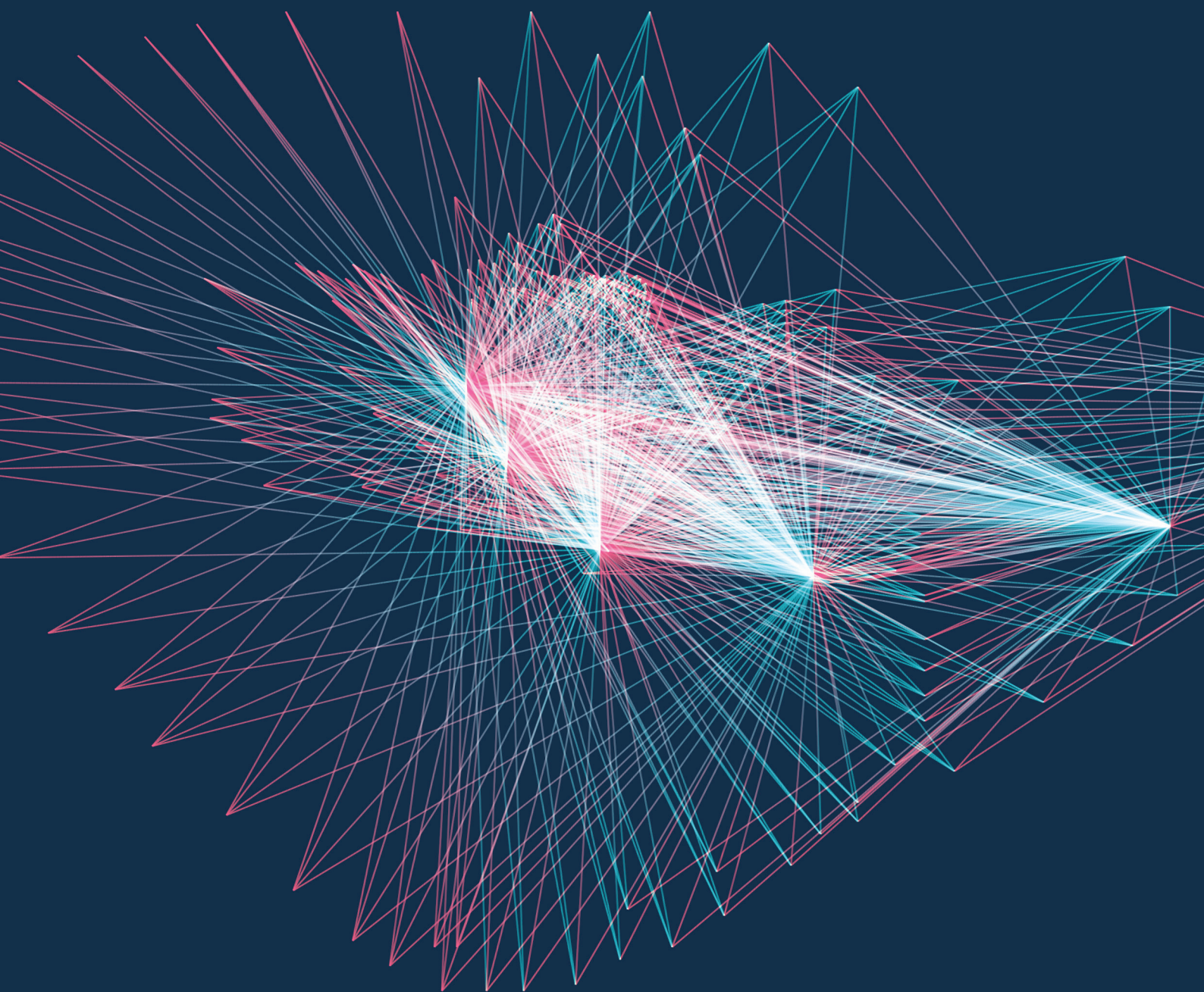


OSR Oral Sciences Reports

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Chiang Mai University's Faculty of Dentistry publishes academic research articles in the newly titled - **Oral Sciences Reports**, which was previously known as *Chiang Mai Dental Journal (CMDJ)*. The journal was originally established for the purposes of publishing academic research articles by the Faculty of Dentistry at Chiang Mai University in 1977. In the current report, editors and experts in their respective fields review articles received from authors prior to being published to ensure that the content of all articles is up-to-date, universal, logical, and in accordance with academic principles so the reader can apply knowledge and cite works in the development of dentistry for the purposes of advancing future research while being beneficial to patients and society.

At present, Oral Sciences Reports openly receives all submissions through an online journal review process system. The new online system also allows reviewers and researchers an ability to read 3 issues each year.

Aims and Scope of the journal

To compile research and content that is up to date and usable to all branches of dentistry and related fields. The articles in Oral Sciences Reports are fundamental research work, including original articles, review articles, case reports/series, short communications, and letters to the editor.

Policy

Accepted articles will be fairly reviewed by the editors and experts with full transparency through the following process.

1. The articles must be correct according to academic principles and not duplicate works that have been previously published.
2. The articles will be considered and reviewed through a non-bias process by concealing the names of authors and related persons in the considered documents while also concealing the names of the experts and reviewers who review the articles (double-blind review).
3. The review process can be tracked online. The article authors can review the status of their article and are able to follow up on the article evaluation through the online process. The duration of each step is closely monitored so that the articles can be published on time.
4. Authors of articles are responsible to review and verify the accuracy of the text, images, tables in the articles before publication.
5. Articles published in Oral Sciences Reports are the copyright of Oral Sciences Reports, which forbids anyone from duplicating published articles for any purpose without explicit permission from Oral Sciences Reports.

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Types of Submission

Oral Sciences Reports invites the following submissions:

1. Original Articles Original contributions of research reports or unpublished recent academic research to the development and applications in dentistry and related fields. The original article must not exceed 4000 words in length and must contain no more than 10 figures and tables in total.
2. Review Articles Comprehensive reviews of special areas of focus in dentistry and related fields. Articles that contain important collected data from numerous books or journals and from the writer's experience. Information should be described, reviewed, compared, and analyzed. The review article must not exceed 4000 words in length and must contain no more than 10 figures and tables in total.
3. Systematic Reviews Clearly formulated reviews that uses systematic and reproducible methods to identify, select and critically appraise all relevant research, and to collect and analyze data from the studies that are included in the review.
4. Case Reports/Series Original findings that highlight novel technical and/or clinical aspects in dentistry and related fields which include clinical symptoms, diagnosis, patient care, treatment, follow-up, and evaluation. The report must not exceed 2500 words in length and must contain no more than 5 figures.
5. Letters to the Editor Commentaries on published papers in the journal and other relevant matters that must not exceed 1000 words in length

Submission Checklist

Authors should ensure to prepare the following items for submission. Failure to complete the required items may contribute to the delay of publication process. Please check the relevant section in this guideline for more details.

1. Title page Must include title of the article, author names and affiliations. One author has been designated as the corresponding author with contact details (e-mail address and full postal address) (see 'Title page' section for more information and an example)

- | | |
|---------------------------------------|---|
| 2. CRediT Contribution | Author will be asked to provide CRediT Contributions as well as their degree of contribution at the time of the original submission. CRediT Contribution is a high-level classification of the diverse roles performed in the work leading to a published research output in the sciences. Its purpose to provide transparency in contributions to scholarly published work, to enable improved systems of attribution, credit, and accountability. |
| 3. Abstract | Must not exceed 250 words. Relevant keywords (up to five keywords) must be included at the end of the abstract. (see the 'Abstract' section for more details) |
| 4. Main Manuscript | Author details and affiliation must not be included. (see 'Manuscript' section for more details) |
| 5. Figures | Should include relevant captions. (see the 'Figures' section for more details) |
| 6. Tables | Should include titles, description, and footnotes. (see the 'Tables' section for more details) |
| 7. Supplementary data (if applicable) | |

Additional considerations the author should confirm before submission:

1. Manuscript must be 'spell-checked', 'grammar-checked', and 'plagiarism-checked'.
2. All figures, tables, and references mentioned in the text should match the files provided.
3. Permission must be obtained for use of copyrighted material from other sources (including the internet).
4. Authors must provide conflicts of interest statement, even if there is no conflict of interests to declare.

Ethical Guidelines

Authors must acknowledge to the following ethical guidelines for publication and research.

A. Authorship and Author Contributions

The policy of Oral Sciences Reports that only ONE corresponding author is accepted. Where there is any uncertainty regarding authorship, the editor of the journal reserves the right to contact the corresponding author of the study for further information. Authors must acknowledge that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal. Authors are required to identify the contributions for which they are responsible. Author will be asked to provide CRediT Contributions as well as their degree of contribution at the time of the original submission. CRediT Contribution is a high-level classification of the diverse roles performed in the work leading to a published research output in the sciences. Its purpose to provide transparency in contributions to scholarly published work, to enable improved systems of attribution, credit, and accountability.

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- (b) Written confirmation (e-mail, letter) from all authors that they agree with the addition, removal, or rearrangement.

In case of addition or removal of authors, these must be confirmed from the author being added or removed. Please be informed that changes of the authorship cannot be made in any circumstances after the manuscript has been accepted.

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All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee's approval for each study. Experimentation involving human subjects will only be published if such research has been conducted in full accordance with the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements or with ethical principles of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above-mentioned principles.

Experimentation involving animal subjects should be carried out in accordance with the guidelines laid down by the National Institute of Health (NIH) in the USA or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

C. Clinical Trials

All clinical trials must register in any of the following public clinical trials registries:

- Thai Clinical Trials Registry (TCTR)
- NIH Clinical Trials Database
- EU Clinical Trials Register
- ISRCTN Registry

The clinical trial registration number and name of the trial register should be included in Materials and Methods of the manuscript. For epidemiological observational trials, authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this must be detailed in Materials and Methods.

D. Systematic Review

The abstract and main body of the systematic review should be reported using the PRISMA for Abstract and PRISMA guidelines respectively. Authors submitting a systematic review should register the protocol in one of the readily-accessible sources/databases at the time of project inception and not retrospectively (e.g. PROSPERO database, OSF registries). The protocol registration number, name of the database or journal reference should be provided at the submission stage in Materials and Methods. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

E. Conflicts of Interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Potential sources of conflict of interest include (but are not limited to) patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If there are no interests to declare, please state 'The authors declare no conflict of interest'. Authors must disclose any interests in the section after acknowledgments.

F. Submission Declaration and Verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright- holder. The conference proceedings are allowed to be part of the article if the contents do not exceed 70% of the article.

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Manuscript Preparation

All texts in the submitted manuscript are required to be inclusive language throughout that acknowledges diversity, conveys respect to all people, is sensitive to differences, and promotes equal opportunities. Authors should ensure that writing is free from bias, for instance by using 'he or she', 'his/her' instead of 'he' or 'his', and by making use of job titles that are free of stereotyping (for instance by using 'chairperson' instead of 'chairman' and 'flight attendant' instead of 'stewardess'). Articles should make no assumptions about the beliefs or commitments of any reader, should contain nothing which might imply that one individual is superior to another on the grounds of race, sex, religion, culture, or any other characteristic.

A. Title page

The title page will remain separate from the manuscript throughout the peer review process and will not be sent to the reviewers. It should include these following details:

- Title should be concise, information-retrieval, and not exceed 30 words. Please avoid abbreviations and formulae where possible.
- Author names and affiliations. Please clearly indicate the given name(s) and family name(s) of each author are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after the author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and the e-mail address of each author.
- Corresponding author will handle correspondence at all stages of refereeing and publication, also post-publication. This responsibility includes answering any future queries about Methodology and Materials. Please ensure that the e-mail address and contact details given are kept up to date by the corresponding author.

