Ferric Sulphate Alterations on Primary Dentin and the Adhesive Interface

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\textbf{Purpose:} The purpose of this study was to test the hypothesis that the pulp medication ferric sulphate (FS) may remain on primary dentin, affecting its microchemical structure and the bond strength of adhesives to primary dentin.

\textbf{Materials and Methods:} The effects of FS and pre-bonding conditioning (37\% phosphoric acid [PA] for 15 s or a self-etching primer, with or without the use of 2\% chlorhexidine [CHX]) on FS-treated primary dentin were characterized using optical microscopy with Masson’s and Perls’ stains, variable-pressure SEM/energy-dispersive x-ray spectroscopy (VP-SEM/EDS), Fourier transform infrared spectroscopy (FT-IR), and x-ray diffraction. Ferric sulphate was applied for 30 s or 1 h for microchemical analysis. The adhesive interface and the bond strength were studied by VP-SEM/EDS and the \( \mu \)TBS test (1 mm\textsuperscript{2} bars, crosshead speed 0.5 mm/min), respectively. The study groups were: etch-and-rinse (E&R, Excite) adhesive (group 1); FS+E&R (group 2); FS+CHX+E&R (group 3); self-etching (SE, Adper Scotchbond) adhesive (group 4); FS+SE (group 5); FS+CHX+SE (group 6).

\textbf{Results:} Ferric sulphate application produced demineralization, gypsum formation, and adsorption of Fe on the dentin surface, and it modified the collagen structure of primary dentin. There were no effects of FS on bond strength of the tested etch-and-rinse adhesive system. FS slightly reduced the bond strength between the primary dentin and the SE adhesive, and the values were not restored by CHX (group 4 = 17.58 ± 5.52 MPa > group 5 = 14.26 ± 7.08 MPa = group 6 = 13.96 ± 4.87 MPa).

\textbf{Conclusions:} Ferric sulphate alters the microchemical structure of primary dentin and can impair the adhesive strength of some self-etching adhesives.

\textbf{Keywords:} ferric sulphate, spectroscopy, Fourier transform infrared spectroscopy, x-ray diffraction, tensile strength, microscopy, dentition, primary.


\textbf{Pulpotomy is still the most common treatment for cariously exposed pulps in symptom-free primary molars.\textsuperscript{11} Currently, there is sufficient evidence for the carcinogenicity of formaldehyde in humans,\textsuperscript{25} which has led to research into alternative products to be used as pulp dressing medication. Ferric sulphate (FS) has proven to be as effective as formocresol\textsuperscript{12,22,24,32} and can be considered a valid and inexpensive solution for pulpotomies in primary teeth.\textsuperscript{11}}

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permanent dentin. In contrast, the adhesion of etch-and-rinse adhesives to permanent dentin do not seem to be affected by FS, or by other astringents. On primary dentin, immersion in FS for 48 h drastically reduced the shear bond strength to dentin of two etch-and-rinse adhesives and two self-etching adhesives.

Two different preparations of ferric sulphate from the same manufacturer (Ultradent Products; South Jordan, UT, USA) are available for use in primary tooth pulpotomy: a 15.5% aqueous solution and a 20% FS viscous gel, Viscostat, which appeared to be more difficult to remove from dentin than the aqueous solution.

We tested the null hypothesis that FS (Viscostat) would not persist on primary dentin and could not affect the microchemical structure of primary dentin. A second null hypothesis is that the prior application of FS does not reduce the bond strength of dentin adhesives to primary dentin.

MATERIALS AND METHODS

This experimental in vitro study was conducted on 101 primary molars, extracted for either pedodontic or orthodontic reasons. Patients were 8 to 12 years old, in their late mixed-dentition period. Informed consent was obtained from the patients and parents or legal guardians for the teeth to be used in this research. All the molars were stored in 0.1% thymol solution for less than one month until use. This research was approved by the Ethics Committee for Human Research of the University of Granada, Spain (Registration number 355).

Optical Microscopy

In order to determine the effects of FS on primary dentin, 6 sound primary molars were used. The pulp chamber was accessed using a high-speed rotary instrument (Kavo Dental; Biberach, Germany) and a diamond bur under water cooling. Any remaining pulp tissue was removed manually. In the experimental specimens, FS was applied in the pulp chamber for 30 s. Specimens were rinsed thoroughly with water spray for 15 s. Two sample specimens were designed to be submitted to Masson’s staining technique, and two to Perls’ staining. One additional molar served as a control for each staining technique and no dentin treatment was applied.

