Effect of 10% Phosphoric acid Conditioning on the Efficacy of a Dentin Bonding System

Jian WU, Kazuo ITOH, Takashi YAMASHITA, Chihiro TANI, Hisashi HISAMITSU and Sadao WAKUMOTO
Department of Operative Dentistry, Showa University, School of Dentistry, 2-1-1, Kitasenzoku, Ohta-Ward, Tokyo 145-0062, Japan

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INTRODUCTION

It is widely known that the smear layer on a ground tooth surface should be removed prior to the application of dentin adhesives because the smear layer disturbs the bonding between the resin material and substrates. The smear layer on the enamel cavity wall is typically removed in clinic by 40% phosphoric acid which is useful both to remove the smear layer and to produce the honeycomb-like micro undercut in the enamel rod structure. The materials and methods to remove the smear layer on the dentin cavity wall are still being discussed. In 1977 an etching technique for both the enamel and the dentin cavity wall using 40% phosphoric acid followed by a dentin bonding agent containing methacryloxyethyl hydrogen phenyl phosphate (phenyl-P) was introduced by Fusayama et al. In 1982 Nakabayashi reported that dentin cleaning by citric acid containing ferric chloride followed by a dentin bonding agent containing 4-methacryloxyethyl trimellitate anhydride (4-META) was effective on bonding. Concerning the bonding mechanism of the dentin bonding system, Nakabayashi suggested that the bonding agent diffused and polymerized in the etched dentin consequently forming a hybrid layer in the superficial dentin. Pyruvic acid containing glycine, citric acid containing cupric chloride, and 10% phosphoric acid were reported to be effective for conditioning both the enamel and the dentin though...
no consistent conclusion was obtained. Furthermore, the effect of priming could not be explained by these bonding mechanisms.

The improvement of bonding by dentin priming was primarily explained by the chemical activation of the protein in the dentin collagen by glutaraldehyde; and the primer was made polymerizable with 2-hydroxyethyl methacrylate (2-HEMA)\(^7\). Recently, the priming effect by biphenyl-dimethyl methacrylate (BPDM) has been proposed by wetting and re-expanding collapsed collagen fibers which have been etched by 10% phosphoric acid. Bonding would thus be established by the impregnation of the monomer into the interfibrous expanded micro space of the dentin collagen\(^8\)\(^\text{11}\). This bonding technique was introduced as the total-etch wet-bonding system. These hypotheses were based on the chemical or physical interaction between the dentin adhesives and the organic components in the dentin.

In 1989 Chiba et al. claimed that the contraction gap width of a commercial light activated resin composite restored into a cylindrical dentin cavity mediated with an experimental dentin bonding system increased in corporation with the degree of the softening on the dentin surface during conditioning\(^12\). This finding suggested that the resin composite paste separated from the decalcified dentin cavity wall during polymerization. The purpose of the present study was to evaluate the efficacy of the commercial total-etch wet-bonding system and to examine the effect of conditioning with 10% phosphoric acid on the efficacy of the experimental dentin bonding system and the Ca-content in the substrate dentin.

**MATERIALS AND METHODS**

The materials tested are listed in the Table 1.

The efficacy of the commercial total-etch wet-bonding system (All Bond 2, Bisco, Itasca, IL, USA) and the effect of 10% phosphoric acid conditioning on the efficacy of the experimental dentin bonding system, which is claimed to completely prevent the contraction gap formation of a light activated resin composite in a cylindrical dentin cavity, was examined by measuring the wall-to-wall polymerization contraction gap

<table>
<thead>
<tr>
<th>Table 1 Components of the dentin bonding system tested</th>
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<tr>
<td><strong>Experimental</strong></td>
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<tr>
<td>Conditioner</td>
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<tr>
<td>Primer</td>
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<tr>
<td></td>
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<tr>
<td>Bonding agent</td>
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<td>Resin composite</td>
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</table>
width of the resin composite filled into a cylindrical dentin cavity. The proximal
eENAMEL of extracted human molars, which were stored in tap water in a refrigerator
for a maximum of four weeks after extraction, was flatly eliminated by using #220
wet carborundum paper. A cylindrical cavity approximately 3 mm in diameter and
1.5 mm in depth was prepared in the exposed dentin.