B. Abstract

Abstract must not exceed 250 words with concise and informative explanations about the article. Authors must prepare an abstract separately from the main manuscript using Microsoft Word processing software (.doc or .docx). Please avoid references and uncommon abbreviations, but if essential, abbreviations must be defined at their first mention in the abstract itself. Abstract structure of the original articles must consist of 'Objectives, Methods, Results, and Conclusions'.

Abstract of other types of submitted articles should be summarized in one paragraph. Up to five keywords relevant to the articles must be provided and arranged in alphabetical order.

C. Manuscript

Oral Sciences Reports adheres to a double-blinded review. The main body of the paper (including the references, figures, tables and any acknowledgements) must not include any identifying information, such as the authors' names. The layout of the manuscript must be as simple as possible with double-spaced, single column format with Sans Serif font and uploaded as an editable Microsoft Word processing file (.doc or .docx). Complex codes or hyphenate options must be avoided, but the emphatic options such as bold face, italics, subscripts, and superscripts, etc. are encouraged.

1. Original article

- *Introduction* should include literature reviews of previous studies, research questions, and the rationale for conducting the study. The Introduction should not be too long and should be easy to read and understand while avoiding a detailed literature survey or a summary of the results.

- *Methods* should provide sufficient details in a logical sequence to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized and indicated by a reference. If quoting directly from a previously published method, use quotation marks and cite the source. Any modifications to existing methods should also be described.

- *Results* should show the data gained from the study's design in text, tables and/or illustrations, as appropriate, and be clear and concise.

- *Discussion* is criticism, explanation, and defense of the results from the standpoint of the author, and comparison with other peoples' reports. The discussion can include criticism of materials, methods and study results, problems, and difficulties, pointing out the benefits of adoption and providing feedback where appropriate. Discussions should explore the significance of the results of the work, not repeat them. Avoid extensive citations and discussion of published literature.

- *Conclusions* refers to a summary of the study or research results.

- *Acknowledgments*: Please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study.

Formatting of funding source:

This work was supported by the 1st organization name [grant numbers xxxx]; the 2nd organization name [grant number yyyy]; and the 3rd organization name [grant number zzzz].

If no funding has been provided for the research, please include the following sentence:

This research did not receive any specific grant or funding from funding agencies in the public, commercial, or not-for-profit sectors.

- *References* should be confined to documents relating to the author's article or study. The number should not exceed 80, placed in order and using numbers which are superscripted and put in parentheses, starting with number 1 in the article and in reference document's name. (see 'References' section for more information regarding reference formatting)

2. Review articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described, and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

3. Systematic review

- Introduction should be focused to place the subject matter in context and to justify the need for the review.
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- Results should present in structured fashion (e.g. results of the search process, characteristics of the included studies, results of primary meta-analysis, additional analysis, publication bias, quality of evidence).
- Discussion should summarize the results, highlighting completeness and applicability of evidence, quality of evidence, agreements and disagreements with other studies or reviews, strength and limitations, implications for practice and research.
- Conclusion(s) should reach clear conclusions and/or recommendations on the basis of the evidence presented.

4. Case reports/series should be divided into Introduction, Case report, Discussion and Conclusions. They should be well illustrated with clinical images, radiographs and histologic figures and supporting tables where appropriate. However, all illustrations must be of the highest quality.

There are some necessary considerations which should be comprehended and consistent throughout the article:

1. Abbreviations: define abbreviations at their first occurrence in the article: in the abstract and in the main text after it. Please ensure consistency of abbreviations throughout the article.
2. Mathematical expressions: the numbers identifying mathematical expressions should be placed in parentheses after the equation, flush to the right margin; when referring to equations within text, use the following style: Eq. (5), Eqs. (3-10), [see Eq. (4)], etc.
3. Nomenclature: abbreviations and acronyms should be spelled out the first time they are used in the manuscript or spelled out in tables and figures (if necessary). Units of measure and time require no explanation. Dental nomenclature in the manuscript should be complete words, such as maxillary right central incisor. Numbering of teeth from pictures or tables should follow the FDI two-digit system.
4. Units: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.
5. Product identification: all products mentioned in the text should be identified with the name of the manufacturer, city, state, and country in parentheses after the first mention of the product, for example, The ceramic crown was cemented on dentin surface with resin cement (RelyXTM U200, 3M ESPE, St. Paul, MN, USA)...

D. Figures

Figures should be prepared and submitted separately from the main manuscript. Color artworks are encouraged at no additional charge. Regardless of the application used other than Microsoft Office, when the electronic artwork is finalized, please 'save as' or 'export' or convert the images to **EPS, TIFF, or JPEG format with the minimum resolution of 300 dpi**. Keep the artwork in uniform lettering, sizing, and similar fonts. Please do not submit graphics that are too low in resolution or disproportionately large for the content. Authors must submit each illustration as a separate file.

Please ensure that each illustration has a caption according to their sequence in the text and supply captions separately in editable Microsoft Word processing file (.doc or .docx), not attached to the figure. A caption should comprise a brief title (not on the figure itself) and a description of the illustration. Keep text in the illustrations themselves to a minimum but explain all symbols and abbreviations used.

E. Tables

Please submit tables as editable Microsoft Word processing files (.doc or .docx), not as images, and avoid using vertical rules and shading in table cells. Each table should be placed on a separate page, not next to the relevant text in the article. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body while ensuring that the data presented in them does not duplicate results described elsewhere in the article.

F. References

Citation in text

Any citations in the text should be placed in order and using numbers which are superscripted and put in parentheses. Please ensure that all citations are also present in the reference list consecutively in accordance with their appearance in the text.

Reference style

All references should be brought together at the end of the paper consecutively in accordance with their appearance in the text and should be in the Vancouver reference format. Please follow these examples of correct reference format below:

1. *Journal article*

1.1. One to six authors

Author(s) – Family name and initials. Title of article. Abbreviated journal title. Publication year;volume (issue);pages.

Example:

Parvez GM. Pharmacological activities of mango (*Mangifera Indica*): A review. *J Pharmacognosy Phytother.* 2016;5(3): 1-7.

Or

Choi YS, Cho IH. An effect of immediate dentin sealing on the shear bond strength of resin cement to porcelain restoration. *J Adv Prosthodont.* 2010;2(2):39-45.

Or

Firmino RT, Ferreira FM, Martins CC, Granville-Garcia AF, Fraiz FC, Paiva SM. Is parental oral health literacy a predictor of children's oral health outcomes? Systematic review of the literature. *Int J Paediatr Dent.* 2018;28(5):459-71.

1.2. More than six authors

Author(s) – Family name and initials of the first six authors, et al. Title of article. Abbreviated journal title. Publication year;volume(issue);pages.

Example:

Vera J, Siqueira Jr JF, Ricucci D, Loghin S, Fernández N, Flores B, et al. One-versus two-visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. *J Endod.* 2012;38(8):1040-52.

1.3. Article in press

Authors separated by commas – Family name and initials. Title of article. Abbreviated journal title in italics. Forthcoming - year of expected publication.

Example:

Cho HJ, Shin MS, Song Y, Park SK, Park SM, Kim HD. Severe periodontal disease increases acute myocardial infarction and stroke: a 10-year retrospective follow-up study. *J Dent Res.* Forthcoming 2021.

2. *Books*

2.1. Book with author (s)

Author(s) – Family name and initials (no more than 2 initials with no spaces between initials)– Multiple authors separated by a comma. After the 6th author add - "et al". Title of book. Edition of book if later than 1st ed. Place of publication: Publisher name; Year of publication.

Example:

Sherwood IA. Essentials of operative dentistry. Suffolk: Boydell & Brewer Ltd; 2010.

Or

Abrahams PH, Boon JM, Spratt JD. McMinn's clinical atlas of human anatomy. 6th edition. Amsterdam: Elsevier Health Sciences; 2008.

2.2. Book with no author

Title of book. Edition of book if later than 1st ed. Place of publication: Publisher name; Year of publication.

Note: Do not use anonymous. Please begin a reference with the title of the book if there is no person or organization identified as the author and no editors or translators are given.

Example:

A guide for women with early breast cancer. Sydney: National Breast Cancer; 2003.

2.3. Chapter in a book

Author(s) of chapter - Family name and initials, Title of chapter. In: Editor(s) of book - Family name and initials, editors. Title of book. edition (if not first). Place of publication: Publisher name; Year of publication. p. [page numbers of chapter].

Example:

Rowlands TE, Haine LS. Acute limb ischaemia. In: Donnelly R, London NJM, editors. ABC of arterial and venous disease. 2nd ed. West Sussex: Blackwell Publishing; 2009. p. 123-140.

3. Thesis/dissertation

3.1. Thesis in print

Author - family name followed by initials. Thesis title [type of thesis]. Place of publication: Publisher; Year.

Example:

Kay JG. Intracellular cytokine trafficking and phagocytosis in macrophages [dissertation]. St Lucia, Qld: University of Queensland; 2007.

3.2. Thesis retrieved from full text database or internet

Author - family named followed by initials. Thesis title [type of thesis/dissertation on the Internet]. Place of publication: Publisher; Year [cited date – year month day]. Available from: URL

Example:

Pahl KM. Preventing anxiety and promoting social and emotional strength in early childhood: an investigation of risk factors [dissertation on the Internet]. St Lucia, Qld: University of Queensland; 2009 [cited 2017 Nov 22]. Available from: <https://espace.library.uq.edu.au/view/UQ:178027>

4. Webpage

4.1. Webpage with author

Author/organization's name. Title of the page [Internet]. Place of publication: Publisher's name; Publication date or year [updated date - year month day; cited date - year month day]. Available from: URL

Example:

American Dental Association. COVID-19 and Oral Health Conditions [Internet]. Chicago: American Dental Association; 2021 Feb 12 [updated 2021 Feb 12; cited 2021 Jun 24]. Available from: <https://www.ada.org/en/press-room/news-releases/2021-archives/february/covid-19-and-oral-health-conditions>

4.2. Webpage with no authors

Title [Internet]. Place of publication (if available): Publisher's name (if available); Publication date or year [updated date (if available); cited date]. Available from: URL

Example:

Dentistry and ADHD [Internet]. 2019 Jan 15 [updated 2019 Jan 15; cited 2020 Apr 8]. Available from: <https://snoozedentistry.net/blog/dentistry-and-adhd/>

4.3. Image on a webpage

Author/organization. Title [image on the Internet]. Place of publication: Publisher's name; Publication date or year [updated date; cited date]. Available from: URL

Note: If the image does not have a title - give the image a meaningful title in square brackets.

Example:

Poticny DJ. An Implant-Supported Denture Offers a Number of Advantages [image on the Internet]. Texas: Office of Dan Poticny; 2018 Nov 21 [updated 2018 Nov 21; cited 2019 Aug 30]. Available from: <https://www.dfwsmlidoc.com/blog/post/an-implant-supported-denture-offers-a-number-of-advantages.html>

5. *Government publications/reports*

5.1. Reports and other government publications

Author(s). Title of report. Place of publication: Publisher; Date of publication – year month (if applicable). Total number of pages (if applicable eg. 24 p.) Report No.: (if applicable)

Example:

Australian Institute of Health and Welfare. Oral health and dental care in Australia: key facts and figures trends 2014. Canberra: AIWH; 2014.

