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1. To disseminate knowledge gained from academic research in dentistry and other related medical sciences;
2. To promote valued research for academic advancement;
3. To create an academic network and to build relationships among dentists and others in related fields in order to keep up with constantly developing knowledge;
4. To enhance the reputation of the Faculty of Dentistry and Mahidol University Dentistry Alumni Association.

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2. **Review Articles** are articles that derive knowledge from new textbooks and journals or from the author’s own work and experience. They should be composed in an analytical, critical, and comparative style for the advancement of knowledge.

3. **Miscellany** encompasses the following:
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   - **Ask the expert** are responses to readers’ academic or clinical problems by experts in particular fields. Interesting questions and answers from conferences may also be published for the benefit of those who have not attended the conferences.

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Editorials

In the globalization there are many competition of invitation submit the manuscript in the journal. We are going to the digital era. So the online manuscript is very quickly and easy. Mahidol Dental Journal is also change to be the online journal in nearby.

I also invited the authors to send the manuscript related the dental, odontostomatology and craniofacial field. This issue is the volume 37 of first issue 2017 everyone who want to read this Journal, please see in this website “http://www.dt.mahidol.ac.th/division/th_Academic_Journal_Unit”

I’m apologize the reader that Mahidol dental journal have not the journal website. This journal have to be in the Faculty website by the policy of the faculty and University.

On January, 9-10, 2017 (2 days) I joined the 3rd Editor and Researcher Day at Kantary Hills Changmai, I got many knowledge from the presenter about the international journal. That day discussed between the well known journal in Thailand, and the big boss (Dr.Jame Testa) The president emeritus editorial development and publisher relations formerly the IP & Science business of Thomson Reuter (Clarivate Analytics), presented about joining Thai Journal and discussion with each journal editor and Dr.Thamasorn showed which articles can stand in the top of Journal. I think every editor of journal should to join and get knowledge I also visited Changmai Journal of Science which got award form Elsevier on Scopus. I also see the processing of Journal including printing the journal by the faculty.

This issue I changed the format of articles and citation in the article with similar the International Journal. The author don’t worry this format. It is the publisher art to management it.

I have to thank reviewers and authors that join us to make this journal in the TCI 1 (Thai Citation Index Tier 1) and I think this journal may be get ACI (Asian Citation Index)

This issue have featured 15 articles which 13 articles are research works (11 English and 2 Thai) and 2 report case in English language.

The articles in this issue are
1. Effect of resin infusion on fracture toughness of dental veneering ceramic. (English article)
2. Fracture resistance of implant supported all ceramic zirconia-lithium disilicate crowns. (English article)
3. Characterization of internal structural integrity of all-ceramic crowns using micro-computed tomography. (English article)
4. The effect of LED light on viability and proliferation of periodontal ligament fibroblast cells. (English article)
5. Effect of curing protocols on degree of conversion and glass transition temperature of a dual-cured resin cement. (English article)
6. Effect of three repairing materials on the flexural strength of repaired heat-cured acrylic resin denture base material. (English article)
7. Allergic contact dermatitis of styrenic thermoplastic elastomers and latex sheets in humans. (English article)
8. Evaluation of marginal and internal gaps of all-ceramic crowns using X-ray micro-computed tomography. (English article)
9. The study of the alveolar antral artery canal using cone beam computed tomography. (English article)
10. Antibacterial effect of herbal plants against three cariogenic microorganisms. (English article)
11. Measurement of anterior loop of inferior alveolar nerve using cone beam computed tomography (CBCT). (English article)
12. Multidisciplinary treatment of a median diastema in a patient with tongue-tie and tongue thrusting: A clinical report. (English article)
13. Guided implant placement and provisional restorations in the aesthetic zone: A case report. (English article)
14. Cytotoxicity and biomineralized nodule formation induced by tinospora crispa crude extract in human bone cells. (English abstract with Thai article)
15. Effect of schizophyllan on proliferation and migration of human gingival fibroblast. (English abstract with Thai article)

We hope you are enjoy the articles.
Hope you follow this journal in the next issue with the new editor-in-chief and editorial board.

Professor N. Wongsirichat
Editor in chief
Mahidol Dental Journal
(E-mail: natthamet.won@mahidol.ac.th)
Marginal and internal gaps of crown and bridge substructure of two all-ceramic systems

Bundhit Jirajariyavej, Daungjan Siangsiaw, Chuchai Anunmana

Department of Prosthodontics, Faculty of Dentistry, Mahidol University

Objective: The objectives of this study were to compare the [1] marginal and internal gaps of two ceramic systems as well as the [2] marginal and internal gaps between single crowns and three-unit fixed dental prostheses (FDPs) substructures.

Materials and Methods: Ceramic substructures were fabricated using CAD/CAM (Lava™ Zirconia) and heat-press technique (IPS e.max® Press) as premolar and molar single crowns and three-unit FDPs (4 groups, n=10). Marginal and internal gap widths were determined and measured using silicone replica technique. Results were analyzed using Mann Whitney U-test ($\alpha = 0.05$), and data were described as median and interquartile range.

Results: For IPS e.max® Press, there was no significant difference of marginal adaptation between the crown and bridge groups, except at the mesial marginal gap of premolar (Crown; 39 µm, Bridge; 106 µm). For Lava™ Zirconia, differences were found at several locations of the premolar and one point of the molar. Significant differences of marginal gap between IPS e.max® Press and Lava™ Zirconia crown substructures were found only in premolar. There were also significant differences of marginal fit of FDPs between two systems in both abutments. Significant differences of internal fit were mostly found in the axial wall and the cusp tip areas.

Conclusions: Most marginal and internal gaps of IPS emax® Press were greater than those of Lava™ Zirconia except at the occlusal locations. In addition, three-unit FDPs revealed larger gap widths than those of single crowns in both ceramic groups.

Key words: marginal fit, internal fit, zirconia, glass-ceramic, all-ceramic

How to cite: Jirajariyavej B, Siangsiaw D, Anunmana A. Marginal and internal gaps of crown and bridge substructure of two all-ceramic systems. M Dent J 2017; 37:135-144

Introduction

Materials for fixed dental restoration can generally be classified into 3 types: all-metal, metal-ceramic and all-ceramic materials. Cast metal restoration exhibits high strength and toughness without risk of fracture; however, it can only be used in unaesthetic zone. Metal-ceramic crown and bridge meet patient’s esthetic more than metallic restorations; nonetheless, the optical properties of this restoration do not mimic the natural teeth. In addition, the opacity of metal under glass veneer may sometimes be problematic. Moreover, the metallic pigmentation of gingiva may occur near the margin of the porcelain fused to metal restoration. [1] Some patients may also be allergic to metal alloys in contrast to all-ceramic materials that are more biocompatible. [2]

For decades, dental ceramic has been used for dental fixed restoration. [3] Interest in all-ceramic application is increasing, both for the anterior and posterior regions. Esthetics is major concern of patients and dentist for the anterior region. In the posterior region, the main purpose is to resist the masticatory force; therefore, the strength of the material is necessary. Ceramics that have been developed for fixed dental restorations include inlays, onlays, crowns, fixed dental prostheses, and implant-supported...
restorations. [2] Metal under veneering glass provides high strength but is inferior in esthetics; therefore, high-strength all-ceramic materials have been developed. Ceramic substructure materials such as lithium disilicate, aluminium oxide, and zirconium oxide for all-ceramic fixed dental prostheses have been developed for more than 10 years and have become more popular in recent years. [3]

To consider the long-term clinical success of all-ceramic restorations, one of the most important factors for restoration longevity is the marginal and internal gaps. The presence of large marginal gap is prone to dental caries, periodontal disease, cement dissolution, and discoloration of the margin. [4,5] Internal gap (occlusal and axial spaces) should provide the optimal luting space, which affects the clinical strength of crown-cement system. [6] Moreover, good internal fit provides appropriate retention and resistance form. [7] The acceptable marginal gap for restoration in the range of 100-150 μm has been proposed. [4]

There are many factors that influence the fit of ceramic restoration such as the type of fabrication systems, span length, veneering, finish line configuration, angulation of the preparation, cement space, and zirconia ageing. [4]

Marginal and internal adaptations of various ceramic materials have been widely studied. [8-10] There were many studies that compared the marginal and internal adaptation of CAD/CAM fabricated and heat-pressed ceramic materials. In one study, it was found that the ZrO₂ fabricated from CAD/CAM technique had smaller marginal gap than that of heat-pressed ceramic technique. [8] On the other hand, heat-pressed ceramics exhibited smaller marginal gap than those of CAD/CAM fabricated zirconia ceramics (Cercon® and LAVA™) in other studies. [9,10] Differences of marginal opening between single crown and fixed dental prostheses were also investigated in many studies. [11-13] Most studies found that long-span fixed dental prostheses exhibited greater marginal opening than that of single-unit restoration. [11-13]

However, there were very few studies comparing the marginal and internal fit between zirconia and lithium disilicate substructures, therefore, the purpose of this study was to compare the marginal and internal gaps between single crown and three-unit substructures fabricated from two all-ceramic systems, Lava™ Zirconia and IPS e.max® Press.

Materials and Methods

Plastic maxillary teeth (Columbia Dentoform Corp, NY, USA) were embedded in type III dental stone platform (Comet 3, Ultima, Thailand) simulating a maxillary second premolar and maxillary second molar for three-unit fixed dental prostheses abutments. A putty-type silicone impression (Express, 3M ESPE, St.Paul, MN, USA) was made as a silicone index prior to abutment preparation to ensure uniformed reduction of abutment teeth. The abutment teeth were prepared as all-ceramic single crown and three-unit fixed dental prostheses (FDPs). The preparation guideline was as follows: 1 mm wide circumferential chamfer, 2 mm occlusal reduction, and 8-degree angle of total occlusal convergence (TOC). The approximate height of each prepared tooth was 4 mm with rounded line angle. TOC was verified using dental surveyor (Paraflex, Bego, Breman, Germany) to ensure the approximate 8 degrees of tooth preparation.

After the preparation was completed, the plastic abutment teeth in dental stone platform were duplicated using polyvinylsiloxane impression material (Wirosil®, Bego, Germany) and casted for a standardized Co-Cr model. The casted model was cleaned, polished, and finished by one operator. The finished standardized Co-Cr model is shown in Figure 1.

Forty impressions were taken on standardized Co-Cr model using customized
Marginal and internal gaps of crown and bridge substructure of two all-ceramic systems

perforated plastic tray and polyether impression material (Impregum™, 3M ESPE, St. Paul, MN, USA). All impressions were poured with type IV die stone (Velmix Kerr Lab, California, USA) as working casts. Forty working casts were randomly assigned according to ceramic materials (Lava™ Zirconia and IPS e.max® Press) and types of restorations (single crown and three-unit FDPs), therefore, ten working casts were prepared for ceramic restorations within four experimental groups as shown in Table 1.

Marginal and internal gaps were determined using silicone replica technique. First, the fitting surface of substructures were filled with low-viscosity polyvinylsiloxane (Express XT, 3M ESPE, St. Paul, MN, USA) and subsequently placed on the abutment teeth of standard Co-Cr model under 50-N load for 10 minutes. After the low-viscosity silicone material set, the substructures were removed from standard Co-Cr model while the thin layer of light body silicone film still conspicuously remained on the abutment teeth. The thin silicone film, which represented the space between abutment tooth and substructure, was stabilized by high-viscosity impression material (Express XT, 3M ESPE, St. Paul, MN, USA) using customized perforated plastic tray. The silicone plastic trays were sectioned using a blade in buccal-lingual and mesial-distal direction on the cutting guide of the tray into four pieces for each abutment as shown in Figure 2. All silicone replicas were examined under optical light microscope (Nikon eclipse E400 POL, Japan) at 50X magnification, and the photograph of the gap was taken using a digital camera (Canon EOS 450D, Japan). Gap measurement was made using Image pro plus software version 7.0 (Media Cybernetics, MD, USA).

For each substructure, the following 6 positions as shown in Figure 3 were used as the references for measuring the marginal and internal gaps according to Holmes et al. [14]

![Figure 1. A standardized Co-Cr model](image)

![Figure 2. Silicone replica technique: Low-viscosity silicone is loaded at the fitting surface](image)

<table>
<thead>
<tr>
<th>Types of materials</th>
<th>Types of restorations</th>
<th>Number of working cast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lava™ Zirconia</td>
<td>Single crown</td>
<td>10 premolar and 10 molar crowns</td>
</tr>
<tr>
<td>Lava™ Zirconia</td>
<td>Three-unit bridge</td>
<td>10 Three-unit bridges</td>
</tr>
<tr>
<td>IPS e.max® Press</td>
<td>Single crown</td>
<td>10 premolar and 10 molar crowns</td>
</tr>
<tr>
<td>IPS e.max® Press</td>
<td>Three-unit bridge</td>
<td>10 Three-unit bridges</td>
</tr>
</tbody>
</table>
The data were reported as median and 25th, 75th percentile values. The differences of marginal and internal gaps were determined by Mann-Whitney U-test statistic at a significant level of 0.05.

Results

1. Marginal adaptation

Marginal fit of substructures was investigated by using the values of absolute marginal discrepancy (AMD) and marginal gap (MG). Figure 4 shows the optical micrograph of the representative gap at 50X magnification. The median and 25th, 75th percentile of AMD and MG of premolar and molar substructures made from IPS e.max® Press and Lava™ Zirconia are listed in Tables 2 to 5.

Median marginal gaps of IPS e.max® Press group ranged between 39 – 82 and 38 – 106 μm while Lava™ Zirconia group ranged between 35 – 96 and 51 – 101 μm for single crown (Cr) and three-unit fixed partial denture or bridge (Br), respectively. There was no significant difference of gap widths between Cr and Br groups of IPS e.max® Press, except the MG at mesial side of premolar substructure (p < 0.05) as shown in Table 2. On the contrary, Lava™ Zirconia showed differences between Cr and Br groups for AMD and MG at mesial and buccal side of premolar and mesial side of molar MG as shown in Table 3.

There was no significant difference in the molar crown substructure between IPS e.max® Press and Lava™ Zirconia; nevertheless, premolar crown presented differences in 5 points as shown in Table 4. For the three-unit fixed dental prostheses, the differences showed in both premolar and molar bridge at mesial side (AMD and MG) of the premolar bridge and palatal and mesial side (AMD and MG) of the molar bridge substructure as shown in the table 5.

2. Internal fitness

The internal gaps were measured as chamfer area (CA), axial wall (AX), cusp tip (CT) and, occlusal adaptation (OA). The median gap width and 25th, 75th percentile are listed in Tables 2 to 5.

The significant differences between Cr and Br substructures fabricated from IPS e.max® Press were found in 5 points for the premolar substructure and 3 points for the molar substructure as shown in the Table 2. However, Lava™ Zirconia presented differences only in the palatal and mesial side of AX of molar substructure (Table 3).

The significant differences between IPS
Marginal and internal gaps of crown and bridge substructure of two all-ceramic systems

Marginal and internal gaps of all-ceramic substructures

Table 2. Median and 25th, 75th percentile (μm) of marginal and internal gap width of IPS e.max® Press premolar and molar substructure compared between single crown and three-unit bridge

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>e.max® premolar substructure</th>
<th>e.max® molar substructure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>p-value</td>
<td>p-value</td>
</tr>
<tr>
<td>AMD</td>
<td>B</td>
<td>78 (64,106)</td>
<td>76 (60,128)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>77 (58,94)</td>
<td>93 (65,119)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>91 (65,108)</td>
<td>116 (84,125)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>102 (63,120)</td>
<td>82 (59,105)</td>
</tr>
<tr>
<td>MG</td>
<td>B</td>
<td>69 (53,98)</td>
<td>67 (50,93)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>56 (34,88)</td>
<td>74 (37,91)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>39 (36,57)</td>
<td>106 (68,127)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>70 (57,116)</td>
<td>75 (28,96)</td>
</tr>
<tr>
<td>CA</td>
<td>B</td>
<td>156 (103,193)</td>
<td>200 (156,238)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>180 (122,213)</td>
<td>152 (127,187)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>166 (103,184)</td>
<td>250 (201,205)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>143 (124,196)</td>
<td>158 (108,212)</td>
</tr>
<tr>
<td>AX</td>
<td>B</td>
<td>68 (51,103)</td>
<td>85 (65,95)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>46 (30,66)</td>
<td>108 (81,135)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>70 (56,117)</td>
<td>173 (157,195)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>89 (67,98)</td>
<td>92 (72,127)</td>
</tr>
<tr>
<td>CT</td>
<td>B</td>
<td>87 (72,113)</td>
<td>80 (59,141)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>108 (96,138)</td>
<td>92 (76,153)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>74 (52,102)</td>
<td>163 (119,182)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>65 (44,106)</td>
<td>101 (45,165)</td>
</tr>
<tr>
<td>OA</td>
<td>B-P</td>
<td>142 (75,267)</td>
<td>119 (87,170)</td>
</tr>
<tr>
<td></td>
<td>M-D</td>
<td>148 (63,242)</td>
<td>96 (87,178)</td>
</tr>
</tbody>
</table>

* Indicates significant difference by Mann Whitney U-test at significant level of 0.05.
AMD = Absolute marginal discrepancy, MG = Marginal gap, CA = Chamfer area, AX = Axial wall, CT = Cusp Tip, OA = Occlusal adaptation, B = Buccal, P = Palatal, M = Mesial, D = Distal

Discussion

These results demonstrated that the marginal gaps of IPS e.max® Press were significantly greater than those of Lava™ Zirconia in premolar substructures, both of crown and bridge groups. The results were similar to another study, which showed that mean marginal discrepancy values of lithium-disilicate (IPS e.max® Press) copings were greater than those CAD/CAM fabricated Lava™ zirconia copings. [15]
The imprecision of heat-press ceramic (IPS e.max® Press) substructures that affected the restoration adaptability could be influenced by many factors; for example, the thickness of die spacer, the shrinkage of wax pattern upon cooling at room temperature, and the thermal shrinkage after pressing. This thermal shrinkage is generally compensated by setting and thermal expansion of an investment material. The CAD/CAM fabrication process such as scanning, design, milling, and sintering could affect the premature contact at the surface of the substructure and internal gap discrepancy. Moreover, several fabricated steps in milling and sintering also cause the internal inaccuracy; for example, mismatch of milling instrument to narrow area, worn cutting instrument form prolonged use, and software inaccuracy to compensate the material shrinkage. In addition, the anisotropic shrinkage after post-sintering of zirconia blank caused the larger shrinkage in the horizontal axis than the tooth long axis. Regarding this

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>Lava™ premolar substructure</th>
<th>p-value</th>
<th>Lava™ molar substructure</th>
<th>p-value</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Crown (n=10)</td>
<td>Bridge (n=10)</td>
<td>Crown (n=10)</td>
<td>Bridge (n=10)</td>
</tr>
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<td>B</td>
<td>54 (33,62)</td>
<td>78 (66,111)</td>
<td>0.004*</td>
<td>66 (49,103)</td>
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<tr>
<td></td>
<td>P</td>
<td>64 (57,84)</td>
<td>81 (59,97)</td>
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<td>45 (37,62)</td>
<td>79 (53,87)</td>
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<td>67 (42,103)</td>
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<tr>
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<td>D</td>
<td>58 (38,81)</td>
<td>79 (55,109)</td>
<td>0.112</td>
<td>104 (68,182)</td>
</tr>
<tr>
<td>MG</td>
<td>B</td>
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<td>70 (42,81)</td>
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<td>50 (32,90)</td>
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<tr>
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<tr>
<td></td>
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<td>51 (37,67)</td>
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</tr>
<tr>
<td></td>
<td>D</td>
<td>35 (26,54)</td>
<td>48 (38,83)</td>
<td>0.071</td>
<td>96 (54,142)</td>
</tr>
<tr>
<td>CA</td>
<td>B</td>
<td>76 (67,89)</td>
<td>79 (51,97)</td>
<td>0.821</td>
<td>106 (82,118)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>99 (83,109)</td>
<td>90 (74,131)</td>
<td>0.762</td>
<td>88 (61,143)</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>65 (58,84)</td>
<td>86 (44,118)</td>
<td>0.496</td>
<td>90 (74,113)</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>71 (62,76)</td>
<td>90 (56,119)</td>
<td>0.364</td>
<td>120 (106,166)</td>
</tr>
<tr>
<td>AX</td>
<td>B</td>
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<td>68 (61,92)</td>
<td>0.597</td>
<td>61 (45,77)</td>
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<tr>
<td></td>
<td>P</td>
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<tr>
<td>CT</td>
<td>B</td>
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<td>172 (125,186)</td>
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<td>178 (162,210)</td>
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<tr>
<td></td>
<td>P</td>
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<td></td>
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<td>121 (98,143)</td>
</tr>
<tr>
<td>OA</td>
<td>B-P</td>
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<td>192 (163,223)</td>
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<td>200 (173,295)</td>
</tr>
<tr>
<td></td>
<td>M-D</td>
<td>203 (176,212)</td>
<td>189 (168,211)</td>
<td>0.406</td>
<td>215 (160,273)</td>
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</tbody>
</table>

* indicates significant difference by Mann Whitney U-test at significant level of 0.05.
AMD = Absolute marginal discrepancy, MG = Marginal gap, CA = Chamfer area, AX = Axial wall, CT = Cusp Tip, OA = Occlusal adaptation, B = Buccal, P = Palatal, M = Mesial, D = Distal
Table 4. Median and 25th, 75th percentile (μm) of marginal and internal gap width of premolar and molar crown substructure compared between e.max® Press and Lava™ Zirconia

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>Premolar crown substructure</th>
<th>Molar crown substructure</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>e.max® (n=10)</td>
<td>Lava™ (n=10)</td>
<td></td>
</tr>
<tr>
<td>AMD</td>
<td>B</td>
<td>78 (64,106)</td>
<td>54 (33,62)</td>
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<td></td>
<td>P</td>
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</tr>
<tr>
<td></td>
<td>M</td>
<td>91 (65,108)</td>
<td>45 (37,62)</td>
<td>0.004*</td>
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<td>D</td>
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<td>MG</td>
<td>B</td>
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<td>38 (27,53)</td>
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<tr>
<td></td>
<td>P</td>
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<td>50 (39,61)</td>
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<td>37 (28,45)</td>
<td>0.496</td>
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<tr>
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<td>D</td>
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<td>35 (26,54)</td>
<td>0.005*</td>
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<tr>
<td>CA</td>
<td>B</td>
<td>156 (103,193)</td>
<td>76 (67,89)</td>
<td>0.001*</td>
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<td>99 (83,109)</td>
<td>0.001*</td>
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<td></td>
<td>D</td>
<td>143 (124,196)</td>
<td>71 (62,76)</td>
<td>0.001*</td>
</tr>
<tr>
<td>AX</td>
<td>B</td>
<td>68 (51,103)</td>
<td>69 (51,88)</td>
<td>0.999</td>
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<td>M</td>
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<td>D</td>
<td>89 (67,98)</td>
<td>49 (30,58)</td>
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</tr>
<tr>
<td>CT</td>
<td>B</td>
<td>87 (72,113)</td>
<td>160 (157,181)</td>
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<td></td>
<td>P</td>
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<td>M</td>
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<td>D</td>
<td>65 (44,106)</td>
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<td>OA</td>
<td>B-P</td>
<td>142 (75,267)</td>
<td>197 (188,221)</td>
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<tr>
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<td>M-D</td>
<td>148 (63,242)</td>
<td>203 (176,212)</td>
<td>0.545</td>
</tr>
</tbody>
</table>

* Indicates significant difference by Mann Whitney U-test at significant level of 0.05.
AMD = Absolute marginal discrepancy, MG = Marginal gap, CA = Chamfer area, AX = Axial wall, CT = Cusp Tip, OA = Occlusal adaptation, B = Buccal, P = Palatal, M = Mesial, D = Distal

Precision of prosthesis adaptation. [19] During the post-machining sintering, the distortion of the framework and shrinkage of the pontic might produce bending stress on the substructure and influence its adaptability. [12]

In this study, IPS e.max® Press had larger gaps than those of Lava™ Zirconia except for the cusp tip and occlusal adaptation. The CAD/CAM fabricated Lava zirconia, which uses the optical scanner with striated projection, might cause the overshoots [20] and rounded edges [19,21] phenomena. These phenomena create the internal gap discrepancies. The overshoots phenomenon is a physical phenomenon, which produces the virtual peak adjacent the edges over the true contour of the die geometry. In reality, there is no elevation and therefore this may increase the internal gap, [20] and the rounded edges phenomenon creates the round angle at the true sharp angle of the object, which results in
shrinkage, the zirconia blanks need to be adjusted in the terms of anisotropic shrinkage for post-machining sintering, including the composition and homogeneity of the pre-sintered zirconia block for more accuracy in milling procedures. [12, 22]

This study demonstrated that both all-ceramic systems revealed the larger marginal gap width in three-unit FDPs than those of single crowns. This finding is similar to other studies. [13,22] Nevertheless, there are many factors that affect the marginal and internal discrepancy of all-ceramic materials such as types of finish line preparation, luting cement, manufacturing process, the porcelain veneering, and effect of zirconia ageing. [4] All of these factors should be considered in clinical application for better marginal and internal adaptation and long-term success of all-ceramic dental prostheses.

<table>
<thead>
<tr>
<th>Location</th>
<th>Side</th>
<th>Premolar bridge substructure e.max® (n=10)</th>
<th>Lava™ (n=10)</th>
<th>p-value</th>
<th>Molar bridge substructure e.max® (n=10)</th>
<th>Lava™ (n=10)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD</td>
<td>B</td>
<td>76 (60,128)</td>
<td>78 (66,111)</td>
<td>0.881</td>
<td>86 (71,105)</td>
<td>74 (41,88)</td>
<td>0.131</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>93 (65,119)</td>
<td>81 (59,97)</td>
<td>0.364</td>
<td>106 (55,113)</td>
<td>112 (92,151)</td>
<td>0.034*</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>116 (84,125)</td>
<td>79 (53,87)</td>
<td>0.007*</td>
<td>65 (52,83)</td>
<td>82 (76,97)</td>
<td>0.013*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>82 (59,105)</td>
<td>79 (55,109)</td>
<td>0.999</td>
<td>109 (93,133)</td>
<td>107 (89,118)</td>
<td>0.651</td>
</tr>
<tr>
<td>MG</td>
<td>B</td>
<td>67 (50,93)</td>
<td>70 (42,81)</td>
<td>0.941</td>
<td>67 (44,80)</td>
<td>54 (35,78)</td>
<td>0.545</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>74 (37,91)</td>
<td>53 (48,79)</td>
<td>0.597</td>
<td>66 (37,92)</td>
<td>101 (79,130)</td>
<td>0.013*</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>106 (68,127)</td>
<td>51 (37,67)</td>
<td>0.001*</td>
<td>42 (36,46)</td>
<td>75 (51,83)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>75 (28,96)</td>
<td>48 (38,83)</td>
<td>0.651</td>
<td>91 (69,137)</td>
<td>90 (67,109)</td>
<td>0.881</td>
</tr>
<tr>
<td>CA</td>
<td>B</td>
<td>200 (156,238)</td>
<td>79 (51,97)</td>
<td>0.001*</td>
<td>199 (147,208)</td>
<td>108 (94,130)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>152 (127,187)</td>
<td>90 (74,131)</td>
<td>0.003*</td>
<td>138 (118,160)</td>
<td>134 (92,154)</td>
<td>0.364</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>250 (201,205)</td>
<td>86 (44,118)</td>
<td>0.001*</td>
<td>175 (143,195)</td>
<td>106 (94,110)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>158 (108,212)</td>
<td>90 (56,119)</td>
<td>0.007*</td>
<td>181 (127,205)</td>
<td>131 (97,147)</td>
<td>0.071</td>
</tr>
<tr>
<td>AX</td>
<td>B</td>
<td>85 (65,95)</td>
<td>68 (61,92)</td>
<td>0.545</td>
<td>99 (86,107)</td>
<td>64 (56,86)</td>
<td>0.006*</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>108 (81,135)</td>
<td>63 (31,75)</td>
<td>0.001*</td>
<td>58 (47,75)</td>
<td>59 (35,69)</td>
<td>0.496</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>173 (157,195)</td>
<td>53 (36,65)</td>
<td>0.001*</td>
<td>96 (58,123)</td>
<td>45 (27,53)</td>
<td>0.002*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>92 (72,127)</td>
<td>47 (30,59)</td>
<td>0.001*</td>
<td>147 (118,194)</td>
<td>37 (30,47)</td>
<td>0.001*</td>
</tr>
<tr>
<td>CT</td>
<td>B</td>
<td>80 (59,141)</td>
<td>172 (125,186)</td>
<td>0.016*</td>
<td>119 (58,145)</td>
<td>211 (185,238)</td>
<td>0.004*</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>92 (76,153)</td>
<td>170 (126,193)</td>
<td>0.071</td>
<td>127 (46,195)</td>
<td>239 (218,250)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>163 (119,182)</td>
<td>103 (81,135)</td>
<td>0.010*</td>
<td>88 (55,146)</td>
<td>232 (178,255)</td>
<td>0.001*</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>101 (45,165)</td>
<td>91 (62,117)</td>
<td>0.597</td>
<td>149 (84,207)</td>
<td>154 (75,206)</td>
<td>0.821</td>
</tr>
<tr>
<td>OA</td>
<td>B-P</td>
<td>119 (87,170)</td>
<td>192 (163,223)</td>
<td>0.034*</td>
<td>168 (120,215)</td>
<td>247 (209,283)</td>
<td>0.019*</td>
</tr>
<tr>
<td></td>
<td>M-D</td>
<td>96 (67,178)</td>
<td>189 (168,211)</td>
<td>0.034*</td>
<td>143 (79,259)</td>
<td>267 (219,286)</td>
<td>0.049*</td>
</tr>
</tbody>
</table>

* indicates significant difference by Mann Whitney U-test at significant level of 0.05.

AMD = Absolute marginal discrepancy, MG = Marginal gap, CA = Chamfer area, AX = Axial wall, CT = Cusp Tip, OA = Occlusal adaptation, B = Buccal, P = Palatal, M = Mesial, D = Distal
groups were in the range of clinical acceptability suggested by McLean and von Fraunhofer. [23] However, median internal gaps in this study ranged from 35 – 267 μm; the lowest gap width was found in the axial wall and the greatest gap width was found in the occlusal adaptation of Lava™ Zirconia, which were greater than the recommended internal space, between 50 – 100 μm. [24]

There are several methods that investigate the gap width of restorations. Micro-CT is a non-destructive and reproducible method that evaluates the marginal and internal gaps of restoration, but it is impossible to demonstrate an accurate analysis when deficient radiographic contrast exists. [25] The most commonly used techniques were direct-view, followed by cross-sectioning and replica technique (47.5%, 23.5%, and 20.2%, respectively). [25] Direct-view technique is less time-consuming because it can proceed without multiple or complex procedures. Moreover, it is low-cost and reproducible method. On the other hand, its disadvantage is that it can only be measured at the margin, not the internal surface. [25,26] Cross-section of the embedded specimen and silicone replica methods are the techniques for marginal and internal gap investigations. The embedded technique is precise as measurement points are repeatable and accurate, but restoration must be sacrificed for measurement, therefore, it is not possible to evaluate at different stages of all-ceramic manufacturing or if further investigation on the same specimen is necessary. [27,28] On the contrary, the replica technique used in this study is non-destructive, easy to carry out, less time-consuming, and inexpensive. Furthermore, the silicone layer, which simulates the gap width, can be sectioned and measured at many locations. For these advantages, many researchers used impression replica method, which is reliable and acceptable when compared with the embedded method. [29]

There was no significant difference between the silicone material and zinc phosphate cement to verify post-cementation space. [30] The thickness of low-viscosity light bodies silicone of replica technique did not demonstrate any significant difference from the Fuji I glass-ionomer cement thickness of embedded method. [29] The mean gap values of impression replica method and cross-sectioning technique were similar. Moreover, light bodies silicone is reliable and accurate for imitation of the existing space. [29]

Conclusion

The gap comparison between two all-ceramic systems with different span length of substructures was evaluated and can be summarized as follows:

1. Substructures from both all-ceramic systems demonstrated in vitro acceptable marginal discrepancy. However, there should be further study on the effect of veneering process on the fit of ceramic restorations.

