Carious Dentin Treatment For Glass Ionomer Cement Adhesion: A Comparative Study

Corresponding Author:
Prof. Davide Zaffe,
Associate Professor, Anatomy and Hystology, Via del pozzo 71, Policlinico, 41124 - Italy

Submitting Author:
Prof. Davide Zaffe,
Associate Professor, Anatomy and Hystology, Via del pozzo 71, Policlinico, 41124 - Italy

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Carious Dentin Treatment For Glass Ionomer Cement Adhesion: A Comparative Study

Author(s): Zaffe D, Botticelli A, Bellincampi M, Chiesa M, Vitale M

Abstract

Carisolv method is a minimally invasive treatment of carious lesions which may improve patient collaboration and allow care of minimal dental cavities, especially in young people. The aim of this study was to compare H3PO4 and Carisolv in vitro treatments of human dentin applying microradiography, SEM examination and X-ray microanalysis to evaluate the capacity of these products to clean dentin surface for glass ionomer cement (GCI) application.

Forty extracted permanent molars with dentin caries were treated with 35% H3PO4 or Carisolv gel according to manufacturer instructions. Ten teeth of each group were filled with GCI and then embedded in methyl-methacrylate (PMMA). The PMMA blocks were sectioned, polished and microradiographed. The remaining teeth were dehydrated and desiccated in a critical point dryer. Sections and teeth were then prepared for SEM and X-ray microanalysis.

H3PO4 treatment produced unpolished surfaces (probably due to mineral re-precipitation), demineralization of dentin, exorbitant tubule widening, highly irregular dentin surface of cavities, and dentin fragments embedded by GCI. Carisolv treatment produced polished dentin surfaces with open tubules, little crystal re-precipitation, good GCI adhesion and penetration inside tubules.

The chemo-mechanical method (Carisolv) seems to be a good technique for treating carious cavities, minimizing dentin injury and providing an appropriate surface for adhesive bonding.

Introduction

The preservation of the bulk of sound tissue in carious teeth is an important goal of modern dentistry and coincides with a less invasive treatment dental lesion. In addition to large dentin removal [1], particularly in young patients rotary instruments may prompt fear and avoidance of treatment [2]. The high pressure, heat and excessive vibration of rotary instruments may cause additional harmful effects [3].

To minimize patient distress, a chemo-mechanically, minimally invasive for softening and removing the carious dentin [Carisolv™] was developed [4]. After an initial formulation, a new Carisolv gel with improved atraumatic removal of carious dentin and shortened treatment times has been proposed [5]. Carisolv is able to soften the carious dentin, leaving healthy dentin intact. Carisolv treatment preserves the maximum amount of sound dentin and opens the surface of dentin. The rough surface produced is adequate for bonding of resins [6]. Several studies of the effects of Carisolv have been performed, but few comparative works include Carisolv. Several authors have tested dentin etching using H3PO4 [7, 8] and many adhesive producers [9, 10] recommend H3PO4 etching before adhesive application.

The aim of this study was to compare in vitro H3PO4 and Carisolv etching of human dentin by means of microradiography, SEM and X-ray microanalysis to define the peculiarity of the two treatments.

Methods

Forty carious, human mandibular molars, extracted for periodontal problems, were selected for study. Teeth were stored until use in 47.5% ethanol (95% ethanol:water - 1:1) at 4°C after debriding all residual tissue. Teeth were treated no later than 30 days after extraction. Teeth were randomly divided into two groups treated with H3PO4 (group A) or Carisolv (group B).

H3PO4 35% (Gel Etch™, Scientific Pharm. Inc., Pomona, CA 91768 USA) was applied for 30 seconds in the carious cavity. Carisolv gel™ (MediTeam Dentalutveckling, Sävedalen, Sweden) was applied on the carious surface following manufacturer instructions; sound enamel and dentin was protected by soluble acetone paint. Teeth were rinsed with an air-water jet and air-dried. Ten teeth of each group were stored in hydroalcoholic solution (95% ethanol:water = 1:1), whereas the cavity of the remaining 10 teeth of each group was filled by glass ionomer cement (GIC - GC Fuji IX GP, GC Europe N.V., Leuven, The Netherlands). Teeth were finally stored in 47.5% ethanol before morphological analysis.