5.2. Government reports available online

Author(s). Title of report. Report No.: (if applicable). [Internet]. Place of publication: Publisher or Institution; Publication date or year [updated date - year month day; cited date - year month day]. Available from: URL

Example:

World Health Organization. WHO mortality database [Internet]. Geneva: World Health Organization; 2019 Dec 31 [updated 2019 Dec 31; cited 2021 Mar 29]. Available from: <https://www.who.int/data/mortality/country-profile>

6. *Tables/Figures/Appendices*

Follow the format of book, journal or website in which you found the table/figure/appendix followed by: table/figure/image/appendix number of original source, Title of table/figure/appendix from original source; p. Page number of table/figure/appendix from original source.

Note: each reference to a different table/figure within the same document requires a separate entry in the Reference list. Please provide permission documents from the original sources.

Example:

Smith J, Lipsitch M, Almond JW. Vaccine production, distribution, access, and uptake. *Lancet* 2011;378(9789):428-438. Table 1, Examples of vaccine classes and associated industrial challenges; p. 429.

7. *Journal abbreviation source*

Journal names should be abbreviated according to the Web of Science - Journal Title Abbreviations.

Peer-review Process

Oral Sciences Reports follows a double anonymized review process. Each manuscript will be assigned to at least three expertises for consideration. The identities of both reviewers and authors are concealed from each other throughout the review to limit reviewer bias. To facilitate this, please ensure that the manuscript keeps anonymity before submission such as affiliation, author's gender, country or city of origin, academic status, or previous publication history. Our peer review process is confidential and identities of reviewers are not released. Letters and technical comments are sent to the authors of the manuscript on which they comment for response or refutation, but otherwise are treated in the same way as other contributions with respect to confidentiality.

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Our online submission system guides you stepwise through the process of entering your article details and uploading your files. Please follow the submission process carefully. The system converts your article files to a single PDF file used in the peer-review process. Editable Microsoft word processing files are required to typeset your article for final publication. All correspondence, including notification of the Editor's decision and requests for revision, is sent to your registered e-mail.

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Digital and Conventional Workflow for Endocrown Fabrication in Pulpotomy Permanent Tooth: Case Report

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Abstract

A higher chance of carrying out a successful full pulpotomy is dependent on the coronal restoration. Preservation of healthy dental structure is essential for providing mechanical stabilization of tooth-restoration integrity and increasing the number of suitable surfaces for adhesion. In this case, endocrown was a suitable restoration due to large coronal destruction. However, the preparation design and material selection affect the manufacturing technique. As shown in this case, the CAD/CAM technique demonstrated technical errors such as marginal chipping and overmilling, for these reasons changing to conventional technique for lithium disilicate endocrown fabrication was adopted. After one week of permanent cementation, the restoration was in good condition and abutment was normal with good gingival health.

Keywords: CAD/CAM, endocrown, lithium disilicate ceramic, pulpotomy, resin nano-ceramic

Introduction

Full pulpotomy in permanent teeth aims to retain pulp vitality and relieve pain from acute pulpitis. Pulpotomy is an alternative treatment for vital pulp therapy and root canal treatment to preserve pulp tissue and retain tooth vitality for the long term.^(1,2) The success rate of full pulpotomy procedures varies from 82.9% to 100% depending on the type coronal restoration.⁽³⁾

For deep carious lesions or teeth with large cavities, indirectly bonded restoration is more suitable than direct restoration.⁽⁴⁾ Teeth that are indirectly restored with resin composite or ceramic have better fracture resistance and marginal integrity, reduced cervical marginal microleakage, less surface roughness, less postoperative sensitivity, and minimal soft-tissue irritation than those directly restored with resin composite.⁽⁴⁾ Overall, indirect restorations have a lower annual mean failure rate than direct restorations in posterior teeth.⁽⁵⁾

Endocrown restorations have been reported as a promising treatment to rehabilitate extensively damaged endodontically treated teeth.^(6,7) The endocrown can be defined as a single piece restoration which contains an extension to the pulp chamber that replaces part of the crown. The macro retention provided by the pulp chamber axial walls associated with the adhesive luting cement makes the endocrown restoration suitable for teeth with short and/or curved roots when the endodontic post cannot be used or when a more conservative approach is planned.⁽⁸⁾

Endocrowns are commonly fabricated using ceramic based on leucite, lithium disilicate, and zirconia ceramics. Even though ceramics show excellent mechanical properties, they are prone to non-repairable fractures extending to the root, owing to their brittle characteristics. In consequence, alternative materials with a more compliant behavior have been introduced for endocrown fabrication, such as resin composites and polymer-infiltrated ceramics as they exhibit higher resilience and more resistance to higher occlusal forces.⁽⁹⁾

However, the endocrown treatment is contraindicated for substrates with insufficient adhesion, or pulp chambers with less than 3 mm deep or cervical margins less than 2.0 mm wide for most of its finishing line.⁽⁶⁾

Case report

A 16-year-old female was referred to the Department of Restorative Dentistry with a chief complaint of large filling and tooth chipping at the lower left region. The medical history was noncontributory. Clinical examination of tooth 36 showed extensive tooth colored restoration at occlusal surface with enamel-dentin fracture (Figure 1). The electric pulp test was positive. The tooth has undergone vital pulp therapy (pulpotomy) for 2 years. The patient had an acceptable oral hygiene and normal occlusion. Pre-operative occlusal scheme when lateral excursion was cuspid protected occlusion.

Radiographic examination showed radiopaque area of restoration from occlusal surface to floor of pulp chamber. The alveolar crest was in normal height with normal periapical tissue (Figure 2).

An endocrown restoration was selected for this tooth because of thin remaining walls and minimal amount of remaining tooth structure. The tooth preparation was done using a sterile high-speed diamond bur under water coolant with at least 2.0 mm occlusal clearance for entire occlusal surface in order to provide appropriate thickness for the ceramic restoration. The central retention was achieved by the height of pulp chamber (at least 3.0 mm.) while maintaining the thickness of mineral trioxide aggregate (Proroot MTA[®], Dentsply Tulsa Dental, Tulsa, USA) at 3.0 mm over the pulpal floor and eliminating undercuts in the access cavity. The cervical finish line has to be supragingival where the bevel finish line is at buccal surface and chamfer finish line is at lingual surface (Figure 3). After preparation, the immediate dentin sealing (IDS) was done with a 3-step etch-and-rinse dentin bonding agent (DBA) (OptiBond FL[™] Kerr Corporation, Orange, USA) in order to increase the dentin bond strength.

A digital impression was obtained via a digital scanner (Cerec[®] Primescan camera Dentsply, Charlotte, United States). Temporary restoration was fabricated by Bis-Acryl composite resin (Protemp 4, 3M ESPE, Beirut, Lebanon) and temporarily cemented by using a spot etch technique at pulpal floor of the cavity. First, the endocrown was designed and manufactured with lithium disilicate block (CEREC[®] Tessera, Dentsply, Charlotte, USA). After milling, the margin of the restoration was chipped, and internal surface was overmilled (Figure 4). Therefore, the type of material was changed to resin



Figure 1: Pre-operative intraoral views: (A) occlusal view of maxillary teeth, (B) right buccal view, (C) anterior view of teeth, (D) left buccal view, (E) occlusal view of mandibular teeth



Figure 2: Pre-operative radiograph

nanoceramic block (GC[®] Cerasmart, Tokyo, Japan) but an overmilling was found at the inner surface and under margin was found at the distal surface of the crown.

The process was finally changed from digital to conventional workflow. The impression was taken by double impression technique. The master model and die were

made from gypsum type IV for fabrication of the lithium disilicate endocrown (IPS e.max Press, Ivoclar vivadent, Schaan, Liechtenstein) using lost wax technique (Figure 5C). Conventional fabrication along with lithium disilicate press material showed the satisfactory results in marginal adaptation and marginal chipping. (Figure 5A-C).

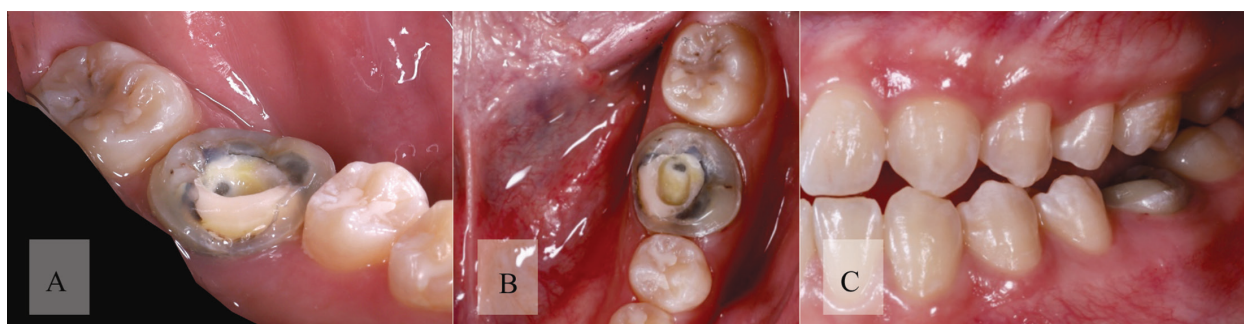


Figure 3: After preparation: (A) lingual view, (B) occlusal view, (C) buccal view

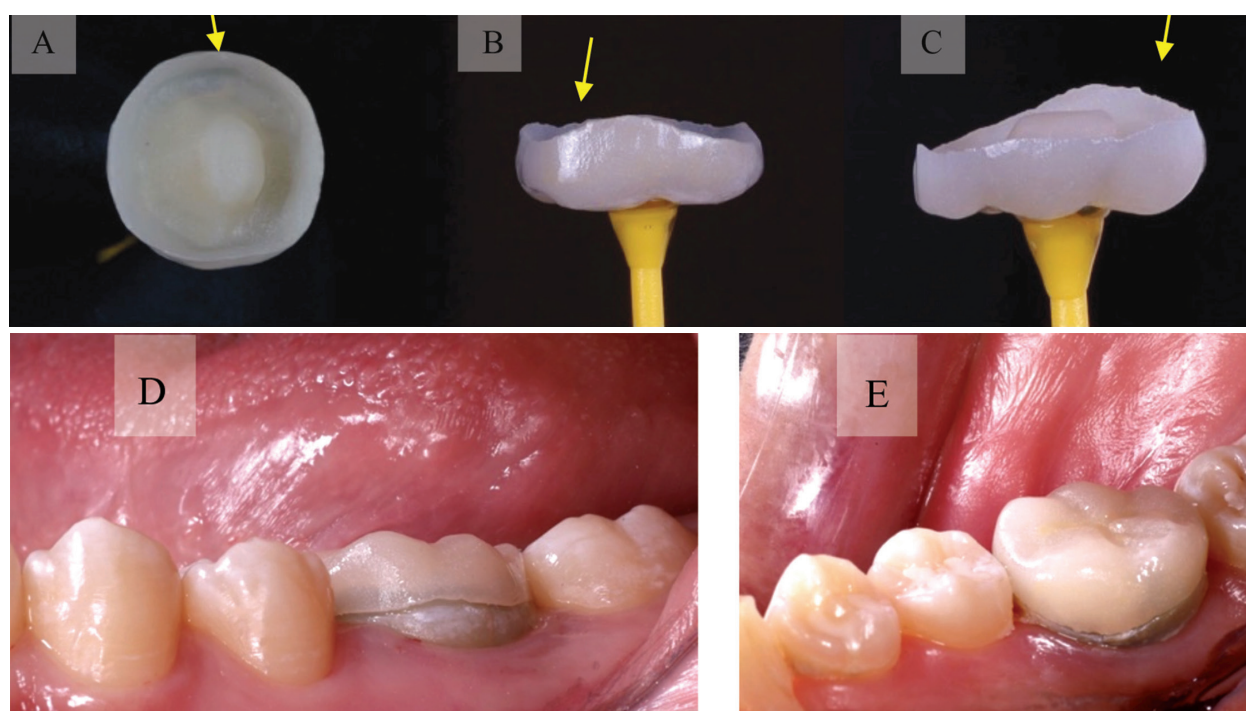


Figure 4: Displays milling lithium disilicate block (CEREC® Tessera, Dentsply, Charlotte, USA): (A) overmilling at inner surface of restoration, (B)-(C) marginal chipping, (D)-(E) try-in endocrown lithium disilicate block shows short margin (CEREC® Tessera, Dentsply, Charlotte, USA)

For the cementation procedure, the restoration was etched with hydrofluoric acid for 20 s, silane was applied before the restoration was cemented on the tooth with a dual cure resin cement (Multilink® N, Ivoclar vivadent, Schaan, Liechtenstein), tack-curing for 3 s and excess resin was removed before 40 s light-curing was applied on all surfaces of the endocrown restoration. A postoperative bitewing radiograph was taken after restoration placement.