2. Given the limitation of this study, most marginal and internal gaps of IPS e.max® Press were greater than those of Lava™ Zirconia. However, Lava™ Zirconia showed larger gap value in occlusal area both of premolar and molar bridge. The proper tooth preparation and case selection with available interocclusal distance should be concerned.

3. Three-unit fixed dental prostheses exhibited the larger gap value than those single crowns in both ceramic systems in this in vitro study, therefore, careful attention is required for more extensive or multiple-unit ceramic restorations.

References

1. Vencikova Z, Benada O, Bartova J, Joska L, Mrklas L. Metallic pigmentation of human teeth and gingiva:


In vitro antibacterial activity of oligomer-based and calcium silicate-based root canal sealers

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2 Department of Operative Dentistry and Endodontics, Faculty of Dentistry, Mahidol University
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Objective: To evaluate antibacterial effects of root canal sealers, oligomer-based (OB) and calcium silicate (BC) in comparison to epoxy-resin (AH Plus) and zinc oxide-eugenol (ZOE), against Enterococcus faecalis.

Materials and Methods: Antibacterial effects of the root canal sealers were evaluated by the modified direct contact method using 96-well plates. Each sealer was filled on the bottom of wells at 1-mm thickness, 20 wells of each sealer. The wells of each group was further divided into five subgroups depending on testing periods after sealer setting, i.e.- 20 min, 24 h, 3, 7, and 14 days. Next, 200-μl aliquot of E. faecalis (5 x 10^5 CFU) was placed in the well containing the set sealer and kept at 37°C for 24 h. The wells containing the bacterial suspension (without any sealer) and sterile culture media were used as positive and negative controls. Survival of bacteria were determined by 10-fold serial dilution in Brain-Heart Infusion (BHI) broth and cultured on BHI agar. In addition, elution test was carried out by incubating the bacterial suspension to culture media that exposed to the set sealers for 20 min, 24 h, and 3 days. Statistical analysis was conducted using Kruskal-Wallis non-parametric test (α=.05).

Results: From the direct contact test, at 20 min and 24h, BC sealer and AH Plus showed strong bactericidal effects while OB sealer did not display any antibacterial effect. At 3 days, antimicrobial effect of BC sealer was significantly reduced while AH Plus did not show the antibacterial effect. At 7 and 14 days, all sealers did not possess any antibacterial activity, except ZOE sealer that had exhibited the potent bactericidal effect until 14 days. For the elution test, eluted substances from the test sealers at all setting periods did not cause any significant reduction of the bacteria.

Conclusion: The root canal sealers showed different antimicrobial activity against E. faecalis after setting. OB sealer showed no antibacterial effects at all periods. ZOE sealer was the most effective sealer with antibacterial activity until 14 days. Antimicrobial effects of AH Plus and BC sealers gradually decreased within 24 h and 3 days after setting, respectively.

Key words: antibacterial, calcium silicate, direct contact, Enterococcus faecalis, oligomer, root canal sealer


Introductions

Root canal obturation with the obturation cone(s) and sealer plays an important role to create apical sealing and entomb remaining bacteria within the root canal. It might be beneficial if survival microorganisms that still remain after the chemo-mechanical disinfection can be further eliminated by antimicrobial effect of root canal sealer [1-3]. This antibacterial effect of sealer could also delay bacterial penetration from coronal leakage overtime if occurs. Sealing ability and antibacterial activity of various root canal sealers have been focused in the literatures [4,5].

Several types of root canal sealers are currently available and proposed to have antibacterial activity [6-11]. Zinc-oxide eugenol sealer exhibits the strong antibacterial
effect due to the eugenol component [1,12]. Other endodontic sealers that consisting of polymer materials contain the antimicrobial effect due to cytotoxicity of the component(s). For example, antimicrobial activity of epoxy resin-based sealer (AH plus) is related to the release of bisphenol-A diglycidyl [6] or formaldehyde [13,14]. For calcium hydroxide-based and calcium silicate-based sealers, the bactericidal action is provided from the alkalinity due to the release of hydroxyl ions during setting [7,15].

Bioceramic (BC) sealer Totalfill® BC Sealer™ (FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) is also known as iRoot SP™ (Innovative BioCeramix, Inc., Vancouver, Canada) or EndoSequence BC sealer™ (Brasseler, Savannah, GA, USA). BC sealer is a biocompatible, calcium silicate-based material that might provide antibacterial activity from slow releasing of calcium hydroxide during and after setting. Setting process of this sealer produces high alkaline pH that is toxic to bacteria [1]. The pH and Ca²⁺ release of BC sealer are significantly higher during setting duration than those of AH Plus [16]. In vitro studies showed that high alkaline pH of BC sealer promotes elimination of E. faecalis [17,18]. In contrast, its antibacterial effect greatly decreased after one week even high pH value still remained [1].

Recently, a new oligomer-based root canal sealer (OB sealer) has been developed to use with a new thermoplastic elastomer-based (TPE) root canal obturation cone. Polyolefin-based TPE showed some promising properties to produce a root canal obturation cone such as chemically inert, highly flexible, light weight and non-toxic in nature [19]. For use with TPE cone, OB sealer is based on synthetic, bi-functional oligomer, mainly consisting of acrylate or methacrylate functional group with back-bone oligomer molecules. The bi-functional group might provide adhesion to TPE cone by cross-linking polymerization between the partial polymerized functional groups of the sealer to the TPE component. From the previous study, the methacrylate-based OB sealer exhibits high biocompatibility to the L929 cultured cells [20]. However, other information of this newly developed sealer, i.e. antimicrobial activity, is limited.

Most freshly mixed sealers exhibit highest antimicrobial activity and cytotoxicity that gradually decrease during and after setting [21,22]. Prolong antibacterial effect of the sealer is desirable to prevent reinfection or recolonization of remaining bacteria in the treated root canals. Results from laboratory studies have been limited to antibacterial activity of the initial-set sealers [7,9,11]. It might be profitable if the persisted bacteria can be further eradicated by prolong antimicrobial activity of root canal sealers. Therefore, long-term evaluation of antimicrobial activity of sealers has been suggested [23,24].

Several methods have been used to evaluate antimicrobial effect of endodontic sealers, such as agar diffusion test (ADT), direct contact test (DCT) or elution test [25-27]. Antimicrobial results from the ADT method does not only depend on antibacterial effect of material, but also relate to diffusion and solubility of material into medium [28,29]. For the DCT, it can be used to evaluate antimicrobial effect of either water soluble or insoluble substances, which overcomes the disadvantage of the ADT [28-30]. Result from DCT is based on observing the growth of bacteria after contact with the testing materials. For root canal sealer, dissolution of the components released from incomplete set materials at the early stage after mixing is possible. The antimicrobial action from these components might inhibited the growth of those bacteria, which survived from the direct contact. Antimicrobial effect from eluted-components of sealers can be evaluated via an elution test.

Among bacteria found in the infected root canals, E. faecalis is the most common species detected from the failed endodontic treated teeth [31]. It participates in the persistent root canal infection and is difficult to eliminate from the infected root canal [32]. Moreover, in vitro studies have shown its ability to invade deep into dentinal tubules [33-36]. Thus, E. faecalis is frequently used as bacteria of choice for antimicrobial testing in endodontic research.

The purpose of this study was to evaluate antibacterial effects of four root canal sealers, i.e. newly developed oligomer-based (OB sealer), bioceramic (BC
sealer), epoxy resin-based (AH Plus), and zinc oxide eugenol (ZOE sealer), against *E. faecalis* using modified direct contact method and elution test.

## Materials and Methods

Four root canal sealers, i.e. - oligomer based sealer (OB sealer), bioceramic sealer (BC sealer), epoxy resin sealer (AH Plus), and zinc oxide eugenol (ZOE) sealers, were tested in this study. Manufacturers, preparations, compositions, setting times and methods of use are summarized in Table 1.

### Direct contact test

Modified direct contact test was conducted to evaluate antimicrobial activity of the endodontic sealers using *Enterococcus faecalis* (ATCC 29212) as bacterial indicator. All sealers were mixed and prepared following the manufacturers’ instructions. Using two 96-well microtiter plates (Sarstedt Inc., Newton, NC, USA), the wells were divided into 4 experimental groups of the

### Table 1: Details of four root canal sealers in this study.

<table>
<thead>
<tr>
<th>Details</th>
<th>BC sealer (TotalFill)</th>
<th>OB</th>
<th>AH Plus</th>
<th>ZOE sealer (MU sealer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manufacturers</td>
<td>FKG Dentaire SA, La Chaux-de-Fonds, Switzerland</td>
<td>M Dent, Bangkok, Thailand</td>
<td>Dentsply-Maillefer, Tulsa, OK, USA</td>
<td>M Dent, Bangkok, Thailand</td>
</tr>
<tr>
<td>Preparations</td>
<td>Pre-mixed syringe</td>
<td>Two pastes</td>
<td>Two pastes</td>
<td>Powder and liquid</td>
</tr>
<tr>
<td>Compositions</td>
<td>Tricalcium silicate, dicalcium silicate, calcium hydroxide, zirconium oxide, phosphate monobasic, filler and thickening agents</td>
<td>Bi-functional oligomer (di-acrylate), light curing initiator, self-cure initiator (peroxide), catalyst, radiopaque (barium sulfate), fillers and oil</td>
<td>Paste A: bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungstate, zirconium oxide, silica, iron oxide pigments</td>
<td>Powder: zinc oxide, resin, bismuth subcarbonate, barium sulfate, sodium borate Liquid: clove oil</td>
</tr>
<tr>
<td>Setting types</td>
<td>Self-curing</td>
<td>Dual-curing</td>
<td>Self-curing</td>
<td>Self-curing</td>
</tr>
<tr>
<td>Setting times</td>
<td>4 h, (can be more than 10 h in very dry root canal)</td>
<td>Immediately after light curing, approximately 1-2 h self curing in anaerobic condition</td>
<td>8 h at 37°C</td>
<td>90 min</td>
</tr>
</tbody>
</table>
| Mixing methods   | No mixing is required | Mixing powder-liquid or equal volume of two pastes, on a glass slab or a mixing pad using a metal spatula, to a homogeneous consistency.
sealers, 20 wells of each, and 2 control groups. Each sealer group was further divided into five subgroups depending on the testing periods after setting at 20 min, 24 h, 3 days, 7 days, and 14 days (Fig. 1). Then, each well was filled with equal amount of freshly mixed OB sealer, BC sealer, AH Plus or ZOE sealer using a cavity liner applicator to obtain approximately 1-mm thickness of sealer. The suspension of 24-h *E. faecalis* in Brain Heart Infusion (BHI) broth was prepared at cell density of $5 \times 10^5$ CFU/ml. An aliquot of 200-μl bacterial suspension was placed on the surface of each sealer at different times after setting, i.e.- 20 min, 24 h, 3, 7, and 14 days. The positive controls were the wells with bacterial suspension but without sealer, and the negative controls were the wells with sterile culture media. The plates were incubated at 37 °C for 24 h. After exposure to the sealers for 24 h, the bacterial suspension from each well was gently mixed with a pipette tip, and 100 μl of suspension was transferred to another 96-well microtiter plates. The suspension was 10-fold serially diluted and cultured on BHI agar plates (*Difco Laboratories, Detroit, MI, USA*). After incubation at 37°C for 24 h, bacterial colonies were observed and calculated into CFU/ml. The experiment was repeated twice to confirm the reliability of results (a total n = 8 of each testing period after setting/ sealer).

**Elution test**

To investigate whether release substances during setting of the sealers had any antibacterial effect, the elution test was performed. In brief, the 96-well microtiter plate were divided into the 4 experimental sealer groups, as previously mentioned. Each sealer was filled at the bottom of the well as previously described and then further divided into three subgroups of setting periods, i.e.- after 20 min, 24 h, and 3 days (n = 2). At each setting period, 200 μl of BHI broth (*Difco Laboratories, Detroit, MI, USA*) was added into each well containing the set sealer and then incubated at 37°C for 60 min. Next, 100 μl of the BHI broth from each well was transferred into another 96-well microtiter plate. The 100 μl of *E. faecalis* suspension ($10^5$ CFU) was added, gently mixed, and incubated at 37°C. The wells filled with the bacterial suspension and the culture media (without elute of sealer) were used as positive control. After 24 h, the suspension from each well was subjected to bacterial count as previously described. The experiment was repeated twice to confirm the reliability of results (a total n =4 of each testing period after setting/ sealer).

**Statistical analysis**

Number of bacterial colonies in CFU/ml was calculated and expressed as $\log_{10}$ CFU/ml. Mean and standard deviation (SD) were calculated. Normal

---

**Figure 1.** Illustrations present the experimental design in the modified direct contact test using 96-well microtiter plates.

Four experimental sealer groups (n = 20 each) divided into 5 subgroups of different testing periods (n = 4 each), and the positive (+ve) and negative (-ve) control groups were included; AH = AH Plus sealer, BC = BC sealer, OB = OB sealer, ZOE = ZOE sealer.
In vitro antibacterial activity of oligomer-based and calcium silicate-based root canal sealers

distribution of data was tested using Shapiro-Wilk test. Since the data were not normally distributed, non-parametric Kruskal-Wallis test was used to compare antibacterial effects among the tested sealers and the periods after setting at a significance level of .05.

**Results**

For antimicrobial activity by the modified direct contact test, the amounts of *E. faecalis* (CFU/ml) after 24-h exposure to the sealers are present in Table 2. Statistical analysis of antimicrobial effectiveness among the sealers at different periods is shown in Table 3. ZOE sealer was considered as a gold standard of antimicrobial activity since it completely eradicated the bacteria through the periods. In contrast, OB sealer did not cause any significant bacteria reduction when compared to the control at all periods (p > .05). Freshly mixed (at 20 min) and early set (at 24 h) AH Plus and BC sealers completely eradicated the test bacteria, which was significantly different from that of the control (p < .05). At 3 days, AH Plus entirely lost the antibacterial activity while BC sealer presented markedly decreased antibacterial activity (Table 3). At 7 and 14 days, the two sealers did not show any bacterial reduction that was not significantly different to that of the control (p > .05).

Comparing the results among the setting periods, OB sealer showed insignificant antimicrobial effect at all setting time. AH Plus had a strong antibacterial effects at 20 min and 24 h after setting that were significantly higher than those at 3, 7, and 14 days (p < .05). BC sealer had potent antibacterial effects after setting up to 3 days that were significantly higher than those at 7 and 14 days (p < .05).

Results of antibacterial activity of the sealers from the elution test are presented in Table 4 and 5. No antimicrobial effect of the eluted media 60-min exposed to the set sealer was observed. Eluted-medium from all root canal sealers did not cause significantly reduction of CFU-counts in comparison with the positive control. No statistically significant antibacterial effect was observed among tested sealers at all setting periods (P > .05)

**Discussion**

None of the sealers was able to sustain its antimicrobial activity up to one week, except ZOE sealer that the effect lasted through the experimental period of 14 days. It may imply that these endodontic sealers could eliminate residual microorganisms that have survived in root canal. However, their effects were sustained for only a limited short period. Thus, these sealers might not prevent microbial re-contamination from coronal leakage if occurs. Initial antibacterial activity of epoxy resin-based sealer might relate to the release of bisphenol-A diglycidyl component, and minor release of formaldehyde [6,13,14]. Using DCT or modified-DCT, freshly-mixed AH Plus had potent antimicrobial effect. Freshly mixed sealers are highly toxic to cells, which is caused by highly initial release of cytotoxic components at early stage of setting [21,22]. However, several studies have shown that its antibacterial effect decreased over time to ineffective level [1,27,37]. In the present study, AH Plus had significant antimicrobial effect until 24 h after setting. Its antibacterial effect was abolished within few days, which is consistent with those of the previous studies [1, 27, 37].

It is believed that bioceramic sealer provides antibacterial effect from the release of calcium hydroxide by-product, causing a very high alkaline pH that is toxic to the bacteria [1,38]. The alkaline pH from BC sealer promotes elimination of bacteria such as *E. faecalis, in vitro* [17,18]. The present experiment showed that BC sealer exhibited immediate, potent antibacterial effect up to 24 h after setting. However, its antibacterial effect considerably decreased within 3 days and was completely diminished at 7 days. This is in agreement to the result of in vitro study that reported a short antibacterial action of BC sealer against *E. faecalis*, using a modified direct contact test [1]. Increase of pH level from the release of calcium hydroxide at early stage of setting creates an unsuitable environment for bacterial growth [39]. Later, calcium hydroxide release might be reduced, so the alkaline pH level decreased. This may explain the decreased antibacterial effect over time and the disappearance after long-term setting.
Table 2: Antimicrobial activity of the root canal sealers against *E. faecalis* at different periods after setting (means ± standard deviation; total n=8).

<table>
<thead>
<tr>
<th>Setting periods</th>
<th>Types of sealer</th>
<th>Number of the bacteria (<em>× 10^9 CFU/ml</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± standard deviation</td>
<td>Mean log&lt;sub&gt;10&lt;/sub&gt; ± range log&lt;sub&gt;10&lt;/sub&gt;</td>
</tr>
<tr>
<td>Freshly mixed sealers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 min</td>
<td>+ ve control</td>
<td>3.26 ± 1.64 1.72–6.80 9.47 ± 0.19 9.24–9.83</td>
</tr>
<tr>
<td></td>
<td>OB</td>
<td>1.16 ± 0.38 0.40–1.64 9.03 ± 0.19 8.60–9.21</td>
</tr>
<tr>
<td></td>
<td>AH Plus</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>ZOE</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Set sealers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>+ ve control</td>
<td>1.32 ± 0.62 0.60–2.24 9.08 ± 0.21 8.78–9.35</td>
</tr>
<tr>
<td></td>
<td>OB</td>
<td>0.91 ± 0.78 0.24–2.40 8.82 ± 0.37 8.38–9.38</td>
</tr>
<tr>
<td></td>
<td>AH Plus</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td></td>
<td>ZOE</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>3 days</td>
<td>+ ve control</td>
<td>1.52 ± 0.44 0.92–2.32 9.17 ± 0.13 8.96–9.37</td>
</tr>
<tr>
<td></td>
<td>OB</td>
<td>0.88 ± 0.44 0.28–1.60 8.89 ± 0.24 8.45–9.20</td>
</tr>
<tr>
<td></td>
<td>AH Plus</td>
<td>1.52 ± 0.56 0.40–2.00 9.14 ± 0.24 8.60–9.30</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>0.27 ± 0.34 0.001–1.00 7.86 ± 0.98 6.16–9.00</td>
</tr>
<tr>
<td></td>
<td>ZOE</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>7 days</td>
<td>+ ve control</td>
<td>1.37 ± 0.30 0.92–1.68 9.12 ± 0.10 8.96–9.23</td>
</tr>
<tr>
<td></td>
<td>OB</td>
<td>1.42 ± 0.45 0.88–2.24 9.13 ± 0.13 8.94–9.35</td>
</tr>
<tr>
<td></td>
<td>AH Plus</td>
<td>0.91 ± 0.24 0.52–1.24 8.94 ± 0.13 8.72–9.09</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>1.00 ± 0.37 0.40–1.60 8.97 ± 0.18 8.60–9.20</td>
</tr>
<tr>
<td></td>
<td>ZOE</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>14 days</td>
<td>+ ve control</td>
<td>1.23 ± 0.20 0.88–1.48 9.08 ± 0.08 8.94–9.17</td>
</tr>
<tr>
<td></td>
<td>OB</td>
<td>0.98 ± 0.34 0.64–1.48 8.97 ± 0.15 8.80–9.17</td>
</tr>
<tr>
<td></td>
<td>AH Plus</td>
<td>0.86 ± 0.26 0.32–1.16 8.90 ± 0.18 8.50–9.06</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>0.79 ± 0.22 0.40–1.16 8.88 ± 0.13 8.60–9.06</td>
</tr>
<tr>
<td></td>
<td>ZOE</td>
<td>0 0 0 0</td>
</tr>
</tbody>
</table>

+ve control = positive control, OB = OB sealer, AH Plus = AH Plus sealer, BC = BC sealer, ZOE = ZOE sealer.
In vitro antibacterial activity of oligomer-based and calcium silicate-based root canal sealers

Table 3: Statistical comparison of antimicrobial effectiveness against *E. faecalis* of the root canal sealers at different periods after setting (median value of log$_{10}$ CFU/ml; total n=8).

<table>
<thead>
<tr>
<th>Type of sealers</th>
<th>Freshly mixed sealers</th>
<th>Testing periods after sealer setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>24 h</td>
</tr>
<tr>
<td>+ ve control</td>
<td>9.44 $^{a,A}$</td>
<td>9.07 $^{a,B}$</td>
</tr>
<tr>
<td>OB</td>
<td>9.09 $^{a,C}$</td>
<td>8.75 $^{b,C}$</td>
</tr>
<tr>
<td>AH Plus</td>
<td>1.27 $^{b,D}$</td>
<td>0.84 – 1.60</td>
</tr>
<tr>
<td>BC</td>
<td>1.51 $^{b,F}$</td>
<td>0.20 – 1.36</td>
</tr>
<tr>
<td>ZOE</td>
<td>0.65 ± 0.53</td>
<td>1.31 ± 0.40</td>
</tr>
</tbody>
</table>

不同的小写字母（在列中）表示在每个测试期的不同封隔器类型的统计学显著差异（p < 0.05）。
不同的大写字母（在行中）表示在每个封隔器的每个测试期的不同统计学显著差异（p < 0.05）。

+ve control = positive control, OB = OB sealer, AH Plus = AH Plus sealer, BC = BC sealer.

Table 4: Antimicrobial activity of eluted-media from the root canal sealers against *E. faecalis* at different periods after setting (mean ± standard deviation; total n=4).

<table>
<thead>
<tr>
<th>Setting periods</th>
<th>Types of sealer</th>
<th>Number of the bacteria ($\times 10^9$ CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td></td>
<td>Mean log$_{10}$</td>
<td>Range log$_{10}$</td>
</tr>
<tr>
<td>Freshly mixed sealers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ ve control</td>
<td>1.58 ± 0.50</td>
<td>0.84 – 1.92</td>
</tr>
<tr>
<td>OB</td>
<td>1.53 ± 0.12</td>
<td>1.36 – 1.64</td>
</tr>
<tr>
<td>AH Plus</td>
<td>1.89 ± 0.34</td>
<td>1.52 – 2.32</td>
</tr>
<tr>
<td>BC</td>
<td>1.64 ± 0.26</td>
<td>1.36 – 1.88</td>
</tr>
<tr>
<td>ZOE</td>
<td>0.50 ± 0.26</td>
<td>0.28 – 0.88</td>
</tr>
<tr>
<td>Set sealers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ ve control</td>
<td>1.61 ± 0.12</td>
<td>1.44 – 1.72</td>
</tr>
<tr>
<td>OB</td>
<td>1.06 ± 0.37</td>
<td>0.80 – 1.60</td>
</tr>
<tr>
<td>AH Plus</td>
<td>1.71 ± 0.59</td>
<td>0.92 – 2.24</td>
</tr>
<tr>
<td>BC</td>
<td>1.60 ± 0.40</td>
<td>1.04 – 1.92</td>
</tr>
<tr>
<td>ZOE</td>
<td>0.40 ± 0.29</td>
<td>0.24 – 0.84</td>
</tr>
</tbody>
</table>

+ve control = positive control, OB = OB sealer, AH Plus = AH Plus sealer, BC = BC sealer, ZOE = ZOE sealer.
Effectiveness of OB sealer against the bacteria was considerably lower than those of the other sealers even at the initial period. This can be explained by the biocompatible characteristic of its structures and components. OB sealer mainly consists of acrylate/methacrylate functional group and back-bone oligomer molecule (Fig. 2). With the stable molecular structure of oligomer, instead of the monomer, it may be possible that the release of cytotoxic components is greatly reduced. The results from an in vitro cytotoxicity test showed the high biocompatibility of OB sealer to the cultured cells [20]. It seems that the synthetic, di-functional oligomer structure makes the sealer become a highly biocompatible material. Nevertheless, OB sealer was light cured in this study, so a further study is required to investigate antibacterial effect in the self-curing mode. Releases of free oligomer and other components may be higher in the self-cured condition, which might affect the biocompatibility and antibacterial activity.

Antibacterial activity of ZOE-based sealer is based on the release of eugenol component, which possesses potent antibacterial effect against microorganisms [40-42]. The result of this study demonstrated that ZOE sealer had the strongest antibacterial effect against E. faecalis and exhibited prolonged antibacterial activity until two weeks. It is indicated that the amount of eugenol release is sustainable and high enough to inhibit bacterial growth. In accordance with the previous findings, this sealer had the greatest antimicrobial effect against microorganisms [5,26,40,43,44].

From the direct contact test, it reported the decrease of antimicrobial activity after setting of the sealers. Reduction of bacteria at the early stage might be related to direct contact between the surface of initial-set sealer and bacterial suspension. In addition, dissolution and releasing of effective components from the initial set materials could improve its antibacterial activity. Nevertheless, the results from the elution assay showed elutes of the sealers had insignificant effect on reduction of bacteria. Thus, it can be concluded that the antibacterial activity of these sealers was primarily due to the direct-contact effect between the sealer and bacteria, rather than elute of sealer.

**Table 5**: Statistical comparison of antimicrobial effectiveness against *E. faecalis* of eluted-media from the root canal sealers at different periods after setting (median value of log$_{10}$ CFU/ml; total n=4).

<table>
<thead>
<tr>
<th>Types of sealer</th>
<th>Freshly mixed sealers</th>
<th>Testing periods after sealer setting</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>24 h</td>
</tr>
<tr>
<td>+ ve control</td>
<td>9.07</td>
<td>9.25</td>
</tr>
<tr>
<td>OB</td>
<td>8.90</td>
<td>9.19</td>
</tr>
<tr>
<td>AH</td>
<td>9.12</td>
<td>9.27</td>
</tr>
<tr>
<td>BC</td>
<td>9.18</td>
<td>9.22</td>
</tr>
</tbody>
</table>

* No statistically significant antibacterial effect among tested sealers at all setting periods.
+ve control = positive control, OB = OB sealer, AH Plus = AH Plus sealer, BC = BC sealer.

Figure 2. The structure of OB sealer.
In vitro antibacterial activity of oligomer-based and calcium silicate-based root canal sealers

In summary, none of test sealers was able to sustain the antimicrobial action against *E. faecalis* through 14 days after setting, except ZOE sealer. BC sealer and AH Plus provided a short antibacterial effect that were diminished within few days after setting. Light-cured OB sealer was not effective at all setting periods. Further investigation is required to verify antibacterial activity of OB sealer in the self-curing condition.

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**Competing interests:** None declared.

**Ethical approval:** Faculty of Dentistry/Faculty of Pharmacy, Mahidol University Institutional Review Board COE. No. MU-DT/ PY-IRB 2016/010.1706.

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**References**


Plasma cell gingivitis associated with dry flower buds of clove: a case report

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Plasma cell gingivitis (PCG) is an uncommon condition of the gingiva characterized by erythematous, edematous and easily-bleeding gingiva. Its etiology is unknown, but hypersensitivity reaction has been proposed. Here we reported a 35-year-old female who used dry flower buds of clove as herbal oral lozenge and later noticed redness of the lips and gingiva. Oral examination revealed fiery red gingiva with easily bleeding. Upper and lower labial mucosa showed moderate erythema with slightly erosive surface and burning sensation. Histopathological examination revealed a hyperplastic epithelium with spongiosis and exocytosis. The lamina propria showed intense infiltration of mature plasma cells with a few lymphocytes and numerous dilated capillaries. These characteristics were compatible with the diagnosis as PCG. Additionally, we proceeded direct immunofluorescent (DIF) study of the lesion. Besides oral hygiene care and suggestion to refrain from causative agents as well as herbal related products, the use of systemic and topical corticosteroids brought successful treatment outcomes with no need for surgical procedures. There was no sign of recurrence during the period of 2-year follow-up.

Key words: clove, corticosteroid, direct immunofluorescent (DIF) study, plasma cell gingivitis


Introduction

Plasma cell gingivitis (PCG) is a rare inflammatory gingival condition with unknown etiology. Clinical features mainly present as diffuse gingival erythema with edematous swelling, as well as smooth, shiny and velvety texture involving free gingiva and attached gingiva. The lesion shows a sharp demarcation and frequently extends to mucogingival border [1,2]. Sometimes desquamation, erosion and ulceration have also been reported [3,4]. Due to the inflammation, this lesion is easily bleeding. PCG is seldom found as an exophytic mass mimicking pyogenic granuloma [5,6]. Most of the cases are asymptomatic, but some cases presented with burning sensation and pain [1,2,4,7-10].

The other names of PCG such as idiopathic gingivostomatitis, atypical gingivostomatitis, plasmacytosis of gingiva and allergic gingivostomatitis are also mentioned in the literatures [11-12]. Although the precise etiopathogenesis is still unclear, the hypersensitivity reaction to some allergens such as components of chewing gums [11,13] and dentifrices [14-17] or specific leaves [3,4,18] were reported.

The differential diagnosis includes the lesions that possess similar clinical characteristics, including mucous membrane pemphigoid, pemphigus vulgaris, HIV gingivitis, and leukemia.
Histopathological features of PCG consist of a dense infiltration of plasma cells in the subepithelial layer, resulting in a disruption to the basement membrane, and dilated capillaries [19]. Hematological examination is one of the important supplementary investigations to rule out other serious plasma cell lesions, including multiple myeloma or solitary plasmacytoma, since the histopathological changes of PCG mimic those lesions [1].

No standard protocol for the management of PCG is available. In general, the management starts with avoidance of known relevant allergens, along with plaque and oral hygiene control [3,4,13-15,20]. Some of cases ended up with surgical treatment as gingivectomy and gingivoplasty [1,9,10,17,18,21,22]. While the benefits of using topical and systemic corticosteroid are still controversy [12,21,23], a few of other medications such as topical antibiotics [8] and anti-allergic drugs [24] are prescribed with successful results.

This report presents a case of PCG concomitantly occurring with mucositis of the labial mucosa in a female who is firstly described to have a relevant cause of using dry flower buds of clove. In addition, the description of direct immunofluorescent (DIF) finding is also reported.

Case report

A 35-year-old female presented to Oral Medicine Clinic, Dental Hospital, Faculty of Dentistry, Mahidol University with the chief complaints of swollen gums and burning sensation. The problems started since 2-3 months back, after she reported about using dry flower buds of clove (figure 1) as herbal oral lozenge with the believe that it might help to improve the oral malodor. She had put a bud onto the oral mucosa around 3 times per day, everyday for a month. During that time, the patient noticed redness of lips and had spicy feeling on oral mucosa. As a result, she then stopped using that herb. However, the reactions further progressed and her gums had more swelling, more redness and severe burning sensation especially when contacting with hot, spicy or sour foods, as well as the feeling of tension on the lips. Around 2 weeks before presenting to Oral Medicine Clinic, she was prescribed with anti-inflammatory and anti-allergic drugs for 10 days with minimal reduction of the edema. At the same time, the patient also received full mouth scaling. Besides the history of prolong contact to dry flower buds of clove, she regularly used herbal toothpaste containing clove.

For medical history, patient had the last medical check-up around 2 years ago and denied any medical problems, except occasionally experiencing mild burning stomach pain. She also denied drug allergy, but had a history of allergic reactions as itching and rash when wearing the Silver-contained accessories. Extra-oral examination was unremarkable except the lower lip that appeared as a slightly swelling. Intra-oral examination revealed generalized edematous swelling of upper and lower gingiva with fiery red color and glistening surface. The margin of lesion at labial and buccal aspect of gingiva extended up to mucogingival junction (figure 2A) and the inflamed gingiva showed easily bleeding up on gently provocation. Both upper and lower labial mucosa showed moderate erythema with slightly erosive surface (figure 2B and 2C). Panoramic radiograph revealed no bony destruction, except lower right lateral incisor (tooth 42) had a periapical radiolucency (figure 3). Tooth 42 showed discoloration, negative to percussion and no response to electric pulp tester, confirming the diagnosis of pulp necrosis with asymptomatic apical periodontitis, and was referred to an endodontist for the root canal treatment.