All teeth were dehydrated through an ethanol series (Fluka Chemie, Buchs, SG, Switzerland). Teeth having an unsealed cavity were desiccated in a critical point dryer (CPD030, Bal-Tec AG, Principality of Liechtenstein) at 40°C and pressure of 7.4 MPa, using
CO2 as intermediate agent. Teeth with GIC-filled cavity were embedded in methyl-methacrylate (PMMA, Fluka) without decalcification. The PMMA blocks were sectioned along the longitudinal axis of the tooth up to the cavity center using a diamond saw microtome (1600, Leica Microsystems GmbH, Wetzlar, Germany). A 200 micron-thick section was obtained from the center of each tooth, and a subsequent 3 millimeter-thick section was obtained using the diamond saw microtome. All surfaces were then polished with sandpaper and diamond compound (3 µm). The polished 200 micron-thick sections were X-ray microradiographed (Italstructures, Riva del Garda, Italy) at 6 kV and 2 mA on high contrast film (EM, Ilford Photo, Mobberley, Cheshire, UK). The microradiographs were analyzed and photographed with a microscope (Axiophot, Carl Zeiss AG, Jena, Germany) under ordinary light. The polished 3 millimeter-thick sections were gold sputtered (SCD004, Bal-Tec), examined by SEM using the back-scattered electron detector (XL40 Philips, FEI Company, Eindhoven, The Netherlands) and analyzed with an X-ray microprobe (EDAX9900 Philips) at 25kV, 0° Tilt, 31° take-off, 0.08 µm spot. An image analyzer (VIDAS, Carla Zeiss) was used to perform measurements of SEM images.

Results

Phosphoric acid

Microradiographs of nearly all GIC-filled teeth, whose cavity had been treated with H3PO4 revealed a radiotransparent layer of dentin in contact with the GIC (Illustration 1 - The arrows point to a dark gray (radiotransparent) layer of the dentin facing the GIC). Cavities were unexpectedly unpolished after H3PO4 etching. Less than one third of the dentin surface had open tubules, whereas most of the surface appeared covered by amorphous material (Table 1 and Illustration 2A - The cavity surface shows open tubules at the center, whereas amorphous material covers the grater part of the surface). Tubules were greatly enlarged with a bore of about 3 µm (Illustration 2B).

Many fragments of material, having the same appearance of that covering most of the dentin surface, was detectable inside the tubules. No collagen component of dentin was found inside the cavities, whereas decalcified dentin with exposed collagen fibers was observed at the border of cervical carious cavities (Illustration 3A - The border of cervical carious cavities often displays decalcified dentin with exposed collagen fibers). Cubic crystals, larger than 1 µm, deposited on the dentin surface were revealed at the enamel-dentin junction (Illustration 3B - Cubic crystals are particularly distinguishable at the enamel-dentin junction of treated cavities) in particular. Backscattered SEM analyses pointed out a layer of dentin with lower backscattering values in contact with the GIC (Illustration 4A - Note the thick layer of the demineralized dentin which faces the GIC). This layer, in some points greater than 250 µm thick, covered one-half to the entire dentin surface of the cavity. The irregular dentin contour often appeared highly indented with large and pitted excavations (Illustration 4C - Note how the carious cavity appears in section highly indented with large and cracky excavations produced by H3PO4 treatment.). Large dentin fragments (greater than 100 µm) were detached from the dentin cavity and embedded with GIC (Illustration 4D - The arrows point to a large dentin fragment detached from the dentin surface and engulfed by GIC).

<table>
<thead>
<tr>
<th>Dentin surface with open tubules in the bottom of the cavity.</th>
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<tr>
<td>%</td>
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<tr>
<td>H3PO4 30.7 ± 3.9</td>
</tr>
<tr>
<td>Carisolv 84.7 ± 2.9</td>
</tr>
</tbody>
</table>

The mean ± SEM (n = 10) values were assessed on SEM images.

Carisolv

Microradiographs did not reveal differences in the morphology of dentin surrounding the carious cavities (Illustration 5 - Uniform dentin surrounds the Carisolv-treated carious cavity filled with GIC). The dentin surface showed a recurrent wave-like aspect with open tubules distinguishable even at low magnification (Illustration 6A - The dentin surface has a wave-like aspect and open tubules). The dentin surface with open tubules exceeded four-fifths of the cavity (Table 1). Tubules had a mean bore of 1.4 µm, and dentin presented scattered small cubic crystals (smaller than 0.6 µm) deposited on the surface (Illustration 6B - The open tubules of the dentin have a small bore and few of them appear clogged with small cubic crystals). The cavity contour appeared finely indented, without excavations (Illustration 7A). The glass ionomer cement perfectly adhered to the dentin surface and penetrated inside tubules (Illustration 7B).