Seven days follow-up showed that the tooth 36 was asymptomatic, negative to percussion, no tooth mobility and good gingival health wherein the restoration was in

good condition and no discoloration of restoration was observed.

Radiographic examination showed radiopaque of endocrown with radiopaque of cement line underneath. Alveolar crest and periapical tissue showed normal condition (Figure 6).

Discussion

A proper planning is necessary for the clinical success of full pulpotomy procedures. The success rate depends on the quality of the coronal restoration due to failure of vital pulp therapy that could be caused by insufficient

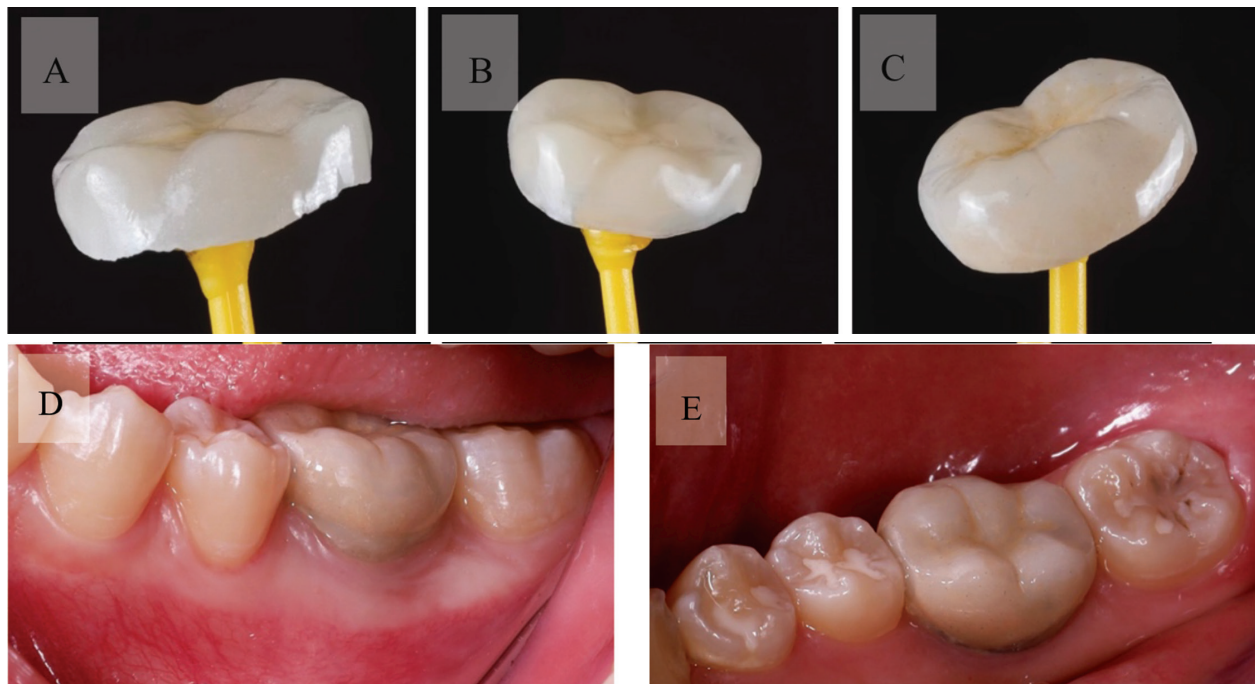


Figure 5: Restoration shows: (A) lithium disilicate block (CEREC® Tessler, Dentsply, Charlotte, USA), (B) resin nanoceramic block (GC® Cerasmart, Tokyo, Japan), (C) lithium disilicate press (IPS e.max Press, Ivoclar Vivadent, Schaan, Liechtenstein), (D)-(E) after cementation of lithium disilicate press



Figure 6: Radiographic examination after insertion for 7 days: (A) periapical, (B) bitewing

sealing between the pulp capping material and the coronal restoration.⁽¹⁰⁾

In this case, tooth 36 was treated by pulpotomy, so post placement could not be done. One of the postless alternatives for treating previously initiated therapy is using the pulp chamber as an extension of crown. This restoration type combines the crown and core build-up in a single element or so called “monoblock”.⁽¹¹⁾ The endocrown requires a simpler and less invasive preparation compared to the multi-step approach of the post-

and-core build-up with full crown, resulting in decreased treatment time and costs.⁽¹²⁾

First, lithium disilicate block was selected (CEREC® Tessler, Dentsply, Charlotte, USA) for endocrown fabrication by CAM technique which presents advantages over the other materials such as aesthetic and ability to bond with resin cement. According to Altier *et al.*,⁽¹³⁾ who compared the fracture resistance of three different endocrowns made of lithium disilicate ceramic and indirect resin composite, they concluded that the

fracture strength of lithium disilicate ceramic endocrown is higher than that of indirect composites. A recent study demonstrated that there was a good stress distribution of lithium disilicate because its elastic modulus is approximate to tooth structure; which were 95, 84.1 and 18.6 GPa for lithium disilicate, enamel, and dentin respectively.^(14,15) Additionally, glass ceramics prevent excessive wear of the opposing dentition due to their similar modulus and hardness to enamel.⁽¹⁶⁾

The preparation design of tooth 36 endocrown is a bevel finish line at buccal surface but lithium disilicate ceramic (CEREC[®] Tessera, Dentsply, Charlotte, USA) is too brittle to mill to a knife-edge⁽¹⁷⁾, so chipping around thin areas of the margin occurred.

Resin-ceramic CAD/CAM blocks such as Lava[™] Ultimate (3M ESPE, Beirut, Lebanon), Cerasmart[®] (GC, Tokyo, Japan) and Vita Enamic (Vita Zahnfabrik, Bad Säckingen, Germany) are highly preferred in chair-side dentistry due to their advantages, including fast and easy production with no need for crystallization or glaze firing after manufacturing, ease of intraoral repair and polish, and better machinability because of their low modulus of elasticity. Moreover, the low hardness values of resin-ceramic materials are found to prevent the wear of opposing dentition and enable rapid milling and to minimize marginal chipping which is associated with better marginal adaptation.⁽¹⁸⁾ The mechanical properties of the resin-ceramic CAD-CAM block materials tested were within the acceptable range for fabrication of single restorations according to the ISO standard for ceramics (ISO 6872:2008). Cerasmart was observed to have superior flexural strength and better internal fit⁽¹⁹⁾; for these reasons, the endocrown was fabricated by resin nanoceramic block (GC[®] Cerasmart, Tokyo, Japan).

Both materials fabricated from CAD/CAM technique still have overmilling problems. Overmilling occurs when the bur is unable to accommodate areas smaller than the size of the bur, especially at cusp tips and sharp line angles, resulting in excess cement space and susceptible restoration.⁽²⁰⁾ So, we decided to change to conventional technique in order to solve this problem by fabricating endocrown with lithium disilicate press (IPS e.max Press, Ivoclar vivadent, Schaan, Liechtenstein) and also wanted to compare between the full digital and full conventional workflow in this kind of problem.

An error in digital workflow, the limitations of the designing software and the size of the cutting tools, can result in the accuracy of the ceramic restorations from the CAD/CAM technique.⁽²¹⁾ The most common causes of errors performing intraoral scanning were the result of improper preparation of teeth, the instability of the scanner in the mouth of the patient, incorrect position, angle of the scanner to the object scanning, contrast spray applied in uneven layer, and the presence of fluid in the scan region and the presence of artifacts in the gingival sulcus region.⁽²²⁾ In this case, the preparation design may be not suitable for digital workflow, as shown from overmilling, marginal chipping and under margin that occurred. For an optimal CAD/CAM endocrown preparation design, clinicians should flatten and round all cusp tips, confirm the absence of undercuts, prepare teeth with 1.0 mm. thick and smooth finish lines. The margin area should be clearly visible for precise milling. Additionally, clinicians can utilize the preparation check and milling simulation steps in CAD/CAM software to verify adequate preparation and identify potential areas that may lead to overmilling.⁽²⁰⁾ In the event that the operator wants to continue using CAD/CAM in this case, the preparation design should be adjusted to deeper finish line such as chamfer or rounded shoulder in order to avoid limitations of milling process.⁽²³⁾

From the study of Carvalho T *et al.*,⁽²⁴⁾ it can be concluded that digital scanning systems were not superior to conventional impression when comparing in fidelity, accuracy, and surface detail reproduction, but have proven to be superior to conventional techniques for clinical chair time, patient and operator preference and patient comfort. Nevertheless, the high cost of these systems still hinders their introduction into the clinical reality.

Conclusions

The preparation design of an endocrown affects the fabrication process of the restoration and material selection. Even though the design was good, manufacturing might not coincide. The digital workflow has many advantages but still has some drawbacks such as overmilling, undermargin and marginal chipping while the conventional workflow provides better clinical outcomes and can decrease error from digital workflow. Therefore, the operator should decide carefully on suitable fabrication techniques and materials for each case.

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Apparent Modulus of Honeycomb Structure: A Guideline for Porous Structure Implant Design

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Abstract

Objectives: To investigate the apparent modulus of honeycomb to be used as a guideline for facilitating porous structure implant design.

Methods: Apparent modulus of each honeycomb model was developed based on finite element analysis. Geometry of honeycomb structures included circumcircle radius of 2 mm, 3 mm, and 4 mm with wall high of 0.5 mm, 1.0 mm, 1.5 mm, and 2.0 mm.

Results: Hexagonal shape of honeycomb structure with circumcircle radius 2 mm was compared with circle with 2 mm diameter, both with wall thickness of 1 mm. The relationship is best described by logarithm equation with coefficient of correlation above 0.99. It was found that reduction of modulus for circular shape is 60 percents. The value is greater than hexagonal pattern which is 50 percents of reduction.

Conclusions: The relationship between height of honeycomb and reduction of apparent modulus of each specific circumcircle radius of honeycomb is best described in logarithm equation and as a guideline for facilitating porous structure implant design.

Keywords: dental implant, finite element analysis, honeycomb structure, modulus of elasticity, porous structure

Introduction

Occlusal load transferring to the surrounding bone is an important factor for the long term success or failure of an osseointegrated implant.⁽¹⁾ The transfer of occlusal load across the interface between osseointegrated implants and surrounding bone tissue is a very relevant issue. The absence of a periodontal ligament around the dental implants, biting force are directly transferred from the rigid implant to the surrounding bone, causing relatively high crestal stress concentrations. Previous studies have shown that the crestal bone loss is associated with unfavorable loading conditions.⁽²⁻⁴⁾ Occlusal load from a restoration part of implant system induces strain through the implant into the surrounding bone, which subsequently affects the bone modeling and remodeling processes.⁽⁵⁾ In biomechanical point of view, Forst *et al.*,⁽⁶⁾ demonstrated that the design of implant is important for several clinical scenario, such as bone densities, jaw regions, implant diameter and length. The macrostructure and microstructure of implant design influence the biomechanical stability of the implant.

