ORIGINAL ARTICLE

The caries-reducing benefit of fluoride-release from dental restorative materials continues after fluoride-release has ended

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Abstract

Objective. This study tested the hypothesis that the benefit of fluoride-releasing restorative materials continues even after their reserve of fluoride has been depleted. Materials and methods. Pits in perspex blocks simulating cavities were filled with either a fluoride-releasing or a non-fluoride-releasing restorative material and a dentin single-section was placed 1 mm from the edge of the filled pit. These combinations were exposed to an acid gel system. Each demineralized dentin section was separated from the adjacent material and immersed in fresh demineralizing solutions. Transversal microradiographs were taken following the two experimental periods. This study defined ΔAZ as the increase of integrated mineral loss (∆L) during the second acid attack. Results. The first acid attack substantially demineralized the near-surface region (depth < 40 μm) in all samples. The second acid attack, however, did not cause further demineralization in this near-surface region. Instead, it demineralized dentin deeper than 40–60 μm. The ΔAZ of the material that did not release fluoride was significantly greater than that of fluoride-releasing materials. Negative correlations were found between ΔAZ and the mineral volume% of the near-surface region and lesion body of the initial lesions. These results indicate that the dentin mineral in the near-surface region is chemically altered to become acid-resistant fluorapatite. In addition, lesion progression during the second period of demineralization, which was fluoride-free, may have been affected for the materials that have high mineral content of the surface layer and lesion body. Conclusions. It is concluded that dentin surrounding fluoride-releasing materials is protected against demineralization even after the fluoride release has diminished.

Key Words: acid resistance, demineralization, dentin, single section, transversal microradiography

Introduction

Fluoride-releasing materials inhibit dentin demineralization and promote remineralization [1–8]. These studies have shown that fluoride-releasing restorative materials contribute to preventing caries in adjacent dentin. However, it is unknown whether the dentin that is protected by this fluoride release remains resistant to demineralization after the fluoride source is exhausted. The aim of this study was to compare simulated cavities in bovine dentin next to various types of restorations, some of which released fluoride. We wished to find out if there was any difference in the caries formation rates after the fluoride release from the fluoride-releasing materials had become negligible. Our microradiographic analyses provided the basis for quantifying the changes in lesion parameters. In this study, the aim was to verify the hypothesis that the benefit of fluoride-releasing restorative materials continues even after their reserve of fluoride has been depleted.

Materials and methods

Preparation of single-sections

Bovine lower central incisors were obtained from the slaughterhouse. Root cylinders (5 mm thick) were cut using a sectioning machine (Isomet Low Speed Saw; Buehler, Rosemont, IL) and from each cylinder four wafers (300 μm thick) were taken by making four
radial cuts with a diamond-coated-wire sectioning machine (Well type 3242; Walter Ebner, Mannheim, Germany). The dentin wafers were embedded in a dentin bonding agent (Adper Scotchbond Multi-Purpose Adhesive; 3M, St Paul, MN) in the following manner. Each wafer was etched with 35% phosphoric acid for 15 s, rinsed with water for 30 s, blotted dry and primed. One drop of bonding agent was placed on a glass plate covered with plastic film (Parafilm M; Pechiney, Chicago, IL) and the primed thin wafer was gently sunk into it. Then another film-coated plate was placed on the first plate and the wafer was sandwiched in the bonding agent. It was light-cured for 20 s on each side (Jetlite 3000; J.Morita USA, Irvine, CA). The glass plates with plastic film were removed. Finally, the fully covered section was ground on one side with a waterproof abrasive paper (Fuii Star #2000, Sankyo Rikagaku, Sairama, Japan), so that an acid solution could directly contact the surface of the bovine root dentin [9,10]. Then the specimens were stored in distilled water to prevent drying of the dentin until use. The mean thickness and standard deviation of our sample were 471.4 µm and 89.0 µm, respectively.

Simulated fillings

A cylindrical pit (diameter: 4 mm, depth: 2 mm) was drilled into a perspex block to simulate a cavity. This pit was filled either with a fluoride-releasing or a non-fluoride-releasing material. The following materials were tested: two fluoride-releasing resin composites, BEAUTIFIL (BF) and UniFil S (US); a fluoride-releasing flowable resin composite, BEAUTIFIL Flow F02 (BFF); and a conventional glass-ionomer cement, Glasionomer-F (GF) (Table I). LiteFil IIA (LF), a non-fluoride-releasing resin composite, was used as a control. All blocks were exposed to R.H. 100% moisture for 24 h and then their surfaces were polished with wool Rif paper (#2000) and rinsed well with distilled water.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Abbreviations</th>
<th>Filler content (wt%)</th>
<th>Manufacturer</th>
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<tbody>
<tr>
<td>Resin composite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lite-fill IIA</td>
<td>LF</td>
<td>84</td>
<td>Shofu</td>
</tr>
<tr>
<td>Beautifil</td>
<td>BF</td>
<td>81.5</td>
<td>Shofu</td>
</tr>
<tr>
<td>UniFil S</td>
<td>US</td>
<td>77</td>
<td>GC</td>
</tr>
<tr>
<td>Flowable resin composite</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beautifil</td>
<td>BFF</td>
<td>55.7</td>
<td>Shofu</td>
</tr>
<tr>
<td>Flow F02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glass-ionomer cement</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Glasionomer-F</td>
<td>GF</td>
<td></td>
<td>Shofu</td>
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All materials are fluoride-releasing except Lite-fill IIA.