At first visit, after history taking and oral examination, the incisional biopsy was done on the labial gingiva around lower left lateral incisor and canine (tooth 32 and 33). Biopsy specimens
were sent for histopathological and DIF investigations. In addition, blood investigation, urinalysis, chest x-ray and stool examination were also performed with insignificant results. The histopathological examination revealed a hyperplastic epithelium with thin elongated rete ridges and suprapapillary thinning. Spongiosis and exocytosis were noticed in the epithelium. The lamina propria showed an extremely intense infiltration of chronic inflammatory cells consisting predominantly of mature plasma cells. Numerous dilated capillaries and a few lymphocytes were also seen (figure 4A and 4B). Taken together, these characteristics were compatible with the diagnosis of PCG. DIF results showed negative (-) to immunoglobulin G (IgG), positive (+) to IgM, IgA and complement 3 (C3) at colloid bodies, + to Fibrinogen (F) at dermal-epidermal junction. Due to burning sensation and redness of the lesion, candida culture swabbed from generalized lesional mucosa was examined to rule out erythematous candidiasis. The result revealed no growth of candida organisms.

The treatment began with systemic corticosteroid as prednisolone 25 mg/day (approximately 0.5 mg/kg). The prescription of omeprazole 20 mg/day was also added to prevent the side effects of corticosteroid since the patient reported experience of mild burning pain stomach if she unable to have a meal on time. A strong advice to refrain from any herbal contained products such as toothpaste or foods and food ingredients was informed. After 6 days, the lesion had dramatically regressed by reducing in redness and swelling of gingiva and labial mucosa. Thereafter, the step for tapering dose of prednisolone was started. Additionally, topical corticosteroid as fluocinolone acetonide 0.1% in oral paste was prescribed to apply at the lesion 3 times/day. Then adjusted dose was performed when the lesion was ameliorated. During the follow-up visits along 2 months, the lesion was

---

Figure 1. Dry flower buds of clove.

Figure 2. (A) Fiery red and edematous swelling gingiva extended to mucogingival junction. (B and C) Moderate erythema with slightly erosive surface on upper and lower labial mucosa.

Figure 3. Panoramic radiograph revealed no bony destruction, except tooth 42 showing periapical radiolucency.
continuously improved. The stimulation of oral hygiene care and periodic periodontal treatment were also constantly proceeded. However, signs of occasionally exacerbations including mild erythema and slightly swelling gingiva with slight burning sensation when the patient tried some foods containing herbs and spices were noticed. Therefore, the management with topical corticosteroid was used to control the lesion. The clinical feature as completely free from the lesion was detected around 8 months after the initial treatment (figure 5A, 5B, 5C), since the patient strictly avoided any kinds of foods or products containing herbs. The periodically recall was scheduled and the oral mucosa was showed the remission of PCG even at the 2 year-visit of follow-up.

Discussion

PCG is a peculiar oral lesion that is mainly believed to relate with the allergic reactions, though the exact cause and mechanism of disease is still unknown [19]. The possible allergens were herbal containing products. The herbs mentioned in the literatures were black pepper, black salt, alum, ajwain [1], cinnamon [7,15], mint [7,17], clove [17], and acasia [22]. Some cases of PCG were associated with a direct contact or chewing the specific leaves such as khat [3,4] and colocacia (arbi) [18]. To the best of our knowledge, this is the first report that dry flower buds of clove is a causative allergen. Clove, an important medical plant, has a scientific name as Syzygium aromaticum or Eugenia cariophylata. The dry flower buds are extracted for essential oil or clove oil in which eugenol is the main compound. Since clove oil has various benefits such as antioxidant, antimicrobial, antinociceptive, and antiviral

![Figure 4A](image1.png) The mucosa shows a hyperplastic epithelium with a heavy plasma cell infiltrate in the lamina propria (hematoxylin-eosin, original magnification x100).

![Figure 4B](image2.png) A high-power view reveals numerous plasma cells and dilated capillaries in the lamina propria (hematoxylin-eosin, original magnification x400).

![Figure 5](image3.png) (A, B, C) Gingiva and labial mucosa appeared normal after 8 months of treatment.
activity, it is widely used in dental products [26,27]. In this case, the herbal toothpaste containing clove was also involved as a causative agent of PCG which similar to the previous report using herbal toothpaste containing mint and clove [17]. Interestingly, even the meticulous history takings were done, many reports could not identify any relevant or causative agents [2,8,9,12,21-24].

The diagnosis of PCG depended on a history of contacting to allergenic causes and heavy plasma cell infiltration in submucosal layer. To rule out other immune mediated disorders that possess similar clinical characters such as pemphigus or pemphigoid DIF investigation was performed. Our DIF results revealed as - IgG, + IgM, IgA and C3 at colloid bodies, + F at dermal-epidermal junction. Only one study reported DIF information of PCG as nonspecific reaction on IgG, IgM, IgA and C3 [23]. Another study showed an immunohistochemical examination revealing of IgG + plasma cells were 90% while of IgA and IgM + cells were less than 5% [8]. These results seem to be different from our study. However, until now there is no standard criteria for the diagnosis of PCG from DIF or immunohistochemical data. Further studies are required to gain more information and knowledge.

The important management of PCG is to absolutely avoid identified allergens or relevant products. Many studies showed the dramatically improvement merely refraining from the causative agents and intensive oral hygiene care [3,4,13-15,20]. Combined treatment using chlorhexidine gluconate mouthrinse was also reported [1,2,17,27]. The effective medication for PCG was scarce. One reported case used topical 2% fusidic acid in a tetracaine-containing adhesive ointment applying 4 times/day [8], while another reported case used topical Fucibet that contained betamethasone and fusidic acid for applying at least 3 times/day [27]. The treatment with modified application of chlorpheniramine maleate 25 mg tablet crusting into powder form over the lesion 3 times/day also led to complete resolution [24]. However, some studies presented that surgical removal of hyperplastic gingival tissue with or without laser technic was necessary after noninvasive treatment was unsuccessful [1,9,10,17,18,21,22].

In this case, the initial dose of prednisolone 25 mg/day (approximately 0.5 mg/kg) could bring dramatically regression within 6 days. After tapering systemic corticosteroid, PCG could be controlled by application of fluocinolone acetonide 0.1% in oral paste. However, the former studies were disagreed with the affects of topical and systemic corticosteroids. One study reported successful treatment of 6 cases from 12 cases by using systemic prednisone with the doses not exceeding 40 mg/day [12]. Another study reported the decrease of symptoms and inflammation, but was unable to reach complete healing by topical 0.05% clobetasol propionate or 0.05% fluocinonide [23]. Although, our case showed complete healing and no recurrence within 2 year-period of follow-up, the time to reach completely normal oral mucosa was long as 8 months. During that time, there were signs of recurrences when patient re-used herbal toothpaste containing clove, or contacting with spices and herb-containing foods. Consequently, the advice for strictly refraining from causative agents and relevant allergens is quite important and should be repeatedly done.

Until present, only a few information regarding the pathogenesis of PCG is available. There is no definite protocol or regimen for treatment PCG. Therefore, our report of PCG in a 35-year-old female added more information of allergic cause, clinical features, DIF findings, and the alternative way for successful treatment without surgical procedure.

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Acknowledgment:

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Assistant Professor Dr. Jira Kitisubkanchana for providing radiographic illustration.

References

26. Chaieb K, Hajlaoui H, Zmantar T, Kahla-Nakbi AB,

The effect of blasting zirconia liner on microtensile bond strength of zirconia to layered and pressed veneers

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Department of Prosthodontics, Faculty of Dentistry, Mahidol University

Objective: To investigate the effect of sandblasting the zirconia liner on the microtensile bond strength of manually-layered and pressed veneers to zirconia

Materials and methods: Bilayered zirconia specimens were prepared from e.max®ZirCAD framework materials and divided into four groups (at least 30 specimens per group). Two veneering techniques were used: I. Layering technique (e.max®Ceram) for group 1 and group 2 and II. Press-on technique (e.max®ZirPress) for group 3 and group 4. ZirLiner was applied onto the zirconia surfaces in group 1 and 3 and fired according to the manufacturer’s instructions. Veneering of these specimens was then performed. The surfaces of group 2 and 4 were treated similarly to group 1 and 3, except that sandblasting with 50-μm aluminum oxide particles was performed on the fired liner material. The bilayered blocks were cut into microbars with 1mm x 1mm in cross-section. All specimens were loaded to fracture using a microtensile tester. The fracture surfaces were analyzed using a scanning electron microscope to identify the mode of failure.

Results: When the liner material was blasted, the mean microtensile bond strength (MTBS) of bilayered specimens in the layering group (14.5±2.7 MPa) and the press-on group (15.5±5.0 MPa) were significantly lower than those of unblasted liner groups (16.5±3.5 MPa for layering and 19.8±6.1 MPa for press-on). Most of the fractures occurring in all groups initiated at the zirconia-veneer interface.

Conclusions: Blasting the zirconia liner material decreased the bond strength between zirconia to layered and pressed veneers. Interfacial failure was predominantly observed in all groups.

Keywords: Layering technique, Microtensile bond strength, Press-on technique, Zirconia liner


Introduction

In the past decades, all-ceramic materials have been widely used in prosthetic dentistry and the improvement of the ceramic materials with different compositions has been reported [1]. All-ceramic fixed dental prostheses in the posterior teeth can be used with the presence of the zirconia framework fabricated by the CAD/CAM technology [2]. Veneering ceramic is used to cover the zirconia framework for esthetic requirement.

Although the manual layering of the veneer ceramic is the conventional technique for dental technician to create the anatomical form of the restoration, this technique may lead to entrapment of air bubbles, voids, microgaps at the core–veneer interface. These structural defects may cause the stress accumulation and lead to delamination or chipping of the veneering ceramics.
The press-on technique is another veneering method. The desired tooth structure is fabricated by wax-up onto the zirconia framework and overpressed with the pressable ceramic. The press-on technique using prefabricated ceramic ingots is performed under controlled condition so it might reduce the possibility of thermal fatigue and lead to less incorporation of structural defects in the veneering ceramic [3]. However, the major complication of zirconia-based restorations is delamination or chipping of the veneering ceramics [4]. It has been reported that the zirconia frameworks that were veneered using conventional technique showed a chipping rate of 13% within 3 years [5] and 15.2% within 5 years [6]. The fracture rate of porcelain fused to metal restorations was reported only 8%–10% within 10 year [7,8]. Both cohesive fracture of veneering ceramics and interfacial delamination of veneering ceramics from the framework material have been reported whereas the fracture of zirconia framework rarely occurred. To overcome these problems, the bond strength between the zirconia framework and veneering ceramic should be improved for favorable clinical performance and long-term clinical success rate of all-ceramic restorations.

The zirconia frameworks are more acceptable in esthetic appearance than the metal frameworks, however, zirconia is still too white and opaque. Different techniques have been used to adjust the color of zirconia frameworks such as adding coloring oxide to the pre-mixed zirconia powder, immersion the milled frameworks in the coloring solution or application of zirconia liner over the sintered white frameworks [4,9]. Most manufacturers recommend applying the zirconia liner onto the white framework prior to veneering to block out the color of the zirconia. However, the use of zirconia liner onto zirconia framework was still controversial. Some studies reported a decrease in bond strength [10]; while some reported an increase in bond strength [11].

The purpose of this study was to investigate the effect of sandblasting the zirconia liner material on microtensile bond strength of zirconia to layered and pressed veneers and the mode of failure of zirconia to layered and pressed veneers.

### Materials and Methods

1. **Fabrication of the bilayered zirconia-veneer specimens and microbars**

Pre-sintered yttrium-stabilized zirconium oxide blocks (IPS e.max® ZirCAD) were cut using a low speed diamond disc (Isomet 1000). The

<table>
<thead>
<tr>
<th>Group</th>
<th>Veneering technique</th>
<th>Core material</th>
<th>Liner</th>
<th>Sandblast over fired liner material</th>
<th>Veneer material</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1. Layering</td>
<td>e.max® ZirCAD</td>
<td>ZirLiner</td>
<td>-</td>
<td>e.max® Ceram</td>
<td>ZLC</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>e.max® ZirCAD</td>
<td>ZirLiner</td>
<td>Sandblast</td>
<td>e.max® Ceram</td>
<td>ZLSC</td>
</tr>
<tr>
<td>3</td>
<td>2. Press-on</td>
<td>e.max® ZirCAD</td>
<td>ZirLiner</td>
<td>-</td>
<td>e.max® ZirPress</td>
<td>ZLZp</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>e.max® ZirCAD</td>
<td>ZirLiner</td>
<td>Sandblast</td>
<td>e.max® ZirPress</td>
<td>ZLSZp</td>
</tr>
</tbody>
</table>
The effect of blasting zirconia liner on microtensile bond strength of zirconia to layered and pressed veneers

Cutting surfaces were polished with 1000-grit silicon carbide paper. The blocks (11mm x 11mm x 5.5 mm) were then sintered and cleaned in an ultrasonic bath for 5 minutes and briefly steam-cleaned. Then, they were randomly categorized into four experimental groups (at least 30 samples per group) according to the veneering porcelain fabrication technique and surface treatments (Table 1).

The ZirLiner was mixed and applied on the prepared zirconia blocks and briefly dried and fired according to the firing program (Ivoclar Programat® P100). After firing the liner, its thickness was measured under the measuring microscope. The ZirLiner thickness should be approximately 0.1 mm according to the manufacturer’s recommendation.

For ZLSC and ZLSZp groups, the fired liner surface of the zirconia block was gently blasted with aluminum oxide particles (Al₂O₃) 50 μm 1.5 bar at a standoff distance of 15 mm for 5 seconds [12] and then steam-cleaned. For ZLC and ZLZp groups (unblasted liner materials), the fired liner surface was prevented from any contamination before veneering.

The conventional layering technique and press-on veneering technique were used according to the veneering materials; e.max® Ceram: Layering technique and e.max® ZirPress: Press-on technique, respectively (Table 1).

The bilayer block was fixed with cyanoacrylate adhesive gel on a resin acrylic base attached to a micro-cutting instrument (Struers). The first section, 1-mm peripheral rims of the specimens were discarded due to the possibility of the absence of ZirLiner at interfaces that might affect the results (Figure 1A). The block was partially sectioned from veneer to zirconia, leaving 1 mm of intact surface at the end of the block (Figure 1B). The cemented block was then rotated 90° (Figure 1C) and continuously sectioned until microbars of 8 mm in length and 1 mm² cross-section were achieved. Only sound microbars with cross sectional area 1.0±0.1 mm² were used for testing.

2. Microtensile bond strength test

The sound microbars were randomly selected from each testing group and attached to the attachment unit on the left and right sides with the adhesive (Model Repair II Blue). The bonded bars were loaded to failure within a microtensile tester machine (Bisco) at a 1 mm/min crosshead speed. The maximum load at failure was recorded and the microtensile bond strength value was calculated. The microtensile bond strength (MTBS) values were calculated using the formula, σ = F/A where ‘F’ is the load at failure (N) and ‘A’ is the cross-sectional area (mm²) at bonded interface measured using a digital vernier caliper prior to the test.

The mean MTBS and standard deviation of each group were calculated and recorded. Shapiro-Wilk test was performed to test normality.

![Figure 1](http://www.ait.ac.th/division/Ih_Academic_Journal_Unit)

Figure 1. Schematic illustration of microbars preparation. (A) 1-mm peripheral rims of specimens were discarded. (B) The block was partially sectioned, leaving 1.0 mm of intact surface at the end of the block. (C) The cemented block was then rotated 90°.
of the data and Levene’s test was done for equality of variances. Independent t-test was used to compare the mean MTBS between unblasted and blasted zirconia liner materials within the layering and press-on groups at a 5% significant level.

3. Scanning electron microscope observations of the ceramic surfaces

The surface morphologies of unblasted and blasted fired liner were observed under a scanning electron microscope (JSM-6610LV) at 500X magnification. Fractured specimens were ultrasonically cleaned, dried and then examined under SEM at 80X and 200X magnification. The failures were classified into two modes: (1) Cohesive in porcelain veneer: veneering porcelain still covered the entire interfacial surface after load to failure and (2) Interfacial failure: the fracture originated at the zirconia-veneer interface. Some veneering porcelain remained attached to the zirconia, but some of the interfacial zirconia was visible.

Results

In this study, only the sound microbars with 1.0±0.1 mm² cross-sectional area were selected. After discarding the oversized and undersized specimens, the number of specimens used in this study were 30, 34, 35 and 34 for the ZLC, ZLSC, ZLZp and ZLSZp, respectively (Table 2). The mean ZirLiner thickness and the mean cross-sectional areas of the four experiment groups are summarized in Table 2. The mean microtensile bond strength (MPa), standard deviation, and failure mode are listed in Table 3.

The data are normally distributed and the equal variances are assumed in each veneering technique (p>0.05). The independent t-test revealed that there was statistically significant difference of the mean MTBS between unblasted and blasted liner material within the layering group (p<0.05) and within the press-on group (p<0.05) (Table 4).

For the layering group (e.max® Ceram), when the liner material was blasted, the mean MTBS (14.48±2.72 MPa) was significantly lower than that of unblasted liner group (16.48±3.52 MPa) (p<0.05). Similarly, for the press-on group (e.max® ZirPress), when the liner material was blasted, the mean MTBS (15.48±4.97 MPa) was significantly lower than that of unblasted liner group (19.83±6.14 MPa) (p<0.05).

Modes of failure of specimens in the four experiment groups are shown in Table 3. Interfacial failure is defined as the fracture originated at the zirconia-veneer interface. Some veneering specimens, the number of specimens used in this study were 30, 34, 35 and 34 for the ZLC, ZLSC, ZLZp and ZLSZp, respectively (Table 2). The mean ZirLiner thickness and the mean cross-sectional areas of the four experiment groups are summarized in Table 2. The mean microtensile bond strength (MPa), standard deviation, and failure mode are listed in Table 3.

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Modes of failure of specimens in the four experiment groups are shown in Table 3. Interfacial failure is defined as the fracture originated at the zirconia-veneer interface. Some veneering

Table 2. Number of specimens (N), mean ZirLiner thickness and mean cross-sectional area

<table>
<thead>
<tr>
<th>Experiment groups</th>
<th>Code</th>
<th>N</th>
<th>Mean ZirLiner thickness (µm) ± SD</th>
<th>Mean cross-sectional area (mm²) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>e.max® ZirCAD/ZirLiner/e.max® Ceram</td>
<td>ZLC</td>
<td>30</td>
<td>145.2±13.1</td>
<td>1.07±0.06</td>
</tr>
<tr>
<td>e.max® ZirCAD/ZirLiner/Sandblast/e.max® Ceram</td>
<td>ZLSC</td>
<td>34</td>
<td>126.3±9.9</td>
<td>1.04±0.04</td>
</tr>
<tr>
<td>e.max® ZirCAD/ZirLiner/e.max® ZirPress</td>
<td>ZLZp</td>
<td>35</td>
<td>149.6±13.8</td>
<td>1.01±0.07</td>
</tr>
<tr>
<td>e.max® ZirCAD/ZirLiner/Sandblast/e.max® ZirPress</td>
<td>ZLSZp</td>
<td>34</td>
<td>117.0±4.8</td>
<td>1.06±0.06</td>
</tr>
</tbody>
</table>
porcelain remained attached to the zirconia, but some of the interfacial zirconia was visible (Figure 2). In cohesive failure, veneering porcelain covered the entire interfacial surface after load to failure (Figure 3). The majority of fractured specimens had interfacial failure (80-100%). Only 3 of 30 specimens in ZLC group and 7 of 35 specimens in ZLZp group fractured cohesively in porcelain veneer.

SEM analysis revealed the different surface morphologies of unblasted fired ZirLiner and blasted with 50-μm aluminum oxide particles (Figure 4). On the unblasted fired ZirLiner surface (Figure 4A), porosities due to ZirLiner application process were visible. Sandblasting led to a distinctly rough surface (Figure 4B).

The SEM image of e.max® ZirLiner/e.max® Ceram (Layering technique) (1000X) shows good contact between the liner-veneer interface without microgaps (Figure 5), while the SEM image of e.max® ZirCAD/e.max® ZirPress (Press-on technique) (1000X) shows poor contact between the two materials (Figure 6A). However, sandblasting the fired liner material before pressing the veneer improved the interface between these two materials and eliminated microgaps (Figure 6B).
Figure 4. SEM images (500X) of ZirLiner surfaces: (A) Unblasted ZirLiner surface (B) The fired liner surface after sandblasting with 50-μm aluminum oxide particles

Figure 5. SEM image of e.max®ZirLiner/e.max®Ceram (1000X) shows good contact between the liner-veneer interface without microgaps. (A) Unblasted liner material and (B) Blasted liner material.

Figure 6. SEM image of e.max®ZirCAD/e.max®ZirPress(1000X): (A) Unblasted liner material created microgaps between e.max®ZirLiner/e.max® ZirPress. (B) Blasted liner material eliminated microgaps between two materials
The effect of blasting zirconia liner on microtensile bond strength of zirconia to layered and pressed veneers

Table 3. Number of specimens (N), mean microtensile bond strength (MPa), standard deviation and failure mode of each experiment group

<table>
<thead>
<tr>
<th>Veneering technique</th>
<th>Group</th>
<th>N</th>
<th>Mean microtensile bond strength (SD)</th>
<th>Mode of failure</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Cohesive</td>
</tr>
<tr>
<td>Layering</td>
<td>ZLC</td>
<td>30</td>
<td>16.48 (3.52)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10%</td>
</tr>
<tr>
<td></td>
<td>ZLSC</td>
<td>34</td>
<td>14.48 (2.72)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0%</td>
</tr>
<tr>
<td>Press-on</td>
<td>ZLZp</td>
<td>35</td>
<td>19.83 (6.14)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>20%</td>
</tr>
<tr>
<td></td>
<td>ZLSZp</td>
<td>34</td>
<td>15.48 (4.97)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0%</td>
</tr>
</tbody>
</table>

The different superscript letter indicates significant difference by t-test at a 0.05 level of significance.

Table 4. Results of the independent t-test for analyzing the difference of the mean MTBS between unblasted and blasted liner material ($\alpha = 0.05$)

<table>
<thead>
<tr>
<th>Mean MTBS</th>
<th>t-test for Equality of Means</th>
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<tbody>
<tr>
<td></td>
<td>t</td>
</tr>
<tr>
<td>Within the layering group</td>
<td>2.563</td>
</tr>
<tr>
<td>Equal variances assumed</td>
<td>3.229</td>
</tr>
</tbody>
</table>

Discussion

The long-term performance of zirconia-based restorations that the success rate over 5 years of service is 85-90% [13-15]. The main cause of failure is chipping or fracture of veneering materials. The fracture of porcelain veneer is either fracture of veneering itself or fracture originated from the zirconia-veneer interface [1]. The reasons for chipping or fracture of veneering materials are the residual stresses generated by the mismatch of coefficients of thermal expansion (CTE) between veneering ceramics and zirconia substructure [16], as well as the insufficient bond strength between these two materials [17]. Zirconia liner which is used to mask the color of the Y-TZP ceramic also influences the bond between Y-TZP and veneering ceramics [11]. Yet, the effect of sandblasting over the fired liner material on microtensile bond strength of zirconia veneered with layered and pressed ceramics was unclear. Therefore, the main purpose of this research is to investigate the effect of sandblasting over the zirconia liner material on microtensile bond strength of zirconia veneered with layered and pressed ceramics.

The microtensile bond strength test has been proven to be a reliable test for determining the core-veneer bond strength [10]. In the present study, the stick-shaped specimen (microbar) of 8 mm in length and 1 mm$^2$ cross-sectional area was achieved. Due to the small cross-sectional area, it was expected that the stress distribution would be uniform. All specimens were loaded to fracture using a microtensile tester. Most of the fracture occurred in all groups initiated at the zirconia-veneer interface (80-100%). Therefore, this test method could demonstrate the actual zirconia-veneer bond strength between these two materials.

The SEM image (1000X) of the liner-veneer interface of sound microbars (Figure 5) showed
that for the manually layering technique, a brushed-on paste procedure showed good wetting of the veneering porcelain (e.max® Ceram) over the unblasted zirconia liner material (e.max® ZirLiner). However, for the press-on technique, the microgaps between the liner-veneer (e.max® ZirLiner/e.max® ZirPress) were observed when the fired liner material was unblasted. These structural defects could interrupt the contact between the unblasted zirconia liner and the pressed ceramics. Aboushelib et al [3] also reported that when the fired liner material was unblasted, the microgaps between liner material and Ceram Express pressable veneer was found. Structural defects were observed when the veneer was pressed over, resulting in poor contact between these two materials and the liner-veneer interface became a potential site for crack initiation. They recommended to sandblast the liner material before pressing the veneer to improve the interface between these two materials. Nevertheless, this method might affect the MTBS value. To enhance the surface contact and increase the surface roughness between the zirconia liner materials and layered ceramics (e.max® ZirLiner) were not presented in both groups. This might be because the layered ceramics were built-up layer by layer using a brush. This technique demonstrated good wetting of the veneering ceramics even in unblasted liner material. Therefore, sandblasting showed no influence on the liner-veneer interface in the layering technique. However, sandblasting liner material significantly decreased the MTBS between zirconia and layered ceramics.

For the press-on technique, the differences in the liner-veneer interface between unblasted and blasted liner materials were identified. Sandblasting over the fired liner material eliminated microgaps between the liner material (e.max® ZirLiner) and the pressed ceramics (e.max® ZirPress). It might be because sandblasting increased surface roughness, modified the surface energy of liner material and improved wettability of pressable ceramics. However, sandblasting liner material also significantly decreased the MTBS between zirconia and pressed ceramics.

As previously mentioned, the MTBS was significantly lower when the liner materials were blasted in both layering and press-on technique. A possible explanation for this phenomenon is that during sandblasting process, the fired liner material may accumulate the stress and generate the microcracks in the liner material itself. This may lead to decreasing the MTBS of zirconia to layered and pressed ceramics.

Regarding the fracture analysis, the present study found that the failure mode of zirconia veneered with both layered and pressed ceramics was predominantly interfacial failure. Fracture originated at the zirconia-veneer interface that left exposed zirconia surface. Harding et al [12] also found 98% interfacial failure in the MTBS test of Kavo Everest® Y-TZP/e.max® Zirpress. A possible explanation for this study is that sandblasting the fired liner material eliminated microgaps and improved the liner-veneer interface in the press-on technique; however, sandblasting could create the microcracks in the liner material and then the zirconia-liner interface became a potential site for crack initiation and propagation. Therefore, the fracture pattern of blasted liner material in both layered and pressed ceramics to zirconia was 100% interfacial failure without cohesive failure in veneering ceramics. On the other hand, an unblasted fired liner material resulted in poor contact between the liner and veneering materials. Consequently, the liner-veneer interface was
susceptible to crack initiation and the fracture pattern was 10-20% cohesive failure in veneering ceramics.

The clinical implication of this study demonstrated that in the zirconia-veneer fabrication process, blasting over the fired liner material before pressing the veneer improved the interface between the two materials. However, it resulted in not only decreasing the MTBS but also increasing the possibility of interfacial failure.

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Competing interests: None declared
Ethical approval: None (Laboratory study)

References

Autoclave monitoring and packaging in Bangkok dental offices, Thailand

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Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University

Objectives: To examine the regular practice of autoclave monitoring and autoclave dental packaging in Bangkok dental offices, Thailand.

Materials and Method: A preliminary questionnaire interview was performed in 52 private clinics in 2006 and a postal questionnaire was sent out to 629 hospital and private clinics in 2013. Questions included practices on 3 modes of sterilization monitoring: mechanical, chemical, and biological, as well as the types of packaging used.

Results: The response rate of the 2013 survey was 18% (n=113). We found improved formal education in infection control but understanding in the significance of autoclave performance monitoring was still low. Mechanical monitoring was performed the most (90.2%) compared with other types of monitoring. External and internal chemical monitoring were applied in 75% and 33% of all clinics, respectively. Biological monitoring was done in 17.9% of clinics surveyed. Only 2% performed all types of monitoring. The disposable paper/plastic pouch was the main packaging material (92.9%) for autoclave, among these 78.1% reused the pouch. Each paper/plastic pouch was reused most frequently 3 times before disposal. Hospital clinics performed better monitoring in all aspects and reused the pouch less than in private clinics.

Conclusion: Sterilization monitoring of an autoclave machine was inadequate among Bangkok dental offices and knowledge could be the contributing factor to poor practices as formal education was low. Reuse of a paper/plastic pouch was a routine practice but its impact was unknown. There is a need of better education on infection control and further study on the validity of pouch reuse.

Keywords: autoclave, infection control, monitoring, paper/plastic pouch, reuse, sterilization


Introduction

In general dental practices, sterilization of dental instruments is an essential process to prevent cross-infection between patients [1]. The most common sterilization method utilized in dental clinics is steam sterilization with an autoclave where the heated vapor touches the surfaces of the instruments under specified time, temperature, and pressure to achieve sterility [2-6]. To ensure the effectiveness of sterilization process, a combination of 3 modes of monitoring need to be regularly applied [7]. Mechanical monitoring involves direct observation of the machine functioning, e.g. the gauges reaching appropriate temperature and pressure [8]. Chemical monitoring involves chemical tapes, strips, or labels that change color when exposed to high temperature [8]. There are 2 types of chemical indicators: internal and external indicators. Both should be applied together because they have different advantages and disadvantages. An internal indicator helps
ensure that the sterilizing vapor reaches the instruments inside the package; however, it may not be clearly visible once inside the package. An external indicator, such as autoclave tape, allows easy inspection right after sterilization process and helps distinguish between processed and unprocessed packages. Mechanical and chemical monitorings do not absolutely guarantee sterilization, they only help detect procedural errors and equipment malfunctions [8,9]. A third mode of monitoring using biological indicator is also required to determine sterilization achievement because it directly detects the killing of a microorganism. A biological indicator or commonly referred to as a spore test involves placing the spores of *Geobacillus stearothermophilus*, a highly

**Figure 1.** Representatives of autoclave dental packaging and chemical indicators. A) A dental instrument in paper/plastic pouch with an internal chemical indicator inside (*) and an arrow pointing the color change of the external chemical label on the pouch. B) An example of internal chemical indicator showing the color change if sterilization is achieved. C) A representative cloth packaging with external chemical indicator (autoclave tape) color change.
resistant microorganism, inside the autoclave. After the usual sterilization cycle, the biological indicator is retrieved for culture to detect the growth if the spore is still alive. A spore test should be determined weekly, while mechanical and chemical monitoring should be performed in every sterilization cycle [7,8].

One important aspect in sterilization process is the packaging of dental instruments. Packaging helps maintain sterility of the instruments in storage before use. An ideal package should be permeable to the vapor during sterilization but impermeable to microorganism after sterilization and in storage [10]. A paper/plastic peel pouch is the most commonly used package for autoclave machine due to its convenience of use and visibility (Fig. 1). It comprises of paper part which is permeable to vapor and the laminated transparent plastic part which is impermeable and provide strength to the package. Sealing of the pouch can be done with heat and single-use of the pouch is recommended. Cloth can also be used to package the instruments. It has the advantage in reusability and the disadvantage in invisibility of the instruments inside [10,11].

A number of studies have surveyed the sterilization practice in dental clinics in many countries [2-5]; however, to our knowledge, a survey in Thailand has not been carried out before. The aim of this study is to survey the regular practice of an autoclave use to achieve sterilization focusing on monitoring and dental packaging in Bangkok dental offices.

Materials and Method

We performed two cross-sectional descriptive surveys using questionnaire interviewing as a preliminary study in 2006 and a postal questionnaire in 2013. The study was conducted in full accordance with the World Medical Association Declaration of Helsinki [12]. The postal survey protocol was approved by Institutional Review Board of Mahidol University and was granted an exemption (COE No. MU-DT/PY-IRB 2012/16.2408). The questionnaire was anonymous. No participant’s identity or confidential information was disclosed or requested. The participants freely chose to take part or stop to take part in the survey.