To visualize the effects of pre-bonding conditioning on FS-treated primary dentin, 24 primary molars, per group, were prepared as described previously and submitted to the procedure assigned to each of the experimental groups 1 to 6 (Table 1). Molars to be stained with Masson’s stain were filled with Tetric EvoCeram (Ivoclar Vivadent; Schaan, Liechtenstein) without any further application of acid etching or adhesive. In specimens designed to be visualized using Perls’ stain, the whole cavity was filled with Fuji II LC (GC; Tokyo, Japan).

All the specimens were stored in distilled water at 37°C for 24 h. Afterwards, two central slices about 1 mm thick were obtained from each molar in a precision cutting machine (Accutom-50, Struers; Bailerup, Denmark) and processed for optical microscopy as described in a previous study. Masson’s stain has a high affinity for cationic elements of normally mineralized type I collagen, which stains in green color. Dentin etching removes these elements from peptidic collagen chains, resulting in a different coloration, generally red. Perls’ stain selectively stains ferric ions blue. The potassium ferrocyanide changes to ferric ferrocyanide or Prussian blue in the presence of ferric iron by the action of the hydrochloric acid that acts to initiate the reaction. Specimens were examined under optical microscopy (BH-2 Olympus; Tokyo, Japan) at 100X magnification. For each specimen, the positive stains were recorded in the three thirds of each optical field, either in intertubular or peritubular dentin, the apertures with the presence of conical formations at the outer extremes of dentinal tubules, and the presence of the dye inside the tubule walls.

VP-SEM/EDS

Three molars were processed, one control (dentin without any treatment) and two treated with FS. The roots were removed, and flat occlusal mid-dentin surfaces were obtained with a diamond bur mounted in a high-speed handpiece (Kavo Dental). They were polished with silicon carbide disks (WS-Flex 18-C, Hermes Abrasives; Virginia Beach, VA, USA) of decreasing grit size (800, 1000, 1200 and 2500). Ferric sulphate was applied as described above. No filling was performed. Specimens were fixed and dehydrated, mounted in aluminum holders, and carbon sputter-coated in an argon atmosphere (0.1 Torr). All analyses were done with a Zeiss scanning electron microscope (DSM 950, Leo 1430VP, Leo Electron Microscopy; Cambridge, UK) equipped with an energy dispersive spectrometer (EDS) (Inca 350 v.17, ISIS, Oxford Instruments; High Wycombe, UK) operating at 20 kV. From each dentin surface, microanalytical spectra were obtained from selected areas of the specimen: three spectra from intertubular dentin and the same number from peritubular dentin and the tubular entrances.

To examine the adhesive interface on FS-treated primary dentin, 12 additional primary molars were used (2 molars per experimental group, 1 to 6). Flat surfaces of mid-coronal dentin were obtained as described above. The dentin was treated as specified in Table 1, and the adhesives were applied and photopolymerized with an Astralis 10 halogen lamp (Ivoclar Vivadent) at 700 mW/cm² for 20 s. One approximately 2-mm-thick increment of composite Tetric EvoCeram (Ivoclar Vivadent) was light cured for 20 s. Specimens were stored in distilled water for 24 h at 37°C. Afterwards, molars to be examined under VP-SEM/EDS were fixed to an acrylic holder with sticky wax (Kerr; Orange, CA, USA) and cut mesiodistally. The medial surfaces of each sample specimen were processed as described. One surface from each molar was selected for microanalysis.