Theoretically, the modulus of Elasticity of an implant material has to be as close as possible to the modulus of bone to assure an optimal occlusal load transfer. Recently, an increasing number of studies focused on the adjustment of the elastic modulus of bone implants by using Ti-based alloys or porous structures of Ti as dental implant materials. The apparent modulus of elasticity is defined as the ratio of stress to strain within the elastic range.⁽⁷⁾

The porous structure is one of the structural design for biomechanical stability of implant as show in Figure 1. Previous studies were suggested that porous structure of titanium implant has biomechanical advantage of load transferring to cortical bone.⁽⁷⁻⁹⁾ Schiefer suggested that the anisotropic behavior of the porous titanium must be considered in the construction of implant devices.⁽¹⁰⁾ Recently, several techniques have been developed to introduce a pore size and a degree of porosity in titanium alloy,⁽¹¹⁻¹³⁾ which has led to the rapid development of various dental implant designs. Additionally the porous structure titanium implant shows a biomechanical behavior of its mechanical properties depending on the pore size and porosity. The knowledge about the biomechanical properties of porous structure dental implants is required for proper design, before fabrication and application. Researchers have investigated biomechanical

properties of different porous structure implant designs such as pore size, porosity and morphology.⁽¹⁴⁾

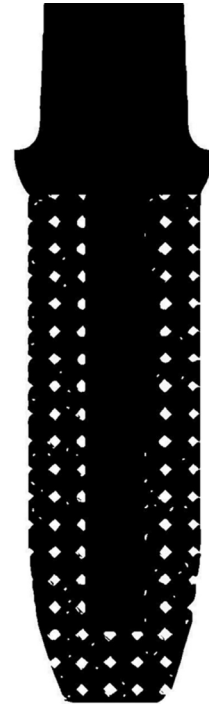


Figure 1: The porous structure implant

Honeycomb structure is commonly used in engineering applications, especially in aircraft and automobile structure^(14,15), due to its lightweight with high strength and energy absorption.^(15,16) Use of honeycomb structure also recently extends to medical devices where it is used as a part to reducing impact reaction producing from physiological loads. Design engineer therefore needs to have a mechanical property guideline.⁽¹⁷⁾ As an important computer tool, the finite element method is particularly convenient for evaluating and improving implant design without the risk and expense of real implantation.⁽¹⁸⁾ Honeycomb geometries were analyzed their apparent modulus by means of finite element (FE) analysis. The objective of this study was to investigate the relation of honeycomb dimensional characteristics to apparent modulus to facilitate the design of porous structure implant.

Materials and Methods

CAD Model

3D representation of Hexagonal shape-honeycomb structure was created using CAD software (VISI 20, Vero software, UK) Hexagonal shape-honeycomb structure was

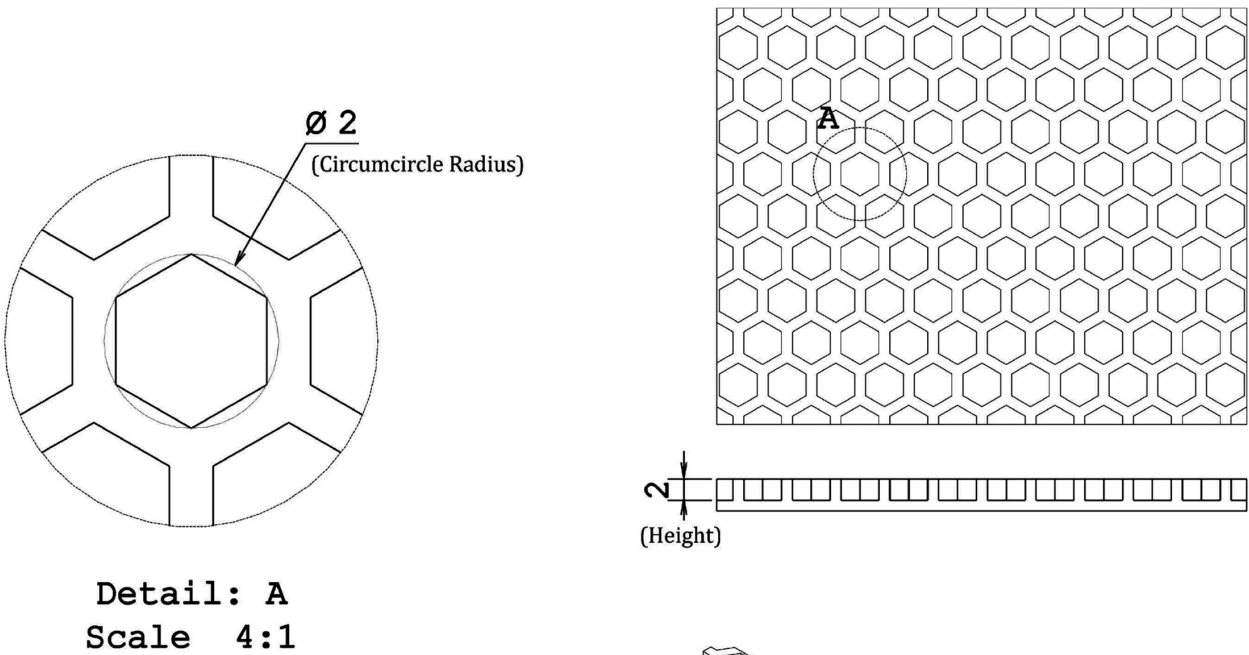


Figure 2: Honeycomb and its parameters

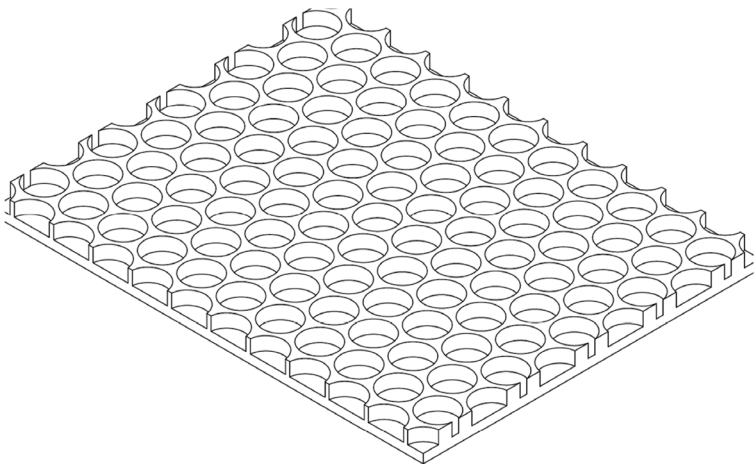


Figure 3: The plate with circle of 2 mm diameter

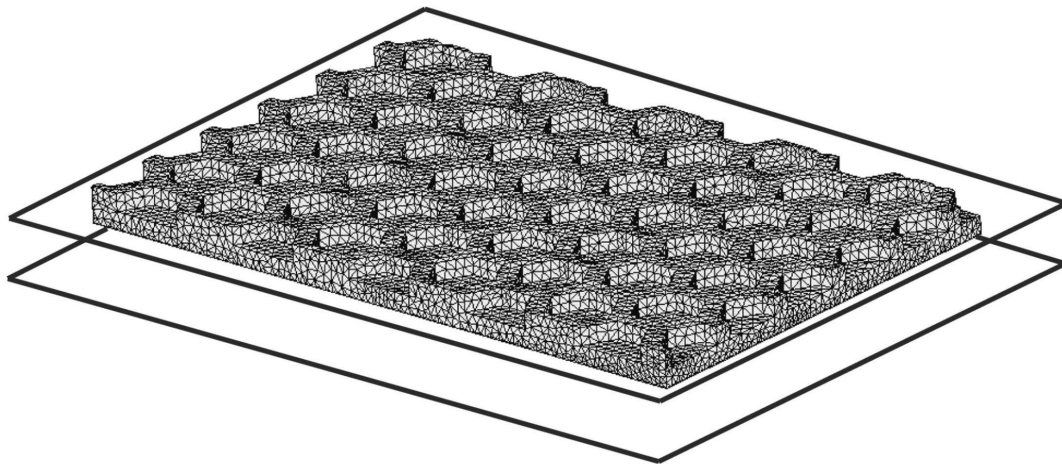


Figure 4: FE Model

under consideration in this study. The material properties assigned to the honeycomb is 5 MPa with Poisson's ratio of 0.3. Honeycomb contains core and facet on one side. Dimension of honeycomb structure included circumcircle radius of 2 mm, 3 mm, and 4 mm with wall high of 0.5 mm, 1.0 mm, 1.5 mm, and 2.0 mm, as shown in Figure 2 and Figure 3.

FE Model

All honeycomb geometries were analyzed their apparent modulus by means of finite element (FE) analysis. In all FE analyses, four-node tetrahedral element was used with total element number ranged from 25,315 to 83,779 (Corresponding to 7,190 to 22,151 nodes). The FE model is compressed with two planes as shown in Figure 4 at distance of 0.1ε. The reaction force exerted on plane was used to analyze the apparent modulus by dividing with multiplying result of effective area and strain. In this case, effective area is 928.00 mm² and strain is 0.1. The finite element analysis was performed using FE software (Patran/Marc Mentat 2005 R2, MSC Software Inc., USA).

Results

The modulus of each honeycomb is as follows in the Table 1. The result of percentage of modulus reduction of each honeycomb dimension shows in Figure 5. It can be seen that the apparent modulus decreases with the increase of height and circumcircle radius. The regression analysis was used to determine the relationship equation between geometric property and reduction of modulus percentage. According to analysis, the relationship is beset described

by logarithm equation as shown in Table 2. In each equation, x is honeycomb height and is reduction of modulus. The mesh convergence is show in Figure 6 that exhibits solution convergence's mesh independences.

Hexagonal shape of honeycomb structure with circumcircle radius 2 mm was compared with circle with 2 mm diameter, both with wall thickness of 1 mm. The relationship is beset described by logarithm equation with coefficient of correlation above 0.99. It was found that reduction of modulus for circular shape is 60 percents. The value is greater than hexagonal pattern which is 50 percents of reduction.