The 2006 study. In this preliminary study, we chose 67 private dental clinics in different districts of Bangkok that used an autoclave to sterilize the instruments and were willing to participate in the study. Exclusion criteria were clinics that did not use autoclave or were not willing to participate. The interview was in accordance to the 41-item fixed-answer questionnaire without advanced appointment with the clinic. Fifteen dental clinics declined participation due to current engagement in the dental work process.

The 2013 study. The list of all 1,410 dental offices in Bangkok was obtained from the national registry of dental practices, Bureau of Sanatorium and Art of Healing, Department of Health Service Support, Ministry of Public health, Thailand. Proportional stratified and cluster random samplings were performed to include hospital and private clinics in different areas of Bangkok. Sample
size was calculated to be 302 using Krejcie and Morgan’s formula [13]. We sent out the questionnaires in the postal mail to 320 clinics. Due to low response rate, we sent out the questionnaires to another 309 clinics. The 41-item fixed-answer questionnaires contained demographic inquiries of the office and the responder and questions concerning sterilization practice and autoclave packaging. Inclusion criteria were clinics that packaged instruments for steam sterilization and the responder was willing to give information anonymously and returned the mail. Exclusion criteria included clinics that the responder was not willing to participate or not return the mail. All participants were assured that their responses were confidential and that the results would be published.

Results

The 2006 study.

Fifty-two private dental clinics participated in the study. Fifty-one clinics had 1-2 dentists treating up to 20 patients per day. Only one clinic had 6-10 dentists caring for 21-30 patients per day. All interviewees were dental assistants with minimum secondary education. None received formal education on infection control and all learned about sterilization method from the dentist or another dental assistant in the clinic.

Sterilization monitoring. All clinics surveyed used an autoclave to sterilize the instruments. From 52 clinics participated, one did not perform any kind of monitoring, one performed only mechanical monitoring, and the rest performed chemical with or without mechanical monitoring. None of the clinics surveyed performed biological monitoring with the spore test. Autoclave tape was applied on every instrument packaging in 49 clinics, one clinic applied the tape on some of the packages, one did not have autoclave tape in the clinic and one did not package the instruments. Regarding the knowledge of the indicator color change, 90% of the dental assistants that applied autoclave tape thought that the color change on the tape equaled sterilization achievement.

Autoclave packaging. Except for one clinic that did not wrap the instruments, all used paper/plastic pouch for autoclave packaging. Most clinics (n=47) also used cloth as packaging material. All clinics reused the paper/plastic pouch. Each paper/plastic pouch was most often reused 3 times before disposal and the autoclave tape would be reapplied every time (n=50) except for 1 clinic that did not reapply the tape. The maximum time of reuse was 6 times (in 2 clinics).

The 2013 study.

From 629 questionnaires being sent out, 113 were mailed back (18% response rate); of these, 15 were hospital clinics and 98 were private clinics. Eight hospitals received Hospital Accreditation (HA). Most responders were dentists (54.9%) and dental assistants (39.8%). Among these, 44% received formal education about infection control and 42% received the training from medical or dental personnel in the workplace.

Sterilization monitoring. There was one missing data regarding this information. All responders (n=112) applied at least one type of monitoring: 22 (19.6%) applied only mechanical
indicator, 6 applied only external chemical indicator, 1 applied only internal chemical indicator, no clinic applied only biological indicator, and 83 (74.1%) performed more than one type of monitoring. However, there were only 12 clinics (10.7%) that applied all 4 types of indicators; 10 were hospital clinics.

Mechanical monitoring was performed in 101 clinics (90.2%). External chemical monitoring (using autoclave tape or observing the label outside the paper/plastic pouch package) was performed in 84 clinics (75%). Internal chemical monitoring (placing the internal indicator strip inside the pouch) was performed in 37 clinics (33%). Biological monitoring (spore test) was performed in 20 clinics (17.9%).

Fig. 2 summarizes and compares the types of monitoring performed in hospital and private clinics. Most hospital clinics performed all types of monitoring while private clinics performed mechanical monitoring the most and only 8.2% of private clinics performed biological monitoring.

Of all the clinics that used the external chemical indicator (n=84), the indicator was applied to every instrument packaging in hospital clinics while the number in private clinic was only 57.1% (Table 1). In clinics that used the internal chemical indicator (n=37), the number of hospital clinics that applied or did not apply the indicator was equal, while most of the private clinics (65.4%) applied the indicator to only some of the packaging (Table 1). For biological monitoring (n=20), 2 clinics performed the spore test daily, 10 clinics performed weekly, 6 performed monthly, and 2 was unknown.

Regarding the knowledge of the indicator color change, only 19.5% of the clinics that used autoclave tape answered correctly, i.e. color change of the tape means that the package has been through autoclave process but does not equal sterilization of the instrument.

Autoclave packaging. Most clinics (61.1%)
used both cloth and paper/plastic pouch to package the instruments. There were 7 dental offices that did not package the instruments for sterilization at all, all of these were private clinics. One clinic used only cloth and 36 clinics used only paper/plastic pouch for autoclave packaging (Fig. 3).

From 105 clinics that used paper/plastic pouch as packaging material, 82 clinics reused the pouch (78.1%). Most private clinics (85.6%) reused the pouch while most hospitals (66.7%) disposed the pouch after single use as shown in Table 2. Each paper/plastic pouch was most often reused 3 times before disposal and the autoclave

**Table 1.** Percentages of clinics that used external and internal chemical indicators

<table>
<thead>
<tr>
<th></th>
<th>Hospital clinics</th>
<th>Private clinics</th>
<th>All clinics</th>
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<tbody>
<tr>
<td><strong>External chemical indicator (n=84)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Applied to all packages</td>
<td>100</td>
<td>57.1</td>
<td>64.3</td>
</tr>
<tr>
<td>Applied to some packages</td>
<td>0</td>
<td>34.2</td>
<td>28.6</td>
</tr>
<tr>
<td>Not answered</td>
<td>0</td>
<td>8.5</td>
<td>7.1</td>
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<tr>
<td><strong>Internal chemical indicator (n=37)</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Applied to all packages</td>
<td>45.5</td>
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<td>59.5</td>
</tr>
<tr>
<td>Not answered</td>
<td>9.1</td>
<td>11.5</td>
<td>10.8</td>
</tr>
</tbody>
</table>

**Table 2.** Percentages of paper/plastic pouch reuse

<table>
<thead>
<tr>
<th></th>
<th>Hospital clinics</th>
<th>Private clinics</th>
<th>All clinics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-use</td>
<td>66.7</td>
<td>14.4</td>
<td>21.9</td>
</tr>
<tr>
<td>Reuse</td>
<td>33.3</td>
<td>85.6</td>
<td>78.1</td>
</tr>
</tbody>
</table>

**Figure 3.** Percentages of packaging material used
tape would be reapplied every time in 50% of the clinics that reused the pouch (Fig. 4). The maximum time of reuse was 6 times (1 clinic).

Discussion

This is the first study examining sterilization practice with an autoclave focusing on sterilization monitoring and packaging in dental clinics in Thailand. Our study involved two surveys done seven years apart in 2006 and 2013 using a questionnaire interview or a postal questionnaire, respectively. We found low levels of monitoring in both surveys. Also, the rates of the disposable paper/plastic pouch reuse were high. This study should prompt more education or monitoring regarding sterilization practice of dental clinics in Thailand.

The response rate in our 2013 survey which used postal questionnaire was only 18% while the response rate in a preliminary 2006 questionnaire interview was 77.6%. When compared with other postal questionnaire surveys on sterilization practices in other countries done between 1995 and 2012, the response rates varied from 40 to 100% [3,6,14-21]. Our low response rate was unexpected. We had to double the sample size in order to get more participants. Despite low response rate, the responses in a 2013 survey were in accordance with those of a 2006 preliminary survey suggesting actual sterilization trend in Bangkok dental clinics. It is possible that clinics with low monitoring might not want to participate in the survey. Other reasons that might contribute to low response rate included not having time to participate, and not understanding the questions. Another approach such as interviewing might be better to really gain the information.

No responders received formal education regarding infection control in a preliminary survey while 44% did in a 2013 survey. This could be due to the fact that all responders in a preliminary survey were dental assistants while in a 2013 survey most were dentists. Nevertheless, the numbers in our study were much less than those in surveys from Ireland and UK which found more than seventy percent of dental assistants receiving formal infection control education [15,22]. Since

![Figure 4. Times reuse of each pouch. X; not answered.](http://www.dt.mahidol.ac.th/division/th_Academic_Journal_Unit)
the person performing the sterilization procedure in a clinic is most likely a dental assistant, poor knowledge on infection control might affect the sterilization practice. It could be performed without clear understanding of the significance of each type of monitoring.

The United States Centers for Disease Control and Prevention (CDC) recommended application of mechanical, chemical, and biological indicators for sterilization monitoring. Mechanical and chemical monitoring should be performed in every cycle of sterilization while biological monitoring should be performed at least weekly [7]. Our survey in 2006 found none of the 52 clinics performed all 3 modes of monitoring. There was even a clinic that did not perform any kinds of monitoring at all. Moreover, mechanical monitoring was performed in only about 50% of the clinics even though it does not require extra equipment. The levels of each type of monitoring all improved in a 2013 survey. About 10% of the clinics performed all types of monitoring and most performed more than one mode of monitoring. Still, most clinics did not perform adequate monitoring.

Less than twenty percent of the clinics surveyed in 2013 applied a biological indicator, among these, 30% performed the spore test every month which was less frequent than the weekly recommendation by the CDC. However, these numbers increased from a finding of zero biological monitoring in all clinics in a 2006 survey. Indeed, a recent review indicated that sterilization monitoring was deficient globally [2]. A survey in Scotland found only 39% and 1% of general dental practitioners to employ chemical and biological monitoring, respectively [23] Only 9.8% of dental clinics in Italy performed both chemical and biological monitoring regularly [24].

It is possible that the significance of doing all modes of monitoring was not realized by most clinics due to inadequate education on infection control as could be exemplified from the misunderstanding in the interpretation of the external indicator color change in more than 80% of the clinics in both surveys. Also, increased cost accompanying some types of monitoring might cause the insufficient monitoring in the majority of the clinics, as found in a previous survey in Romania that cost was an important factor for infection control practice [25].

Regarding autoclave dental packaging, more clinics in a 2013 survey used paper/plastic pouches than cloth compared to the survey in 2006. A paper/plastic pouch is recommended for single use; however, reuse of a pouch is a common practice in Thailand. To our knowledge, there was no study in other countries examining whether the pouch is reused or not. Most private clinics as well as one-third of hospital clinics reused the pouches for many times before disposal. Of note was that the external chemical indicator was also reapplied only in half of the clinics that reused the pouches.

As expected, hospital clinics performed better monitoring than private clinics in all aspects. Hospital clinics with HA showed appropriate monitoring according to the guideline recommendations. Reuse of a paper/plastic pouch was also less in hospital clinics compared with private clinics.

The effectiveness of a sterilizer performance was not explored in our surveys. Previous studies in many countries have found the failure rates of
Autoclave monitoring and packaging in Bangkok dental offices, Thailand

autoclave performance as monitored with a biological indicator to vary from 0 to 57.9% in studies done between 1976 and 2004 [4,14,15,26-28]. These results pointed toward the importance of regular maintenance of the machine as well as of monitoring autoclave performance to ensure proper functioning. Knowing the sterilization failure rate of an autoclave in dental clinics in Thailand would be of great importance and should be examined in future studies.

In summary, our study indicated inadequate monitoring of an autoclave performance in Bangkok dental offices. Most clinics performed the monitoring without clear understanding of the methods used. Reuse of a paper/plastic pouch was a regular practice which should actually prompt more rigorous monitoring. The impact of such a reuse was not known. Our findings suggested improved education on infection control especially in dental assistants as well as other possible measures to increase proper sterilization practice of dental offices in Thailand. The limitations of this study included the low response rate and the possibility of misunderstanding of a questionnaire. Also, we surveyed only clinics in Bangkok, a capital city of Thailand. The situations in rural clinics could be different. Future studies should explore the effectiveness of sterilization performance and factors affecting sterilization practice in dental clinics across the country.

Acknowledgement

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References


http://www.dt.mahidol.ac.th/division/th_Academic_Journal_Unit 181


The effects of airborne abrasion on micro-shear bond strength of resin cements on zirconia

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Objective: To evaluate the effect of various air abrasion treatments on micro-shear bond strength (μSBS) of zirconia bonded with different self-adhesive resin cements.

Methods: Fully-sintered Y-TZP block specimens (IPS e.max® ZirCAD, Ivoclar Vivadent) were prepared to 5 types of surface treatments: no treatment [CON], air abrasion with different particle sizes and pressures: 50 µm & 1 bar [50.1], 50 µm & 3 bar [50.3], 110 µm & 1 bar [110.1], and 110 µm & 3 bar [110.3]. Then, the specimens were divided into two subgroups for further cementation with self-adhesive resin cements (Rely X Unicem Aplicap, 3M ESPE and Panavia SA, Kuraray). These cements were placed into tygon tubes over the zirconia specimens. After water storage at 37°C for 24 hours, micro-shear bond strength (μSBS) was measured. Failure mode was observed under SEM. Statistical analysis was performed by ANOVA and Tukey’s test (α = 0.05).

Results: The air abrasion treatment with various particle sizes and pressures affected μSBS (p<0.05). The higher pressure and larger particle size improved zirconia bond strength. Panavia SA performed superiorly in μSBS than Rely X Unicem (p<0.05).

Conclusions: The air abrasion with larger particle size (110 µm) & higher air pressure (3 bar) in combination with MDP-containing resin cement (Panavia SA) could create the optimal adhesion to zirconia.

Key words: air abrasion, micro-shear bond strength, resin cement, surface treatment, zirconia

strength and maintaining durable bond against the aging processes. Various studies emphasized on the mechanical treatments on zirconia: SIE (selective infiltration etching) [8], Tribocchemical silica coating [3], Nd:YAG laser [9], or air abrasion. Also, the chemical retention to zirconia bonding was acquired by the applications of 10-MDP-containing agents [3-4] (10-methacryloyloxydecyl dihydrogenphosphate), silane coupling agents [10], Radio-Frequency plasma spraying [5], alloy primer [10], universal primer (Monobond® Plus) [11], and metal/zirconia primer [12]. Nevertheless, the mechanical retention was most likely strengthen the zirconia adhesion, which played a better role over the chemical retention [13]. One of the most common surface treatments claimed to improve the mechanical retention is the air abrasion with various particle sizes and pressures [10-11]. These variables should be performed thoroughly because an improper altitude such as an inadequate pressure significantly affected zirconia bonding [7,13]. Also, the treatment could yield some defects on the zirconia surface, which could lead to the micro-cracking, inducing the detrimental to bond strength and durability [8]. Therefore, the process should be carefully handled [14].

Still, an air abrasion has been widely chosen to perform due to many advantages: removing contaminations, increasing wetting, reducing surface tension, creating micromechanical interlocking, and providing long-term optimal bond strength to zirconia [10-11].

Zirconia bonding depends on not only the surface treatments, but also the selection of resin luting types [1,4]. Consequently, proper luting determines the longevity of indirect restorations especially in all-ceramics [15]. Indeed, resin cements are the material of choice for ceramic bonding due to many desired properties required in all-ceramic restorations such as: great marginal sealing, improved fracture toughness, low solubility, low marginal leakage, high compressive strength, great retention, and varieties of colors & translucency, inhibiting crack formation and superior strengthening effect [16-18]. Some studies claimed that both conventional and self-adhesive resin cements performed similarly in term of the bond strengths as long as they both contained suitable compositions that chemically reacted to zirconia oxide [4,19]. The 10-MDP (10-methacryloyloxydecyl dihydrogenphosphate) component in resin cements offered the chemical bond to zirconia restorations and increased surface wettability and maintained adequate chemical bond strength [8-9,16,20].

Even though, many studies on airborne abrasion treatments have been published as previously mentioned, there is limited knowledge regarding the effects of definite methods for the air abrasion protocols on zirconia surface [21]. Also, there has not been many studies regarding to bonding of IPS e.max ZirCAD (Ivoclar Vivadent, Schaan, Liechtenstein). Therefore, this present study aimed to find the proper air abrasion treatments and selection of self-adhesive resin cements to optimize the adhesion of IPS e.max ZirCAD. The null hypotheses were: (1) there were no significant difference on μSBS on zirconia surfaces luting with different resin cements, and (2) There were no significant differences on μSBS of pretreated zirconia with different particle sizes and air pressures of air abrasion.

Material & Methods

Pre-sintered Y-TZP block with size of 15.4×19×39 mm³ (IPS e.max® ZirCAD, Ivoclar Vivadent, Schaan, Liechtenstein) were cut into smaller dimensions of 2.5×4.5×15 mm³ by low speed diamond saw (Isomet, BUEHLER, Illinois, USA). The specimens were ground-finished with a 180 SiC papers on 300 rev/min for 10 seconds under running water by grinding machine (Buehler Metaserv, Buehler Germany) in order to stimulate as-milled surface, then cleaned in ultrasonic cleanser (Q210H, L&R manufacturing, USA) with 95% ethanol for 2 minutes to eliminate the debris particles. Then, the specimen were sintered to its final density in the furnace (Sirona in Fire HTC, Sirona Dental Systems, USA) at 1500°C with the total period of 7 hours.

The zirconia specimens were divided into 5 groups (n=20 in each group) according to the different air abrasion treatments with aluminum oxide particles (Al₂O₃) (Cobra, Renfert, Hilzingen, Germany). The specimens were perpendicularly and uniformly
The Effects of Airborne Abrasion on Micro-Shear Bond Strength of Resin Cements on Zirconia

sandblasted across the specimens by sandblast machine (Bash M.B.L., Dentalfarm, Torino, Italy) in a circular motion, equipped with the nozzle of 1.2 mm in diameter, 5-mm distance from the nozzle, for 10 seconds with various particles sizes and pressures: 50 μm & 1 bar [50.1], 50 μm & 3 bar [50.3], 110 μm & 1 bar [110.1], and 110 μm & 3 bar [110.3]. No treatment group [CON] represented as controls, which represented the zirconia surface after milling. After sandblasting, the specimens were cleaned in ultrasonic cleanser (Q210H, L&R manufacturing, USA) with 95% ethanol for 5 minutes, then cleaned with Steam Clean Machine (Evolution steam line, EV4, Silfradent, Italy) for 5 seconds, and dried with the compressed air for 30 second. Surface topography in each group (n=1) was evaluated under Scaning Electron Microscope (SEM: JSM-5410LV, JEOL Ltd., Tokyo, Japan) images with magnification of 2,000.

Micro-shear bond strength test

All treated specimens were subdivided to 2 subgroups (n=10) as the self-adhesive resin cement used. The cementation were proceeded immediately after the surface treatments to prevent any possible contamination. RelyXTM Unicem AplicapTM and PanaviaTM SA CEMENT (Table 1). were loaded into the plastic tube (Tygon®, Norton Performance Plastic Co, Cleveland, OH, USA) (Ø 0.8 mm, height 1 mm). After that, the specimens were photo-cured by LED light-curing unit, Bluephase® (Ivoclar Vivadent, Schaan Leichtenstein) for 20 seconds with 1200 mW/cm² prior to the water storage at 37°C for 24 hours. Voids or gaps were evaluated under Polarize light microscope examination at the magnification of 25 (E400 POL), which was excluded before the bonding test. The interfacial areas of each luting cylinder were measured by the digital vernier caliper (± 0.1 mm²) (Model CD-15CW, Mitsutoyo Corp., Japan).

The specimens were attached on the testing device using the cyanoacrylate adhesive (Model repair II Blue, Dentsply-Sankin, Japan) on the universal testing machine (Lloyd™ Testing Machine, Model LR 10K, Lloyd Instruments, Fareham Hanth, UK). The wire of 0.2 mm-diameter were looped around luting cylinder, which were aligned to parallel for the accuracy of μSBS. The crosshead speed (1 mm./min) was loaded until failure and recorded.
<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition</th>
<th>Manufacturer</th>
<th>Instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPS e.max ZirCAD Batch # (T42432)</td>
<td>ZrO$_2$, Y$_2$O$_3$, HfO$<em>2$, Al O$</em>{2.2}$</td>
<td>Ivoclar Vivadent; Schaan, Liechtenstein</td>
<td>-</td>
</tr>
<tr>
<td>RelyX™ Unicem Aplicap™ (3M ESPE, St. Paul, MN, USA) Batch # (568880)</td>
<td>Powder: Alkaline (basic) fillers, Silanated fillers, Initiator components, Pigments Liquid: Methacrylate monomers containing, phosphoric acid groups (phosphoric ester), Methacrylate monomers, Initiator components, Stabilizers</td>
<td>3M ESPE (St Paul, MN, USA)</td>
<td>Triturated for 10 seconds</td>
</tr>
<tr>
<td>Panavia™ SA CEMENT (Kuraray, Osaka, Japan) Batch # (620080)</td>
<td>PASTE A: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), Bisphenol A diglycidylmethacrylate (Bis-GMA), Triethylene glycol dimethacrylate, Hydrophobic aromatic dimethacrylate, Silanated barium glass filler, Silanated colloidal silica, dl-Camphorquinone, Benzoyl peroxide, Initiators PASTE B: Bisphenol A diglycidylmethacrylate (Bis-GMA), Hydrophobic aromatic dimethacrylate, Hydrophobic aliphatic dimethacrylate, Silanated barium glass filler, Silanated colloidal silica, Surface treated sodium fluoride, Accelerators, Pigments</td>
<td>Kuraray Noritake, Osaka, Japan</td>
<td>Equal amounts of pastes were extruded, and then hand-mixed for 10 seconds</td>
</tr>
</tbody>
</table>
Failure mode analysis

The fractured patterns were evaluated under polarized light microscope (E400 POL) at the magnification of 10. Any unclear patterns were sputtered coated with gold-palladium (E-1030, Hitachi, Tokyo, Japan) and re-examined under SEM at the magnification of 90-100x. The fracture patterns were classified as cohesive failure, adhesive failure and mixed failure.

Statistical analysis

The means and standard deviations of μSBS (MPa) were evaluated by Kolmogorov Smirnov test (K-S test) to determine the distribution of the data. Levene’s test was then used to test the homogenous of variance. Two-way ANOVA and Tukey’s test were used to determine statistically differences among the μSBS to examine the effects of particle sizes, pressures on each resin cement (p<0.05). T-test was used to compare the μSBS between self-adhesive resin cement in any surface treatment. For the failure modes, the results were analyzed by chi-square test (p<0.01).

Results

For SEM assessment of surface topography, the irregularities of horizontal-line pattern produced by the SiC paper were shown in the SEM image (Figure. 3a). After the air abrasion with different particle sizes and pressures, the irregularities of polished-line pattern were less visible to air-abraded specimens. Then, some defects were more apparent after the air abrasion treatments (Figure. 3b-f). Although, the high pressure completely eliminated the initial horizontal-line pattern, the high impact of deep grooves also mostly presented as well (Figure. 3c&f). Whereas, the specimens encountered with the lower pressure of 1 bar inadequately eliminated the initial irregularities. These presented rougher surfaces and the horizontal-line patterns were partially visible (Figure. 3b&d).

Figure 2 showed the means and standard deviations of μSBS with two self-adhesive resin cements. All each independent factor (pressure, particle size) had a significant effect on μSBS (p<0.05). Moreover, the interactions between particle size & pressure were significantly affected (p = 0.043).

 Obviously, all of the air-abraded zirconia groups were significant higher in the bond strengths than the non-treated one. Panavia SA cement performed significantly higher bond strength than Rely X Unicem cement, regardless of particle sizes or pressure. The highest bond strength was found in the zirconia treated with 3-bar air pressure and 110 μm-sized particle when cemented with Panavia SA (31.001 ±3.5633 Mpa),

![Figure 2](http://www.dt.mahidol.ac.th/division/th_Academic_Journal_Unit)

Figure 2. Means μSBS (μm) of cemented zirconia surfaces with different surface treatments (n=10). The different capital letters represented the significant differences within Rely X Unicem groups, while the different small letters represented the significant differences within Panavia SA groups (p<0.05). There was significant difference on μSBS between 2 resin cements* (p<0.0001).
while the control group in Rely X Unicem performed the lowest bond strength (6.638 ±1.076 MPa).

Considering the particle size, all groups treated with the 110 μm-sized particle produced significantly higher bond strength than the 50 μm-sized particle, regardless of the air pressures and resin cements (p<0.05). Furthermore, the 3-bar air pressure also produced significantly higher bond strength than the 1-bar air pressure, regardless of the particle size and resin cement.

The fracture mode distribution was outlined in Figure 4. All groups revealed significantly different on fracture mode patterns (p<0.05). The most failure mode was the adhesive failure between resin cement and zirconia surface. Those groups cemented with Rely X Unicem (with same particle sizes and pressures) presented higher adhesive failure pattern than those cemented with Panavia SA. The mixed failure was found more when the pressure increased in both resin cements. However, there were no significant differences in failure mode when the particle size was increased.

Figure 5 showed SEM image (90x-100x) on the right side that represented a fracture specimen of ConX with the clear visual of an adhesive failure pattern. The zirconia surface revealed more than 75% throughout the surface. On the left side, the image of the light microscope (10X) correlated to the SEM image (Figure 4a-b). The representative of cohesive pattern was presented in ConSA specimen. The remnant cement exhibited more than 75% (Figure 4c-d). The other specimen of ConSA was calibrated the two different surfaces, which neither unveiled more than 75% of zirconia nor cement surface (Figure 4e-f). Therefore, it was categorized as the mixed failure pattern.
Discussion

Nowadays, dental zirconia is used extensively in dentistry as it acquires many superior properties such as higher toughness, greater flexural strength, and better-disguised discolored abutments, comparing to glass ceramics [2,17]. Nonetheless, the bonding to zirconia is crucial as no efficient protocol to treat an intaglio of zirconia fixed prosthesis prior to cementation is convinced. Obviously, both mechanical and chemical pre-treatments to zirconia surfaces have been studied inevitably to render durable bond strength [22]. However, no many studies have studied about the $\mu$SBS of various variables of air-abraded-treated zirconia to self-adhesive resin cements. Consequently, this present study was conducted to investigate the effect of airborne abrasion on the $\mu$SBS of resin cements bonded to zirconia.

![Figure 4](image1.png)

**Figure 4.** Representative light microscope (10X) and SEM micrograph (90-100X) of each fractured pattern. (a,b) adhesive failure pattern, (c,d) cohesive failure pattern, (e,f) mixed failure. The letter “Z” stands for zirconia surface and “R” stands for attached cements on the bonding interface.

![Figure 5](image2.png)

**Figure 5.** The fracture mode distribution in all group tested.
conducted to establish the effect of various airborne abrasion protocols on zirconia in micro-shear bond strength with different self-adhesive resin cements.

Moreover, it was known that selecting types of resin cement was mandatory for longevity of zirconia bonding [4]. Our study demonstrated that Panavia SA, MDP-containing cement, provided significantly higher μSBS than Rely X Unicem at 24 hours, regardless of any air abrasion treatments. Therefore, our first hypothesis, there were no significant difference in μSBS between different luting agents on zirconia surface, was rejected (p<0.05). Many studies highly favored the performance of Panavia over Rely X Unicem in the long-term bond strength [3,8,16]. The main reason was due to the component of MDP, bonding directly to the metal oxides [21,23], which positively affected the adhesion. The monomer increased surface wettability and created methacrylate cross-linkages & siloxane bond with OH group of zirconia [4,13,20]. The other favorable property was the greater flexural strength of Panavia SA (81 MPa) compared to Rely X Unicem (48 MPa), which positively affected the bond strength.

In this study, the μSBS of surface-treated zirconia was significant higher than non-treated groups as anticipated [24], which was consistent to the study of Gome et al. (2013). This study conducted the μTBS of pretreated Cercon with various air abrasion treatments. It found that the microbar of untreated specimens failed prematurely prior to the conduction of the bond strength test [20]. Consequently, such mechanical treatment was mandatory for the zirconia bonding [16]. Nonetheless, great care must be taken not to perform excessive pressures or particle sizes, so such treatment must be performed wisely within normal range to prevent any damage [25].

In the bond strength test with zirconia, most studies evaluated the bond strength by polishing zirconia specimens with 600 or 1200-grit SiC paper to create flat surfaces and eliminate excess or uneven spots on zirconia surfaces prior to the bond strength test. Therefore, their control surfaces were considered the smoothest compared to other air abraded groups [24]. Nevertheless, our present study attempted to evaluate the bond strength of an actual milled zirconia surface, which matched the roughness of the zirconia surfaces polished with the 180-grits of SiC paper on our pilot study. For this reason, the irregular surface of the control group in this present study was more obvious compared to some of the air-abraded groups.

As results has shown, both larger particle size and higher pressure positively affected on the bond strength (Figure 2). Thus, our second hypothesis, there are no significant difference in μSBS affected by either in different particle sizes or air pressures of air abrasion, was rejected (p<0.05). The 110 μm-sized particle increased significantly the bond strength values compared to the 50 μm-sized particle. According to our SEM micrographs on treated zirconia surfaces, the grooves were more prominently in 110 μm-sized particle groups than the 50 μm-sized particle groups (Figure 3b, d, c, f). This characteristic would increase the more mechanical retention to rougher zirconia surfaces. Unlike this present study, Valentino et al. (2012) evaluated the influence of different surface treatment for promoting a bond between zirconia-based ceramic (Cercon Smart Ceramics, Degudent, Hanau, Germany) and a dual-cure resin luting agent (Enforce, Dentsply, Caulk, Milford, DE, USA). No significant differences in surface morphology and μSBS between the air abrasion with particle sizes of 50 and 110 μm was found [26].

Also, the 3-bar air pressure also produced significantly higher bond strength than the 1-bar air pressure, regardless of the particle size and resin cement. While, the 1-bar air pressure was not enough to create the adequate retention on zirconia surfaces [27], which was consistent to our SEM micrograph of surface topography (Figure 3b,d). Therefore, the treated zirconia with any particle sizes in 3-bar air pressure (50.3 & 110.3) provided higher bond strength than those in 1-bar air pressure (50.1 & 110.1).

The higher pressure of 3 bar and larger particle size of 110 μm were more capable to remove the superficial defects and the horizontal line pattern created by the initial polishing of 180-grit SiC paper (Figure 3). When the Al2O3 particle attacked on the zirconia surface, the larger particle size and higher air pressure, the more
kinetic energy presented. This energy could transfer to the rough surface, and groove, chamfering the sharp angle and subsequently shallowing the deep groove, smoothening and leveling out these rough surfaces [28-29]. This shallow groove may facilitate the resin infiltration on this treated zirconia, increasing the adaptation of resin cement on surface. Therefore, this protocol could increase the bond strength. Moreover, this protocol was more capable to remove contamination, increase surface wettability [24].

The adhesive failure was mostly found in the non-treated zirconia. The air-abraded zirconia, on the other hand, demonstrated greater amounts of the cohesive and mixed failures [20]. Moreover, Oyague et al. (2009) found the bond strengths of air-abraded specimens remained stable after 6 months water storage due to the micromechanical interlock of resin penetration into roughened zirconia [4]. Furthermore, Panavia SA (MDP-containing cement) also presented less in adhesive failure, because the MDP was proposed to chemically interact with oxides of zirconia [3,8,20].

This study selected performing μSBS test in order to avoid hassle and error of the specimen preparation [22], because μTBS tended to cause the pre-mature failure during cutting the microbars [19]. Furthermore, unstable temperatures and moisture could degrade the zirconia bonding [6,30]. Therefore, the clinical simulation of thermocycling should be conducted for the future research in order to investigate long-term bond strengths on zirconia bonding.

**Conclusion**

1. MDP-containing resin cement significantly provided higher μSBS to zirconia bonding than non-MDP resin cement.

2. The different air abrasion treatments on the zirconia surfaces and zirconia bonding with two resin cements affected on the bond strength. The zirconia treated by the air abrasion with larger particle size (110 μm) & higher air pressure (3 bar) and cemented with MDP-containing resin cement (Panavia SA) created the optimal adhesion to zirconia.