For the specimens of the commercial dentin bonding system, cavity conditioning,
priming and bonding agent application were performed according to the manufact-
urer’s instruction. In the positive control group, the dentin cavity wall was condi-
tioned with 0.5 mol/L neutralized EDTA solution for 60 sec followed by rinsing and
drying. The cavity wall was then primed with 35 vol% glyceryl mono-methacrylate
solution for 60 sec and the cavity was dried completely. A commercial dual-cured
dentin bonding agent containing 10-methacryloxydecyl dihydrogen phosphate (10-
MDP) was applied to the cavity followed by 10 sec irradiation using a lamp unit. In
both specimens of the commercial and experimental dentin bonding systems, a com-
mercial light-activated resin composite (Silux Plus, 3M, St Paul, MN, USA) was
slightly over-filled in the cavity and irradiated for 40 sec after the resin composite
surface was flatly pressed on a glass plate mediated with a plastic matrix. After
storing the specimens in water at a room temperature of 24 ± 1 °C for 10 min, the
over-filled resin composite was removed and the cavity margin was exposed with
#1000 wet carborundum paper. The resin surface and surrounding dentin was then
polished on a linen cloth mediated with 0.3 μm alumina slurry. The marginal adap-
tation of the resin composite was inspected under a light microscope at a magnifica-
tion of 1024, and the width of any contraction gap was measured with a screw micrometer
mounted on the ocular lens of the microscope at eight points every 45 degrees along
the cavity margin. The contraction gap was presented by the sum of the diametri-
cally opposing gap width as a percentage of the cavity diameter. The maximum of
four gap values was recorded as the contraction gap value of the specimen. In addi-
tion, the combination of the conditioner, primer, and bonding resin of the commercial
and experimental dentin bonding system was modified by exchanging for other mate-
rials as shown in Table 2 and the contraction gap was measured.

To examine the effect of the 10% phosphoric acid conditioning on the bonding ef-
cicacy of the experimental dentin bonding system, the cavity wall was conditioned
with 10% phosphoric acid solution for 5, 10, 15, 30 or 60 sec and the cavity was rinsed
and dried completely. The experimental dentin primer, aqueous solution of 35 vol%
of 2-hydroxyethyl methacrylate (2-HEMA) (Merck, Darmstadt, Germany) or glyceryl
mono-methacrylate (GM) (Nippon oil and fat, Tokyo, Japan) was filled in the cavity
for 60 sec and blown away completely with a compressed air blast. The commercial
dual-cured dentin bonding agent described above containing 10-MDP was filled in the
cavity and irradiated for 10 sec using a lamp source after removing the excess mate-
rials with a compressed air blast. For the positive control, the cavity wall was con-
ditioned with 0.5 mol/L EDTA neutralized to pH 7.4 for 60 sec, and for the negative
control, the dentin conditioning was omitted though other procedures were performed
with the same methods as in the experimental groups.
The Ca-content in the conditioned dentin was analyzed using an energy dispersion electron microanalyser (EDS). The flat dentin was prepared by grinding the occlusal enamel of the extracted human molar perpendicular to the tooth axis. Half of the dentin surface was covered with adhesive tape and the other half was cleaned with 10% phosphoric acid for 5, 10, 15, 30 or 60 sec. The specimens were then dehydrated with gradual ethanol solutions and vacuum evaporated with carbon. The Ca-content was analyzed at ten points both in the conditioned and covered dentin surfaces with the EDS at an accelerating voltage of 10 kV. The residual Ca-content by the conditioning was presented as the mean value of Ca-content in the dentin after conditioning as a percentage of that in the covered dentin. For the positive control, the dentin surface was treated with neutralized 0.5 mol/L EDTA (pH 7.4) for 60 sec. Five specimens for each group, 30 in total, were prepared and the data were analyzed by ANOVA (p<0.05).