FT-IR and XRD

Slices about 300 μm thick were obtained from the coronal dentin of 10 sound primary molars (Micromtome Accutom 50, Struers). Dentin was examined under 40X magnification (Olympus PT stereoscope, Olympus...
Optical; Tokyo, Japan) and any remaining enamel was removed with a high-speed handpiece. Slices were hand ground with silicon carbide paper disks down to approximately 120 μm (Digital Caliper 350-MHN1-25-DM, Mitutoyo; Tokyo, Japan). Two or three slices were randomly assigned to one of the experimental groups (groups 2, 3, 5, or 6; Table 1). Some slices remained untreated and served to obtain the primary dentin spectrum. In addition, two spectra were included as controls: FS and 2% CHX. In all the FS-treated groups, specimens were immersed in a glass beaker containing FS for 1 h. The treated specimens were washed in distilled water under mechanical agitation (magnetic stirrer MS3000, BOECO; Hamburg, Germany) 3 times for 1 min each. The water was changed after each agitation period. In addition, two spectra were included as controls: FS and 2% CHX. In all the FS-treated groups, specimens were immersed in a glass beaker containing FS for 1 h. The treated specimens were washed in distilled water under mechanical agitation (magnetic stirrer MS3000, BOECO; Hamburg, Germany) 3 times for 1 min each. The water was changed after each agitation period. In groups 2 and 3, 37% phosphoric acid (Dentalux, José Ripoll SL; Madrid, Spain) was applied for 15 s, rinse with water for 15 s, gently dry. In groups 4 to 6, Adper Scotchbond self-etching acidic adhesive (3M ESPE; St Paul, MN, USA) was applied for 1 min, no rinsing. 

For microchemical assays (FT-IR and XRD), Viscostat was applied for 1 h. *Astralis 10 lamp (700 mW/cm²; Ivoclar Vivadent). Abbreviations: FS: ferric sulphate; E&R: etch-and-rinse; CHX: chlorhexidine; SE: self-etching adhesive.

### Table 1: Study groups, materials and application procedures

<table>
<thead>
<tr>
<th>Group</th>
<th>Dentin treatment</th>
<th>Adhesive</th>
<th>SEM/EDS and optical microscopy</th>
<th>SEM/EDS and μTBS</th>
</tr>
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<tbody>
<tr>
<td>FS</td>
<td>30 s, wash with water spray for 15 s.</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Group 1 (E&amp;R)</td>
<td>No</td>
<td>No</td>
<td>Groups 1 to 3: 37% phosphoric acid (Dentalux, José Ripoll SL; Madrid, Spain) Apply for 15 s, rinse with water for 15 s.</td>
<td>Groups 1 to 3: E&amp;R adhesive Excite (Ivoclar Vivadent; Schaan, Liechtenstein) Apply, gently dry, polymerize for 20 s* Composition: &lt; 11% phosphoric acid acrylate; &lt; 15% HEMA &lt; 53% dimethacrylates; &lt; 20% alcohol; SiO₂, initiators, stabilizers</td>
</tr>
<tr>
<td>Group 2 (FS + E&amp;R)</td>
<td>No</td>
<td>No</td>
<td>Rub CHX-soaked cotton pellet on dentin for 1 min, no rinsing.</td>
<td></td>
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<tr>
<td>Group 3 (FS + CHX + E&amp;R)</td>
<td>30 s, wash with water spray for 15 s.</td>
<td>Rub CHX-soaked cotton pellet on dentin for 1 min, no rinsing.</td>
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<tr>
<td>Group 4 (SE)</td>
<td>No</td>
<td>No</td>
<td>Groups 4 to 6: Adper Scotchbond self-etching (3M ESPE; St Paul, MN, USA) (primer), Liquid A: Apply, stir for 10 s, blow gently for 10 s. Composition: water, HEMA, surfactant, pink colorant).</td>
<td>Groups 4 to 6: Adper Scotchbond self-etching acidic adhesive: Liquid B: Apply for 10 s until pink color (liquid A) disappears. Polymerize for 10 s*. Composition: UDMA, TEG-DMA, TMPTMA (hydrophobic trimethacrylate), HEMA phosphates, MHP (methacrylated phosphates), bonded zirconia nanofiller, initiator system based on camphorquinone.</td>
</tr>
<tr>
<td>Group 5 (FS + SE)</td>
<td>30 s, wash with water spray for 15 s.</td>
<td>Rub CHX-soaked cotton pellet on dentin for 1 min, no rinsing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 6 (FS + CHX + SE)</td>
<td>30 s, wash with water spray for 15 s.</td>
<td>Rub CHX-soaked cotton pellet on dentin for 1 min, no rinsing.</td>
<td></td>
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</table>

For microchemical assays (FT-IR and XRD), Viscostat was applied for 1 h. *Astralis 10 lamp (700 mW/cm²; Ivoclar Vivadent). Abbreviations: FS: ferric sulphate; E&R: etch-and-rinse; CHX: chlorhexidine; SE: self-etching adhesive.