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**References**


Fracture mechanics approach to determine bonding quality of two ceramics

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² Research office, Faculty of Dentistry, Mahidol University

Objective: To evaluate bonding quality of bilayered and monolithic dental ceramics using chevron-notch specimens.

Materials and methods: The monolithic blocks of ceramic core materials (IPSe.max®Press) and ceramic veneering material (IPSe.max®Ceram) were fabricated. In addition, the blocks of bilayered ceramic (IPSe.max®Press/IPSe.max®Ceram) was also fabricated. The triangular notch shape were created at the middle of the prepared bars and at the interface between two ceramics (3x4x16 mm) by using the specific devices and loaded in tension until failure. Optical microscope and Scanning Electron Microscope (SEM) were used to determine the crack propagation and ensure the mode of failure. Then, the bonding quality of the bilayered and monolithic specimens were analyzed.

Results: All of the specimen failures were found at the interface. The optical microscope revealed that the crack was initiated at the tip of the triangular notch and the failure occurred along the interface. Accordingly, the interfacial failure pattern was classified.

Conclusions: The test reveals the different approach to evaluate the bonding effectiveness of the ceramics. Specimen size and cutting angle of the triangular notch can be control. The sample can be readily prepared. Therefore, this test can be used as a valid alternative to analysed the bonding quality of the ceramics because the approach ensured the interfacial failure.

Key words: all ceramic, bonding, interfacial, fracture toughness, chevron-notch, bilayered


Introduction

All-ceramic restorations have been widely used as fixed prostheses due to their superior esthetics and excellent biocompatibility. Several types of ceramic are commercially available such as lithium disilicate, zirconia, alumina and their matching veneering materials. The high strength ceramic core materials are used as alternatives to metal. However, failures of all-ceramic restorations were fracture of the core materials, chipping and fracture of veneering layers [1,2]. The bond between ceramic core material and the corresponding veneering material might be one of the weakness that can affect the long-term success of all-ceramic restoration [3-5]. The strength of the core-veneer bond is influenced by the residual stresses developed from the CTE mismatch or from the rapid or slow cooling rate during firing [6-8]. The difference in elastic modulus of ceramics core and veneering material is also the affecting factor [9,10]. To evaluate the effectiveness of the ceramic/ceramic bonding, shear bond test, tensile bond test, micro shear...
bond test (μSBS) and micro tensile bond test (μTBS) have been used [11-13]. However, the results presented the variability of the fracture pattern, mostly found cohesively in the veneering layers [12]. This means that it represents the strength of the veneering material instead of the bond strength of the core-veneer interface.

In order to obtain an appropriate bonding test method, the chevron-notch test has been developed for testing the interfacial fracture toughness of ceramics or other brittle materials [14-17]. This method provides some advantages. The pre-crack is not needed because high stress concentration presents at the tip of chevron-notch. The tip of chevron-notch, which is the weakest part, can cause the crack at the low applied loads. Fracture toughness can be easily calculated from the maximum test loads and calibration factor, depending on specimen geometry and loading configuration [18,19]. Moreover, it uses small, simple dimension and inexpensive [20]. Consequently, the measurement of fracture resistant at the interface is possible.

The objective of this study is to evaluate bonding quality of bilayered and monolithic dental ceramics using chevron-notch specimens.

**Materials and methods**

Three groups of specimens were established with 15 specimens per group.
- Group 1: IPS e.max® Ceram (monolithic)
- Group 2: IPS e.max® Press (monolithic)
- Group 3: IPS e.max® Press/IPS e.max® Ceram (bilayered)

**Preparation of monolithic specimens**

Dentine porcelain powder (IPS e.max® Ceram) and liquid were mixed and condensed into plastic mold. The first layer was fired according to manufacturer’s instructions. The additional layers were added with the same technique to gain the monolithic blocks with final dimension of 3 mm x 4 mm x 16 mm.

For IPS e.max® Press, resin blocks (3 mm x 4 mm x 16 mm) were prepared. After that, the lost-wax technique was done to gain the final dimension of the monolithic specimens (3 mm x 4 mm x 16 mm).

**Preparation of bilayered lithium disilicate core ceramics-veneer specimens.**

Resin blocks (3 mm x 4 mm x 8 mm) were prepared, sprued and invested according to manufacturer’s instruction. Then, the investing process was completed. The completed lithium disilicate blocks were placed in plastic mold for veneering process. IPS e.max® Ceram dentine porcelain powder and the modeling liquid were mixed and condensed on the IPS e.max® Press block, which placed in plastics mold using load transferring device with 10 kg load and the load was maintained for 5 minutes. The three cycles of veneering were done and fired according to manufacturer’s instruction to achieve the bilayered specimens. Finally, the specimens were finish and polished to derive the specimens with the dimension of 3 mm x 4 mm x 16 mm (Figure 1).

**Cutting process**

The prepared bilayers and monolithic blocks were cut in to triangular notch shape (3 mm x 3 mm x 3 mm.) at the interfacial zone or at the middle of the monolithic blocks using a low-speed diamond saw (Isomet, Buehler, Illinois, USA) under water cooling. The specimen was secured using a holding device to ensure that all the specimens were prepared with the same cutting angle (Figure 2).

The finished specimens were tested in...
Fracture mechanics approach to determine bonding quality of two ceramics

Figure 2. Cutting process
(a) chevron-notch bar specimens geometry
(b) Preparation of specimens with the same cutting angle
(c) Prepared specimens after cutting process

Tension at a crosshead speed of 0.1 mm/min using a universal testing machine (EZ-S, Shimadzu, Japan). Specimen was tightened on stainless steel wire at the circular notch and attached to the grips of testing machine. Specimen was paralleled to the long axis of the device to avoid bending stress. After that, the samples were loaded until failure occurred (Figure 3).

The loads at failure were recorded and calculated using the equation.

$$K_{IC} = \frac{P_{\text{max}} \times \sqrt{a}}{W \times L^{1/2}}$$

$K_{IC}$ is the apparent fracture toughness.
Results

The mean and standard deviation of apparent fracture toughness were summarized in Table 1. Data analysis was performed using One-way ANOVA and Dunnett’s test at a significant level of 0.05.

The mean apparent fracture toughness of the monolithic IPS e.max® Ceram was significantly greater than that of the bilayered IPS e.max® Press/IPS e.max® Ceram, whereas, the apparent fracture toughness of IPS e.max® Press was significantly greater than those of the other groups.

After testing, specimens were observed using magnifying glass and optical microscope to determine if the crack was initiated at the tip of chevron-notch and the failure occurred along the interface. All of the specimen failures were found at the prepared triangular notch. The optical microscope revealed that the crack was initiated at the tip of the triangular notch and the failure occurred along the interface (Figure 4).

The fracture patterns were examined under SEM to ensure the modes of failure. It was found that the fracture originated between core ceramics (IPS e.max® Press) and veneering half (IPS e.max® Ceram). Accordingly, the interfacial failure pattern was classified (Figure 5 & 6).

Discussion

Microtensile bond strength test is one of the most practical test methods to study the bond strength of core-veneer ceramic restorations. However, the stresses obtained from these studies might not represent the actual bond strength since the failure did not occur at the interface [12]. Moreover, micro tensile bond strength is influenced by specimen geometry, substrates and thickness of the substrates [16]. Chevron Notch Beam (CNB) was developed and used to evaluate the interfacial fracture toughness [21,22]. This test was first developed for fracture toughness test of high strength metal or ceramics, and other brittle materials [23,24]. Anunmana et al. [20] used this test to investigate the interfacial fracture toughness of bonded core-veneer bilayered dental ceramics by initiating the crack propagation through the bonded interface of the core ceramics and their corresponding veneer using the chevron-notch short bars.

In this study, the bonded interface was modified from the previous study [20] to be 3x4mm in order to obtain smaller specimens and to decrease the internal defects. It was much simpler for this specimen geometry and no specimens failed during preparation. According to the effects of surface bonding area, the smaller bonding area produced the higher bond strength than the larger bonding area [24]. Although the preparation of the

<table>
<thead>
<tr>
<th>Ceramic groups</th>
<th>Number of specimens</th>
<th>Interfacial toughness (MPam^{1/2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>IPS e.max® Ceram</td>
<td>15</td>
<td>0.75±0.06a</td>
</tr>
<tr>
<td>IPS e.max® Press</td>
<td>15</td>
<td>2.34±0.28b</td>
</tr>
<tr>
<td>IPS e.max® Press/IPS e.max® Ceram</td>
<td>15</td>
<td>0.68±0.05c</td>
</tr>
</tbody>
</table>

Groups with the same superscript are not significantly different.
Fracture mechanics approach to determine bonding quality of two ceramics

Figure 4. The representative image of fractured specimens of IPS e.max®Press/IPSe.max® Ceram. The ceramic core materials were on the left side and their corresponding glass veneers were on the right. Failure pattern was classified as interfacial failure.

Figure 5. The representative SEM image of fractured surface of monolithic blocks of glass veneer (IPS e.max®Ceram) reveal critical crack of specimen that originated from the tip of chevron-notch.

Figure 6. The representative SEM image of fractured surface of core-veneer (IPS e.max®Press/IPSe.max® Ceram) showed different surface between exposed surface of core material and veneering side, indicating the interfacial failure.

chevron-notch bars require a specific technique and devices, the sample preparation could be carefully prepared. The tensile mode was used to avoid the bending stress. The slow loading rate was suggested to propagate the stable crack growth. Therefore, the crosshead speed 0.1 mm/min was chosen. The load-displacement curve of a specimen tested in this study is shown in (Figure 7).

The curve peak represented a stable crack growth, which typically obtained from the chevron-notch specimens in this study. After the test started, there was a non-linear increasing load pattern, then, it turned into the linear mode. After that, the slope of loading curve was decreased to almost zero which was attributed to the stable

Figure 7. The load-displacement of a chevron-notch specimens.
crack growth.

In calculation of the apparent interfacial fracture toughness, the value of $Y_m$ was calibrated from the material which known fracture toughness. Based on a previous study [20], the monolithic glass veneer was used as a control group to calculate the $Y_m$ constant. It was approximately 5 for these specimens. We recognized the triangular notch as the one of the weakest part since all of the specimens fracture occurred at the interfacial area. The pre-crack loads were not required due to the high stress concentration propagated at the tip of the chevron notch. Correlatively to the studies in 2016 [25,26], the short rod chevron-notch was used to assess the enamel bonding effectiveness. The overall failure of specimens propagated through the interface. Moreover, the finite element analysis model revealed the mainly tensile stress found at the tip of the triangular notch. Likewise, all specimens in this study fracture at the prepared triangular notch when they were observed using a stereomicroscope. The crack propagated between or near the core-veneer layer. Correspondingly, the interfacial fracture toughness value of the bilayered group IPS e.max® Press/IPS e.max® Ceram was significantly lower than the fracture toughness of the monolithic veneer IPS e.max® Ceram. This study suggested that the fracture toughness of the interface was not greater than the fracture toughness of the veneering material.

The failures of bilayer ceramic restoration might occur due to the residual stresses. Taskonak et al. [7] reported that the residual stress values were detected in bilayer lithium disilicate core-veneer specimen. The incompatibilities of CTE cause the residual stress to develop between core and veneering ceramics [8,27]. Mismatch of CTE also generates stress gradients within the veneer and affects the crack propagation, likewise, it affects the interfacial adhesion between core ceramics and their veneer layers. Although, the previous studies [6,10] suggested that the minimal difference in CTE can increase the chipping resistance of bilayered ceramic restorations in the clinical application, Dehoff et al. [9] suggested that the CTE difference in the range of $-0.61 \times 10^{-6} \cdot K^{-1}$ to $+1.02 \times 10^{-6} \cdot K^{-1}$ is acceptable. Based on the present result in this study, the chevron-notch test can be used as an alternative test to determine the apparent interfacial fracture toughness of bilayered dental ceramics. However, in preparation of the triangular notch at the interface using the diamond blade might cause the microcracks near the cutting area and these cracks might interfere with the crack propagation during testing. Further improvement in preparation of triangular notch at the interface such as a gentle cut using the laser technology should be used if possible. The size of the specimen needs to be verified. The further studies in different type of ceramics are useful. In addition, the study about stress distribution between core and veneer of bilayered specimens may be useful in explaining the fracture propagation and pattern for this test.

**Conclusion**

In this study, the chevron-notch approach was used to evaluate the effectiveness of the ceramic/ceramic bonding. Fracture surface examination indicated the crack propagated within the interface. Specimen size and cutting angle of the triangular notch could be controlled. Therefore, this test can be used as a valid alternative to analyse the bonding quality of the adjoining ceramic materials.

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**Competing interests** : None  
**Ethical approval** : No requirement  

**Reference**
Fracture resistance of four dental computer-aided design and computer-aided manufacturing glass-ceramics

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Department of Prosthodontics, Faculty of Dentistry, Mahidol University

Objective: The objective of this study was to compare the flexural strength of four dental computer-aided design and computer-aided manufacturing (CAD/CAM) glass-ceramics, i.e., lithium-disilicate-based (IPS e.max CAD; EMX), leucite-based (IPS Empress CAD; EMP) and two zirconia-reinforced lithium silicate glass-ceramics (CELTRA DUO; CD and VITA SUPRINITY; VS).

Materials and methods: Thirty bar-shaped specimens (1.5mm x 4mm x 18mm) were prepared for each material by cutting from rectangular blocks, and finishing their surfaces by glazing. All specimens were loaded to fracture at a crosshead speed of 0.5 mm/min using a three-point bending fixture mounted on a universal testing machine. The flexural strength values were calculated and statistically analyzed by one-way ANOVA and Dunnett T3 (α=0.05). The strength data of all groups were also analyzed using the Weibull Statistics.

Results: The result from a statistical analysis showed that VS (319±42MPa) had the highest flexural strength following by CD (278±49MPa) and then EMX (236±20MPa). EMP (157±14MPa) showed the lowest flexural strength. The Weibull moduli and the characteristic strength were 9.02 and 336.97 for VS, 6.55 and 298.87 for CD, 13.90 and 244.69 for EMX, 12.77 and 164.02 for EMP.

Conclusions: For CAD/CAM glass-ceramics examined in this study, the zirconia-reinforced lithium silicate glass-ceramics had higher flexural strength and characteristic strength than a lithium-disilicate-based and leucite-based glass-ceramics.

Key word: flexural strength; leucite-based; lithium-disilicate-based; three-point bending; zirconia-reinforced lithium silicate


Introduction

All-ceramic restorations are widely used due to their acceptable mechanical properties and esthetics [1,4]. Lithium-disilicate-based glass-ceramic is an all-ceramic material currently used in the fabrication of single- and multi-unit dental restorations. It can be customized to match the appearance of natural teeth such as color and translucency. This material is fabricated by the hot-pressing technique. For IPS e.max Press (Ivoclar Vivadent, Schaan, Liechtenstein), its microstructure consists of approximate 70% lithudisilicate crystals (Li2Si2O5), which are embedded in a glassy matrix. IPS e.max CAD (Ivoclar Vivadent, Schaan, Liechtenstein) was introduced in 2005 for a computer-aided design and computer-aided manufacturing (CAD/CAM) technique [5].

Leucite-based glass-ceramic consists of a glassy matrix and leucite crystals. The glass matrix is based on an alumino-silicate glass. Leucite crystal is used to reinforce a glass-based material and to improve its mechanical properties. IPS Empress CAD (Ivoclar Vivadent, Schaan, Liechtenstein) is a CAD/CAM machinable ceramic. It was introduced in 2006 with a flexural strength of
about 160 MPa and designed to be used either with chairside or laboratory-side CAD/CAM systems. This material can be used for veneers, inlays, onlays, and anterior crowns.

For zirconia-reinforced lithium silicate glass-ceramics, CELTRA DUO (Dentsply, Hanau-Wolfgang, Germany) was introduced in 2012 and VITA SUPRINITY (VITA Zahnfabrik H. Rauter GmbH&Co.KG, Bad Säckingen, Germany) was introduced in 2013. These materials contain a large number of very fine-grained lithium crystallites, which high glass content gives the material its excellent optical and mechanical properties. Their compositions contain approximate 10 percent by weight of ZrO2. It has a comparable flexural strength to lithium-disilicate-based ceramic. They can be used for single-unit anterior and posterior crowns, inlays, onlays and veneers. VITA SUPRINITY’s strength is approximate 420 MPa by three-point flexural method. Weibull modulus is approximate 8.9.

The Weibull distribution is the most widely used function in describing strength data of ceramics. Ceramic strength data are generally not normally distributed, but often skewed in the high strength portion [6]. The Weibull characteristic strength is the strength occurring at a probability of failure at 63.2% [7]. The Weibull modulus (m) is the parameter describing the shape of the distribution of strength as a function of failure probability [8]. Higher values of Weibull modulus relate to greater materials structural reliability, consistent flaw distribution and their strengths would be narrowly distributed. The Weibull moduli of most ceramics are reported in the range of 5-15 [9].

The objective of this study was to compare the flexural strength of leucite-based (IPS Empress CAD), lithium-disilicate-based glass-ceramic (IPS e.max CAD) and zirconia-reinforced lithium silicate glass-ceramics (CELTRA DUO and VITA SUPRINITY) using three-point bending method. The characteristic strength (σf) and Weibull modulus (m) of the materials were determined using the Weibull statistics.

### Materials and methods

#### Specimen preparation

Four dental ceramics (IPS e.max CAD, IPS Empress CAD, CELTRA DUO and VITA SUPRINITY) were examined in this study. Thirty ceramic bar-shaped specimens were fabricated following the manufacturer’s instructions for each ceramic. Bar specimens were cut into the dimension of 1.5 mm x 4 mm x 18 mm using a low-speed diamond-coated disk with water cooling. The four sharp edges of the specimens were chamfered to the length of between 0.09 to 0.15 mm and at 45° angle with a 600-grit SiC paper. IPS e.max CAD and VITA SUPRINITY were further crystallized according to the firing program provided by the manufacturer. All bars were coated by their glazing materials and fired according to their firing schedules to obtain glazed surfaces which were used as a control surface condition for all experimental groups.

#### The three-point bending test

For the flexural strength (σf) measurement, all specimens were loaded in three-point bending at a cross-head speed of 0.5 mm/min using a universal testing machine (LF Plus Lloyd instruments, Ametek, Inc., USA). Failure loads were recorded and the flexural strength values were calculated using the following equation [1].

\[
σ_f = \frac{3Pl}{2wb^2}
\]

Where \(σ_f\) is the flexural strength (MPa), \(P\) is the load at fracture (N), \(l\) is the center-to-center distance between the support rollers (mm), \(W\) is the width of the specimen (mm), \(b\) and \(I\) is the thickness of the specimen (mm). The flexural strength values of all groups were statistically
analyzed using one-way ANOVA and Dunnett T3 at the significance level of .05.

The Weibull modulus \((m)\) and the characteristic strength \((\sigma_0)\) were calculated relative to the cumulative probability of failure \((P_f)\) as shown in Equation [2]. [6]

\[
P_f = 1 - \exp\left[-\left(\frac{\sigma}{\sigma_0}\right)^m\right]
\]  

[2]

The fractographic analysis

The fractured specimens were ultrasonically cleansed in 35% ethanol solution for 5 minutes and mounted on a metal stub. They were coated with a 5-nanometer-thin gold-palladium using a sputter-coating machine (SPI Module®, SPI Supplies, Structure Probe Inc., Canada). Then, the crack initiation flaw of each specimen was located and the patterns of the fractured markings were observed using a light microscope (Nikon SMZ1000, Japan).

Results

The mean flexural strengths of all groups are summarized in Table 1. The flexural strength values of the all groups were significantly different from each other. VITA SUPRINITY had the highest flexural strength while IPS Empress CAD possessed the lowest strength. The two estimated parameters of the Weibull statistics, the modulus \((m)\) and the scale \((\sigma_0)\) of all groups are described in Table 2. The Weibull probability plots of four dental ceramics are demonstrated in Fig1. Even the characteristic strength of VITA SUPRINITY was also the highest but its modulus \((m)\) was lower than that of IPS e.max CAD. CELTRA DUO had the lowest modulus \((m)\) value.

Discussion

The CAD/CAM system is a popular processing technique that is used for fabricating

Table 1. The flexural strength of the four experimental groups.

<table>
<thead>
<tr>
<th>Material</th>
<th>Flexural Strength (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITA SUPRINITY</td>
<td>319.0 ± 42.8\textsuperscript{a}</td>
</tr>
<tr>
<td>CELTRA DUO</td>
<td>278.9 ± 49.7\textsuperscript{b}</td>
</tr>
<tr>
<td>IPS e.max CAD</td>
<td>245.3 ± 23.5\textsuperscript{c}</td>
</tr>
<tr>
<td>IPS Empress CAD</td>
<td>157.1 ± 14.9\textsuperscript{d}</td>
</tr>
</tbody>
</table>

Values with different superscript letter are statistically different \((P < 0.05)\).

Table 2. Weibull modulus and characteristic strength of the four experimental groups.

<table>
<thead>
<tr>
<th>Material</th>
<th>Weibull modulus ((m))</th>
<th>Characteristic strength ((\sigma_0))</th>
</tr>
</thead>
<tbody>
<tr>
<td>VITA SUPRINITY</td>
<td>7.2</td>
<td>336.9</td>
</tr>
<tr>
<td>CELTRA DUO</td>
<td>5.3</td>
<td>298.8</td>
</tr>
<tr>
<td>IPS e.max CAD</td>
<td>9.7</td>
<td>254.7</td>
</tr>
<tr>
<td>IPS Empress CAD</td>
<td>10.4</td>
<td>162.4</td>
</tr>
</tbody>
</table>
Figure 1. The Weibull probability plots of four dental glass-ceramics.

Figure 2. The representative fracture surfaces of VITA SUPRINITY dental ceramic showing the failure origin on the surface at 30x (a) and 120x (b).

Figure 3. The representative fracture surfaces of IPS e.max CAD dental ceramic showing the failure origin on the surface at 30x (a) and 120x (b).
numerous of all-ceramic restorations in dentistry. One of the main advantages of using CAD/CAM is its fast processing speed and many types of restoration can be delivered in one-visit appointment. The prefabricated blocks that used to fabricate dental restorations are made from their manufacturers. Therefore, flaws and defects formation would be less compared with the laboratory-made restorations [10].

A new dental ceramic material is developed and aimed to match the appearance of natural teeth such as color, translucency. It should have excellent mechanical strength and toughness to obtain structural reliability during dental functions [11]. New innovation and technique such as addition of small and tough crystal particles is frequency used. Zirconia-reinforced lithium silicate glass-ceramics (CELTRA DUO and VITA SUPRINITY) are examples of these new dental ceramic materials. According to the manufacturers, these materials offer mechanical strengths ranging from 370 to 420 MPa which are comparable to lithium-disilicate-based glass-ceramics (IPS e.max Press and IPS e.max CAD) [12].

Flexural strength is a significant mechanical property that is frequently used to evaluate the fracture resistance of brittle materials. [9] In this study, the flexural strength of leucite-based glass-ceramic, lithium-disilicate-based glass-ceramic and zirconia-reinforced lithium silicate glass-ceramics were determined. The results showed that the flexural strength values of these materials are significantly different from each other. VITA SUPRINITY had the highest strength (319 MPa) which was lower than those obtained from a manufacturer and a previous study [13]. Its

Figure 4. The representative fracture surfaces of CELTRA DUO dental ceramic showing the failure origin on the surface at 30x (a) and 120x (b)

Figure 5. The representative fracture surfaces of IPS Empress CAD dental ceramic showing the failure origin on the surface at 30x (a) and 120x (b)
strength was higher than that of CELTRA DUO which had comparable composition and microstructure. A lithium-disilicate-based glass-ceramic (IPS e.max CAD) had lower strength than those of zirconia-reinforced lithium silicate glass-ceramics but its strength was higher than that of leucite-based glass-ceramic (IPS Empress CAD).

From the fracture surface analysis, the failure origins of most specimens were at the glazed surfaces (Fig.2-5). For zirconia-reinforced lithium silicate glass-ceramics, the glaze layer of these materials was more homogeneous and resulted in a very small failure origin when compared with a lithium-disilicate-based glass-ceramic. A large defect in the glaze layer of a lithium-disilicate-based glass-ceramic could result from the heterogeneity of material compositions or porosity created during glazing material application. This large defect in the glaze layer was also observed in leucite-based glass-ceramic (IPS Empress CAD). This large defect in the glaze layer might be the reason for the lower strength for dental glass-ceramic materials. Glazing is a procedure supposed to increase the mechanical strength of all-ceramic restorations due to the reduction in porosity and surface flaws [14]. However, the major fault occurred in this step is the formation of porosities during condensation procedure or from an inappropriate powder/liquid mixing ratio [15]. These porosities could be the failure origin sites [16] and this idea was confirmed in this study especially when the glaze layer was thick. In this study, the thicknesses of the glaze layer ranged between 40-60 µm.

After crystallization, zirconia-reinforced lithium silicate glass-ceramics consist of a glassy matrix and very fine lithium metasilicate and lithium disilicate crystals with submicron-size ranges and high glass content [12,17]. Because of this high glass content, the fractured surfaces of zirconia-reinforced lithium silicate glass-ceramics were clearly observed with evident fracture markings. Unlike a lithium-disilicate-based glass-ceramic which had larger crystals and lower glass content, its fractured surface was rough and it was more difficult to observe the fracture origin and markings.

The flexural strength of IPS e.max CAD obtained in this study was closely to that reported by Albero A. et al [18], but it was lower than that reported from Coldea A et al [19]. This difference could be attributed from the specimen preparation with or without glazing for these studies. The flexural strength of IPS Empress CAD was comparable to the results obtained from previous works [18, 20, 21].

The Weibull modulus and the Weibull characteristic strength are generally used to describe the cumulative probability of failure corresponded to the failure stresses of dental ceramics. The characteristic strength ($\sigma_0$) is the strength at a probability of failure of 63.2 %. The Weibull modulus ($m$) is a parameter demonstrating the shape and width of strength distribution as a function of failure probability. Materials with high Weibull moduli are more predictable and less likely to break at a stress much lower than a mean value. A typical Weibull modulus for ceramics is reported to be 5-15 [9,13].

In this study, the two estimated parameters, the Weibull modulus ($m$) and the characteristic strength ($\sigma_0$) were determined. Ideally, a clinically reliable dental ceramic should tolerate high chewing forces during a long service period. This means that it can be used for a posterior restoration (high loading area) and survived for many years of service (excellent reliability). In order to accomplish these goals, materials with high characteristic strength ($\sigma_0$) and Weibull modulus ($m$) should be utilized. For four CAD/CAM dental glass-ceramics investigated in this study, the Weibull modulus was the highest for IPS e.max CAD, while VITA SUPRINITY possessed the highest characteristic strength. The values obtained for these two parameters were not consistent for any material. Therefore, there should be long-term clinical
researches to assure the benefit of use of these materials corresponding to their clinical applications claimed by the manufacturers.

**Conclusion**

Within the limit of this study, these conclusions could be drawn;

For CAD/CAM glass-ceramic blocks, the zirconia-reinforced lithium silicate glass-ceramics had higher flexural strength and the characteristic strength than a lithium-disilicate-based and leucite-based glass-ceramics. The characteristics of a glaze layer had an effect on the flexural strength of all dental glass-ceramics used in this study.

**Acknowledgements**

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Effect of resin volume fraction on fracture toughness of resin-infiltrated ceramic.

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Objective: The objective of this study was to investigate effect of resin volume fraction on fracture toughness of resin-infiltrated ceramic (IPS e-max® Ceram).

Materials and Methods: Forty bar specimens (2x4x24 mm) were divided into 4 groups. The control group was IPS e-max® Ceram specimens. They were prepared following the manufacturer’s recommendation. The other groups were IPS e-max® Ceram infiltrated with 0.5%, 1% and 2% by volume of Silane/UDMA/TEGDMA resin mixture. They were prepared by mixing IPS e-max® Ceram glass powder with polymeric fiber (0.5%, 1% and 2% by volume) until homogenous and added liquid to form slurry. Then, the mixture was packed into the mold and fired according to the firing schedule without vacuum. After that, resin mixture was infiltrated into the specimens under vacuum at 0.01 MPa for 2 hours then cured by dry heat in the oven at 100°C for 6 hours. Vickers hardness tester was used to create pre-crack indentation on the specimen. Four-point bending test was performed using a universal testing machine with a crosshead speed of 0.5 mm/min until fracture. Fracture surfaces of all specimens were examined under optical light microscope. Critical flaw sizes were measured using the fractographic approach, and fracture toughness (K_Ic) was calculated. One-way ANOVA was used to determine and analyze a significant difference of the mean K_Ic between control group and 0.5%, 1% and 2% by volume of resin-infiltrated ceramic groups at α=0.05.

Results: The mean fracture toughness of control group, 0.5%, 1% and 2% of resin-infiltrated ceramic were 0.69±0.05, 0.71±0.08, 0.77±0.11 and 0.80±0.11 MPa·m^1/2 respectively. Only 2% resin-infiltrated ceramic specimen showed the significant increasing on fracture toughness comparing with the control group, (p < 0.05).

Conclusions: The amount of resin volume fraction of resin infiltrated ceramic had an influence on its fracture toughness.

Key words: resin volume fraction, resin infiltrated ceramics, fracture toughness, fractography, asymmetrical crack pattern, cracked deflection mechanism


Introduction

Nowadays, the trend to use all ceramic restorations increases due to their excellent biocompatibility, good mechanical property and higher esthetic compared with porcelain fused to metal crown [1]. However, the most common complication of all - ceramic restoration is chipping or cracking on veneering ceramic because of its brittleness, crack propagation and low tensile strength [2]. Recently, the application of translucent monolithic zirconia has increased. The advantage of this material is high strength that can solve the problem of cracking of veneering ceramic. On the other hand, it is still more opaque than conventional ceramic. In order to achieve the esthetic, substructure core is still made by zirconia and veneering with feldspathic ceramic [3].

The important mechanical property that defines the capability to prevent fracture or catastrophic failure of a material is fracture toughness [4]. In addition, it represents the serviceability of dental material in the oral cavity [5].
Urethane dimethacrylate monomer (UDMA) is the most common resin monomer using in many dental products due to its relatively low viscosity and high flexibility of its structure linkage that may promote high fracture toughness [6,7]. UDMA can be used alone or mixed with the other low viscosity diluent monomer, for example, Triethyleneglycol dimethacrylate (TEGDMA) [6,7].

In resin composite, it consists mixture of ceramic filler particles to strengthen the soft and more flexible resin materials to improve its mechanical properties to be a long-lasting serviceable restorative material. It was found that ceramic filler can raise its strength, fracture toughness and can resist crack propagation [8,9].

Like composite resin materials, many researchers attempt to increase fracture toughness of dental ceramic by using the flexible resin infiltrated into the porous ceramic to increase its flexibility leading to increase its fracture toughness. Chaiyabutr et al [10] investigated effect of UDMA/TEGDMA resin-infused alumina. They found that the resin infusion technique can improve the strength and fracture toughness of alumina matrix [10]. Moreover, Coldea et al [11] attempted to create novel material called polymer-infiltrated-ceramic-network (PICN) by infiltration of UDMA/TEGDMA resin mixture into porous ceramic. They reported that the high volume of polymer fraction of polymer infiltrated ceramic group has greater flexural strength and stain resistance but lesser elastic modulus and hardness than these of the low volume of polymer fraction group [11, 12].

However, those researches were aiming to increase the fracture toughness of the core materials with a special industry preparing porous ceramic. From the initial study of in-laboratory technique to produce the resin infiltrated veneering ceramic by Urapepon et al [13]. They found the feasibility of this technique. Therefore, the objective of this study was to investigate the effect of resin volume fraction on fracture toughness of resin-infiltrated ceramic.