RESULTS

The contraction gap values of the combination of the commercial and experimental dentin bonding systems measured are listed in Table 2. Complete marginal integrity was obtained only in the positive control in which the dentin cavity wall was conditioned with EDTA for 60 sec followed by GM priming and dentin bonding agent application containing 10-MDP. When the resin composite was filled after the cavity

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Efficacy of the commercial and experimental dentin bonding system</th>
<th>EDTA</th>
<th>10% H₃PO₄ gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>35% GM, Clearfil P.B.</td>
<td>0 (10)</td>
<td>0.019±0.033 (7)</td>
<td></td>
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<tr>
<td>AB primer, Clearfil P.B.</td>
<td>0.071±0.050 (1)</td>
<td>0.068±0.028 (0)</td>
<td></td>
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<tr>
<td>35% GM, All Bond</td>
<td>0.078±0.063 (1)</td>
<td>0.078±0.025 (1)</td>
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<tr>
<td>AB primer, All Bond</td>
<td>0.105±0.047 (0)</td>
<td>0.096±0.032 (0)</td>
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</table>

Clearfil P.B.: Clearfil Photo Bond
mean±SD and number of gap-free specimens of the Silux Plus restored into the cylindrical dentin cavity (N=10).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Contraction gap of Silux Plus in cylindrical dentin cavity</th>
<th>35% GM priming</th>
<th>35% 2-HEMA priming</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>0 (10)</td>
<td>0.015±0.029 (8)</td>
<td></td>
</tr>
<tr>
<td>10% H₃PO₄, 5 sec</td>
<td>0.016±0.025 (7)</td>
<td>0.039±0.042 (5)</td>
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<tr>
<td>10% H₃PO₄, 10 sec</td>
<td>0.011±0.019 (7)</td>
<td>0.045±0.044 (4)*</td>
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<tr>
<td>10% H₃PO₄, 15 sec</td>
<td>0.052±0.041 (3)*</td>
<td>0.048±0.052 (4)*</td>
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</tr>
<tr>
<td>10% H₃PO₄, 30 sec</td>
<td>0.019±0.033 (7)</td>
<td>0.056±0.056 (4)*</td>
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<tr>
<td>10% H₃PO₄, 60 sec</td>
<td>0.145±0.096 (1)*</td>
<td>0.073±0.070 (3)*</td>
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</tbody>
</table>

without cleaning | 0.198±0.056 (0)* | 0.222±0.044 (0)* |

n=10, mean±SD of the contraction gap, the number of gap-free specimens is given in ( ).
GM: glycerol mono-methacrylate.
Silux Plus was filled in the dentine cavity after EDTA or 10% phosphoric acid conditioning, GM or HEMA priming and Clearfil Photo Bond application. The difference was not significant when the eight groups identified by *were excluded by ANOVA (p<0.05).
treatment according to the commercial total-etch wet-bonding system, a contraction gap was observed in all of ten specimens prepared. Furthermore, it was impossible to completely prevent the gap formation when any materials of the experimental contraction gap-free system were exchanged with those of the commercial total-etch wet-bonding system.

The contraction gap values of the EDTA and 10% phosphoric acid conditioning group, which was followed by the GM or 2-HEMA priming and the commercial dentin bonding agent application containing 10-MDP, are presented in Table 3. The contraction gap formation was could not be prevented completely when the cavity was conditioned with the 10% phosphoric acid or primed by 2-HEMA solution. When the

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**Fig. 1** Light microscope photograph of the marginal integrity of the resin composite to the dentine cavity wall conditioned with EDTA.