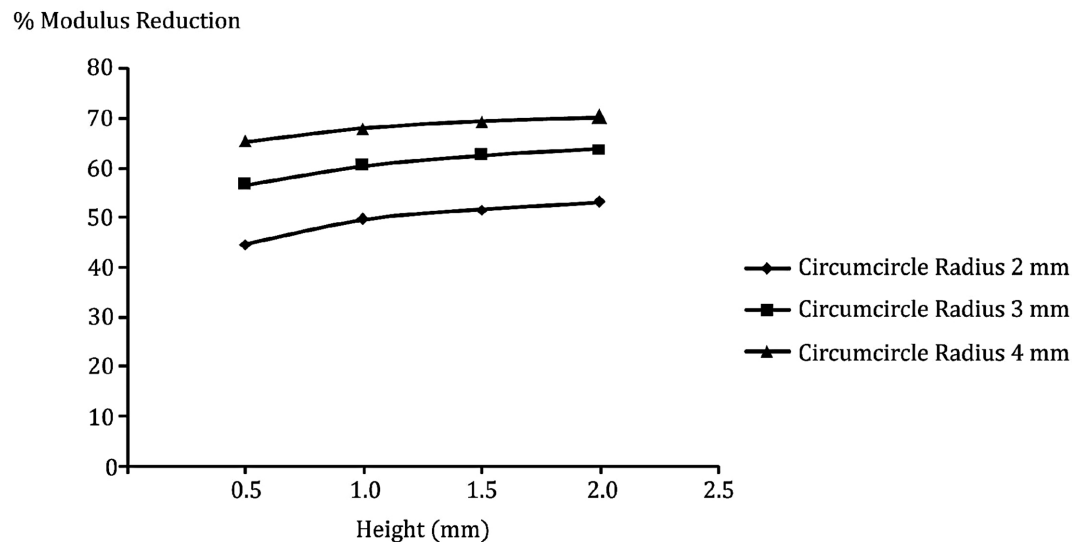
Discussion

The result from this study may not be able to directly compare with elastic modulus of human bone. Since the study aims to assess how the dimension of geometry affects the apparent modulus. Since the material properties assigned here in this FE study were intended to polymer, it cannot be used for bone application unless this honeycomb structure is made from metallic materials. The reduction of modulus from these metallic materials potentially close to the bone modulus.

This study provides a relationship between wall thickness and modulus reduction of honeycomb structure as a guideline for facilitating porous structure implant design. The apparent modulus decreases with the increase of height and circumcircle radius. Beside the hexagonal shape honeycomb structure, circular shape is also the common used pattern. In order to investigate how different honeycomb structure affect to the apparent modulus,

Table 1: Modulus of the honeycomb

No.	Inscribed Radius (mm)	Wall Height (mm)	Stiffness (MPa)	% Modulus Reduction
1	2.0	0.5	2.77	44.70
2	2.0	1.0	2.51	49.78
3	2.0	1.5	2.42	51.66
4	2.0	2.0	2.34	53.28
5	3.0	0.5	2.16	56.70
6	3.0	1.0	1.98	60.47
7	3.0	1.5	1.87	62.63
8	3.0	2.0	1.81	63.81
9	4.0	0.5	1.73	65.34
10	4.0	1.0	1.60	67.95
11	4.0	1.5	1.53	69.38
12	4.0	2.0	1.48	70.30

**Figure 5:** Percentage of modulus reduction of each honeycomb dimension

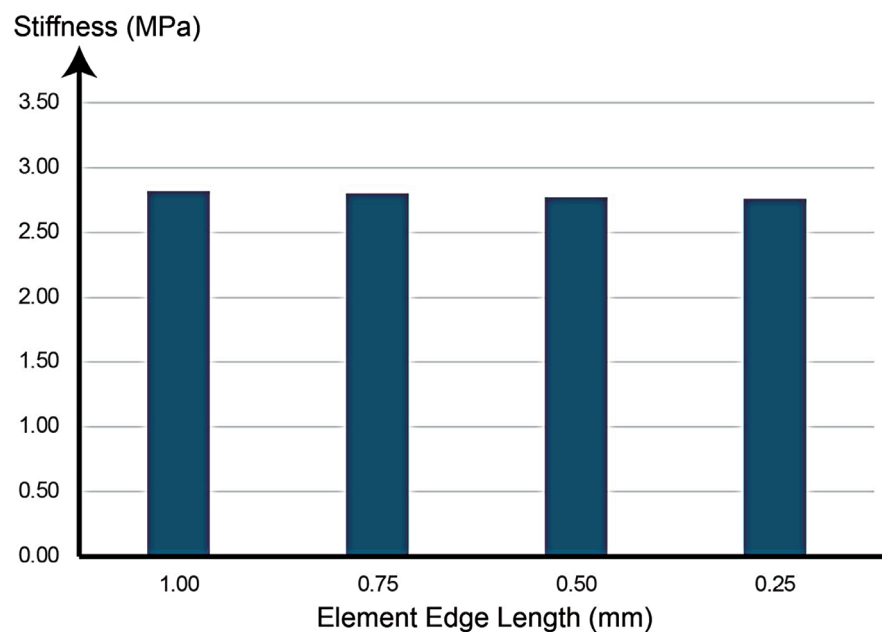
hexagonal with circumcircle radius 2 mm was compared with circle with 2 mm diameter, both with wall thickness of 1 mm. It was found that reduction of modulus for circular shape is 60 percents. The value is greater than hexagonal pattern which is 50 percents of reduction.

The result of this study was in agreement with the previous study, which the porous structure of implant effectively reduced crestal cortical bone and enhanced the load-bearing capacity.⁽⁸⁾ The long-term success of implant depended upon the biomechanical stability; therefore, the proper relationship between wall thickness and modulus reduction of honeycomb structure may be an alternative design for dental implant.

The finding of this novel study is beneficial for designing the implant macrostructure. Our implant design provided optimal biomechanical stability, which could reduce of risk of biomechanical complications. The previous study demonstrated that the success of osseointegrated implant therapy depends on a combination of biological and biomechanical factors.⁽¹⁹⁾ Current study suggested that some conditions with percent of porosity and pore size offer an optimal balance between biomechanical and biofunctional properties.⁽²⁰⁾ However, further *in vitro* and *in vivo* studies are required. It is worth noting that the mechanical performances analyzed by finite element could have been different depending upon materials and properties assigned.

Table 2: Equation between honeycomb height and reduction of modulus of each circumcircle radius

Honeycomb Dimension	Equation	Coefficient of Correlation
Circumcircle radius 2 mm	$y = 5.19\ln(x)+60.37$	0.999
Circumcircle radius 3 mm	$y = 6.16\ln(x)+49.23$	0.998
Circumcircle radius 4 mm	$y = 3.59\ln(x)+67.87$	0.999

**Figure 6:** The mesh convergence figure

Conclusions

The relationship between height of honeycomb and reduction of apparent modulus of each specific circumcircle radius of honeycomb is beset described in logarithm equation with high coefficient of correlation so the honeycomb structure base on apparent modulus analysis might have application potential in the porous design of implants. Further studies are needed to justify this novel porous structure implant designs.

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Conflicts of interest

The authors declare no conflicts of interest.

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Effect of Self-etch Silane Contamination on Dentin Bond Strength to Resin Composite

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Abstract

Objectives: This study aimed to investigate the effect of self-etch silane contamination on dentin bond strength to resin composite when using different adhesive systems.

Methods: 40 flat occlusal dentin surfaces were prepared and randomly divided into 4 groups (n=10): group ER (Optibond FL); group SE (Clearfil SE bond); group SiER and group SiSE (Monobond Etch and Prime (MEP) followed by Optibond FL and Clearfil SE bond, respectively). Microtensile bond strength (μ TBS) data was analyzed by two-way and one-way ANOVA followed by the post hoc Tukey honest test. The failure mode data was analyzed using Pearson Chi-square test. After undergoing different contamination procedures (distill water, phosphoric acid, and acidic primer with and without MEP contamination), 6 additional specimens were analyzed by scanning electron microscopy (SEM).

Results: The two-way ANOVA indicated that the adhesive system and silane contamination significantly influenced the μ TBS. μ TBS (MPa) of all (ER 47.79 \pm 3.48; SiER 41.16 \pm 11; SE 39.77 \pm 3.16; SiSE 35.10 \pm 4.12) groups were significantly different from each other, except for the SE versus SiER group. The silane contamination significantly decreased the μ TBS for both adhesive systems. Adhesive failure was the most common failure mode for the SiER, SE, and SiSE groups.

Conclusions: Self-etch silane cross-contamination on dentin negatively impacted the μ TBS of etch-and-rinse and self-etch adhesive systems. However, the etch-and-rinse adhesive system may be more effective in mitigating the effects of dentin contamination than the self-etch adhesive system.

Keywords: ammonium polyfluoride, bond strength, contamination, dentin, self-etch silane

Introduction

The silane coupling agents are used as adhesion promoters in dentistry between the resin matrix of the resin-based materials and an inorganic substrate. Acid-etching and silane priming of etchable glass ceramic restorations have been mandatory for strong and long-lasting bonding to tooth structures.⁽¹⁾ Despite the variety of etchable ceramics used in dentistry, acid-etching with hydrofluoric acid (HF, 5-10 wt%) and silanization with hydrolyzed 3-methacryloxypropyl trimethoxysilane (MPS) have been instructed for surface pretreatment, before resin luting agents' application.⁽¹⁻³⁾

The latest generation of silane primer is known as a self-etch one-bottle system, self-etch silane primer, or single-step pre-hydrolyzed silane solutions. The example of this single bottle ceramic primer is Monobond Etch & Prime (MEP) (Ivoclar Vivadent, Schaan, Liechtenstein). Because it contains a MPS for silanization and a new ammonium polyfluoride, tetrabutyl ammonium dihydrogen trifluoride (TADF), for the etching step. The product also contains a methacrylated phosphoric acid ester.⁽⁴⁾

The fracture of dental ceramics remains a major concern in restorative dentistry. The primary causes of ceramic fracture include microdefects in the material, impact and fatigue loads, improper design, mastication, parafunction, and intraoral occlusal pressures that induce persistent dynamic loading.⁽⁵⁾ In addition, cervical recession, microleakage, caries, or discolorations may occur at the margins of restorations.⁽⁶⁾ In some situations, ceramic repair may be a more cost-effective and time-saving than re-making the entire ceramic restoration or surgical procedure. In many cases, exposed tooth tissues, such as enamel and dentin, are included in the intraoral repair of ceramics.

It may be difficult to avoid contamination of the dental tissue substrate with self-etch silane during etching, rinsing, or drying procedures. According to ceramic repair protocol, dental application with different adhesive systems may influence the results. Soontornvatin *et al.*,⁽⁷⁾ investigated the effect of silane contamination on the μ TBS of 3-step etch-and-rinse adhesives on dentin. The study concluded that silane contamination on dentin before the etching step did not affect the dentin bond strengths. However, contamination after etching and priming had a significant negative influence on dentin bond strengths. Lührs *et al.*,⁽⁸⁾ investigated the impact of surface contamination on the μ TBS of universal adhesives during

repair procedures and concluded that contamination with HF acid or an MEP results to a significantly lower bond strength after aging only, but there were no significant differences in the immediate μ TBS. In the present, it is unclear whether a MEP would affect dentin bond strength. It is interesting to investigate how MEP contamination affected dentin morphology and dentin bond strength to resin composite when using different adhesive systems.

Therefore, the objective of this study was to investigate the effect of self-etch silane contamination on dentin bond strength to resin composite when using different adhesive systems.

Materials and Methods

Specimen preparation

After approval by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University No. 053/2022, 46 extracted human third molars that were free of caries, restorations, or cracks were carefully washed under running water and removed blood clots and attached soft tissues by scalpel and ultrasonic scaler (Branson, Germany). Then, teeth were immersed in a 0.5% chloramine-T aqueous solution at 4°C and were used within one month after extraction.⁽⁹⁾

All specimens were prepared by sealing root ends with wax and mounting root in a cold-curing acrylic resin base leaving the clinical crown exposed (Figure 1A). The occlusal central groove of the teeth was drilled 1.5 mm in depth using a high-speed cylindrical diamond bur (Jota, Switzerland), ensuring that the dentin exposure was located at bur-end level using a stereomicroscope evaluation (ML9300[®], MEIJI, Japan) at 40x magnification. Then, the occlusal enamel was removed perpendicularly to a tooth axis using a low-speed cutting machine (IsoMet 1000, Buehler; Lake Bluff, IL, USA) and stereomicroscope evaluation at 40x magnification to ensure all central area show dentin exposure (Figure 1B). A standardized smear layer on dentin was made using 600-grit silicon carbide paper (TOA, Thailand) with a polishing machine (Nano 2000, Pace technologies, USA) at 100 rounds per minute with 2.27 kg for 30 seconds in one direction under running water, rinsed, and stored in 37°C distilled water for 24 hours (Contherm 160M; Contherm Scientific Ltd., Lower Hut, New Zealand) (Figure 1C).⁽¹⁰⁾ Teeth presented with pulp exposure were excluded.