Materials and methods

Veneering glass ceramic powder (IPS e.max Ceram, Ivoclar-Vivadent, Schann, Liechtenstein) 1.5 g, density at 3 g/ml was initially mixed with 0.00375g, 0.0075g and 0.015g (0.5, 1 and 2 vol %, respectively) of chopped polymeric fiber, density at 1.51 g/ml, 75 μm in diameter and 3.5 mm long, to prepare a space for resin infusion. The ceramic liquid (Ceram liquid, Ivoclar-Vivadent, Schann, Liechtenstein) was added to the powder to form a slurry. The ceramic slurry was filled and condensed into the bar shape mold, size 2x4x24 mm. The condensation of the powder was done using plugger and vibrator. The specimens were removed from the mold and placed on the firing tray until dry. The control specimen, veneering glass ceramic powder without polymeric fiber added was prepared as same as the experimental specimen. Ten bars for each were prepared.

After drying, the specimens were fired in a ceramic furnace according to the company recommendation for firing schedule. However, the experimental specimens were fired without vacuum in order to relieve the polymeric vapor pressure during firing. After firing and cool down, the experimental specimens were immersed in a mixture of urethane dimethacrylate (UDMA), triethylene glycol dimethacrylate (TEGDMA) and Benzoyl peroxide (BPO) on the ratio 74.5:24.5:1 wt%. After that 2% by weight of 3-acryloxypropyl trimethoxysilane were added. The resin mixture was infiltrated into the specimens under vacuum at 0.01 Mpa for 2 hours, and subsequently cured by dry heat in the oven at 100°C for 6 hours. All specimens were grinded and polished with silicon carbide paper (# 400, 800, 1000 and 1500) and finally finished with 0.05 μm alumina particles.

The fractographic analysis method (FTA) was used to identify the critical crack size for fracture toughness calculation. A well-defined controlled flaw for fractographic analysis was made by indentation on the surface using a Vickers indenter (FM-700, Future-Tech Corp., Tokyo, Japan) at 9.8 N load for 15 sec.

All indented specimens were tested using four-point flexure on universal testing machine (LF Plus, LLoyd instruments, Ametek Inc., USA). The specimens were placed with the pre-cracked side under tension and located centrally on the bearers (20 mm supporting span, 10 mm loading span). The bars were loaded until fracture
Effect of resin volume fraction on fracture toughness of resin-infiltrated ceramic.

at a crosshead speed of 0.5 mm/min. and the flexural strength was calculated.

The critical flaw sizes on fracture surfaces of all specimens were measured under optical light microscope (Nikon Eclipse E400 Microscope, Fukuoka, Japan) at 100X magnification. Fracture toughness, $K_{IC}$, is calculated using the equation

$$K_{IC} = Y \sigma_f c^{1/2}$$

Where $Y$ is the geometric factor for sharp cracks that are induced by Vickers indentation (1.65), $\sigma_f$ is the flexural strength (MPa), $c$ is the critical crack size (m) calculated from the equation $c = (ab)^{1/2}$ where $a$ is the crack depth and $b$ is the half crack width.

One-Way ANOVA and Tukey’s test were performed to compare the mean fracture toughness of each group at 95% confidence level ($p < 0.05$).

The fracture surfaces of all specimens were examined under optical light microscope (Nikon Eclipse E400 Microscope, Fukuoka, Japan) at 25X, 50X magnification and under scanning electron microscopic (Quanta 250, FEI, Oregon, USA) at 60X, 500X magnification.

**Results**

The mean fracture toughness of control group, 0.5%, 1% and 2% resin-infiltrated ceramic were $0.69 \pm 0.05$, $0.71 \pm 0.08$, $0.77 \pm 0.11$ and $0.80 \pm 0.11$ MPa·m$^{1/2}$, respectively.

It was found that only fracture toughness of 2% resin-infiltrated ceramic ($0.80 \pm 0.11$ MPa·m$^{1/2}$) was significantly higher than that of control ($0.69 \pm 0.05$ MPa·m$^{1/2}$) while the other were not significant ($p > 0.05$).

The optical light micrograph of fracture surfaces of specimens at magnification of 25X and 50X were presented in figure 1. The crack patterns of the control group are symmetry while the other resin-infused ceramic groups, the cracks have asymmetrical pattern and greater deflection than that of control group.

The scanning electron micrographs of fracture surfaces of specimens at magnification of 60X and 500X are presented in figure 2.

Resin-infiltrated ceramic groups have porosities distribution greater than the control group and the high resin volume fraction in ceramic group has porosities more than the low resin volume fraction group.

**Discussion**

This study intended to create the method of resin-infiltration into veneering ceramic during the process in dental laboratory. The dental technician can add the polymer fiber into veneering ceramic to produce the space for resin infusion. Then they can perform a conventional technique to veneer the glass on its substructure. After the firing, the polymeric fiber will burn out and left the replica space in ceramics [13]. This space can be filled by resin mixture to create interpenetrating phase [14] by using vacuum method and this process can be used as a grazing process to smoothen the glass ceramic surface.

From the result of this study, the resin infiltration had effect on the fracture toughness of the glass ceramic. Although only the highest load of resin infiltration (2%) group had fracture toughness greater than the control group.

From the optical light microscope examination at Vickers’ indentation point on resin-infiltrated ceramic specimen, the crack extensions from indentation diagonals crack run though ceramic parts had the deflection at polymer-ceramic interfaces (Figure 3) and found greater asymmetrical pattern than control group.

This conformed to the study by He and Swan [12], which reported that the polymer infiltrated ceramic had higher fracture toughness as a result of a crack deflection mechanism. In dense ceramic, the crack propagation occurred around of ceramic crystalline but in polymer infiltrated ceramic, the crack propagation occurred pass into the polymer phase. Moreover, from the investigation of Coldea et al [11], they reported that the crack extension form indentation diagonals of dense ceramic was greater than polymer infiltrated ceramic and the crack run through ceramic part but the defect occurred at polymer-ceramic interface. It can imply that the interpenetrating...
Figure 1. Fracture surface at the crack initiation point of specimens at magnification of 25x and 50x. (Images a-b) IPS e-max Ceram (control), symmetrical crack patterns (red arrows) are presented. (Images c-h) 0.5%, 1% and 2% resin-infiltrated ceramic, asymmetrical crack patterns with deflection (yellow arrows) are presented.
Effect of resin volume fraction on fracture toughness of resin-infiltrated ceramic.

Figure 2. SEM micrograph of fracture surface of specimens at magnification of 60x and 500x. (Images a-b) IPS e-max Ceram (control), small round porosities distribute. (Images c-h) 0.5%, 1% and 2% resin-infiltrated ceramic, larger porosities and tube-like porosities are presented.
phase of resin infiltrated ceramic can increase crack resistance [14]. These effects can improve the durability of the material and decrease chipping of veneering ceramic [14].

Although the polymer infiltration has an effect to the fracture toughness of veneering ceramic, the effect also depends upon the amount of volume fraction. This study, the effect was statistically significant when the volume fraction was up to 2%. This result corresponds with the findings by Coldea et al [11], they reported that the high volume of polymer fraction in polymer infiltrated ceramic group had high flexural strength and stain resistance but lower elastic modulus and hardness.

In this study, the maximum volume of resin infiltrated ceramic was end up at 2% although the fracture toughness result showed promising higher when the volume fraction increase more than 2%. The preliminary study found that the specimen was dimension, after firing difficult to hold their dimension. Moreover, the color of the resin infiltrated ceramic seemed to change a little bit grayish. This may be due to the residual acrylic fiber that was unable to burn out completely or burn out vapor was incorporated in the ceramic and also the higher amount of resin infiltrated might showed some affected on the original color of ceramic. Therefore, the highest volume fraction of this study was end up at 2% by volume. There should be further studies to increase more volume fraction and improve the properties of this material, including color are required.

Figure 3. Illustration of optical light micrograph of Vickers’ indentation on resin-infiltrated ceramic specimen that shown the crack deflection at polymer-ceramic interfaces.(red arrow)

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Competing interests: None
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References


Fluoride Release from Different Powder Liquid Ratios of Fuji VII

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Introduction: The quantity of fluoride ions released from glass-ionomer cements is of major importance in the prevention of dental caries in children. Fuji VII is a glass-ionomer that releases more fluoride ions than other fluoride releasing materials.

Purpose: To evaluate the concentration of fluoride ions released from the Fuji VII with differing powder liquid (P/L) mixing ratios.

Methods: Eight cylindrical specimens from four groups with different P/L ratios were prepared and immersed independently in 10 mL of deionized water. The fluoride release was evaluated on days 1-7 using a fluoride ion specific electrode. Statistical analyses of the difference between fluoride concentrations were analyzed using one-way ANOVA and Tukey’s multiple comparison test.

Results: The fluoride released by the glass ionomers (GIs) was found to be highest during the first 24 h and decreased significantly; lower levels were obtained on day 7. Fuji VII P/L ratio 4:4 and Fuji VII P/L ratio 3:4 showed similar patterns and quantity of fluoride release, which were significantly lower than Fuji VII P/L ratio 2:4 and Fuji VII P/L ratio 1:4.

Conclusions: Fuji VII P/L ratio 2:4 and Fuji VII P/L ratio 1:4 released more fluoride than Fuji VII P/L ratio 4:4 and Fuji VII P/L ratio 3:4.

Keywords: fluoride release, glass ionomer, Fuji VII, fluoride releasing materials, powder liquid (P/L) ratio.


Introduction

Dental caries, a common oral disease among Thai children, can result in pain and chewing difficulties. Recently, the Thai Dental Health Division (2012) [1] showed the prevalence of caries in Thai children in their report based on the findings of the Thai National Oral Health Survey. They found that more than 45% of Thai children aged 3-12 years with dental caries did not receive dental treatment. This situation is a cause of concern for pediatric dentists as they recognize the importance of and have the responsibility for oral health of children. Dental treatment today not only removes caries lesions but also changes the demineralization process caused by acid fermentation by bacteria into the remineralization process in enamel, helping the prevention of initial caries in children³. The role of fluoride in preventing dental caries is well-established [3-5]. It is well understood that fluorides have an anti-cariogenic property and they prevent initiation and progression of caries [2]. Various restorative materials containing fluoride in their formulation are currently available on the market. These materials, including composite, compomers, and glass ionomers are able to release fluoride ions. The amount of fluoride release has been found to be consistently higher in glass ionomer materials than in other restorative...
Woranun Prapansilp, et al.

Glass ionomers (GIs) have been used for more than 20 years and their major advantage is their potential to inhibit caries in children [9,10]. There are many types of glass ionomers, including Fuji II LC, Fuji IX and Fuji VII. Fuji VII is a glass ionomer that was developed from conventional glass ionomers. It was developed to correct the disadvantageous properties of conventional glass ionomers. It is used as sealant and is a surface protectant material that can prevent dental caries. Among fluoride releasing materials, Fuji VII has shown the highest fluoride release [11]. This property may be useful in prevention of initial caries in Thai children. Fuji VII has is used as a hand-mixed preparation; some studies have shown that by varying the powder/liquid (P/L) mixing ratio of GIs it is possible to change their mechanical and physical properties [12,13]. Moreover Torabzadeh et al (2015) [14] has demonstrated that changing the P/L mixing ratio has an effect on the amount of fluoride release. Considering the significant the effect of P/L ratio on the fluoride release from glass ionomers, there is a lack of adequate studies and many of the issues in controversial studies have not been addressed. Therefore, this in vitro study aimed to investigate the amount of fluoride release from the glass ionomer Fuji VII, changed with varying P/L ratios.

**Materials and Methods**

This experimental study was conducted using the GI Fuji VII (GC Corporation, Tokyo, Japan).

Four groups of 8 specimens were prepared. Each group used a different P/L ratio

- **Group P/L ratio 4:4**, mixed as recommended by the manufacturer
- **Group P/L ratio 3:4**, this is 25% less powder than the ratio recommended by the manufacturer
- **Group P/L ratio 2:4**, this is 50% less powder than the ratio recommended by the manufacturer

The powder and liquid were mixed according to the Fuji VII manufacturer’s instructions (GC Corporation) within the instructed time period. To make the specimen the Fuji VII specimen was transferred to a plastic mold measuring 3 mm in diameter and 5 mm in depth. A piece of thin thread was placed inside the molds in such way that one end of the thread was out of the mold. This thread was used to suspend the specimens in the container which prepared for immersed the specimen. A plastic strip and a glass slab were placed on the molds to better pack the Fuji VII into the mold and allow any excess material to leak out. All specimens were polymerized for 20 s on both sides with a curing unit (3M™ ESPE™ Curing Light XL3000, 3M, Germany). After hardening, the specimens were removed from the mold and transferred into 10 mL of deionized water and stored at 37 °C. Specimens were transferred to new plastic containers with fresh deionized water every 24 h.

**Fluoride analysis**

Concentrations of released fluoride ions were measured using a fluoride-specific ion electrode (ORION EA™ 940 expandable, ORION, USA) connected to a ORION digital ion analyzer (ORION MODEL 96-04, 96-09, USA). Prior to each measurement, the electrode was calibrated using five standard fluoride solutions of 0.1, 1, 10, 20 and 100 ppm fluoride. The slope of the calibration curve varied between -54 to –60 at 25 °C.

Measurements were performed by pipetting 10 mL of each sample solution into a clean plastic test tube, adding 1 mL of TISAB II (Total ionic strength adjustment buffer, 940911, USA) and stirring for 3 min by magnetic stirrer (Clifton Cerastir™, CHARAN Associateds CO, LTD.) before measurement. The measurement was repeated three times, the mean (±SD) of the
fluoride concentrations were recorded. Fluoride concentrations were automatically displayed on the analyzer and converted to parts per million (ppm).

**Statistical Analysis**

The difference in fluoride concentrations among experimental groups at the same time points were analyzed using one-way ANOVA and Tukey’s multiple comparison test, with the level of significance set at p < 0.05. Differences in fluoride concentrations in the same group at the different time points were analyzed using one-way repeated measures ANOVA follow by Tukey’s multiple comparison test, with the level of significance set at p < 0.05.

**Results**

Mean (±SD) concentration of fluoride released (ppm) from specimens in the four groups day 1-7 are shown in Table 1 and Fig 1. One-way ANOVA indicated that, at the same time point, the maximum fluoride release for days 1-7 was found in P/L ratio 1:4 group (75% less powder than recommended by the manufacturer). The P/L ratio 2:4 group had significantly higher fluoride release than the P/L ratio 3:4 group and the P/L ratio 4:4 group. Between day 1 and 7 there was no statistically significant differences in fluoride release between the P/L ratio 3:4 group and the P/L ratio 4:4 group, the ratio recommended by the manufacturer (p > 0.05).

![Figure 1](http://www.dt.mahidol.ac.th/division/th_Academic_Journal_Unit)

**Table 1.** The mean (±SD) of the amount of fluoride (ppm) released from Fuji VII with different P/L mixing ratios. Differences in superscript letters indicate statistically significant differences within columns, and differences in superscript numbers indicate significant differences within rows (p < 0.05)

<table>
<thead>
<tr>
<th>Group P/L ratio</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; day</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; day</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; day</th>
<th>4&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>5&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>6&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>7&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr 1 4:4</td>
<td>1.35 ± 0.30&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.59 ± 0.13&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.35 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.24 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30 ± 0.06&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.34 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.26 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gr 2 3:4</td>
<td>1.51 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81 ± 0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.40 ± 0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gr 3 2:4</td>
<td>3.23 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.85 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.15 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.83 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.81 ± 0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gr 4 1:4</td>
<td>8.78 ± 0.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.83 ± 0.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.38 ± 0.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.54 ± 0.27&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.82 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.59 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.03 ± 0.21&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Discussion

Dental caries is one of the most prevalent chronic dental diseases affecting Thai children. Its progression or control depends on the balance between the demineralization and remineralization processes. One strategy for caries prevention focuses on stopping demineralization and improving the remineralizing process by using fluoride.

Many studies have showed that GI materials were the most suitable materials for prevention dental caries in children because of their continuous fluoride release. This continuous fluoride release promotes reduction of the mineral dental structure solubility. Because fluoride ion concentrations are essential for caries prevention and treatment, measuring of the amount of fluoride released from GIs is significant in understanding the cariostatic properties of GIs.

We found that the pattern of fluoride release from specimens in all groups released the highest amount of fluoride during the first 24 h and the levels of release decreased sharply in the following 7 days. This result was similar to the finding of Vermeersch et al. (2001) and Bayrak et al. (2010) and Torabzadeh et al. (2015). Two mechanisms have been proposed by which fluoride may be released from glass-ionomers. One mechanism is an initial high fluoride release over the first 24 h, likely due to the burst effect of fluoride released from the glass particles when reacting with the polyalkenoate acid during the setting reaction; this mechanism is short-term and rapid. The second mechanism is more gradual and results in the sustained diffusion of ions through the bulk cement. Since fluoride is released from glass ionomer cements, it has been suggested that they will be clinically cariostatic.

The specimens with P/L ratio less than the manufacturer’s recommended ratio appeared to release significantly more fluoride than specimens prepared as recommended by the manufacturer. Similar results were reported by Torabzadeh et al. (2015) and Muzynski et al. (1988) and Perrin et al. (1994). The reason for decreased P/L ratios increasing the amount of fluoride released may be explained by the fact that an increase in solubility increases the dissolution of the exposed surface areas of the cement being release to higher amounts of fluoride ions.

With an increased P/L ratio the solubility of the glass ionomer increases and consequently its constituents, including the fluoride ions, also become more soluble. The high initial release and followed by a low but sustained release of fluoride ions from Fuji VII are vitally important in the remineralization of dental enamel and dentine. This release-remineralization mechanism may have clinical therapeutic implications in vivo. This short-term in vitro study can be used to inform in future in vivo studies.

Conclusion

The results showed that the release of fluoride from the glass ionomers, Fuji VII, was time-dependent and decreased over-time. Decreasing the P/L mixing ratio had a significant effect on increasing fluoride release.

Acknowledgements

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Ministry of Public Health, (cited 2015 Sep 11)


Flexural strength of relined denture base using different thickness of self-cured relining material

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Objectives: To evaluate the flexural strength of a heat-cured denture base polymer relined with different thickness of self-cured relining materials.

Materials and methods: Two different thickness ratios (1.5:1.5 and 2:1) of one heat-cured denture base polymer (ProBase Hot) to three self-cured hard relining materials (Kooliner PEMA, Tokuyama Rebase II PEMA and Unifast Trad PMM) were examined. Three point bending flexural test was applied on the relined specimens (64mm x10mm×3mm) and on intact specimens of similar dimension with 3, 2 and 1.5 mm thickness. Each specimen was immersed in 37±1°C distilled water for 43±2 hours prior to relining and testing. Statistical analysis was performed using one-way ANOVA and Tukey's HSD post-hos test (α = 0.05).

Results: There were significant differences in flexural strength among denture base relined with different materials, in both thickness ratios. The specimens relined with Unifast Trad possessed the highest flexural strengths (60.77 ± 5.88 MPa), while those relined using Tokuyama Rebase II displayed the lowest strength (40.55 ± 1.04 MPa). No significant differences were found when ProBase Hot was relined with different thickness (1.5mm and 1mm) of Tokuyama Rebase II (40.55 ± 1.04 MPa and 44.25 ± 1.79 MPa), Kooliner (45.80 ± 2.25 MPa and 50.20 ± 2.95 MPa), and Unifast Trad (60.77 ± 5.88 MPa and 60.77 ± 5.88 MPa).

Conclusions: Self-cured hard relining materials have an effect on the flexural strength of relined denture base. Flexural strength of denture base relined with PMMA is higher than that relined with PEMA. Different thickness ratio of denture relining polymer had no effect on the flexural strength of relined denture base.

Key words: Flexural strength, Denture base polymer, Relining denture base, Thickness ratio


Introduction

Existing removable prostheses dentures become loose over the period of time because of the continuous progressive alveolar bone resorption and bone remodeling of edentulous ridge [1-3]. In addition, the processing of alveolar bone resorption may compromise the adaptation of a dental prosthesis in some areas of the oral mucosa. The poor-fitting denture will move to any direction resulted in mucosal trauma, contribute to compromised function and rapid residual ridge reduction [4-6]. For this reason, denture must be evaluated periodically. Denture relining material could be used to recover the existing prosthesis adaptation to the patient’s oral mucosa by the technique which performed directly in the patient’s mouth (with auto-polymerizing resin) or indirect technique in a laboratory (with heat-polymerizing resin). Direct relining of denture base is carried out...
with the auto-polymerizing acrylic resin to improve the fit of denture base and maintaining the prosthesis-tissue relationship [7]. Generally, removable prosthesis made of poly (methyl methacrylate) (PMMA), which has reliable with resins containing MMA. Some studies reported that acrylic monomers with different chemical compositions presented lower bond strength of PMMA denture base polymer [8-10]. The widely use of the materials to reline denture is an acrylic resin polymer that similar to the original denture base material. An important property of denture relining materials is adequate mechanical strength [11,12].

Over the last two decades, there were many numbers of studies [11-19]. have been investigated the effect of relining materials on flexural strength of denture base by using different types of relining materials. Measurement the flexural strength of denture base material is always taken through flexural test and using a three point bending test, specimen was bend under one loading nose [11-19].

Some studies have demonstrated the flexural strength of relined denture bases lower than the intact denture base and significantly decreased flexural strength of denture base after relining [11,14-16]. However the information that showed flexural strength value of relined denture base by using the different thickness ratio of relining material to existing denture base are still limited.

Objective of this present study was to evaluate the flexural strength of heat-cured denture base when relined using three self-cured hard relining materials with 2 thickness ratios.

Materials and Methods

One heat-cured denture base polymer and three self-cured hard relining materials were used in this investigation. The manufacturers, fabrication process, mixing proportions of powder to liquid, mixing and working time, and curing procedures of these materials are listed in Table 1.

Four different size of the metal molds (64.5 mm × 10.5 mm × 3.5 mm, 64.5 mm × 10.5 mm × 2.5 mm, 64.5 mm × 10.5 mm × 2 mm, and 64.1 mm × 10.1 mm × 3 mm) were used and mounted with dental stone type III into the dental flasks. Three different thickness of intact specimens of ProBase Hot (Ivoclar Vivadent, Schaan, Liechtenstein), Tokuyama Rebase II (Tokuyama Dental, Tokyo, Japan), Kooliner (GC America Inc., IL, USA) and Unifast Trad (GC Corporation, Tokyo, Japan) in size of 64mm×10mm × 3mm, 64mm×10mm×2mm and 64mm×10 mm×1.5mm were fabricated by using the metal mold size 64.5mm×10.5mm×3.5mm, 64.5mm×10.5mm ×2.5mm and 64.5×10.5×2mm, respectively. The intact specimens were served as control groups (n=3). All materials were manipulated according to the manufacturers. After polymerization, each specimens was polished with standard metallographic paper number p500, p1000, p1200 to make all surfaces of specimen smooth before testing. Then all those specimens were immersed in 37 ±1°C distilled water for 43 ± 2 hours before testing the flexural strength.

Thirty-six specimens in the size of 64mm×10mm×2mm and the another thirty-six specimens in the size of 64mm×10mm×1.5mm of ProBase Hot were fabricated from stainless steel molds with cavity 64.5mm×10.5mm×2.5mm and 64.5mm×10.5mm×2mm, respectively. Each thirty-six denture base specimens was divided into three sub-groups (n=12). Each sub-group specimens was prepared for relining with three different self-cured hard relining materials. After polymerization, the specimens were wet polished with p500 grinding paper to obtain the desired dimension. The superior surface of specimen which to be relined was polished with p120 grinding paper to create retention for the relining materials. Six groups of denture base specimens were immersed in 37 ± 1°C distilled water for 43 ±
Table 1. Materials used in this investigation

<table>
<thead>
<tr>
<th>Material</th>
<th>Manufacturer</th>
<th>Processing procedure</th>
<th>Powder-liquid ratio</th>
<th>Mixing time</th>
<th>Working time</th>
<th>Curing procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProBase Hot (PMMA)</td>
<td>Ivoclar Vivadent Schaan, Lichenstn</td>
<td>Heat-cured compression molding</td>
<td>2.25g : 1ml</td>
<td>10 mins. (including time for leaving mixture to become dough stage)</td>
<td>20 mins.</td>
<td>Standard procedure (recommended procedure). Place mold in cool water, heat up 100 °C and boil it for 45 minutes.</td>
</tr>
<tr>
<td>Tokuyama Rebase II Fast (PEMA)</td>
<td>Tokuyama Dental Corporation, Tokyo, Japan</td>
<td>Self-cured</td>
<td>2.4g : 1ml</td>
<td>5-10 seconds</td>
<td>20 -60 seconds</td>
<td>Apply adhesive to the surface of denture. Mix powder into liquid and then apply the mixture on denture surface. It will be set in 6-8 minutes. Then Prepare Tokuyama Resin Hardener II and immerse the relined denture for 3 minutes.</td>
</tr>
<tr>
<td>Kooliner (PEMA)</td>
<td>GC America Inc, IL, USA</td>
<td>Self-cured</td>
<td>2.5ml : 1ml</td>
<td>No more than 30 seconds</td>
<td>1-2 mins</td>
<td>Mix powder into liquid slowly and then spread the mixture over the area to be relined, wait about 10 mins until it set.</td>
</tr>
<tr>
<td>Unifast Trad (PMMA)</td>
<td>GC Corporation, Tokyo, Japan</td>
<td>Self-cured</td>
<td>1g : 0.5ml</td>
<td>10-15 seconds</td>
<td>2 mins</td>
<td>Mix powder into liquid and then apply the mixture on denture surface. Then wait until it reaches setting time.</td>
</tr>
</tbody>
</table>

2 hours. After water storage, each specimens was placed into the metal mold with a cavity 64.1mm×10.1mm×3mm in dimensions, relined with hard relining material to made 3mm thickness specimen. Each self-cured hard relining materials (Tokuyama Rebase II, Kooliner and Unifast Trad) was mixed according to the manufacturer’s instruction. The mixture of each relining materials was applied to the superior surface of ProBase Hot which polished with p120 grinding paper. Then, each relined specimens was polished with 500, 1000, and 1200 grinding paper on the grinding machine to get smooth surfaces. The dimension of relined specimens were 64mm×10mm×3 mm. The relined specimens were stored in 37±1°C distilled water for 43 ± 2 hours.
Flexural strength testing was carried out by applying a three-point bending test in a Universal testing machine (Model EZ-S, SHIMADZU, Japan, Load Cell<500N). Each specimen was taken from the distilled water, measured with a digital caliper (Mitutoyo, Japan) to verify the dimension, then immediately placed on the flexural test supporters which immersed in 37±1 °C water bath. The testing was designed by placing the surface of the ProBase Hot face down to receive the tensile stress as showed in Figure 1. A vertical load was applied at the midpoint of each specimen at a crosshead speed of 5mm per minute on a load test machine until the specimen break down. The highest load at the time of specimen break down was recorded in the computer program and used equation below to calculate the value of flexural strength.

$$\sigma = \frac{3FL}{2bh^2}$$

$\sigma$ was the flexural strength of specimen (MPa). $F$ was maximum load exerted on the specimen at the time of fracture (N), $L$ was the distance between two supports (mm), $h$ was the height of specimen measured immediately prior to water storage (mm) and $b$ was the width of specimen measured immediately prior to water storage (mm).

The statistical analysis was done by SPSS for windows version 16.0. Normality of the data was determined by the Shapiro-Wilk test. The homogeneity of variances was carried out by using Levene’s test. One-way analysis of variances (ANOVA) was applied to analyze all data at $\alpha = 0.05$. Tukey’s HSD post-hoc test was used for comparing the mean of flexural strength of each relined groups at $\alpha = 0.05$.

**Results**

The mean and standard deviation values of flexural strength of six relined groups ($n=12$) are presented in Table 2. Flexural strength of all control groups ($n=3$) are showed in Table 3. Data distribution of all groups were normal ($p>0.05$). Therefore, One-way ANOVA was utilized to detect the effect of differences in thickness ratio and relining materials on the flexural strength of relined denture base. There was significant difference in flexural strength among six relined...
Tukey’s comparison test revealed that the flexural strength of three relined materials were significantly different (p<0.05). There were no significant differences for different thickness of ProBase Hot (1.5mm and 2mm) when relined with different thickness (1.5mm and 1mm) of Tokuyama Rebase II (40.55 ± 1.04 MPa and 44.25 ± 1.79 MPa), Kooliner (45.80 ± 2.25 MPa and 50.20 ± 2.95 MPa), and Unifast Trad (60.77 ± 5.88 MPa and 60.77 ± 5.88 MPa) as presented in Table 2 and Figure 2. The relined group with Unifast Trad possessed the highest flexural strengths (60.77 ± 5.88 MPa), while Tokuyama Rebase II displayed the lowest strength (40.55 ± 1.04 MPa) as shown in Table 2.

### Discussion

The strength of relined denture base polymer depended on the strength of both denture base polymer and relining material, and the bonding strength between denture base and relining material [11]. Many commercial self-cured hard relining materials have been investigated and claimed for their superior physico- mechanical properties [20-22], including their bond strength to acrylic resin denture base [8,23,24]. Nowadays,
a newly developed elastic and resilient relining resin, which consists of non-MMA-based monomers, generates less heat during polymerization and produces less irritation to oral mucosa when compared to conventional relining materials. [14,25] In this study, the flexural strength of relined denture base was investigated by using three brands of self-cured hard relining material commercially available in Thailand. They were Tokuyama Rebase II, Kooliner and Unifast Trad to reline on one brand of heat-cured acrylic resin denture base, ProBase Hot. Previous study reported that Tokuyama Rebase II presented lower heat generation and higher flexural strength than its predecessor, Tokuso Rebase [26]. Kooliner is a self-cured hard relining material with lower polymerization temperature than other autopolymerizing acrylic resin, can be used more favorable to direct relining denture base in patient’s mouth [22,27-29]. Unifast Trad is a hard relining material with a fast setting time (3min). It is difficult to manipulate in patient with tissue undercut. And the polymerizing state of Unifast Trad produced heat that might be irritate to the oral mucosa.

Results from table 2 showed the flexural strengths of ProBase Hot relined with Unifast Trad were higher than those of Probase Hot relined with Kooliner and Tokuyama Rebase. A possible explanation for this phenomenon is the higher ability of ProBase Hot to bond to Unifast Trad than to Kooliner and Tokuyama Rebase II. ProBase Hot is a high cross-linked polymer composed of Poly (methyl methacrylate) (PMMA) as the powder and methyl methacrylate (MMA) as the liquid. Unifast Trad is also a high cross-linked polymer which its composition was similar to ProBase Hot. Probably to the fact that Unifast Trad is more structurally compatible to ProBase Hot (both Polymethyl methacrylate based) than Kooliner and Tokuyama Rebase II (Polyethyl methacrylate with non-monomer based). High bond strengths of relined denture base were obtained when relining material chemically similar to the denture base. [30-32] Minami H et al. suggested that greater cross- linking occurred between base material consist of similar composition, so similarly for PMMA denture polymer, higher bond strength was reported with MMA-based resin as compared to non-MMA-based resin [31]. The manipulation procedure in this study, Unifast Trad presented higher temperature during polymerization than other two relining materials. Unifast Trad was the

![Figure 2](image-url)

Figure 2. Flexural strength of relined denture base specimens in both thickness ratios
only one PMMA relining material; the rest were PEMAs. The previous studied on hard relining denture base reported that PMMA resins process stronger exothermic heat reactions than PEMA [33]. This result was the same as the other vitro studies on autopolymerizing resin used for direct fabrication of interim restorations [34]. The high temperature of Unifast Trad during reline on ProBase Hot might increase diffusion rate of relining material into the base polymer resulting in good bond strength. Adhesive failure was displayed for all relined specimen in the Kooliner groups (Figure 3). The phenomenon of de-bonding surface of Kooliner to denture base polymer revealed in this study is similar to some previous studies [10,30,35,36]. Adhesive bonding of relined denture base depends on the penetration of polymerizable monomer of relining material into the denture base network [21]. For self-cured hard relining material, higher molecular mass of isobutyl methacrylate (IBMA) monomer might have limited the monomer penetration to denture base [21,23,31]. Kooliner is one of the self-cured hard relining material consist of IBMA. The failure bond strength of Kooliner to denture base could cause the low flexural strength of relined denture base.