Initial lesion formation and subsequent acid resistance test (Figure 1)

We placed a root dentin single-section into a groove cut into each block, which was ~ 1 mm from the cavity filled with the test material. The top of each experimental single-section was positioned to be flush with the surface of the block. The blocks were fastened to the bottom of a cylindrical plastic container and immersed in 8% methylcellulose gel (Methocel MC; Fluka, Buchs, Switzerland). After 24 h, a 0.1 M lactic acid buffer (pH 4.8) was poured on the gel. All experimental samples were maintained at 37°C. After 7 days, we removed the sections from the grooves and took transversal microradiographs (TMR).

Next, the single-sections by themselves were immersed in 10 mL of a solution containing 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 0.2 ppmF as NaF, and 0.1 M acetic acid buffer (pH 5.0) at 37°C for 3 days. To maintain the surface of the dentin lesion, a small amount of fluoride was added to the second acid. We feel that the influence of this added fluoride was negligible and did not essentially affect the results of this experiment.

Transversal microradiography (TMR)

Microradiographs were taken of each specimen after the first period of acid exposure produced the initial lesion and then again after the second period of acid exposure (Figure 1). We designate these two time points AE1 and AE2, respectively. For this the single-sections were placed on a perspex holder in a drop of distilled water and covered with a thin polyester sheet to prevent the dentin sample from shrinking [11]. Together with an aluminum step-wedge of 13 steps, the sections were radiographed on a high-resolution glass film plate (High Resolution Plate; Konica Minolta, Tokyo, Japan) with a nickel-filtered Cu-Kα source operated at 15 mA and 25 kV for 20 min (PW3830; Spectris, Surrey, UK). Radiographic images of the sections and aluminum step-wedge were analyzed with a system including a microscope, videocamera, microcomputer and software (TMR2000; Inspektor Research Systems, Amsterdam, The Netherlands). The output parameters obtained were the mineral content profiles of the lesions, lesion depth (defined as the point at which mineral content is 95% of the sound dentin value), and integrated mineral loss (ΔZ). We defined ΔZ as the difference between the Z of the initial lesions and that of the advanced lesions (ΔZ = ΔZAE2 - ΔZAE1, where ΔZAE2 and ΔZAE1 are the values of Z at time points AE2 and AE1, respectively). In addition, the value of the integrated mineral content was determined for the surface layer and the body of each lesion.
Lesion characteristics formed with fluoride

Single-section: placed in the groove leaving 1mm from the materials

Demineralization (0.1M lactic acid & MC gel system, pH4.8, 7days, 37°C)

Initial lesion ——> (1st TMR analysis)

Subsequent acid resistant test (0.1M acetic acid, 1.5mM Ca, 0.9mM P, 0.2ppmF, pH5.0, 3days, 37°C, without the materials)

Advanced lesion ——> (2nd TMR analysis)

Figure 1. The series of transversal microradiography (TMR): after initial lesion formation and advanced lesion formation.

Statistical analysis

The data was analysed by using SPSS 11.0.1 software package. One-way ANOVA and Tukey's test were performed to compare ΔAZ between the groups. The differences were considered to be statistically significant when 𝑝 < 0.05. In addition, the correlations between ΔAZ and the mineral volume of the surface layer, between ΔΔZ and the mineral volume of the lesion body and between ΔAZ and ΔZ of the initial lesions were found using Pearson correlation coefficients.

Results

Figure 2 shows average profiles of the initial lesions (time AE1) and those of the advanced lesions after the second period of acid exposure (time AE2). For the control material LF, there was a small and indistinct peak near the surface in the mean profile of the initial lesion. In addition, the lesion body was severely demineralized. After subsequent demineralization, we observed no further mineral loss in the near-surface region. We observed a different pattern of demineralization in all the samples exposed to one

Figure 2. Average mineral profiles formed at the initial lesion (black line) and the advanced lesion (grey line).
of the fluoride-releasing materials. The profiles of the initial lesions in samples of BF, US and BFF at time AE1 were similar. These showed comparatively high mineral content in the near-surface region with a lesion body of 15-30 vol% at 40–50 μm deep. The profiles of the advanced lesions recorded at time AE2 in these three fluoride-releasing materials showed significant mineral loss in deeper regions. For the samples of GF, the mean profile of the initial lesion had a well-defined peak in the near-surface region. In addition, the GF samples lost less minerals in the second period of acid exposure than did any of the other three fluoride-releasing materials.