**Fig. 2** Light microscope photograph of the cervical dentine cavity margin treated with 10% phosphoric acid for 60 sec. An etched dentine approximately 5 μm in width (indicated with £) and the contraction gap (indicated with *) formation were observed.
Table 4  Residual Ca-content after conditioning with EDTA or 10% \( \text{H}_3\text{PO}_4 \)

<table>
<thead>
<tr>
<th></th>
<th>83.55±8.27</th>
<th>62.12±6.93</th>
<th>44.85±5.39</th>
<th>35.83±4.84</th>
<th>22.28±4.98</th>
<th>11.33±6.55</th>
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</thead>
<tbody>
<tr>
<td>EDTA</td>
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<tr>
<td>10% ( \text{H}_3\text{PO}_4 ) 5 sec</td>
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<tr>
<td>10% ( \text{H}_3\text{PO}_4 ) 10 sec</td>
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<tr>
<td>10% ( \text{H}_3\text{PO}_4 ) 15 sec</td>
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<tr>
<td>10% ( \text{H}_3\text{PO}_4 ) 30 sec</td>
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<tr>
<td>10% ( \text{H}_3\text{PO}_4 ) 60 sec</td>
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</table>

Mean±SD of the residual Ca-content (%). The residual Ca-content after conditioning is presented as a percentage of the untreated dentine surface. N=5

conditioning time of the 10% phosphoric acid was prolonged to 60 sec, decalcified dentin approximately 5 \( \mu \)m wide was observed (Fig. 1 and 2). By a statistical analysis of ANOVA, there were significant differences in the 35% GM priming group, between the EDTA condition group and the 10% phosphoric acid for 15, 60 sec conditioning groups. In the 35% 2-HEMA priming group, significant differences were found between the EDTA conditioning group and conditioning by 10% phosphoric acid for 10, 15, 30, 60 sec group.

The Ca-content after 10% phosphoric acid conditioning (Table 4) was reduced even when the conditioning time was as short as 5 sec (62.12±6.39%). However, the relatively high Ca-content remained the same after EDTA conditioning for 60 sec (83.55±10.99%). Furthermore, a significantly high correlation was recognized between the contraction gap width and the residual Ca-content after conditioning as presented in Fig. 3. \( y=0.265-0.144\log(x), R^2=0.706 \) for the GM priming groups, \( y=0.136-0.058\log(x), R^2=0.906 \) for the 2-HEMA priming groups.
DISCUSSION

As demonstrated in this study, the efficacy of the commercial total-etch wet-bonding system was not remarkable. Furthermore, the efficacy of each component of this system, conditioner of 10% phosphoric acid, primer of BPDM and NTG-GMA and inter
terdent resin of Bis-GMA, UDMA and HEMA was not reliable because the mar-
ginal integrity of the experimental contraction gap-free dentin bonding system
deteriorated significantly by changing the materials to those of the commercial sys-
tem. This finding suggests that the bonding mechanism reported of wetting and re-
expanding the dentin collagen for the dentin bonding agent to penetrate and polymer-
ize is ineffective in obtaining marginal adaptation of the resin composite in the dentin
cavity.

In many reports concerning hybrid layer formation or the wet-bonding mecha-
nism, it is considered essential to remove the hydroxyapatite on the substrate dentin
using an acid or an acidic monomer. During conditioning with acid, the sound dentin
beneath the smear layer was possibly decalcified and the exposed dentin collagen fiber
collapsed; this collapsed collagen fiber was reported to re-expand by dentin
priming\textsuperscript{13\textendash{}17}. Thus the microspace between the collagen fiber in which the monomer
was able to impregnate and polymerize was enlarged and the bonding between the
resin composite and the superficial dentin collagen network would improve.

By atomic force microscopic observation, Igarashi et al. described dentin etched
by 10% phosphoric acid to have re-expanded to the same level as that before condi-
tioning by HEMA priming for 9 min, though this priming time is too long to be of
practical value\textsuperscript{8}.

However, Ohhashi et al. defined the momentary priming with a GM and HEMA
solution on the EDTA-conditioned dentin cavity as adequately effective because the
contraction gap width of the commercial light activated resin composite in the cylin-
drical dentin cavity was not significantly affected by the priming time between mo-
mentary and 60 sec\textsuperscript{20}. In particular, the momentary priming with a GM solution
after 60 sec EDTA-conditioning exhibited complete marginal integrity of the light-ac-
tivated resin composite restored into the cylindrical dentin cavity mediated with the
MDP-containing dentin bonding agent\textsuperscript{20}.