Microtensile Bond Strength (μTBS) Test

Seven primary molars per group (groups 1 to 6) were processed in the same way as described for the SEM/EDS analysis. To test the bond strength, two additional increments of composite were added to form a build-up about 5 mm high (shade A1) and another similar build-up (shade...
A4) was made on the apical side after the pulp debris was removed with an excavator. The methodology followed in this test has been described previously.4,29 Cylinders containing the specimens to be submitted to the μTBS test were fixed in an acrylic holder and cut in the microtome Accutom-50 in bars about 1 mm². Using a cyanoacrylate adhesive gel (Henkel Adhesivos; Barcelona, Spain), the composite/dentin/composite bars were glued to a test probe and submitted to tensile stress (Instron 3345, Instron; Norwood, MA, USA) at a constant crosshead speed of 0.5 mm/min until failure. Fractured surfaces were examined at 40X magnification with a stereomicroscope (Olympus PT, Olympus Optical) to determine the mode of failure. Fractures were classified as adhesive, mixed, cohesive in resin, or cohesive in dentin. Only adhesive or mixed fractured surfaces were considered for statistical analysis.42

Statistical Analysis
After verifying the normality (Kolmogorov-Smirnov test) of the data distribution, a full factorial ANOVA model was applied (adhesive system: etch-and-rinse or self-etching; dentin treatment: none, FS, or FS + CHX). One-way ANOVA was used to compare between groups using the same adhesive system. Tukey’s test allowed for post-hoc multiple comparisons between groups. Student’s t-test was used to compare means between adhesives. The chi-square test was applied to compare failure mode between study groups. Significance was set at p < 0.05.

RESULTS
Effects of Ferric Sulphate on Primary Dentin
With respect to untreated control (Fig 1a), after applying FS, a demineralization layer could be observed on primary dentin as a continuous red band in all the optical microscopic images (n = 6) with Masson’s stain. Two-thirds of the tubule entrances were open, and the red stain continued inside the tubule walls, with the typical funnel forms (Fig 1b). Perls’ stain (n = 6) revealed a pronounced blue band indicating the presence of Fe³⁺ on intertubular and peritubular dentin in FS-treated specimens. The blue stain was frequently (2/3 of each microscope field) seen to have spread inside the tubular lumen. Fe³⁺ distribution was irregular (Fig 1d). These findings suggest that ferric ions were not washed away by water spray. No blue color was visible in the Perls’ staining control without FS treatment (Fig 1c). The VP-SEM/EDS analysis revealed the absence of smear layer. The tubule entrances were

Fig 1 Optical microscopic and SEM images of characterization of primary dentin treated with FS for 30 s. (a) Masson’s stain, control without treatment; (b) Masson’s stain, FS-treated primary dentin with a continuous red layer indicating exposed collagen mesh; (c) Perls’ stain, control without treatment; (d) Perls’ stain; blue stain reveals the presence of Fe³⁺ on the dentin surface; (e) VP-SEM image of FS-treated dentin; (f) back-scattered image and EDS elemental mapping, highlighting Fe distribution in red; (g) sum spectrum.
clearly visible, although partially occluded by smear plugs (Fig 1e). Fe was found on the entire dentin surface, but accumulations were observed at the entrances of dentin tubuli (Fig 1f). EDS showed Ca, C, P, Mg and Fe to be the main elements present on the FS-treated primary dentin surface, although Na and Zn were also detected and mapped (Fig 1g).

In the control FT-IR spectrum (primary dentin without any chemical treatment), different bands were observed which correspond to chemical groups of hydroxyapatite, whose main wavenumbers were related to the constituent dentin molecules. The peaks used in this study corresponded to the components: ν (OH−) at 3500–2900 cm−1; organic component: amide I, amide II, and amide III at 1655, 1560 and 1280–1380 cm−1, respectively; inorganic components: carbonates ν (CO32−) at 1459–1420 cm−1 and phosphates, ν3 (PO43−) and ν1 (PO43−) at wavenumbers 1097, 1035 and 960 cm−1, respectively. The FS spectrum showed the typical sulphate bands to be ν3 (SO42−) at 1104 cm−1 and ν4 (SO42−) at 613 cm−1.