The specimens were randomly divided into 4 groups (n=10 for each group): Group ER and SE: The dentin was subjected to distill water application for 1 minute served as the control followed by water rinsing for 10 seconds and drying with oil-free compressed air for 10 seconds. Then, the ER group was subjected to Optibond FL™ application (Kerr, Orange, CA, USA) as well as the SE group to Clearfil SE bond™ (Kuraray, Kurashiki, Japan). Group SiER and SiSE: 1 coat of MEP (Ivoclar Vivadent, Schaan, Liechtenstein) was applied to dentin according to the manufacturer's instructions followed by OptibondFL™ application for SiER group and Clearfil SE bond™ application for SiSE group. To specify the bonding region of the central dentin, a piece of adhesive tape with a 6x6 mm square shaped hole was located and firmly adhered in the most central area of dentin (Figure 1D). All materials were used strictly according to the manufacturer's instructions as described in Table 1.

Bonding procedures

Dentin surfaces that were have been etched, primed, and bonded were dried using oil-free compressed air at 0.2 MPa air pressure from 5 cm above the dentin surface using a three-way syringe. An LED light-curing unit (Demi™ LED light-curing system, Kerr, Orange, CA, USA) with an irradiance of 1,000 mW/cm² was used to light-polymerize the resin-based materials for 10 seconds at a standardized distance of 2 mm from the bonding surface.

After light-curing of the bonding agents, resin composite buildup with a height of 4.5 mm was created in the central area of each tooth. To form and hold the resin composite onto the dentin surface, a transparent acrylic plate mold 6x6 mm square shaped with 1.5 mm in hight was employed. The resin composite was placed and compacted into the mold (Filtek Z350XT; 3M Oral Care, St. Paul, USA). The excessive material was removed with glass plate. It was made for three increments, each of which was light-cured for 20 seconds with the tip of light curing unit located at a standard distance of 1 mm from the resin composite surface. Before light-curing process of every one specimen, the light energy output was verified at more than 800 mW/cm² with a radiometer (Demetron L.E.D Radiometer, Kerr, Orange, CA, USA) throughout the procedure.

After the restorative operation, the specimens were immersed in distilled water and kept in an incubator for

24 hours at a temperature of 37°C.

μTBS testing

After storage, ten specimens were used for each group (n=10). Theses restored teeth were sectioned occluso-gingivally across a bonded interface into stick shaped specimens with 1 mm×1 mm cross-sectional area using a low speed cutting machine (IsoMet® 1000, Buchler, USA).⁽¹⁴⁾

The four central sticks from each tooth were obtained (Figure 1F, 1G). In contrast, the peripheral area was excluded. Pre-test failures also were excluded from statistical analysis.

According to ISO technical specification 11405:2015, specimens were fixed by their endings to Ciucchi's jig with cyanoacrylate glue (Model Repair II Blue, Dentsply, Ohtawara, Japan) and stressed at a crosshead speed of 1 mm/min until failure in a universal testing machine (EZ-S, Shimadzu, Japan), with a load of 10 kg (Figure 1I). The movement was automatically stopped at the fracture point. The μTBS values were recorded and calculated to the average μTBS (MPa) of each tooth for statistical analysis. Premature failures were excluded from the statistical analysis.

At the ending of the test, the two parts of the sample were stored to investigate the fracture pattern and failure mode.

SEM analysis

SEM was used to examine the specimens' surface morphology of six teeth after undergoing different contamination procedures (distill water or self-etch silane) and after the etching procedure described in Table 1. (Figure 2).

One extra specimen from each group, after contamination procedure and etching procedure, was dehydrated in a series using ethanol solution (Scientific and Technological Research Equipment Centre, Chulalongkorn University, Bangkok, Thailand) (70% for 10 minutes, 95% for 10 minutes and 100% for 20 minutes) then mounted on aluminum stubs, dried in a desiccator, and finally sputter-coated with gold coating. Then, their surfaces were evaluated at magnification of 500X and 3,000X at an acceleration voltage of 15 kV (JSM-6610LV Scanning Electron Microscope JEOL, Japan).

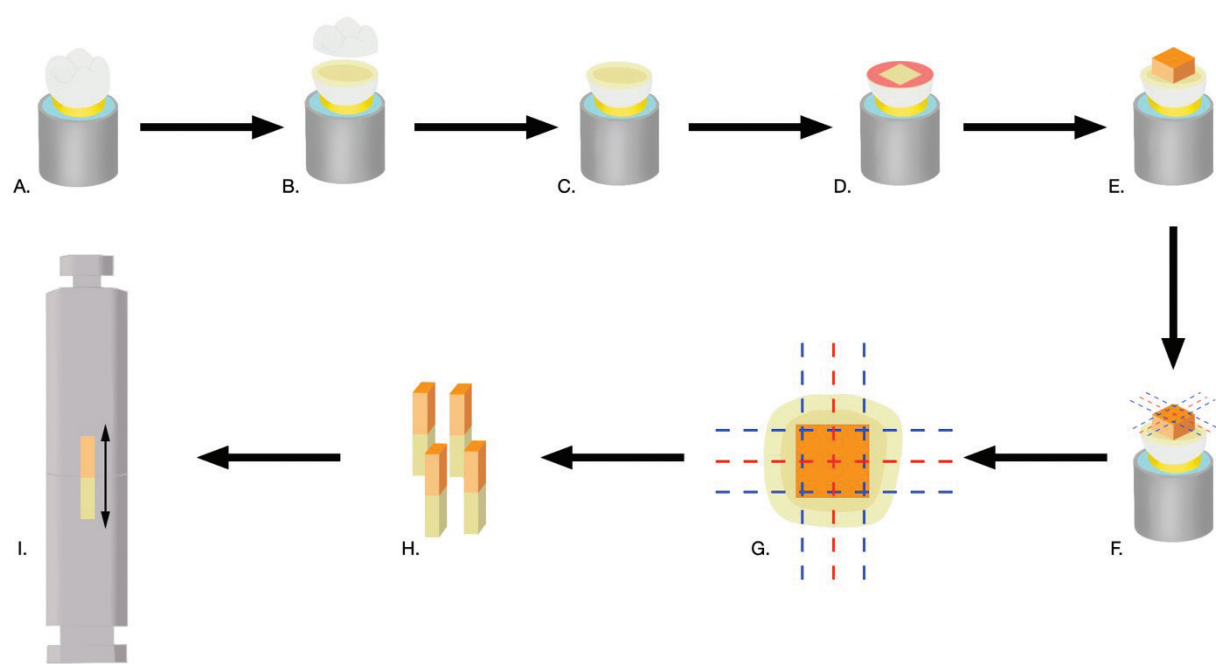


Figure 1: Schematic representation of the procedure for measuring the μ TBS of dentin. (A) root mounted in cold-curing acrylic resin, (B) occlusal third removal, (C) standardized smear layer on dentin, (D) 6x6 mm square shaped hole adhesive tape on dentin, (E) composite buildup, (F) serial sectioning, (G) serial sectioning, top view (red dash line: the central reference lines, blue dash line: the adjacent lines), (H) square specimen (1 x 1 x 8-9 mm³), (I) specimen testing

Table 1: Materials’ detail, composition, and manufacturer’s instructions

Material and manufacturer	Composition	Manufacturer’s instructions
Optibond FL (Kerr, Orange, CA, USA) LOT No. 8308256	Etchant: 37.5% phosphoric acid, silica thickener Primer: HEMA, GPDM, PAMM, ethanol, water, photoinitiator (pH 1.8) Adhesive: TEGDMA, UDMA, GPDM, HEMA, bis-GMA, filler, photoinitiator	Etch: Apply Etchant 15 s, rinsed with water 15 s, gently air dry 3 s Prime: Apply primer with light scrubbing motion for 15 s, gently air dry 5 s Bond: Apply bonding agent with light scrubbing motion for 15 s, remove the excess with a gently air 5 s and light cure for 10 s
Clearfil SE Bond (Kuraray, Kurashiki, Japan) LOT No. 000079	Primer: 10-MDP, HEMA, water, photoinitiator (pH1.9) Bond: 10-MDP, bis-GMA, HEMA, hydrophobics dimethacrylates, photoinitiator	Prime: Apply a layer of primer, wait 20 s, gently air dry 5 s Bond: Apply the bonding agent, remove the excess with a gently air 5 s and light cure for 10 s
Monobond Etch&Prime (MEP) (Ivoclar Vivadent-Schaan, Lichtenstein) LOT No. Z03024	Butanol, tetrabutyl ammonium dihydrogen tri-fluoride, methacrylated phosphoric acid ester, bis(triethoxysilyl)ethane, silane methacrylate, colourant, ethanol, water	Actively apply on the ceramic surface for 20 s, let it react for 40 s and wash it with water for 10 s, strong stream of water- and oil-free air for 10 s
Filtek Z350XT (A2 Body) (3M Oral Care, St. Paul, USA) LOT No. NE45910	Bis-GMA, UDMA, TEGDMA, Bis-EMA, non-agglomerated/ non-aggregated 20 nm silica filler, non-agglomerated/ non-aggregated 4 to 11 nm zirconia filler, and aggregated zirconia/ silica cluster filler	Insert the composite in 2 mm increment and light-cure for 20 s

Abbreviations: HEMA: 2-hydroxyethyl methacrylate; PAMM; Methacroyloxyethyl Phthalate; TEGDMA: triethylene glycoldimethacrylate; Bis-GMA: bisphenol A diglycidyl ether dimethacrylate; 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; GPDM; glycerol phosphate dimethacrylate Bis-EMA; Ethoxylate biphenol A glycol diamethacrylate^(7,11-13)