Table 2

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Mineral composition of materials of Illustration 3B

Phosphorous Calcium
Dentin 12.5 26.0
Crystals 8.2 24.4

Quantitative X-ray microanalysis performed using CaHPO4.2H2O as standard. Values are expressed as Wt %.

X-ray microanalyses
Analysis of cubic crystals deposited on the cavity surfaces did not reveal differences in composition after H3PO4 and Carisolv treatments. Both crystals were formed of the same calcium-phosphate compound. Semi-quantitative X-ray evaluation showed that this compound differed greatly from enamel or dentin (Illustration 3C - Graphs plotted to the same full scale. The main peak of gold [Au-M 2.15 KeV] was not indicated in graphs. Note how the dentin of the image [cyan graph] contains more phosphorous and calcium [tallest peak and greater peak-area] than the crystals [yellow graph]. Note also how the two P and Ca peaks reach a similar height in dentin [cyan graph] whereas the height of the Ca peak is about double that of the P peak in the crystals [yellow graph]), with a Ca/P ratio of 3 (Wt/Wt), much greater than that of hydroxyapatite (Ca/P = 2.15). This compound also showed a lower mineral content than dentin hydroxyapatite (Illustration 3C). Quantitative X-ray analyses, referenced to a standard compound (a thin CaHPO4.2H2O tablet of the same stub as the sample), confirmed both the higher Ca/P ratio and mineral content of crystals, lower than that of dentin hydroxyapatite (Table 2). Additional X-ray analyses highlighted the substantial overlap in the composition of crystals and amorphous mineral material covering the dentin surface treated with H3PO4 (Illustration 2).

X-ray analyses of demineralized dentin treated with H3PO4 (Illustration 1 and 4A) revealed a lower phosphorous and calcium content than that of sound dentin, and the presence of a very small amount of aluminium (Illustration 4B - Graphs plotted to the same full scale. The main peak of gold [Au-M 2.15 KeV] was not indicated in graphs. Note how the sound dentin [cyan graph] contains more phosphorous and calcium than demineralized dentin [yellow graph] and how aluminium is contained in the latter). Moreover, quantitative X-ray analyses, referenced to a CaHPO4.2H2O standard, revealed both a lower mineral content and a lower Ca/P ratio (1.79) of demineralized dentin (Table 3) than those of sound dentin.

X-ray analyses of the tubule filling (Illustration 7B) showed a decreasing aluminium and silicon content proceeding from the GIC layer towards the deep tubule (Illustration 7C - Graphs plotted to the same full scale. The main peak of gold [Au-M 2.15 KeV] was not indicated in graphs. Note how aluminium and silicon gradually decrease from the proximal [cyan graph] to the distal part [yellow graph] of the filled tubule). Due to the unavoidable inclusion of the dentin wall in the analysis, calcium and phosphorous were detected together with the tubule filler elements (Illustration 7C). Table 3

Discussion

Acid etching is a quick and easy technique for removing unwanted mineral coatings of dentin surfaces formed by mechanical (smear layer) [11, 12, 13, 14] or chemical (caries) activities [9, 15, 16]. Most authors [11, 12; 13, 16] point out the capacity of H3PO4 etching to open the tubules, and only a few of them stress the erosive effects of this acid treatment [11, 13]. Our results show that H3PO4 treatment affects not only the superficial dentin lining the carious cavity but also a conspicuous layer of dentin wall, more than one hundred microns thick. Though this may be a small detrimental effect, since dentin uniformly loses about 1/3 of its mineral content, it underlines the erosive properties of H3PO4. This effect is particularly pronounced in the outer layer of the dentinal cavity, where dentin contacts the GIC. Here, in line with the findings of Takeda et al. [11] and Ayad [13], the tubules present enlarged openings and a highly eroded wall. A similar appearance can be seen in images (Figs. 3 and 5) by Takeda et al. [11] where the intertubular dentin is thinned and eroded. The superficial dentin of H3PO4-treated carious
cavities does not appear to be a vigorous material, and it may not be the best mechanical support for the bonding agent. Moreover, we detected detachment of fragments of the dentinal wall, suggesting dentin brittleness.