After 1 h of FS application, some interesting observations were made. As shown in Fig 2, with respect to non-treated dentin, the spectra of all the treated dentin groups demonstrated increased absorbance in the amide I (1655 cm−1) and amide II bands (1560 cm−1) and a slight decrease in absorbance of the amide III band (1280–1380 cm−1). There were also new shoulder peaks at around 1104 cm−1 and 613 cm−1, corresponding to free sulphate (SO42−) bands, more pronounced in specimens treated with CHX (groups 3 and 6) than in groups 2 and 5, without CHX. The amide II band was also more evident in groups 3 and 6, overlapping with CHX spectral bands (Fig 2).
XRD made it possible to explore any possible alteration of the crystalline structure of primary dentin under the studied experimental conditions. Dehydrated ferric sulphate was identified as parabutlerite. After 1 h of exposition to FS, the most relevant finding was the presence of gypsum along with hydroxyapatite crystals. Gypsum was evident in FS-treated dentin, and in a lesser amount in the rest of the groups, although it was scarcer in group 3 (Fig 3).

A highly demineralised surface was evident under optical microscopy with Masson’s stain (red-stained dentin surface, and open tubular apertures) in all the experimental groups. In the etch-and-rinse groups, Perls’ stain revealed the presence of Fe$^{3+}$ in group 2, but it was almost undetectable in group 3. In the self-etching groups (5 and 6) using optical microscopy with Perls’ stain, a thick, continuous blue layer was seen to stain both intertubular and peritubular dentin (Fig 4). No blue stain appeared in control specimens.

Using VP-SEM/EDS in the etch-and-rinse groups, the formation of the tags and the demineralizing effects of 37% PA (15-s etching) on primary dentin were evident, producing thick interfaces with scalloped edges. The elemental distribution captured at the bottom of the hybrid layer revealed a high degree of demineralization. C, Ca, P, O and a discrete presence of Si were the most frequently found elements. In group 3, clear peaks of S were seen in spectra recorded at the bottom of the hybrid layer. When the self-etching adhesive was used, a regular, thin hybrid layer was visible with VP-SEM/EDS (Fig 4). The presence of Fe was a constant in group 5 at the top of the hybrid layer on the intertubular dentin, at the tag/peritubular dentin interface, and even inside the tubular lumina (Fig 4; group 5, images a, b, and c). In group 6, the Fe peak was not as high as in group 5, and its distribution was more irregular. Cl and S were frequently present at the top of the dentin. Other elements were Mg, O, and Si. Pt from the SE adhesives’ bonding component was frequently detected in groups 4, 5, and 6; the elements W, Yb, and Ba were also present.

**Effects of FS on Bonding**

Results of the μTBS test are shown in Table 2. Pre-test failures were infrequent (11.8%) and mainly produced in the pulpal adhesive surface, as identified by a darker composite shade. They were not included in the statistical analysis. The full-factorial ANOVA model demonstrated the influence of the adhesive system (etch-and-rinse or self-etching) (p < 0.05) and of the interaction of adhesive system x treatment (p = 0.001) on μTBS. Post-hoc comparisons showed that the self-etching system gave higher bond strength values than the etch-and-rinse system (p < 0.05). Tukey’s test showed significantly greater bond strength (p < 0.02) when CHX was used (group 3) with respect to the control group (group 1). Comparisons between self-etching adhesive groups showed significant differences, with a better adhesive performance in the control group (group 4) with respect to groups 5 (p < 0.05) and 6 (p < 0.05), which presented similar bond strength values. Comparisons between adhesive systems in the etched group revealed significant differences only in the control group, with better bond strength values when the self-etching system was used (p < 0.001).

Distribution of failure type (adhesive or mixed) is shown in Table 2. Specimens treated only with FS before bonding did not show any adhesive failure, regardless of the adhesive used.
Figure 4 Microscopic analysis of FS-treated experimental groups (2, 3, 5 and 6). OM (original magnification 100X) shows demineralization (Masson’s stain) (a) and presence of Fe (Perl’s stain) (b). VP-SEM/EDS characterization (c) of the adhesive interface. Back-scattered SEM images (c) and representative micro-analytical spectra from selected points (+, *). Symbols correspond to spectra on the right. Group 2 (d): O, Si and Mg are present. No Fe is detected. Group 2 (e): Note the relatively high peaks of Ca, C, and P, indicating demineralization. Group 3 (d) and (e): S is frequently detected, with Mg and O. In Group 2 (c) and Group 3 (c), the aggressive effect of 37% phosphoric acid etching for 15 s is evident on primary dentin. Group 5 (d) and (e) show the presence of Fe at the tubular lumen. Group 6 (d): Fe is also present at the bottom of the hybrid layer and at the tubular entrance (e); spectra show the presence of S and Cl.
DISCUSSION

The main concerns with respect to the influence of ferric sulphate and other astringents on bonding to dentin are two-fold: its persistence on dentin surface, related to staining and filtration of the adhesive restorations, and its acidity, which is able to alter the dentin surface.