Methyl methacrylate monomer (MMA), ethyl acetate (EA) and acetone (AC) are primer and adhesives provided by manufacturers to use as chemical agents for surface treatment of denture base polymer before relining procedure [37-40]. Tokuyama adhesive was applied on ProBase Hot specimen surface before relining with Tokuyama Rebase II. The composition of Tokuyama adhesive consists of 47% ethyl acetate (EA) and 47%. acetone (AC) However, the effect of EA and AC that contained in Tokuyama has not been well established. The difference between the chemical composition ProBase Hot denture base resin and Tokuyama Rebase II Fast denture reline may leads to absence chemical interaction between them, and this seems to be the main reason for the bond failure observed in relined specimen (Figure 4). Thus, the result of this experiment suggest to study how to improve bond strength of Tokuyama adhesive and the proper management of denture base surface before relining procedure.

The present study revealed that 3mm thick intact denture base showed higher flexural strength (72.00 ± 2.80 MPa) than all relined denture base groups (40.55 ± 1.04MPa to 60.77 ± 5.88 MPa). Many studies also reported that the flexural strength of relined denture base was lower than intact denture base [14,41-43]. The reason of decreasing in flexural strength of the relined denture base could be mainly related to the
adhesive failure under load between the relining and the denture base material or from the low strength of relining material. We clearly knew that a relined denture base consists of two different materials with an interface between them and resulting in creation a composite laminate structure. If the composite interface structure is weak, then delamination will be occurred during function [44]. A weak bond will decrease the flexural strength of the relined denture base [8,11]. It may be probably explained by molecular interaction of active site between two surfaces. The active site of denture base to be relined was occupied by the previous curing material, while the added relining material has fully activated active sites. As a result, the compatibility between the two resins is incompetent which leads to weakening of the molecular interaction between the two resins, consequently decreasing the mechanical properties of the relined denture base [45]. This result is in agreement with Baily who reported that the cross linkage between the existing denture base and the new reline resin was not as complete as the initial polymerization process [46].

This current study investigated the flexural strength of two different thickness ratio of denture base to reline material (1.5:1.5 and 2:1). The result displayed that the different thickness ratio of denture base to relining materials had no significant effect on flexural strength of the relined denture base. This result is in agreement with the study of Hatim et al which concluded that there were no significances of relining thickness (0.5mm, 1mm, and 1.5mm) on the flexural strength of denture base polymer [47].

**Conclusion**

Within the limitations of this study, it can be concluded that;

1. Different thickness ratios of denture base to relining materials had no significant effect on flexural strength of the relined denture base.

2. Different self-cured hard relining material had significant effect on the flexural strength of the relined denture base.

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**Competing interests:** None declared  
**Ethical approval:** None (Laboratory study)

**References**


10. Leles CR, Machado AL, Vergani CE, Giampaolo ET, Pavarina AC. Bonding strength between a hard chairside reline resin and a denture base material as


Masking ability of two ceramics with different thicknesses on various substrates

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Objective: The objective of this study was to examine the influence of material type, thickness, and substrate color on the masking ability of two ceramics on various substrates.

Materials and methods: In total, 36 disc-shaped specimens (15 mm in diameter × 0.5- and 1.0-mm thicknesses) were fabricated from lithium disilicate glass ceramic (IPS e.max Press, n = 6), high-translucent zirconia (Lava Plus, n = 6), and high-translucent zirconia with liner material (Lava Plus/Liner, n = 6). Contrast ratios were measured over white and black substrates. Color differences were measured over different substrates: white, black, metal, and resin composite shades A2, A3, and C4. White and A2 substrates were used as reference groups. Contrast ratio and color difference values were analyzed with linear regression (P<0.05).

Results: Contrast ratios in the IPS e.max Press group at 0.5 and 1.0 mm showed the highest values (0.73 ± 0.04 and 0.87 ± 0.01) when compared with those in the Lava Plus and Lava Plus/Liner groups. IPS e.max Press at both thicknesses showed the highest masking ability over various substrates. Higher contrast ratio and masking ability were significantly related to thicker material. Material type, thickness, and substrate were significantly related to masking ability.

Conclusion: Ceramic type, thickness, and substrate color are strongly associated with contrast ratio and masking ability, both of which increase as thickness increases.

Clinical implications: Increased ceramic thickness could benefit masking ability. For improved masking ability, IPS e.max Press is recommended over Lava Plus and Lava Plus/Liner for the masking of dark substrates.

Key words: contrast ratio, lithium disilicate ceramics, masking ability, zirconia ceramics

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Introduction

Over the years, ceramics have been increasingly used for the tooth-colored restoration of anterior teeth [1]. Many factors are involved in the final color of all-ceramic restorations, for example, thickness and translucency of the ceramic, color of the luting resin cement, and color of the supporting substrate [2-5]. The supporting substrate, such as a tooth or artificial materials, plays a major role in the final color of a ceramic restoration [2]. Previous studies have reported that the final color of a veneer was affected by the color of the supporting substrate [6]. The use of a dark or high-opacity substrate resulted in a detectable change of the final color after cementation when compared with that achieved with a light or low-opacity substrate [7]. The thickness of the material regulates its translucency [8,9]. In addition, luting resin cement also influences the final color of a restoration [10]. Therefore, matching the final color of all-ceramic restorations to that of natural teeth is still considered to be a difficult and largely subjective task [11]. Ceramic selection is considered to be crucial for optimization of the aesthetic outcome [1].
The translucency of all-ceramics varies among selected systems and depends strongly on the amount of light-scattering, which is affected predominantly by their microstructure and thickness [8,9,12]. When compared with glass-based ceramics, zirconia is considered to be less translucent [13,14].

Contrast ratio (CR) is considered to be one method for measuring the translucency of all-ceramic systems and has been used in previous studies [15,16]. The relative opacity of ceramics can be measured by the differences between specimens over black and white backgrounds. The space system Yxy was used to measure the contrast ratio as a ratio of reflectance (Yb/Yw), with the value from the specimen placed over a black background (Yb) relative to the value from the specimen placed over a white background (Yw). In contrast, when CR decreases, the translucency of the specimen increases [13,17,18].

The masking ability of all-ceramic systems can be measured by the color differences (ΔΕ) when the specimen is placed over different substrates. There will be no color difference (ΔΕ = 0) if the masking ability is perfect [18]. A color difference in the range of 3.3 to 3.7 was considered to be clinically acceptable, as has been reported by one or more operators, while some studies reported higher values to be clinically acceptable [19, 20].

Previous studies reported that different types of materials and thicknesses resulted in different contrast ratios and levels of masking ability [2,5,6,21]. Unfortunately, none of the previous studies has reported the influence of association across material type, thickness, color of the substrate, and reference color on masking ability. This current study aimed to investigate the influence of material choice and associated variables on contrast ratio and masking ability. The null hypothesis in this study was that ceramic type, thickness, and substrate would have no significant effect on the material's masking ability.

IPS e.max Press and Lava Plus have been introduced as an alternative material for anterior restorative region in recent years. IPS e.max Press (lithium disilicate) represents level of translucency similar to natural tooth, while the ability of masking the color of the underlying substrate may not be as good as those made from zirconia. Lava Plus, the new version of zirconia, in addition with various liners, occupies a higher level of translucency as compared to its predecessors. However, the masking ability of the underlying dark substrate of Lava Plus is still questionable.

Therefore, High Opaque (HO) IPS e.max Press was chosen to compare with Lava Plus and shade MO liner among various substrates, in order to investigate material of choice for anterior restorative in terms of the ability to mask the underlying substrate color, while also to represent similar translucency with tooth structure in order to achieve optimum esthetic outcomes.

Materials and methods

In total, 36 disc-shaped specimens were fabricated from three types of ceramics: IPS e.max Press HO 0 ingot (Ivoclar Vivadent, Schaan, Liechtenstein), Lava Plus (3M ESPE, St. Paul, MN, USA), and Lava Plus/Liner shade MO W2 (3M ESPE). Each group consisted of 12 specimens based on material type and was further divided into two groups (n = 6) according to thickness (0.5 or 1.0 mm), yielding a total of six groups. A post hoc power analysis revealed, on the basis of the mean, a between-groups comparison effect size in the present study (d = 0.91).

The specimens were tested over six substrates: white, black, metal, and resin composite shades A2, A3, and C4 (Z350; 3M ESPE). A spectrophotometer (Ultrascan XE, HunterLab, Reston, VA, USA) with a wavelength range from 360 to 750 nanometers and a view area size of 9.53 mm was used in this study for color measurement.
Fabrication of ceramic specimens

Plastic sheets of 0.5 mm and 1.0 mm were cut into circular discs of 15 mm diameter by means of a heated metal pipe. The specimens were fabricated by the lost wax technique according to the manufacturer’s instructions and were later subjected to air abrasion with two bars of 50 µm aluminum oxide (Renfert GmbH, Hilzingen, Germany) and cleansed ultrasonically (IPS e.max Press Invex liquid; Ivoclar Vivadent) (Figure 1). The ceramic specimens were immersed in distilled water at 37°C ± 1°C for 24 h and polished with 600-, 800-, 1000-, and 1200-grit abrasive papers (Figure 1).

The pre-sintered blocks of Lava Plus were cut into discs of 18 mm diameter with thicknesses of 0.6 mm and 1.2 mm to compensate for 20% shrinkage. After being sintered, the Lava Plus specimens were immersed in distilled water at 37°C ± 1°C for 24 h, then polished with aluminum oxide paper (3M ESPE) of 320, 500, and 1000 grit.

Five measurements were made at five different locations around the center of each disc with a Praecimeter (Aura-Dental GmbH, Aura an der Saale, Germany) to confirm the thickness at 0.5 ± 0.05 mm or 1.0 ± 0.05 mm. All ceramic specimens were immersed in distilled water at 37°C ± 1°C for 24 h before color measurement.

Fabrication of backgrounds

Five different substrates of 37.80-mm diameter and 1.94-mm thickness were studied: white, black, metal, and shades A2, A3, and C4 of resin composite. White and A2 substrates were used as reference groups.

A metal substrate was cast from non-precious metal and sandblasted with two bars of 50-µm aluminum oxide (Renfert GmbH) to eliminate shininess and simulate a metal post in endodontically treated teeth.

To simulate dentin color, the substrates of resin composite shades were fabricated with a metal substrate as a reference. Vinylpolysiloxane (VPS) putty (Variotime, Heraeus, Germany) and light silicone (Silagum, DMG, Hamburg, Germany) were used as duplication materials. Resin composite was preheated to facilitate flow into the silicone mold. It was then pressed onto a glass slab and light-cured with a visible-light-polymerization unit (Demi Plus, Kerr Corporation, Orange, CA, USA) at 750 mW/cm² for 40 s. To prevent penetration of excess light, additional resin composite of 15 mm in diameter and 1.0 mm in thickness was added to the specimen. Clear resin compensated for the excess circumferential space around the specimen.

All resin composite substrates were polished under water coolant in an automatic polishing machine (DPS 3200, IMTECH, Durban, South Africa) with 600-, 800-, 1000-, and 1200-grit abrasive papers. The substrates were immersed in distilled water at 37°C ± 1°C for 24 h before color measurement.

Spectrophotometric analysis

Color measurements of all Lava Plus samples were performed by spectrophotometer (Ultrascan XE, HunterLab). Then, Lava Ceram liner shade MO W2 was applied to the discs with 0.1mm thickness, according to the manufacturer’s recommendations, and the color difference was measured again.

Before each measurement, the spectrophotometer was calibrated with standard black and white substrates according to the manufacturer’s instructions. First, the white control substrate was used as a control group. Later, A2 substrate was used as a control group against black, metal, A3, and C4 substrates, to simulate the colors of natural teeth. Measurements were done for each specimen with various substrates. The equation $\Delta E_{ab}^* = (\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2$ was used to calculate the color differences between and among groups.
Figure 1. Plastic sheets with thicknesses of 0.5 mm and 1.0 mm and diameter of 15 mm (A). Plastic sheets were attached to a ring mold with sprue (B). Investment was removed with a carborundum disc (C). Remaining investment was sandblasted with aluminum oxide (D) and ultrasonically cleansed with IPS Invex Press liquid (E).

Statistical analysis
The data were analyzed by STATA software, version 10. Multiple linear regression analysis was used to determine if any correlation of contrast ratio and masking ability existed among ceramic type, thickness, and substrate. The level of significance was determined at 5% (P<0.05).

Results

Contrast ratio
As shown in Figure 2, specimens in the IPS e.max Press group presented the highest mean contrast ratio at 0.5 and 1.0 mm thicknesses. In addition, the study found that the contrast ratio of specimens in the Lava Plus/Liner group was comparable with that of those in the Lava Plus group and lower than that in those of the IPS e.max Press group. In terms of thickness, the 1.0-mm-thickness sample revealed a contrast ratio higher than that of the 0.5-mm-thickness sample.

The analyses of the associations between and among contrast ratio, masking ability and material type, thickness, substrate, and reference group are shown in Table 1. With regard to thickness, higher contrast ratios were significantly related to thicker material (P = 0.05). Ceramics with a thickness of 1.0 mm showed significantly higher contrast ratios than did 0.5-mm specimens. In addition, mean contrast ratios of specimens in the IPS e.max Press group revealed significantly higher contrast ratios than those in the other groups.

Masking ability
With respect to masking ability, the mean color difference values (ΔE) were likely to be lowest in the IPS e.max Press group, followed by the Lava Plus/Liner group, and were highest in the Lava Plus group (Table 2). In terms of thickness, 1.0-mm-thickness samples were significantly correlated with lower color difference values when
compared with the 0.5-mm-thickness samples (Table 1). In addition, regarding the substrate color with A2 as a reference, A3, C4, metal, and black were less likely to show color difference values. In comparison, with white as a reference, A2, A3, C4, metal, and black, the color difference values were more likely to be higher \((P = 0.05)\).

In this study, we found that the color difference values were significantly related to substrate shade. The A3 substrate revealed the significantly lowest color difference values, followed by C4, metal, and black substrates. In addition, the reference group of A2 substrate presented significantly lower color difference values when compared with the white substrate \((P = 0.05)\).

**Discussion**

In our study, the findings showed that specimens in the IPS e.max Press group presented with significantly higher contrast ratio values than those in the Lava Plus/Liner and Lava Plus groups. In addition, contrast ratio values were strongly correlated with color difference values. As thickness increases, both contrast ratio and color difference values decrease. The findings in this study are also consistent with those of previous studies that reported strong correlation between contrast ratios and masking ability [22,23]. Lava Plus tended to exhibit more translucency when compared with previous Lava materials. Moreover, the liner applied to the Lava Plus ceramic exhibited low opacity, which explained the results showing no significant differences between the Lava Plus/Liner and Lava Plus groups. In addition, IPS e.max Press and Lava Plus samples in this study had no color impregnated into the materials. Meanwhile, a previous study reported that a colored zirconia framework with proper veneering material showed increased masking ability over an underlying dark substrate [24]. Color difference values increase as material changes from IPS e.max Press to Lava Plus/Liner and Lava respectively. In addition, color difference values decrease as substrate changes from black to metal and resin composite shade respectively.

All three ceramic groups in this study demonstrated the strong influence of thickness on increasing contrast ratios, in agreement with the results of previous studies [12,17,25,26]. The findings of this study are also consistent with those of other studies suggesting that thickness and contrast ratios demonstrated a direct linear relationship [6,27,28].

In this study, we found that IPS e.max Press tended to have the highest degree of masking

![Graph showing mean contrast ratio values for all ceramic specimens.](http://www.dt.mahidol.ac.th/division/Th_Academic_Journal_Unit)
Table 1. Association of (A) contrast ratio, (B) color difference values ($\Delta E$), and (C) underlying variables (material, thickness, substrate, reference group)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta coefficients (95% CI)</th>
<th>Unadjusted Odds ratios</th>
<th>Adjusted Odds ratios</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.) Contrast Ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lava Plus</td>
<td>-8.28 (-13.07, -3.48)</td>
<td>(-10.29, -6.26)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- Lava Plus/Liner Lava Ceram</td>
<td>-7.38 (-12.18, -2.59)</td>
<td>(-9.39, -5.37)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- IPS e.max Press (as reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0.5 mm</td>
<td>-10.06 (-13.09, -7.03)</td>
<td>(-11.70, -8.42)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- 1.0 mm (as reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.) Color difference value by white substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lava Plus</td>
<td>2.4 (1.61, 3.20)</td>
<td>(2.14, 2.67)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- Lava Plus/Liner Ceram</td>
<td>1.65 (.86, 2.44)</td>
<td>(1.38, 1.92)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- IPS e.max Press (as reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0.5 mm</td>
<td>3.36 (2.86, 3.87)</td>
<td>(3.15, 3.58)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- 1.0 mm (as reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substrates</td>
<td></td>
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</tr>
<tr>
<td>- Metal</td>
<td>-1.27 (-2.25, -0.28)</td>
<td>(-1.61, -.92)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- A2</td>
<td>-3.04 (-4.03, -2.06)</td>
<td>(-3.39, -2.70)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- A3</td>
<td>-3.13 (-4.11, -2.14)</td>
<td>(-3.47, -2.79)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- C4</td>
<td>-2.33 (-3.31, -1.34)</td>
<td>(-2.67, -1.98)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- Black (as reference)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.) Color difference value by A2 substrate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Lava Plus</td>
<td>0.62 (.11, 1.13)</td>
<td>(.41, .84)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- Lava Plus/Liner Ceram</td>
<td>0.26 (-.25, .77)</td>
<td>(.04, .47)</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>- IPS e.max Press (as reference)</td>
<td></td>
<td></td>
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<tr>
<td>Thickness</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- 0.5 mm</td>
<td>.75 (.34, 1.16)</td>
<td>(.57, .93)</td>
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<tr>
<td>- 1.0 mm (as reference)</td>
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<tr>
<td>Substrates</td>
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<td></td>
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<tr>
<td>- Metal</td>
<td>-1.26 (-1.58, -0.93)</td>
<td>(-1.50, -1.01)</td>
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<tr>
<td>- A3</td>
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<td>(-3.03, -2.54)</td>
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<td>- A4</td>
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<td>- Black (as reference)</td>
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Table 2. Distribution of color difference value (ΔE), (mean±SD) across different materials, thickness, and substrate.

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<th>Metal</th>
<th>White</th>
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<td>2.20±0.42</td>
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<tr>
<td></td>
<td>1.0</td>
<td>5.61±0.29</td>
<td>1.54±0.22</td>
<td>5.08±0.27</td>
<td>1.04±0.20</td>
<td>4.23±0.28</td>
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<td>12.48±0.50</td>
<td>3.90±0.42</td>
<td>10.98±0.51</td>
<td>2.29±0.37</td>
<td>8.93±0.52&lt;br&gt;0.58±0.34</td>
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<tr>
<td></td>
<td>1.0</td>
<td>9.29±0.16</td>
<td>2.73±0.11</td>
<td>8.07±0.28</td>
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<tr>
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<td>Lava Plus/Liner</td>
<td>Lava Plus</td>
<td>IPS e.max Press</td>
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<td>Reference Substrate</td>
<td>Reference Substrate</td>
<td>Reference Substrate</td>
<td>White</td>
<td>White</td>
<td>Reference Substrate</td>
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</tr>
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<tr>
<td>1.0</td>
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<td>7.04±0.32</td>
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</table>
ability, followed by Lava Plus/Liner and Lava Plus. The color differences in Lava Plus/Liner and Lava Plus also showed significant differences in masking ability. This result could be explained by the increased thickness of Lava Plus after liner was applied. In addition, the liner conferred slight opacity, which influenced masking ability on the underlying substrates. Therefore, we can assume that the liner was responsible for the increased masking ability.

In addition, thickness is one of the factors that influence masking ability. Samples with 1.0-mm thickness tended to have higher masking ability than those with 0.5-mm thickness. This was probably a simple direct result of the increased 0.5-mm distance that light must penetrate [29]. Moreover, substrate color also influenced final color perception: every substrate but the A2 and A3 showed color difference values that differed significantly from each other.

Translucency reinforces natural tooth characteristics. A low-translucency material is able to mask underlying dark backgrounds but might not create natural tooth characteristics. To achieve ideal esthetic outcomes, restorative materials should have proper opacity that can mask the underlying substrate color and offer optimum translucency to represent that of the teeth [30]. Therefore, the core material should be chosen carefully, since it affects the final color outcome [31].

The clinically acceptable color difference in dentistry ranges from 3.3 to 3.7 [18-20,32]. In this study, the smallest color difference of samples over a black substrate, with white substrates as a reference group, was 5.61, which is worse than the clinically acceptable value. The results revealed that none of the materials tested was able to mask the underlying dark substrate in the clinically acceptable range when a white substrate was used as the reference. In contrast, when A2 was used as a reference, the results showed that the color difference values of all materials tested at 0.5- and 1.0-mm thickness over metal substrate were in the clinically acceptable range. In addition, in 1.0-mm-thick samples over black substrates, the color difference values of IPS e.max Press and Lava Plus/Liner exceeded the clinically acceptable range. The materials tested in this study showed lower color difference values when A2 was used as a control substrate, compared with the white control group. The results showed that samples over yellowish substrates had a high tendency to lower color difference values when compared with those over white substrates. The possible explanation for the results could be that the A2 substrate exhibits yellowish pigment, while white substrate has no color impregnated. Therefore, color difference values between control groups were drastically changed when the substrate was changed from white to A2 substrate. In addition, optimum thickness of both IPS e.max Press and Lava Plus with or without liner under C4 substrate were all capable to mask the underlying substrate color in clinically acceptable range. Both A2 and C4 shades exhibit yellow pigments. Consequently, the results show slight difference in number on C4 substrate when A2 was used as a control group.

It is important to take into account the limitations of ceramics in masking ability, in terms of influencing factors. The first issue to be considered is that different materials have different microstructures and masking abilities. Second, a thicker material tends to have a higher degree of masking ability than a thinner material. In addition, substrates also play an important role in the final color outcome. Further, to achieve optimum esthetic outcomes, interactions between and among factors should be strongly considered. Finally, a yellowish substrate contributes a higher degree of masking ability when compared with a white substrate.

Therefore, within the limitations of this study, the following conclusions can be drawn:

1. The factors ceramic type, thickness, and substrate color had a strong influence on the
masking ability of lithium disilicate and zirconia ceramics.

2. A higher masking ability of the ceramic was significantly related to its thickness.

3. A darker substrate color was significantly related to a lower masking ability of ceramics when compared with that achieved with a lighter-color substrate.

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Competing interests: None declared
Ethical approval: Not require

References


Effect of orthodontic forces on human dental pulp: A systematic review

Kanok-on Tantipanichkul, Kanin Nimcharoensuk, Suwannee Luppanapornlarp

Department of Orthodontics, Faculty of Dentistry, Mahidol University

Introduction: Orthodontic force application stimulates a biological response of the dental pulp. The pulpal response to orthodontic force involves cell damage, inflammation, and wound healing. The pulpal repair rate, pulpal obliteration by secondary dentin formation, root resorption, and pulpal necrosis have all been associated with orthodontic treatment.

Objective: The aim of this systematic review was to investigate the influence of orthodontic force on human pulpal reaction.

Methods: Electronic search was made until July 15, 2016. Additional studies were identified by hand search of reference list of relevant articles from the electronic search. Search terms were the following keywords: “orthodontic force”, “tooth movement”, orthodontic treatment”, “pulpal blood flow”, “vitality loss”, “necrosis”, “pulpal reaction”, “pulpal cellular response”, “pulpal alteration”, and “inflammatory response”. Two independent reviewers assessed the eligibility for inclusion, extracted the data, apply quality indicators, and grade level of evidence.

Results: Forty-one studies matched the inclusion criteria. The outcome concerned the effect of orthodontic force on pulpal blood flow (PBF) in 9 studies, the influence of orthodontic force on human pulpal cellular responses in 23 studies, and the pulpal reaction of orthodontic force on previously traumatized teeth in 5 studies.

Conclusion: There is a lack of high-quality scientific evidence to prove that orthodontic forces affect in alteration of human dental pulp. However, applying orthodontic force on traumatized teeth is considered a risk factor for vitality loss of dental pulp.

Keywords: orthodontic force, tooth movement, dental pulp, pulpal blood flow, pulpal reaction, human


Introduction

After orthodontic tooth movement, there is a series of changes in dental pulp. Because pulpal tissue is located in a rigid dentinal cover, its vitality depends on blood vessels passing through the apical foramen. Changes in pulpal blood flow or vascular tissue pressure can endanger the health of dental pulp.[1] The pulpal response to orthodontic force involves cell damage, inflammation, and wound healing. Force application from orthodontic tooth movement evokes an acute inflammatory response in the PDL, which is presented by vasodilatation and migration of leukocytes out of the capillaries. [2] These migratory cells produce various local biochemical signal molecules and cytokines, which interact with the population of native periodontal cells. A day or 2 later, the acute phase is replaced by a chronic process involving fibroblasts, endothelial cells, osteoblasts, and alveolar bone marrow cells. During this phase, leukocytes continue to migrate into strained periodontal tissues and modulate the remodeling process. [3,4]

Many researchers have examined the effect...
of orthodontic forces on the oral tissues. [5-9] The majority of these researchers were concerned with the reactions of the alveolar bone and periodontal ligament, [7-9] while fewer have dealt with pulpal changes. [5,6]

There are various methods to evaluate the pulpal response after orthodontic force application. Human pulpal blood flow has been shown with the use of laser Doppler flowmetry (LDF), which is a noninvasive method that can repeatedly measure pulpal blood flow (PBF) without causing damage to the pulp. [10] Moreover, electrical pulp testing (EPT) and thermal pulp testing provide simple methods of acquiring information, which is helpful in evaluating the sensitivity of a tooth. [11]

Furthermore, the histologic study has reported depression of pulp tissue respiration, vacuolization, circulatory disturbances, hemorrhage, fibro-hyalinosis and even necrosis as the major pulpal changes following orthodontic force application. [12-14] The prolonged and excessive orthodontic forces may result in loss of pulp vitality. [5]

Orthodontic treatment of traumatized teeth in several studies showed root resorption. [15-17] Only few studies have been made to analyze the effect of orthodontic tooth movement on the pulpal vitality of previously traumatized teeth. There was the incidence of pulp necrosis on previously traumatized teeth after orthodontic force application. [18,19]

Understanding the effects of orthodontic force on the pulp is importance, especially because altered pulpal reparation rate, pulpal obliteration by secondary dentin formation, internal root resorption, and pulpal necrosis have all been associated with orthodontic treatment. Therefore, the purpose of this systematic review was to investigate the relationship between orthodontic force and pulpal reaction in both traumatized and non-traumatized humans’ teeth. The results of this systematic review could help orthodontists to understand whether the pathology of the pulp, which might occur in response to force-induced therapeutic tooth movement, is transient or permanent and could help them in determining long-term prognosis of the teeth.

Materials and methods

Focusing on this question, “Does orthodontic force affect to the reaction of human dental pulp?”, this systematic review was undertaken by following the guidelines provided by the PRISMA statement. [20]

Data sources and searches

Electronic searches were conducted for published studies up to July 2016. The databases searched are shown in Table 1. The reference lists of the articles eligible for inclusion in this investigation were also manually reviewed. Citations of articles published in journals, dissertations, and conference proceedings were located by searching several electronic databases, using a search strategy appropriately adjusted for each individual database (Table 1).

No restrictions were applied concerning publication year, language, or status. Grey literature was not excluded from our search. If additional information was needed, the authors were contacted. Hand-searching of potentially relevant original and review articles was also performed. This was done to identify any studies that could have remains unidentified in the previous step and checked for disagreement via discussion among the authors.

Study selection

Two review authors (K.T. and K.N.) independently screened all titles and abstracts obtained from the database searches. Duplicate records, such as published articles also presented in conferences, studies with multiple publications, and dissertations also published as journal articles, were excluded. The same authors reviewed the
full texts of the potentially relevant titles and abstracts against the inclusion criteria. The eligibility of the trials was assessed independently. Any disagreement was resolved by consultation with the third and the fourth author (S.L. and N.T.) until a final consensus was achieved. Appropriate studies to be included in this systematic review fulfilled specific predefined inclusion criteria; only randomized controlled clinical trials (RCTs), prospective and retrospective controlled clinical trials (CCTs), and prospective cohort studies were included in current investigation (Table 2).

Data extraction
Two reviewers (K.T. and K.N.) independently extracted relevant data in a pre-designed collection form. Any disagreement was resolved by discussion with the third and the fourth author (S.L. and N.T.) until a final consensus was achieved.

Quality assessment
Two reviewers (K.T. and K.N.) evaluated independently the methodological quality of the included studies according to a grading system developed by the Swedish Council on Technology Assessment in Health Care, [21] which was based on the criteria for assessing study quality from the Centre for Reviews and Disseminations in York, United Kingdom. [22] The methodological quality criteria are listed as follows.

Grade A (High) – Randomized controlled trial or prospective study is composed of a well-defined control group; defined diagnosis and end points; diagnostic reliability tests and reproducibility tests described; and blinded outcome measurements (all criteria should be met).

Grade B (Moderate) – Cohort study or retrospective case series is composed of a defined control or reference group; defined diagnosis and end points; and diagnosis reliability tests and reproducibility tests described (all criteria should be met; if not, grade C).

Grade C (Low) – one or more of the following conditions are found: large attrition of the sample, unclear diagnosis and end points, and poorly defined patient material.

Table 1. The electronic databases searched, the search strategies used, and the corresponding results

<table>
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<tr>
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<th>Extend of search</th>
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<td>All fields</td>
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Table 2. Eligibility criteria used in this systematic review

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<td>Interventions</td>
<td>• Studies investigating any orthodontic treatments except orthognathic surgery and any orthodontic treatments with surgical intervention</td>
<td>• Distraction osteogenesis</td>
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<td></td>
<td>• Auto tooth transplant</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Debonding orthodontic braces</td>
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<td>Outcomes</td>
<td>• Studies investigating on the effect of orthodontic forces on pulpal blood flow or pulpal responses</td>
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Results

Effect of orthodontic forces on pulpal blood flow (PBF)

Nine studies reported the effect of orthodontic force on PBF (Table 3). Laser Doppler flowmetry was used in all of the studies to measure the PBF. Orthodontic force application had a significant effect on basal blood flow in every study. The number of study samples ranged between 8 and 30 patients. Types of orthodontic force were applied to achieve maxillary canine retraction in 1 study, intrusion of maxillary incisors in 5 studies, intrusion of maxillary first molars in 2 studies, and rapid maxillary expansion (RME) in 1
study. Eight studies [14,23-29] reported the orthodontic force applied to teeth ranged between 25 and 4,400 grams. Another study of RME did not report the amount of force. The duration for orthodontic force application ranged between 4 minutes and 6 months. Three studies [14,23,25] reported that intrusive force can reduce PBF temporarily, and the next three studies [24,26,27] found that decreasing of PBF after intrusive force application is a reversible effect. PBF decreased at day 3, continued to remain suppressed until 3 weeks, and it tended to return to baseline values in about 3 weeks. Moreover, Sabuncuoglu and Ersahan [28] found that after intrusion of incisors, PBF decreased significantly in 24 hours. Then, it continued to increase gradually in 3 days and 7 days, and finally returned to control level at 3 weeks. Sabuncuoglu and Ersahan [29] also reported that PBF underwent a significant decline at 24 hours after canine retraction, then returning to near-baseline levels within one month, and Babacan et al. [30] found that PBF increased in the first week, and decreased by the third week of RME because of separation of the median palatal suture. Then, PBF tended to return to baseline level after 3 months of retention.

