Effect of different intrapulpal pressure simulation on bond strength and resin tags length in primary incisors

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Abstract

Objective: To evaluate effect of different intrapulpal pressure on bond strength and the length of resin tags in primary incisors in vitro.

Material and methods: Sixty prolonged retention lower primary incisors were collected and tested within 24 hours after extraction. The root was cut off 1.0 mm below the CEJ. The tip of incisal edge was cut off to the level 1 mm deep into dentin. The samples were randomly divided into 0, +15, +30 cmH₂O group (n=20 each). The preset hydrostatic pressure system was connected to the tooth specimen. The cut tooth surface and the composite rod surface were treated with acid etching and applied bonding agent as recommended by manufacturer. Both surfaces were fixed with thin layer of flowable composite resin. Half of each group was used for tensile bond strength testing and another half was cut longitudinal and examined under SEM for measuring resin tags length. The p value less than 0.05 was considered significant.

Results: The mean±SD of 0, +15, +30 cmH₂O groups for tensile bond strength were 10.78±2.17, 10.70±3.40, 7.37±2.93 MPa respectively, and for the resin tags length were 8.74±1.55, 6.92±0.76 and 6.38±0.72 μm, respectively. The tensile bond strength of the +30 cmH₂O group was significant lower than the other groups. The resin tags length was significant different among 3 groups while the longest and the shortest groups were presented in 0 cmH₂O and +30 cmH₂O groups, consecutively.

Conclusion: This study found that increasing of simulated intrapulpal pressure resulted in decreasing of tensile bond strength and the length of resin tags, which might affect the ability of dental adhesive.

Keywords: intrapulpal pressure, dentin, dentin adhesive, tensile bond strength, resin tags lengths, primary teeth

Introduction
Composite resin is one of the most popular restorative materials in the present day. It bonds to dentin by dental adhesive resin. In general, the dentin surface is treated with acidic primer or acid etchant to demineralize peritubular and intertubular dentin, and clean the smear layer from the dentinal tubules. The adhesive resin is applied onto the demineralized dentin and infiltrated between the collagen networks to form the hybrid layer which then formed micromechanical interlock after polymerization\(^1\). The quality of the bond interface in dentin depends on various factors such as the hybrid layer, resin tags\(^2\) and the intrapulpal pressure.

In vital tooth, there is the continuous outward flow of dentinal fluid presented on the cut dentin surface\(^3-5\). The rate of dentinal fluid flow through dentin depends upon the changing of pulpal pressure\(^6\) and the permeability of dentin\(^7\). Normal pulpal pressure is range between 14.1-32.6 cmH\(_2\)O\(^6,8,9\) while the permeability of dentin depends upon the number of dentinal tubules per unit area and diameter of dentinal tubules. However, the effects of the alternated intrapulpal hydrostatic pressure on the bond strength and the resin tags in primary teeth are still unclear. Therefore, this experiment was designed to step up this knowledge by investigated the effect of different simulated intrapulpal pressure on the bond strength and the resin tags length of an adhesive in primary incisors in vitro.

Materials and methods
This study has been approved by The Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand. (No. 8/2016). The inform consent form was signed by the parent to allow us to collect the tooth sample for experiment.

Sixty primary mandibular incisors from 5-8-year-old healthy children, which were collected immediately after extraction and been tested within 24 hours. There were non-carious and was diagnosed as prolonged retention teeth. The root was cut off at the level of 1.0 mm. below the cemento-enamel junction using the diamond disk. The enamel on tip of incisal edge was reduced until exposed dentin. Further 1 mm. of dentin was then cut off using the same diamond disk. The remaining pulp tissue was removed then connected the tooth sample to silicone tubing of the hydrostatic pressure system under water to prevent tapping air bubble.