Although previous studies reported a greater susceptibility of primary dentin to acid etching, the mineral content changes in primary teeth might be greater than in permanent teeth. As a large number of first and second primary molars were used in the present study, a wide variability in composition and crystallinity could be expected.

The product Viscostat used in this study has a pH of about 0.7, similar to that reported by Land et al. Acidic treatment prior bonding could increase the discrepancy between the depth of acid demineralization and monomer infiltration during the adhesive procedures, leaving the collagen fibrils exposed mainly at the hybrid layer base, which may be exposed to long-term degradation. The effects of FS on permanent dentin are shown to be time dependent. In this study, the application time of FS (30 s) is longer than recommended in primary-tooth pulpotomies, intentionally maximizing its effects and the possibility of contamination, yet reflecting a clinical situation in which a further pulp amputation or FS application could be necessary. Other studies regarding the influence of FS on bonding to primary dentin immersed the dentin specimens in 15% FS aqueous solution for 48 h. On permanent dentin, different exposure periods to 15% FS, ranging from 30 s to 5 min, or to 20% FS gel, from 20 s to 1 min, have been explored.

The use of microscopy and analytical techniques sheds light on different aspects of the problem. In regard to the demineralizing effects of FS, optical microscopy in conjunction with Masson’s stain revealed a red-stained layer corresponding to exposed collagen mesh. In the present study, no conditioner or adhesive was applied, meaning this effect was due exclusively to FS action on primary dentin. The manual elimination of pulp debris could allow some debris of the odontoblastic layer and predentin to remain, which is rich in collagen and could be more sensitive to differential Masson staining. In EDS spectra, demineralization is also visible as an increase of the C peaks, representing the organic component of dentin with respect to the relative height of Ca and P peaks. Consequently, the first null hypothesis of this study is rejected, as FS demineralizes primary dentin and affects the microchemical composition.

When the dentin surface is polished before treating, as in the present research, only a very thin smear layer (if any) is present. Along with the short exposition time (30 s), the lower mineral content of primary dentin may explain the discrepancy with respect to a previous SEM study, in which an amorphous precipitate layer was evident on a ground permanent-dentin surface even after 1 to 5 min of application of FS. Crystallite formation from peritubular dentin treated with FS and etched with PA2 was not detected in the current study.

In order to verify the persistence of FS on the primary dentin surface after washing, optical microscopy with Perls’ stain was performed, as well as VP-SEM/EDS analysis and mapping of the contaminated primary dentin. Because no other source of Fe was added in these in vitro experiments, when present, Fe indicates that at least this component of Viscostat remains on the dentin surface after washing with water spray.

Changes in the dentin molecular structure or the formation of new chemical compounds were explored by FTIR and XRD, respectively. The tests were performed on thin slices of dentin submerged in FS gel for 1 h because in a previous study, no spectral differences were detected between untreated primary dentin and that exposed to FS for 30 s. While this does not mean that no molecular changes took place, they may have gone undetected. Under such conditions, the main change in FS-treated vs untreated primary dentin found with FT-IR was new spectral absorbance bands corresponding to free sulphate, adsorbed on dentin, and an increase of amide I and amide II with a relative reduction of the amide III band. Amide bands of dentin structure are related to the collagen helix. Changes in amide I and amide III bands have been described after plasma treatment, and might indicate

| Table 2 | μTBS test results (MPa) and comparisons |
| Treatment | Etch-and-rinse Mean (SD) | PTF | Group (n) | Self-etching Mean (SD) | PTF | A/M | A/M |
| Control 1 (44) 13.73 (4.12)a | 2 4 (34) | 17.58 (5.52)b | 7 26/18 a,1 | 10/24a,1 |
| FS 2 (36) 12.76 (5.36) | 5 5 (38) | 14.26 (7.08) | 7 0/36 | 0/38 |
| FS+CHX 3 (46) 15.80 (7.61) | 1 6 (40) | 13.96 (4.87) | 5 5/41 a,2 | 15/25a,1 |
| Total 1 (126) 13.73 (6.11)a | (112) | 15.16 (6.06)b | Chi-square = 62.78; p < 0.001 |