Our findings also highlight some recurrent findings: the inability of H3PO4 to clear the entire dentin surface. Most works concerning H3PO4 treatment of dentin [11, 12, 13, 14, 16] report medium-to-high-magnification (x1000 up to x5000) SEM images that show a clean but rather limited portion of the dentin surface. We studied the entire carious cavity at low SEM magnification to evaluate the amount of polished surface. The use of H3PO4 gel, as indicated in the bonding agent manufacturer instructions, produced a polished dentin surface over about one-third of the carious surface.

An explanation of this result must consider several facts. First, H3PO4 activity seems to be consistent, as shown by the uniform demineralization of the dentin wall. Second, calcium phosphate crystals were observed inside the cavities. These crystals have a slightly different composition than that of sound dentin but almost identical to that of the material lining most of the treated carious surface. We can therefore speculate that if the composition of the crystal and this material is the same, the only difference consists in the degree of crystallization of calcium phosphate: high in the recovered crystals, totally absent in the amorphous material covering the surface. Since crystal formation (also detected by Haznedaroğlu [17]) is probably due to re-precipitation of dissolved ions, we may suggest that the amorphous material could also form by re-precipitation of large amounts of dissolved ions.

Carisolv treatment of carious cavities appears to provide good performance. Contrary to Yazici et al. [18] and Kinoshita et al. [19] that found wide unclean surface preparations, more than 4/5 of the dentin surface was polished after Carisolv treatment. In agreement with Banerjee [20], the surface had a roughened (wave-like) aspect, but contrary to Yazici et al. [18], Kinoshita et al. [19] and Banerjee [20] all tubules were opened in the cleaned surfaces. The mean bore of the tubules was only slightly enlarged, with small impact on peritubular dentin. The fine pitting without excavations of the cavity contour has confirmed this weak erosive effect. Also treatment with Carisolv allows formation of crystals with the same composition as those observed after H3PO4 treatment, but smaller in size. Some small dirty fragments of amorphous material were detected, but very scanty distributed as compared to that after H3PO4 treatment. Primer or GIC will engulf these formations without compromising seize. The detection of these materials both in Carisolv and H3PO4 specimens, not reported by previous authors, is probably due to the protocol we adopted. Preservation of samples in 47.5% ethanol and the use of CPD treatment probably maintained the materials and their aspect similar to that most likely present in vivo, obviously not displayed. The good GIC engagement inside tubules of dentin treated with Carisolv is reflected in the samples by the decrease in aluminosilicate content of the embedding material. This component corresponds to minute particles of the glass component that cannot reach the deeper parts of tubules, where only plastic components (polycrystalline acid, HEMA, dimethacrylate) penetrate.

Conclusion(s)

In conclusion, conventional excavator or drills may not be the best choice to treat carious cavities since they remove intact dentin [21]. On the other hand, our results show hard chemical aggression may compromise dentin robustness, despite the recommendation of many adhesive producers to use H₃PO₄ etching [9,10]. The Carisolv method provides highly satisfactory results. The special tools and chemical aggression by Carisolv components minimize dentin injury and provide a surface suitable for adhesive bonding [22]. Therefore, to achieve the best results Carisolv treatment may replace etching with H₃PO₄, required before primer or GIC application.

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Authors Contribution(s)

D. Zaffe, M.C. Vitale contributed to the research design. M. Bellincampi, M. Chiesa, M.C. Vitale contributed to the teeth harvesting and treatments. D. Zaffe performed the morphological and chemical-physical analyses. D. Zaffe, A.R. Botticelli, M.C. Vitale contributed in drafting the paper. D. Zaffe, A.R. Botticelli, M.C. Vitale worked on the critical revision of the paper.
References

Illustrations

Illustration 1

H₃PO₄ - Microradiograph of a crown section of a tooth embedded in PMMA.

[Image of a microradiograph showing a tooth section with arrows indicating caries]

1 mm

Illustration 2

H₃PO₄ - SEM images of carious cavities.

[Image of SEM images showing carious cavities labeled 'Pulp cavity' with two panels A and B]
Illustration 3

H3PO4 - SEM images of teeth (A-B) and X-ray microanalyses of dentin and crystals (C).

Illustration 4

H3PO4 - SEM images of sections of GCI filled teeth (A,C,D) and X-ray microanalyses of dentin (B).
Illustration 5

Carisolv - Microradiograph of a crown section of a tooth embedded in PMMA.

Illustration 6

Carisolv - SEM images of carious cavities.
Illustration 7

Carisolv - SEM images of GCI filled teeth (A,B) and X-ray microanalyses of tubule content (C).
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