**Effect of orthodontic forces on the cellular responses of the dental pulp**

There are 23 prospective studies reported the influence of orthodontic force on human pulpal cellular responses (Table 4). Patients’ age ranges from 10 to 29 years. Types of orthodontic force included extrusion (8 studies), intrusion (7 studies), tipping (6 studies), and rapid palatal expansion (2 studies). All of them performed on premolar teeth. The magnitude of force ranged between 25 to 300 g for duration ranging between 21 minutes and 180 days. Almost all studies, including study of Veberiene et al., [31] reported even if orthodontic treatment can cause temporary metabolic changes in dental pulp, they are reversible. However, only one study [32] stated some of alterations, such as root anatomy and root resorption, were permanent in teeth which the root was not completed but pulp alteration, such as vacuolization, seemed to improve after removing intrusive force. Hamersky et al. [5] found that the orthodontic forces decrease pulpal respiration rate and increasing age causes a larger lowering rate. Han et al. [12] reported dental pulp still has vitality and no necrotic observed after intrusive treatment. Caviedes Bucheli et al. [33] reported CGRP expression increased in dental pulp of teeth submitted severe orthodontic forces. Four studies of Derringer [34-37] confirmed that extrusive forces stimulated angiogenic growth factor and subsequently increased numbers of microvessels in the pulp. However, Mostafa et al. [38] found circulatory disturbances, including vacuolization and edema of pulpal tissues.

**Influence of orthodontic force on the pulpal responses in traumatized teeth**

Five retrospective studies [18,19,39-41] on upper incisors investigated the pulpal reaction of orthodontic force on previously traumatized teeth (Table 5). Four studies [19,39-41] reported the type of tooth movement such as tipping, intrusion, and extraction. Three studies [19,39,40] reported the duration of orthodontic force application in previously traumatized teeth range from 3.2 to 7.2 months. All studies showed that there was a higher frequency of pulp necrosis of orthodontically treated previously traumatized teeth. [18,19,39-41] Incidence of pulp necrosis was especially found in teeth with fracture of enamel-dentine, teeth with subluxation, extrusion, lateral luxation and intrusion. [39] Moreover, traumatized teeth with total pulp obliteration had higher susceptibility to pulpal complication such as pulp necrosis during orthodontic intrusion than that without or with partial pulp obliteration. [19]
Discussion

Grading the evidence is complicate and numerous scales have been proposed to use. Using a scale to allocate points to individual quality items has proved to be inadequate. [53] From currently indexed literature, there is still no agreed goal standard quality assessment system. [54,55] Lack of high-quality scientific evidence such as randomized controlled trials studies and a disharmony in the study protocols are limitations of this systematic review. Most of the studies were graded moderate to low methodological quality (Table 3-5). Low grading was mainly based on no random assignment to experimental and control treatment groups and no description of reliability tests.

This systematic review was performed to evaluate the effect of orthodontic force on dental pulp tissues. It was hypothesized that long duration or high magnitude of orthodontic force is harmful for human pulp vitality. In the studies that assessed the influence of orthodontic force to PBF, there were various magnitude and duration of orthodontic force application (25 - 4,400 g and 4 minutes – 6 months, respectively). It is presumed that the application of light orthodontic force about 50 grams for a short duration might not cause any change or make just a minor change in PBF comparing with longer duration. However, there is no scientific evidence in the magnitude and duration of orthodontic force to PBF. Furthermore,
<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study subjects</th>
<th>No. of teeth</th>
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<th>Magnitude of force (g)</th>
<th>Duration of force (days)</th>
<th>Conclusion</th>
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<td>Babacan et al. (2010)</td>
<td>21 10-15 (13.1)</td>
<td>42 Upper central incisors, 28 Upper canines, 42 Upper first molars</td>
<td>Before expansion</td>
<td>RME (bonded type: upper first premolars to second molars) Measuring PBF on upper central incisors, canines and first molars</td>
<td>N/A (1 turn/day)</td>
<td>33.6 (4.8 ±0.7 weeks)</td>
<td>PBF increased in the first week, and decreased by the third week of RME. PBF tended to return to baseline level after 3 months of retention.</td>
</tr>
<tr>
<td>Barwick and Ramsay. (1996)</td>
<td>8 25-49 (34.8)</td>
<td>8</td>
<td>Upper central incisor intrusion</td>
<td></td>
<td>0.0028 (4 minutes)</td>
<td>• Baseline PBF values did not differ among session. • Force level had no effect on PBF. • PBF drop after administration of the vasoconstrictor (L.A.).</td>
<td>A</td>
</tr>
<tr>
<td>Ikawa et al. (2001)</td>
<td>17 24-29 (N/A)</td>
<td>17</td>
<td>Upper left central incisors intrusion</td>
<td></td>
<td></td>
<td>• Transient apical displacement (intrusive force) can reduce PBF temporarily. • Increase in intrusive force, PBF both with and without dam deceased significantly.</td>
<td>B</td>
</tr>
<tr>
<td>Ersahan et al. (2016)</td>
<td>20 20-40 (27.6)</td>
<td>20</td>
<td>Upper first molars intrusion</td>
<td></td>
<td></td>
<td>• PBF decreased at 3 days and continued to remain suppressed until 3 weeks, after which a gradual trend of recovery was observed until 3 months, when the levels returned to near those measured before intrusion. • Despite slight regressive changes in pulp tissue over the short term, PBF values tend to return to their initial levels within 3 months.</td>
<td>A</td>
</tr>
<tr>
<td>Authors (Year)</td>
<td>Study subjects</td>
<td>No. of teeth</td>
<td>Type of force</td>
<td>Magnitude of force (g)</td>
<td>Duration of force (days)</td>
<td>Conclusion</td>
<td>Quality grade</td>
</tr>
<tr>
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</tr>
<tr>
<td>Sabuncuoglu and Ersahan (2014)</td>
<td>16</td>
<td>18-25 (21.7)</td>
<td>20</td>
<td>Upper first molar intrusion</td>
<td>100</td>
<td>180 (6 months)</td>
<td>Intrusive forces made short-term reduced PBF (3 days and 3 weeks) and it tended to return to baseline values, indicating that they are reversible effects.</td>
</tr>
<tr>
<td>Sabuncuoglu and Ersahan (2014)</td>
<td>20</td>
<td>1-25 (20.3)</td>
<td>E1:20 (light force) E2:20 (heavy force)</td>
<td>Upper central and lateral incisors intrusion</td>
<td>• 40 • 120</td>
<td>• 3 • 21 (3 weeks)</td>
<td>PBF values decreased in teeth subjected to 3 days of either light or heavy intrusive force, and tended to return to initial levels after 3 weeks.</td>
</tr>
<tr>
<td>Sabuncuoglu and Ersahan (2015)</td>
<td>30</td>
<td>18-25 (21.7)</td>
<td>E1:40 (MIA) E2:40 (utility arches)</td>
<td>Upper central and lateral incisors intrusion</td>
<td>25</td>
<td>• 1 (24 hours) • 3 • 7 • 21 (3 weeks)</td>
<td>Mean PBF in experimental group decreased significantly in 24 hours, continued to increase gradually at 3 days and 7 days, and then returned to control level at 3 weeks.</td>
</tr>
<tr>
<td>Sabuncuoglu and Ersahan (2016)</td>
<td>24</td>
<td>19-25 (21.91)</td>
<td>24</td>
<td>Maxillary canine retraction</td>
<td>100</td>
<td>• 1 (24 hours) • 3 • 7 • 30 (1 month) • 90 (3 months)</td>
<td>PBF underwent a significant decline at 24 hours after canine retraction, then returning to near-baseline levels within one month.</td>
</tr>
<tr>
<td>Sano et al. (2002)</td>
<td>13</td>
<td>27-31 (N/A)</td>
<td>8</td>
<td>Upper left central incisors intrusion</td>
<td>• 50 • 99 • 101.97 • 203.94</td>
<td>6</td>
<td>PBF reduced during force application and followed by recovery to normal at the end.</td>
</tr>
</tbody>
</table>

No., Number; E, Experiment group; N/A, Non-available
<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study subjects</th>
<th>No. of teeth</th>
<th>Type of force</th>
<th>Control group</th>
<th>Force applied (g)</th>
<th>Duration of force (days)</th>
<th>Pulpal response</th>
<th>Conclusion</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caviedes-Bucheli et al. (2011)</td>
<td>33 N/A, 18-37 (N/A)</td>
<td>10 E1:10 (moderate force, 56 g) E2:10 (severe force, 224 g)</td>
<td>Premolars tipping and extrusion</td>
<td>No orthodontic force</td>
<td>56, 224</td>
<td>1</td>
<td>Increased CGRP expression in both severe- and moderate-force group (greater in severe-force) compared with control group</td>
<td>CGRP expression in human dental pulp increases when teeth are submitted to severe orthodontic forces.</td>
<td>C</td>
</tr>
<tr>
<td>Derringer et al. (1996)</td>
<td>15 11-14 (N/A)</td>
<td>15 N/A, Fixed appliance at premolars</td>
<td>No orthodontic force</td>
<td></td>
<td>51-102</td>
<td>14</td>
<td>There were greater numbers of microvessels of culture in the pulp explants from orthodontically treated teeth compared with those from the pulps of control teeth.</td>
<td>There is an increase in angiogenic growth factors in the pulp of orthodontically moved teeth.</td>
<td>B</td>
</tr>
<tr>
<td>Derringer and Linden (2003)</td>
<td>14 11-14 (N/A)</td>
<td>10 8 Upper second premolars extrusion</td>
<td>No orthodontic force</td>
<td></td>
<td>51-102</td>
<td>14</td>
<td>Angiogenic growth factors (VEGF, FGF2, PDGF, EGF and TGF β) are released in the pulp following orthodontic force. (NAs reduced microvessel numbers in the human dental pulp and rat-aorta co-culture assay)</td>
<td>Growth factors play a role in pulpal angiogenesis.</td>
<td>B</td>
</tr>
<tr>
<td>Derringer and Linden (2004)</td>
<td>20 11-14 (N/A)</td>
<td>80 80 Upper and lower second premolars extrusion</td>
<td>No Ab (anti h VEGF, anti h FGF2, anti h-PDGF, anti h TGF β) in co-cultures</td>
<td></td>
<td>51-102</td>
<td>14</td>
<td>VEGF, FGF2, PDGF and TGF β which are released after orthodontic force application causes the reduction of microvessel numbers.</td>
<td>Growth factors were released following orthodontic force application and play a role in the angiogenic response of the pulp. These factors may be more effective in combination.</td>
<td>B</td>
</tr>
<tr>
<td>Derringer and Linden (2007)</td>
<td>10 11-14 (N/A)</td>
<td>10 10 Upper second premolars extrusion</td>
<td>Co-culture without anti-h EGF</td>
<td></td>
<td>51-102</td>
<td>14</td>
<td>Orthodontic forces stimulate the release of EGF in pulp tissues. (Co-culture with anti-h EGF resulted in a reduction in pulpal and rat aorta microvessels numbers)</td>
<td>Human epidermal growth factor (EGF) released following orthodontic force application plays a part in the angiogenic response of the pulp.</td>
<td>B</td>
</tr>
<tr>
<td>Authors (Year)</td>
<td>Study subjects</td>
<td>No. of teeth</td>
<td>Type of force</td>
<td>Control group</td>
<td>Force applied (g)</td>
<td>Duration of force (days)</td>
<td>Pulpal response</td>
<td>Conclusion</td>
<td>Quality grade</td>
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</tr>
<tr>
<td>Hamersky et al. (1980)</td>
<td>5</td>
<td>17 11.7-25.7 (15)</td>
<td>Upper and lower first premolars extrusion</td>
<td>No orthodontic force</td>
<td>170</td>
<td>3</td>
<td>Orthodontic force decreased the pulpal respiration rate. Increasing age causes a larger depression in the pulpal respiration rate. The greater occurrence of root resorption and pulpal pathosis observed in adult orthodontic patients may be related to this greater depression in pulpal tissue respiration.</td>
<td>Orthodontic forces cause biochemical and biologic pulpal tissue alterations and orthodontic forces may be less biologically safe as the age of the patient increases.</td>
<td>C</td>
</tr>
<tr>
<td>Han et al. (2013)</td>
<td>12</td>
<td>27 14-24 (17.9)</td>
<td>Upper first premolars intrusion</td>
<td>No orthodontic force</td>
<td>50, 300</td>
<td>7, 28, 56, 84</td>
<td>Odontoblast disruption, vacuolization, and moderate vascular congestion without necrosis (in both light- and heavy-force groups) Pulp stones in heavy-force group</td>
<td>Dental pulp still has vitality and no necrotic is observed after intrusive treatment.</td>
<td>C</td>
</tr>
<tr>
<td>Kayhan et al. (2000)</td>
<td>42</td>
<td>N/A 15-17 (N/A)</td>
<td>Upper premolars</td>
<td>No orthodontic force</td>
<td>N/A (1/2 turn/day)</td>
<td>21</td>
<td>Vessel area, minimum vessel diameters and maximum vessel diameters showed significant differences between control and 3-month groups. Maximum vessel diameters showed significant differences between 1-month and 3-month groups. Teeth in 3-month group found more fibrosis in pulp.</td>
<td>Force applied by RME caused an adaptive vascular tissue response and fibrotic changes.</td>
<td>B</td>
</tr>
<tr>
<td>Lazzaretti et al. (2014)</td>
<td>17</td>
<td>12-19 (N/A)</td>
<td>Upper first premolar intrusion</td>
<td>No orthodontic force</td>
<td>60</td>
<td>21</td>
<td>Fibrous tissue and pulpal nodules were increased significantly in the experimental group.</td>
<td>Intrusive force caused vascular changes and increased the presence of fibrosis and the number of pulp calcifications without pulp necrosis.</td>
<td>B</td>
</tr>
</tbody>
</table>

Table 4. con't
<table>
<thead>
<tr>
<th>Study subjects</th>
<th>No. of teeth</th>
<th>Age range (Mean age)</th>
<th>Type of force</th>
<th>Control group</th>
<th>Force applied (g)</th>
<th>Duration of force (days)</th>
<th>Pulpal response</th>
<th>Conclusion</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mostafa et al. (1991)</td>
<td>18</td>
<td>16-21 (18)</td>
<td>18</td>
<td>18</td>
<td>Upper first premolar extrusion</td>
<td>48-57</td>
<td>7, 14, 28</td>
<td>The pulpal reactions involve circulatory disturbances, odontoblastic degeneration, vacuolization and edema of the pulp tissues and fibronectic changes.</td>
<td>Many characteristic pulpal reactions arise from orthodontic extrusion.</td>
</tr>
<tr>
<td>Parris et al. (1989)</td>
<td>20</td>
<td>11-29 (14.6)</td>
<td>44</td>
<td>36</td>
<td>Premolar tipping force</td>
<td>120-245</td>
<td>0.015-0.054 (21-78 minutes)</td>
<td>Concentrations of ir-ME and ir-SP each correlated negatively with the magnitude of the orthodontic force.</td>
<td>Orthodontic forces and pulpal ir-ME and ir-SP concentrations are interrelated.</td>
</tr>
<tr>
<td>Perinetti et al. (2004)</td>
<td>17</td>
<td>14-19 (N/A)</td>
<td>17</td>
<td>17</td>
<td>Upper first premolar tipping force</td>
<td>30-90</td>
<td>7</td>
<td>The AST activity in the test teeth is higher significantly than in the control teeth.</td>
<td>Application of mechanical load to teeth can cause metabolic changes in the pulp.</td>
</tr>
<tr>
<td>Perinetti et al. (2005)</td>
<td>16</td>
<td>15-19 (17)</td>
<td>16</td>
<td>16</td>
<td>Upper first premolar tipping force</td>
<td>30-90</td>
<td>7</td>
<td>ALP activity is significantly decreased in dental pulp tissue, explained by damage of the pulp cells responsive to the synthesis of this enzyme.</td>
<td>Orthodontic treatment can lead to significant early-phase reduction in ALP activity in pulp tissue.</td>
</tr>
<tr>
<td>Ramazanizadeh et al. (2009)</td>
<td>13</td>
<td>14-24 (16.8)</td>
<td>E1:20 intrusion</td>
<td>E2:20 extrusion</td>
<td>75; extrusion, 25; intrusion</td>
<td>21 (3 weeks; evaluated at 3 days and 3 weeks)</td>
<td>More fibrous tissue formation is seen from extra force.</td>
<td>Histologic pulpal changes of intrusive force after 3 days and 3 weeks showed no significant difference.</td>
<td>Intuive forces cause histologic pulp tissue alterations.</td>
</tr>
<tr>
<td>Stenvik and Mjor (1970)</td>
<td>6</td>
<td>N/A</td>
<td>10-13 (N/A)</td>
<td>60</td>
<td>Premolar intrusion force</td>
<td>35-250</td>
<td>4-35</td>
<td>Vacuolization of the pulp tissue and circulatory disturbances occurred.</td>
<td>Intrusive forces cause histologic pulp tissue alterations.</td>
</tr>
<tr>
<td>Stenvik (1971)</td>
<td>32</td>
<td>N/A</td>
<td>12-13 (N/A)</td>
<td>35</td>
<td>Premolar extrusion and jiggling force</td>
<td>100-200</td>
<td>7-14</td>
<td>Minor reactions related to the circulatory system in the pulp were observed in experimental group.</td>
<td>The effect of extrusion on dentin and pulp seemed to be less than that of intrusive forces of the same magnitude.</td>
</tr>
<tr>
<td>Stenvik and Mjor (1971)</td>
<td>47</td>
<td>N/A</td>
<td>10-13 (N/A)</td>
<td>35</td>
<td>Premolar extrusion force</td>
<td>35-250</td>
<td>4-35</td>
<td>Alterations in apical anatomy were found only in teeth in which the root was not completely removed, while resorption defects were noted in most teeth in the experimental group.</td>
<td>Intuive forces cause histologic pulp tissue alterations. Some were of permanent change, while others were partly resolvable.</td>
</tr>
</tbody>
</table>

Table 4. con't
<table>
<thead>
<tr>
<th>Study subjects</th>
<th>No. of teeth</th>
<th>Type of force</th>
<th>Control group</th>
<th>Force applied (g)</th>
<th>Duration of force (days)</th>
<th>Pulpal response</th>
<th>Conclusion</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subay et al. (2001)</td>
<td>48</td>
<td>15-18 (N/A)</td>
<td>40</td>
<td>0</td>
<td>Premolar extrusion</td>
<td>None</td>
<td>75</td>
<td>10, 40</td>
</tr>
<tr>
<td>Taspinar et al. (2003)</td>
<td>N/A</td>
<td>13-17 (N/A)</td>
<td>20</td>
<td>8</td>
<td>Upper first premolar RME</td>
<td>No orthodontic force</td>
<td>N/A (heavy force)</td>
<td>22</td>
</tr>
<tr>
<td>Veberiene et al. (2009)</td>
<td>21</td>
<td>11-21 (15.5)</td>
<td>21</td>
<td>21</td>
<td>Premolar intrusion</td>
<td>No orthodontic force</td>
<td>61</td>
<td>7</td>
</tr>
<tr>
<td>Veberiene et al. (2010)</td>
<td>13</td>
<td>14-22 (16.5)</td>
<td>26</td>
<td>21</td>
<td>Premolar intrusion</td>
<td>No orthodontic force</td>
<td>85</td>
<td>7, 14</td>
</tr>
<tr>
<td>Veberiene et al. (2015)</td>
<td>16</td>
<td>N/A (25.7)</td>
<td>20</td>
<td>11</td>
<td>Upper premolar alignment</td>
<td>No orthodontic force</td>
<td>150-200 (per arch)</td>
<td>180 (6 months)</td>
</tr>
<tr>
<td>Walker et al. (1987)</td>
<td>17</td>
<td>N/A (N/A)</td>
<td>20</td>
<td>14</td>
<td>Upper premolar tipping</td>
<td>No orthodontic force</td>
<td>41-174</td>
<td>0.014-0.104 (20 minutes - 2.5 hours)</td>
</tr>
</tbody>
</table>

No., Number; E, Experiment group; N/A, Non-available; CGRP, Calcitonin gene-related peptide; VEGF, Vascular endothelial cell growth factor; FGF2, Fibroblast growth factor 2; PDGF, Platelet derived growth factor; EGF, Epidermal growth factor; TGF β, Transforming growth factor β; NAs, Neutralising antibodies; Ab, Antibody; RME, Rapid maxillary expansion; ir-ME, Immunoreactive-methionine enkephalin; ir-SP, Immunoreactive-substance P; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase; EPT, Electrical pulp testing; ME, Methionine enkephalin.
Table 5. Studies on the influence of orthodontic force on the pulpal responses in traumatized upper incisors teeth.

<table>
<thead>
<tr>
<th>Authors (Year)</th>
<th>Study groups</th>
<th>Type and magnitude of force (g)</th>
<th>Duration of force (months)</th>
<th>Results</th>
<th>Quality grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brin et al. (1991)&lt;sup&gt;41&lt;/sup&gt;</td>
<td>Traumatized teeth without OT</td>
<td>Tipping, removable appliance</td>
<td>N/A</td>
<td>The highest prevalence of loss of pulp vitality was found in group 3 while low sensibility (EPT 6.5-10) was prevalence in group 2.</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>Non-traumatized teeth with OT</td>
<td>41</td>
<td>11.3±2.1</td>
<td>9.6±1.6</td>
<td>14.3±2.1</td>
</tr>
<tr>
<td></td>
<td>Traumatized teeth with OT</td>
<td>41</td>
<td>11.3±2.1</td>
<td>9.6±1.6</td>
<td>14.3±2.1</td>
</tr>
<tr>
<td></td>
<td>Non-traumatized teeth with OT</td>
<td>41</td>
<td>11.3±2.1</td>
<td>9.6±1.6</td>
<td>14.3±2.1</td>
</tr>
<tr>
<td>Authors</td>
<td>Study groups</td>
<td>Type and magnitude of force (g)</td>
<td>Duration of force (months)</td>
<td>Results</td>
<td>Quality grade</td>
</tr>
<tr>
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<td>------------------------------------------------------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Bauss et al. (2009)&lt;sup&gt;16&lt;/sup&gt;</td>
<td>Traumatized teeth during OT</td>
<td>Non-traumatized teeth during OT</td>
<td>N/A</td>
<td>Higher frequency of pulp necrosis of group-1-teeth than group 2 or control.</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>• Subject = 46</td>
<td>• Subject = 200</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• n=59 (CI=43, LI=16)</td>
<td>• n=800 (CI=400, LI=400)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• YOA at the beginning of OT = 11.2 (9.5-16.7)</td>
<td>• YOA at the beginning of OT = 12.7 (9.7-17.5)</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Traumatized teeth without OT</td>
<td>Non-traumatized teeth without OT</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Subject = 173</td>
<td>• Subject = 146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• n=193 (CI=400, LI=47)</td>
<td>• n=200 (CI=146, LI=47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• YOA at trauma = 9.3</td>
<td>• YOA at trauma = 9.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.6-16.4)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Non-traumatized teeth during OT</td>
<td>None</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Subject = 173</td>
<td>• Subject = 146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• n=193 (CI=400, LI=47)</td>
<td>• n=200 (CI=146, LI=47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• YOA at trauma = 9.3</td>
<td>• YOA at trauma = 9.3</td>
<td></td>
<td></td>
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<td>(6.6-16.4)</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Non-traumatized teeth without OT</td>
<td>None</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Subject = 173</td>
<td>• Subject = 146</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• n=193 (CI=400, LI=47)</td>
<td>• n=200 (CI=146, LI=47)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• YOA at trauma = 9.3</td>
<td>• YOA at trauma = 9.3</td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>(6.6-16.4)</td>
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<td></td>
<td>Traumatized teeth with OT</td>
<td>Non-traumatized teeth with OT</td>
<td>Extrusion (20 g/tooth)</td>
<td>• Pulp necrosis (group 1 &gt; group 2)</td>
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<td></td>
<td>• Subject = 66</td>
<td>• Subject = 100</td>
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<td></td>
<td>• n=77 (CI=50, LI=27)</td>
<td>• n=400 (CI=200, LI=200)</td>
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<td></td>
<td>• YOA at trauma = 10</td>
<td>• YOA at trauma = 15.9 (13.5-19.0)</td>
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<td>(7.3-16.7)</td>
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<td></td>
<td>Traumatized teeth without OT</td>
<td>Non-traumatized teeth without OT</td>
<td>Extrusion (20 g/tooth)</td>
<td>• Pulp necrosis (group 1 with periodontal injuries &gt; group 2, control with periodontal injuries)</td>
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<td></td>
<td>• Subject = 173</td>
<td>• Subject = 146</td>
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<td></td>
<td>• n=193</td>
<td>• n=200 (CI=146, LI=47)</td>
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<td></td>
<td>• YOA at trauma = 9.3</td>
<td>• YOA at trauma = 9.3</td>
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<td>(6.6-16.4)</td>
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<td>YOA, Year of age; OT, Orthodontic treatment; EPT, Electrical pulp testing; CI, Central incisors; LI, Lateral incisors</td>
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it is predisposed that application of high magnitude of orthodontic forces for a long duration can influences PBF significantly greater than short duration. Therefore, it is difficult to consider the association between PBF and magnitude of force.

Barwick and Ramsey [23] found that PBF in human maxillary central incisors was not altered significantly by the application of a transient intrusive force (4 minutes) at the maximum level of approximately 4,500 grams. This meant that there was no strangulation of PBF during a brief heavy intrusive force application. According to the study of Parfitt, [56] human central incisors could move 0.028 mm after applying 1,000 gram intrusive force for 15 seconds. Therefore, intrusive force of 4,500 grams would displace incisor for at least 0.028 mm. The average width of PDL space at the apex of teeth is 0.18 - 0.21 mm. [57] Then, there was a PDL space reduction after intrusive force application only about 16% which might be insufficient to compress the apical vasculature. In addition, Miura [58] indicated that blood circulation was not altered by the compression of PDL of 1/3 or less. Moreover, brief heavy forces might not easily cause excessive apical tooth movement because of the mechanical properties of the PDL are oriented to resist intrusive force and remain rigid when force application is transient. Furthermore, the finding of Goz and colleagues [59] could confirm the adequate PDL circulation during brief orthodontic intrusive tooth movement. They found that there was no histologic evidence of circulatory disturbance in PDL after applying 2,000 grams intrusive force to the third premolars of Beagle dogs for a brief duration less than 3 hours.

It has been reported that the effect of orthodontic force on PBF is associated with various factors such as patients’ age, size of apical foramen, and dentinogenic activity. [5,23] None of the studies in this systematic review assessed the influence of size of apical foramen and dentinogenic activity on PBF. However, it is pertinent to mention that in these studies [14,23-30] age of the study participants was also markedly unclear. The study by Sano et al., [14] individuals with age ranging between 27 and 31 years were included, whereas Sabuncuoglu and Ersahan. [27,28] assessed the effect of orthodontic forces on PBF among 18–25 year old patients with the same type of treatment, intrusion of the incisors. Further clinical studies with standardized parameters, particularly the magnitude and duration of force application, are needed to clarify the effect of orthodontic forces on PBF.

Being surrounded by the hard tissue, i.e., dentine, the pulp does not have collateral circulation and is therefore the most sensitive part of the human body, to various forms of stimuli. Many studies have reported that orthodontic force application may lead to significant pulpal reactions such as hyperemia, margination of white blood cells, stasis, vacuole formation in the odontoblastic layer, cyst formation, and hemorrhage. [60] These indicate inflammation and an adaptive process of pulpal tissue.

Cellular response will be discussed separated by type of orthodontic treatment. First of all, intrusive force, Proffit and Fields considered a range of 10-20 grams to be the optimum force magnitudes for intrusion, furthermore Woodside, Berger and Hanson considered 50-100 grams as light forces. [61,62] Most experiments used low to moderate intrusive force and found odontoblast disruption, vacuolization, presence of fibrosis, pulp calcification, and moderate vascular congestion. In studies of severe intrusive force (250-300 grams), they found stasis of pulp vessels, destruction of vessel walls, and pulp stones. Pulp necrosis was not found in any experiment. The results demonstrated that supplying vessels in the severe force group caused a reduced ability of the pulp to react to the impairment of pulpal blood, however, they maintain a sufficient blood supply compared with the low and moderate-force group. [12]
Reitan and Vanarsdall [63] recommended that the extrusive force for adults must be between 25 to 30 grams to prevent pulpal damage. However, Profitt and Fields [2] considered that a range of 50 to 75 grams force is the optimum force magnitude for extrusion. Force used in experiments was lower than 75 grams except the study of Stenvik [32] that applied 100 and 200 g only 10 days. Orthodontic extrusion causes odontoblastic degeneration, circulatory disturbances with congested blood vessels, vacuolization and edema of the pulp tissues, and appearance of fibrotic changes, with no necrosis. Moreover, the effect of extrusion on dentin and pulp seemed to be less than that of intrusive forces of the same magnitude.

Rapid maxillary expansion (RME) is used to correct maxillary constriction and posterior cross-bite by separate the mid-palatine suture. RME exerts a powerful force (7.54-15.8 kg) on the crown of the tooth that is transmitted, via the root, to the bone. [64] After expansion, a 3 to 6-month retention period is required. Taspinar and Kayhan found fibrosis and vessel diameter was significantly increased in 3 months, then disappeared in 18 months after RME. [42,49] This indicates that the vascular changes are reversible and orthodontic treatments using large forces in a short period are safe.

In clinical situations where fixed orthodontic treatment is performed, the teeth are affected by various types of force such as intrusion, extrusion, and tipping. It is almost impossible to define and reproduce the single tooth movements involved in specific clinical situations. The inability to completely control the magnitudes and directions of forces applied can be considered a limitation of these studies. Experimental and clinical techniques are usually limited with regard to applying known complex force systems. [31] Moreover, it can be hypothesized that application of heavy orthodontic force for long duration may cause a magnification of the pulp inflammatory process that could lead to irreversible pulpitis and necrosis. Therefore, it is important to point out the clinical relevance of using moderate and intermittent orthodontic forces, which are capable of generating an adequate tooth movement, limiting the damage, and allowing pulp to recover from the injury. [65] In addition, it is advisable to use controlled movements and long resting periods in order to achieve the esthetic and functional objectives of orthodontic treatment, without triggering severe inflammatory reaction capable of inducing irreversible damage to the dental pulp and periapical tissues. [33]

The quality grading of the studies on the influence of orthodontic force on the pulpal responses in traumatized upper incisors teeth is low. It is mainly because of the limited field of study and no description of reliability tests. However, it might state that severe periodontal injury, such as lateral luxation extrusion and intrusion, revealed a significantly higher rate of pulp necrosis during orthodontic treatment, especially intrusive force, than teeth with only slight periodontal or hard tissue injury. All previously traumatized teeth showed a positive reaction to sensibility test prior to orthodontic treatment, implying an adequate vascular supply to the pulp. As mentioned above that orthodontic tooth movement can affect the blood supply to the dental pulp which decreases in PBF followed by a pulpal hyperemia that compensates for the lack of tissue perfusion. Therefore, it might be concluded that in teeth with severe periodontal injury, the capacity of the blood vessels supplying the pulp was insufficient for maintenance of an adequate pulpal blood flow during orthodontic treatment. Moreover, severe periodontal injury might cause permanent damage and reduction of the apical vessels. These previously traumatized teeth are more prone to pulp necrosis. In addition, previously traumatized teeth with total pulp obliteration have a higher risk for pulp necrosis than traumatized teeth without or only partial obliteration during...
orthodontic force application. Pulp obliteration is caused by progressive hard tissue apposition along the pulp chamber that gradually decreases the pulpal lumen. [66] Therefore, it might imply that this hard tissue formation of total pulp obliteration leads to progressive compression and finally to constriction the existing pulpal vessels, resulting in an impaired blood supply. Progressive hard tissue apposition might lead to constriction of the apical foramen, and thus to compression of the neurovascular bundle. This may cause strangulation or even rupture of the apical vessels during orthodontic tooth movement. In contrast, a narrow apical foramen in teeth with partial obliteration might allow the pulpal circulatory system of these teeth to function adequately and to maintain sufficient pulpal perfusion during orthodontic treatment.

Conclusion

Temporary reduction of PBF after intrusive force application is a reversible effect, and it tends to return to baseline values in about 3 weeks. Although there is a lack of high-quality scientific evidence to prove that orthodontic forces affect in irreversible alterations of human dental pulp, short-term application of orthodontic forces can provoke a reversible biological response. However, previously traumatized teeth applying orthodontic force are considered a risk factor for vitality loss of dental pulp. The recommendation is to use appropriated optimum force, controlled tooth movement, and long resting period to accomplish goal of orthodontic treatment and avoid temporary side-effects.

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