The samples were equally and randomly divided into 3 groups (n=20 each). The applying hydrostatic pressure for group I, II and III were 0, +15, +30 cmH\(_2\)O, respectively. The hydrostatic pressure system was set to the designated test level. The composite rods with tip diameter of 1 mm. were prepared with Filtek Z350 (3M ESPE, USA) using the same molding. The cut tooth surface and the flat end of composite rod were treated with 35% phosphoric acid (Scotchbond™ Etchant, 3M ESPE, USA) for 15 seconds then rinsed with plenty of water spray. Two layers of dentin adhesives (Adper™ Single Bond 2-step total-etch adhesive, 3M ESPE, USA) were applied on the surfaces, gently blown with air from triple syringe and cured with light for 10 seconds as recommended by manufacturer. The tooth sample and the composite rod were held firmly in the holding device to be able to apply constant force when bonded these two layers together. A tiny drop of flowable composite resin (Filttek Z350XT Flowable, 3M ESPE, USA) was added to the flat surface of composite rod before placing on the cut tooth surface by the gravity (Figure 1) then light cured for 40 seconds. The excess composite was wiped out from the edge of composite rod. The specimen was stored in 37°C distilled water for 24 hours. Ten of these were used for testing tensile bond strength in a Universal testing Machine (UTM) (Instron\(^\text{®},\))
Massachusetts, USA) with a cross-head speed of 0.5 mm/min until fracture (Figure 1). The rest of specimen were cut longitudinal through the interface, treated with 35% phosphoric acid and 5.25% sodium hypochlorite and cleaned in the ultrasonic cleanser and examined under Scanning Electron Microscopy (SEM). The SEM image was taken at x2,000 for further analysis. The resin tags were measured using ImageJ program (Java-based image processing program, the National Institutes of Health, USA).

The one way ANOVA statistical analysis was used to compare the difference mean of tensile bond strength and the length of resin tags to the experimental groups. Moreover, the Pearson’s correlation was tested between tensile bond strength and resin tags. The $p<0.05$ was considered as significant difference.

**Results**

The tensile bond strength tended to decrease when increasing hydrostatic pulpal pressure. The mean±SD of tensile bond strength for 0, +15, +30 cmH$_2$O groups were 10.78±2.17, 10.70±3.40 and 7.37±2.93, respectively (Table 1). In detail, there was no significant different among group I and group II. The tensile bond strength of the +30 cmH$_2$O group was significant lower than the other groups ($p<0.05$).

The length of resin tags significantly decreased when increased intrapulpal pressure. The mean±SD of the length of resin tag for group I, II, and III were 8.74±1.55, 6.92±0.76 and 6.38±0.72, respectively (Table 2). The SEM evaluation indicated that the length of resin tags was significant different among 3 groups ($p<0.05$). Group I presented the longest resin tags while the shortest resin tags were found in group III (Figure 2).

Pearson’s test indicated that there was significant correlation between the length of resin tags and the tensile bond strength when alternated the intrapulpal pressure ($R^2=0.311$, $P<0.01$) (Figure 3). In other word, increasing of intrapulpal pressure has an effect on both the reduction of tensile bond strength and the length of resin tags.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tensile bond strength (MPa) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cmH$_2$O</td>
<td>10.78±2.17</td>
</tr>
<tr>
<td>+15 cmH$_2$O</td>
<td>10.70±3.40</td>
</tr>
<tr>
<td>+30 cmH$_2$O</td>
<td>7.37±2.93</td>
</tr>
</tbody>
</table>

*indicated the significant different ($P<0.01$)

**indicated the significant different ($P<0.05$)

<table>
<thead>
<tr>
<th>Group</th>
<th>Length of Resin tags (µm) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 cmH$_2$O</td>
<td>8.74±1.55</td>
</tr>
<tr>
<td>+15 cmH$_2$O</td>
<td>6.92±0.76</td>
</tr>
<tr>
<td>+30 cmH$_2$O</td>
<td>6.38±0.72</td>
</tr>
</tbody>
</table>

*indicated the significant different ($P<0.001$)

**indicated the significant different ($P<0.05$)
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Figure 2 SEM image of resin tags at a magnification of x2,000 (A) a sample in the 0 cmH2O group (B) 15 cmH2O group (C) 30 cmH2O group

Figure 3 Correlation between the length of resin tags and the tensile bond strength when alternated intrapulpal pressure ($R^2=0.311$, $P <0.01$).
Discussion

The outward flow of dentinal fluid during simulated intrapulpal pressure condition might not only affect the polymerization of bonding agents but also has an effect on the etching process. The intrapulpal pressure settings used in this study were simulated the normal condition and the inflamed condition of the dental pulp as suggested by Vongsavan and Matthews\textsuperscript{6} and others\textsuperscript{9,10}.