Most of the hypotheses about dentin bonding system, including the above men-
tioned bonding mechanism based on the hybrid layer or interdiffusion zone formation
in the acid-etched dentin layer, were based on the measurement of bond strength of
the dentin adhesive to a flat dentin surface or the ultrastructural observation of a
dentin rod coated with the dentin adhesive. With respect to bond strength measure-
ment, an adhesive fracture in the dentin and a cohesive fracture in the resin compos-
ite cylinder were frequently experienced whereas these two failures were never
observed in contraction gap measurement nor in clinical dentin cavities. It should be
noted that a contraction gap is always formed between the resin composite and the
dentin cavity wall. Regarding the specimens for bond strength measurement, the
resin composite contracts toward the flat dentin surface during polymerization
maintaining the bond to the dentin surface mediated with dentin adhesives. Remarkably high bond strength is obtained regardless of the degree of decalcification in the substrate dentin. The most important effect for the dentin bonding system is not to obtain high mechanical stress to destroy the specimen for the bond strength measurement after the polymerization of the resin composite, but to maintain the attachment between the dentin cavity wall and unpolymerized resin composite paste until the polymerization of the composite is finished and to compensate for any volume lost during the polymerization contraction by the flow of the composite from the free surface toward the cavity. In order to clinically practice, it is most important for the dentin bonding system to be ensured as contraction gap free in vitro because Yanagawa et al. previously defined a high correlation of the frequency of gap formation observed between cavities restored in the extracted teeth and those restored in the vital teeth in vivo and then extracted and observed in as short a period as 10 min after restoration\textsuperscript{20}. Contraction gap formation can be completely prevented only when the dentin bonding system minimises polymerization contraction stress of the composite during polymerization, and when contraction is completely compensated for by the flow of the composite from the free surface into the cavity.

As demonstrated in this study, the resin composite paste separated from the dentin cavity wall conditioned with 10% phosphoric acid or primed with 2-HEMA solution during polymerization. Such a marginal discrepancy caused by the polymerization contraction stress of the composite can be detected neither by bond strength measurement nor by observation of the ultrastructural morphology of a resin-coated dentin rod. Since the interaction between the efficacy of the dentin bonding system and the polymerization contraction stress can not be detected by such measurements or by observations, the efficacy of the dentin bonding system is easily misunderstood.

The GM solution exhibited a complete priming effect in the EDTA-conditioned dentin cavity whereas the HEMA priming effect was incomplete even when the Ca-content in the dentin cavity wall was optimized by EDTA conditioning. The difference in the priming effects between the HEMA and GM might be explained by the degree of impregnation of the functional monomer in the dentin bonding agent. Chigira et al. reported that a high electron density zone beneath the substrate dentin primed with glyceryl mono-methacrylate may be explained by the high concentration of phosphate ester in the dentin bonding agent\textsuperscript{21}. Consequently, the functional monomer concentration was kept high at the adhesive interface.

As demonstrated in this study, the primary requirement for dentin bonding is to maintain a high Ca-content in the substrate dentin. Furthermore, it is possible to speculate that contraction gap formation is prevented by priming which is useful to keep a high concentration of the functional monomer at the adhesive interface and by firm polymerization of the Ca-monomer compound at the adhesive interface.

It was also recognized that functional monomers, such as methacryloxyethyl trimellitate anhydride (4-META), IO-methacryloxydecyl dihydrogen phosphate (MDP) or methacryloxyethyl hydrogen phenyl phosphate (phenyl-P) had a carboxylate or a phosphate group which was thought to bond to the inorganic component and not to
the organic component in the dentin\textsuperscript{23}. As demonstrated in this study, the loss of calcium in the substrate dentin deteriorated the efficacy of the dentin adhesives containing the MDP. Therefore, it is possible that the quantity of Ca-monomer compound at the adhesive interface decreased with the decalcification of the dentin by the dentin conditioner.

As discussed above, conditioning of the dentin cavity wall with 10% phosphoric acid should be avoided even when the conditioning time is limited to as short as five sec because the Ca-content in the cavity wall is reduced rapidly and the resin composite paste separates from the decalcified dentin cavity wall during polymerization.

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