Note: Group (n), mean (SD). PTF: pre-test failures. Different superscript letters indicate statistically significant differences in rows. Different superscript numbers indicate statistically significant differences in columns. A: adhesive failure; M: mixed (adhesive and cohesive in resin) failure.
secondary structure changes of dentin collagen and/or breaking of some intrafibrillar bonding. The increase of the amide-II band peak must be cautiously interpreted, because it overlaps with CHX spectral peaks at around 1450–1645 cm⁻¹ without frequency shifts or new peaks, as shown in a previous study on CHX. We speculate that CHX could act as an external source of sulphate, either due to its own composition or, eg, the solution’s artificial colorant (light red), which may contain sulphur.

Under XRD, in FS-treated dentin, new peaks appeared when compared to the untreated dentin and were identified as gypsum (calcium sulphate dihydrate). Such a compound might correspond to the amorphous precipitate described by Ayo-Yusuf et al. No ferric compounds were identified by XRD; this appears to corroborate the adsorption of Fe on the dentin surface. Gypsum was less evident in group 3 than in the rest of the FS-treated specimens, probably due to the additive cleaning effects of CHX and phosphoric acid. No detrimental effects of FS on the µTBS of one etch-and-rinse adhesive system to primary dentin were found in this study, similar to a report on permanent teeth.

In contrast, the deleterious effects of FS on the bond strength to primary dentin of a mild self-etching adhesive have been previously reported. One can speculate that a “strong” self-etching adhesive, such as that used in this study, could dissolve the smear layer as well as the FS and its reaction products, forming a hybrid layer similar to etch-and-rinse adhesives. This hybrid layer could be thicker in groups in which FS – which has an acidic pH – was applied prior to the self-etching adhesive, resulting in “over-etching”. Nevertheless, a thin hybrid layer was created and FS was not completely removed, which could be responsible for the lower bond strength in groups pre-treated with FS with respect to the self-etching control. Mine et al described the behavior of Adper Scotchbond SE to be more like that of the “intermediate-strength” self-etching adhesives, with a pH between 1 and 2. Along with the pH, other factors related to the composition of the adhesives, the substrate, or the dentin treatment can influence the bond strength and stability.

Etching time was 15 s in our study. Some studies recommend shorter etching times for primary dentin because of its lower mineral content. Although there is not complete agreement on the benefits of reduced etching time in immediate bond strength to primary dentin, it may improve the long-term bonding results.

When the SE primer was rubbed on the FS-treated primary dentin, we found a significant reduction of bond strength. The main findings of the analytical examinations were the presence of Fe and gypsum formation, which was more evident in groups 5 and 6 (self-etching adhesive) than in etch-and-rinse specimens. In group 5, Fe accumulated on the tubular entrances, while in group 6 this accumulation was patent, but weak. It is likely that Fe was dispersed by the CHX scrub, which is known to act by a cation-chelating mechanism. Those findings support the adsorption of Fe on the dentin surface, without chemical binding to dentin components. In our study, the effect of CHX in restoring the µTBS values obtained with the SE adhesive on FS-contaminated dentin was negligible, contrary to a previous report. Better removal through longer washing of FS could explain these differences. On the other hand, CHX was not rinsed off in group 6, and could remain bound to demineralized dentin during resin-dentin bonding. Once bound, CHX is relatively resistant to the displacing effects of HEMA or ethanol, but is more easily removed by water. Thus, the resulting CHX concentration in the hybrid layer could be much higher than that reported to inhibit MMPs and may also affect the stability of the hybrid layer. The real concentration of CHX in the dentin of the current study is unknown.

CONCLUSIONS

Ferric sulphate demineralized the primary dentin, remained on the dentin surface after washing, and persisted even after scrubbing with CHX for 1 min or conditioning for the bonding process. Elemental changes demonstrate the presence of Fe adsorbed on the dentin surface. Microchemical changes consisted of demineralization of dentin surface, and after a long application time, resulted in free sulphate and gypsum. The collagen molecular structure was also affected. FS diminished the bond strength of a self-etching but not an etch-and-rinse adhesive to dentin.

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REFERENCES


