser fluorescence enhanced by fluorescent dyes proved effective in detecting initial demineralization around the brackets. In this study, the porphyrin was dissolved in water and added to a lesion produced by submerging the enamel in an acetic acid solution with the pH adjusted to 4.8 for 16 days, without pH cycling. The enamel lesion obtained produced mean LF values of 10.3 ± 11.8. In our study, we used pH cycling to produce enamel lesions and laser fluorescence with the help of protoporphyrin IX dissolved in dimethyl sulfoxide to assess the processes of demineralization and remineralization. The values that we obtained after the pH cycling for the enamel lesions around brackets were higher (15.15 ± 4.2896). These higher LF values may be because the lesion produced by demineralizing gel in the pH cycling process produces a subsurface lesion, whereas using only one demineralizing solution causes erosion. We recommend that this demineralization process model be considered for future experiments.
The data from the present study confirm the effects of fluoride remineralization on human enamel in terms of LF values. The three fluoride compounds tested had the ability to remineralize enamel lesions produced by pH cycling. Protoporphyrins are a valuable, indeed indispensable aid in detecting demineralization by LF in vitro. Treatment with 900 ppm fluoride produces the highest remineralization values in lesions around brackets bonded with resin or GIC.

Conclusions

The researchers declare not have funding or conflict of interest with any commercial entity.

Authors’ Contributions

JFGC designed the study, analyzed the data and drafted the manuscript. DA and AP carried out all the laboratory procedures and drafted the manuscript. All authors read and approved the final manuscript.

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References