Our results in this study were conformed to the results of previous studies from our groups\textsuperscript{11-13}. The mean values of tensile bond strength tested with alternated intrapulpal pressure in this study were similar. The increase alternated intrapulpal pressure resulted in the increase rate of outward fluid flow, has weaken the bond strength of dental adhesive in permanent teeth\textsuperscript{14-18}.

Even if most bonding agents claim to have hydrophilic properties and can be bonded to moist dentin surface but the excessive moisture on the dentin surface under the simulated intrapulpal pressure might overcome the hydrophilic properties of the adhesives and might be responsible for the reduction of diffusion rate of resin monomers and mechanical properties of the adhesive layer\textsuperscript{10,19,20}.

Moreover, the results in this study showed that the length of resin tags were shorten when increased intrapulpal pressure similar to the study of Rahal and colleagues\textsuperscript{2}, but slight lower than other studies which studied in the extracted permanent molar\textsuperscript{21,22}. Either the etching agent was less effective for dissolving the peritubular dentin or the dentinal fluid impeded the diffusion of the resin monomers to form the resin tags\textsuperscript{7,23}.

The depth of dentin demineralization depends on the types, etching time and the concentration of the acids\textsuperscript{24,25}. Perdigão and colleagues\textsuperscript{26} found the depth of intertubular demineralization after using 35% phosphoric acid was 1.9-5.8 μm. The interaction of etching agents on dentin is limited by the buffer effect of hydroxyapatite and other dentin components. Moreover, the etching agents used in dentistry are hypertonic, when they oppose the outward fluid, they absorb some fluid and result in a dilution of the acid itself and a reduction in their acid penetration into dentin\textsuperscript{23}.

Similar to our results, the shorter resin tags length was found when increased the simulation intrapulpal pressure\textsuperscript{20}. The outwards flow of dentinal fluids possibly affected the bond strength by changing the viscosity of the adhesive system\textsuperscript{27}. An alcohol based dental adhesives such as Single bond seem to have less water chasing ability so the resin infiltration into the collagen network was not effective compared to acetone based dental adhesives\textsuperscript{10,20}.

The correlation between the tensile bond strength and the length of the resin tags when alternated intrapulpal pressure might enhance some useful information to the observation of Anchieta and colleagues\textsuperscript{22} whose found the strong correlation between hybrid layer thickness and bond strength but not related to the length of resin tags. However, this present results was contrasted to the study of Rahal and colleagues\textsuperscript{2} which microtensile bond strength of Single bond did not depend on the thickness of the hybrid layer and length of resin tags.

Our present study investigated in primary tooth which has less mineralization in both peritubular and intertubular dentin\textsuperscript{28}, which might result in the higher formation of an intratubular hybrid layer than permanent tooth that has highly mineralized peritubular dentin. The length of resin tags is also crucial not only it provided the mechanical lock but also the hybrid layer was formed along the wall of dentinal tubules. These could influence on the strength of adhesive bonding as found increasing in this study.
The pulpal pressure in vital teeth is varies upon the pulpal condition. It can be increased during pulpal inflammation due to deep caries or certain stage of pulpitis. The vasodilatation and the increasing in membrane permeability of the vessels increase intrapulpal pressure. These is one of the self-defenses mechanism helping to protect the pulp against the invasion of bacteria and its toxin. The increasing of pulpal pressure and increasing outward flow may influence the infiltration of the dental adhesives to the demineralized dentin. Moreover, the higher water content of deep dentin may also prevent the infiltration of adhesive resins into the networks of organic matrix left after etching that may be critical for optimal bonding.

In conclusion, this study found that increasing of simulated intrapulpal pressure resulted in decreasing tensile bond strength and shortening the length of resin tags. These raise a possibility that the ability of etching agents and dentin adhesives in the bond strength was affected by increasing of intrapulpal hydrostatic pressure in vitro.

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Competing interests: None
Ethical approval: This study has been approved by The Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand. (No. 8/2016)

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