Table II shows the mean ΔAZ of the five groups. The ΔAZ of the non-fluoride-releasing material was significantly greater than that of any of the fluoride-releasing materials (BF, US, BFF and GF) \((p < 0.05)\). Similarly, the ΔAZ of BF and BFF was greater than that of GF \((p < 0.05)\). There were no significant differences between BF, US and BFF.

The correlations between ΔAZ and the mineral volume of the surface layer, between ΔAZ and the mineral volume of the lesion body and between ΔAZ and AZ of the initial lesions are shown (Figure 3). The ΔAZ and the mineral volume% of both surface and lesion body of the initial lesions were negatively correlated (correlation coefficients: -0.87 and -0.73, respectively). The ΔAZ and AZ of the initial lesions were positively correlated (correlation coefficient: 0.78).

Discussion

Clinically it is of interest to study whether dentin lesions previously formed adjacent to fluoride-releasing materials will resist subsequent acid attacks when the source of external fluoride supply is exhausted. This situation occurs in vivo since fluoride-containing materials typically release fluoride only for a short period after placing the restoration.

We developed an in vitro model consisting of a pit filled with the restoration material of choice placed in close proximity of a removable dentin single-section. After making lesions in a microenvironment with fluoride-releasing materials present, we placed the dentin sections into a fresh acidic solution, thus simulating the supply of fluoride from restorative materials having become negligible.

The hypothesis of this study was validated; dentin surrounding fluoride-releasing materials was protected against demineralization even after the fluoride release had diminished. There were no significant differences for ΔAZ between BF, US and BFF as fluoride-releasing resin composites. However, BF and BFF contain surface reaction-type pre-reacted glass-ionomer (S-PRG) filler. Restorative materials containing the filler show significant levels of fluoride release and are rechargeable [12,13].

Lesion progression (expressed as ΔAZ) correlates positively with initial lesion severity (ΔAZ) and negatively with the mineral content (both in the near-surface layer and in the lesion body) of the initial lesions. On the topic of lesion progression, Mellberg [14] reported that small lesions tended to demineralize faster in vivo than deep lesions due to the presence of soluble mineral available near the surface. However, his studies did not take into account fluoride taken up by lesions.

Previous studies where white spot lesions were re-subjected to a cariogenic challenge showed small amounts of tissue loss near the surface of the white spots [15]. Similarly in our study, mineral loss in the near-surface regions during the second acid attack was small in the fluoride-releasing materials in comparison with the non-fluoride-releasing material. Also, the mineral profiles in the near-surface region were essentially unchanged after the second acid attack. However, in the deeper regions significant differences in the degree of demineralization were observed between the experimental materials after the second acid challenge.

Several explanations may be put forward to explain these findings: We hypothesize that the more highly mineralized near-surface region of the samples that had been exposed to fluoride-releasing materials reduces the rate of acid penetration and thus reduces the amount of mineral dissolved. In addition, if the demineralization process takes place with fluoride present, fluoride is deposited not only in the near-surface layer, but also at the lesion front, protecting this zone during subsequent periods of exposure to acid. Silverstone [16] reported that local fluoride-induced remineralization is evident from the size of the crystallites in the respective zones. The relative inertness of the original lesions during subsequent demineralization is seen as laminations found in natural and in vitro lesions [17]. Fluoride taken up during the first period of acid exposure may also be redistributed to the sites of acid attack or co-precipitate with calcium and phosphate dissolved in the deeper layers, leading to local remineralization.

<table>
<thead>
<tr>
<th>Materials</th>
<th>ΔAZ (mean (SD)) (vol%–μm)</th>
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<tbody>
<tr>
<td>LF</td>
<td>3648 (446)(^a)</td>
</tr>
<tr>
<td>BF</td>
<td>2578 (345)(^b)</td>
</tr>
<tr>
<td>US</td>
<td>2071 (329)(^bc)</td>
</tr>
<tr>
<td>BFF</td>
<td>2479 (698)(^b)</td>
</tr>
<tr>
<td>GF</td>
<td>1421 (162)(^c)</td>
</tr>
</tbody>
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\(^{abc}\) Values of the materials with the same letters are not significantly different at \(p > 0.05\).
In conclusion, the current findings show that developing dental restorative filling materials that have superior fluoride-releasing characteristics will help to protect the vicinity of a restoration against caries even after the amount of fluoride released from the restoration has become negligible.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References