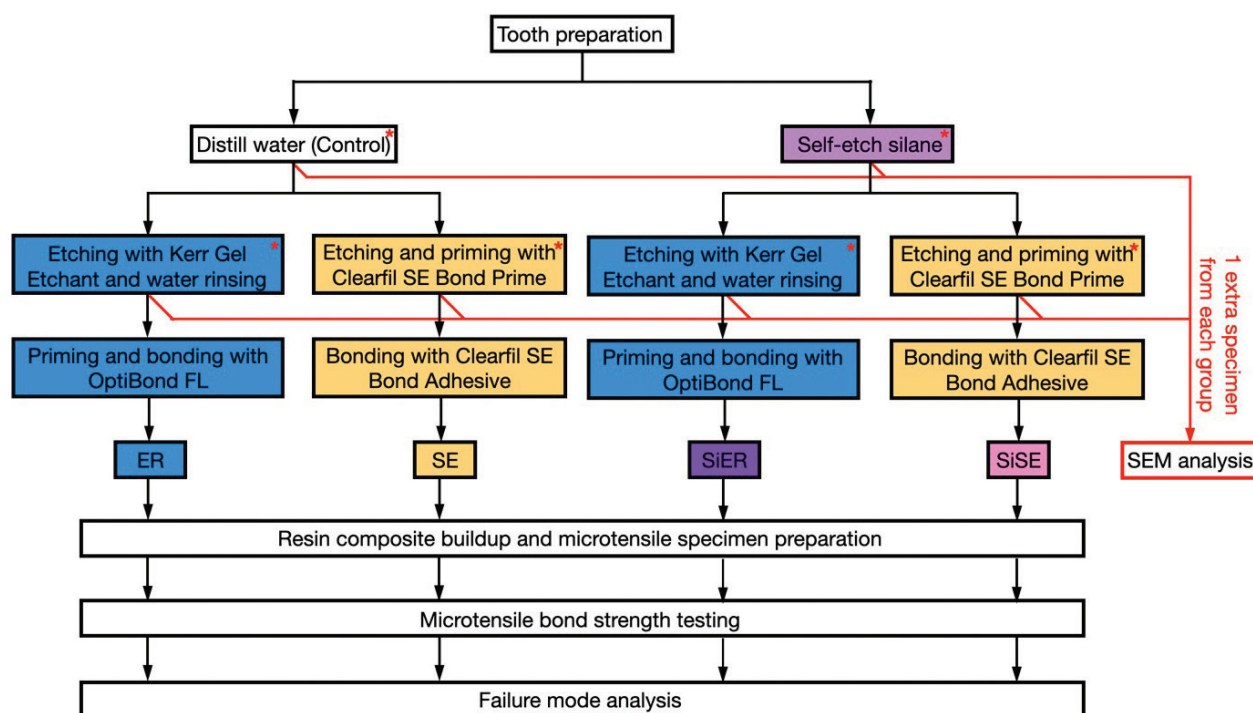


Figure 2: Diagram of study design

Failure mode analysis

After the μ TBS testing, the debonded surfaces of all specimens were examined under a stereomicroscope (ML9300[®], MEIJI, Japan) at 40x magnification to determine the failure mode. Each specimen was classified into 1 of 4 types as following:

1. Adhesive failure (AF; >80% of the failure area occurred between resin and dentin);
2. Cohesive failure in resin (CFR; >80% of the failure area occurred in dentin/resin, but the majority is in adhesive resin or composite resin);
3. Cohesive failure in dentin (CFD; >80% of the failure area occurred in dentin/resin, but the majority is in dentin);
4. Mixed failure (MF; mixed with adhesive failure between dentin and resin, cohesive failure in resin and/or dentin).⁽¹⁵⁾

Statistical analysis

Means and standard deviations were calculated and presented in MPa. The Kolmogorov-Smirnov test and Levene's test were used to determine homogeneity. The data was statistically analyzed by 2-way ANOVA and 1-way ANOVA followed by the post hoc Tukey honest significant difference (HSD) test ($\alpha=0.05$), and the

failure mode data was analyzed using the nonparametric Pearson Chi-square test with a spreadsheet (Excel Microsoft Office 2010; Microsoft Corp) and a statistical analysis software (SPSS 22.0; SPSS Inc, Chicago, IL, USA.).

Results

μ TBS test

The Kolmogorov-Smirnov and Levene's tests indicated that the μ TBS data had a normal distribution and homogeneous variances ($p=0.54$). Mean μ TBS data for dentin with and without silane contamination were presented in Table 2. Mean \pm standard deviation μ TBS of ER was 47.79 ± 3.48 MPa, SiER was 41.16 ± 3.11 MPa, SE was 39.77 ± 3.16 MPa, and SiSE was 35.10 ± 4.12 MPa. The box plots of the μ TBS data are shown in Figure 3.

As shown in Table 3, the results of one-way ANOVA indicated that μ TBS of all groups were significantly different ($p < 0.001$). Two-way ANOVA revealed that both the adhesive system ($p < 0.001$) and silane contamination ($p < 0.001$) had a significant influence on the μ TBS. However, there was no statistically significant interaction between the adhesive system and self-etch silane contamination ($p = 0.381$). Further analysis using the post hoc Tukey HSD test revealed that all groups were signifi-

cantly different from each other, except for the SE versus SiER group ($p=0.811$) (Table 4). The silane contamination significantly decreased the μ TBS for both adhesive systems ($p<0.001$ for ER versus SiER and $p=0.024$ for SE

versus SiSE). Additionally, the μ TBS of the SiER group was significantly higher than the SiSE group ($p=0.002$), although it was insignificantly higher than the SE group ($p=0.811$).

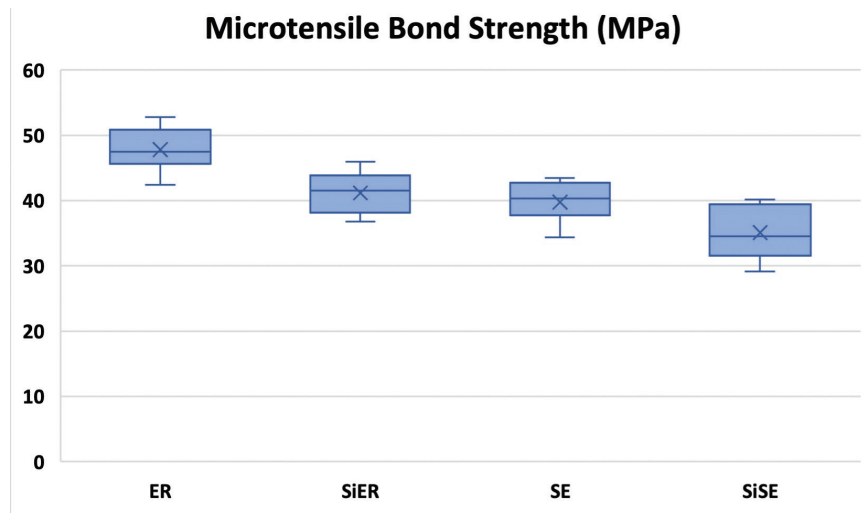


Figure 3: Summary of box plots of μ TBS (MPa). Box plot shows the median (+), 25% quartile ([box] bottom line), 75% quartile ([box] top line), maximum (plus error bar), and minimum (minus error bar)

Table 2: Mean μ TBS (MPa) on dentin with and without silane contamination

Group	Etch-and-rinse	Self-etch
Control (distill water)	47.79±3.48 ^a	39.77±3.16 ^b
Silane contamination	41.16±3.11 ^b	35.10±4.12 ^c

Values are means \pm standard deviation (n=10). Means with different superscript letters are statistically different ($p<0.05$).

Table 3: Summary of 1-way and 2-way ANOVA

Source of Variation	Sum of Squares	df	Mean Square	F	p	Partial Eta Squared
1-way ANOVA						
Between groups	824.051	3	274.684	22.556	<0.001	
2-way ANOVA						
Silane contamination	319.300	1	319.300	26.220	<0.001	0.421
Adhesive system	495.180	1	495.180	40.662	<0.001	0.530
Interaction	9.570	1	9.570	.786	0.381	0.021

Table 4: Tukey HSD results

Treatment Pair	Tukey HSD Q statistic	Tukey HSD p value	Tukey HSD Inference
ER versus SE	8.015	<0.001	* $p<0.05$
ER versus SiER	6.629	<0.001	* $p<0.05$
ER versus SiSE	12.688	<0.001	* $p<0.05$
SE versus SiER	-1.386	0.811	Insignificant
SE versus SiSE	4.672	0.024	* $p<0.05$
SiER versus SiSE	6.059	0.002	* $p<0.05$

ER, etch-and-rinse control; SiER, etch-and-rinse with silane contamination; HSD, honest significant

Failure mode analysis

The analyzed failure mode data of the different groups, as shown in Figure 4, revealed that the most common failure mode for the SiER, SE, and SiSE groups was AF. Particularly, the SiSE group had the highest percentage of adhesive failures at 85%. On the other hand, the ER group predominantly exhibited CFR at a rate of 40%. There were significant differences in failure mode distribution among the groups ($p < 0.001$). The failure mode was confirmed by stereomicroscope images, as shown in Figure 5 (original magnification $\times 40$).

SEM analysis

Representative SEM images of dentin surfaces with and without self-etch silane contamination are displayed in Figure 6.

Discussion

According to Van Meerbeek *et al.*,⁽¹⁶⁾ OptiBond FL (Kerr) and Clearfil SE Bond (Kuraray Noritake) were identified as the gold-standard adhesive systems for etch-and-rinse and self-etch techniques, respectively. This designation was based on a comprehensive analysis of laboratory⁽¹⁷⁾ and clinical data⁽¹⁸⁾ through meta-analysis, as well as their exceptional performance in a

thirteen-year randomized clinical trial.⁽¹⁹⁾

This study found that the immediate dentin μ TBS of the control ER group was significantly higher than that of the control SE group, which aligns with a previous study.⁽²⁰⁾ The results indicated that the presence of self-etch silane contamination on dentin leads to a significant decrease in the μ TBS for both adhesive systems. However, Liang Chen *et al.*,⁽¹⁵⁾ examined whether the contamination of silane primer would adversely affect tooth adhesion and concluded that silane contamination did not have a negative impact on dentin shear bond strength. According to the use of universal adhesive in self-etch or etch-and-rinse mode, the shear bond strength of the ER and SE groups was insignificantly different. This finding suggests that the choice of adhesive mode does not significantly affect the bond strength in this context.

Furthermore, Soontornvatin *et al.*,⁽⁷⁾ investigated the effect of silane primer contamination on the μ TBS of two commercial 3-step etch-and-rinse adhesives on dentin. The study indicated that silane contamination on dentin before the etching step did not affect the dentin bond strengths of the 3-step etch-and-rinse adhesives. However, contamination after etching and priming had a significant negative influence on dentin bond strengths.

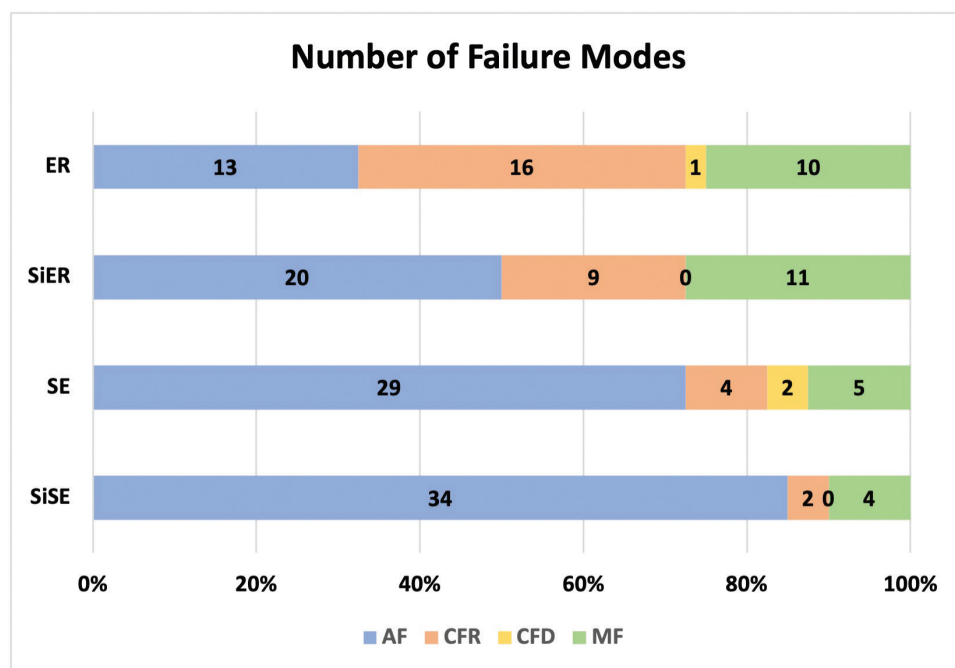


Figure 4: Number of failure modes of resin-dentin bond in each group (n=40). Numbers in each bar represent number of fractional failure modes in each group

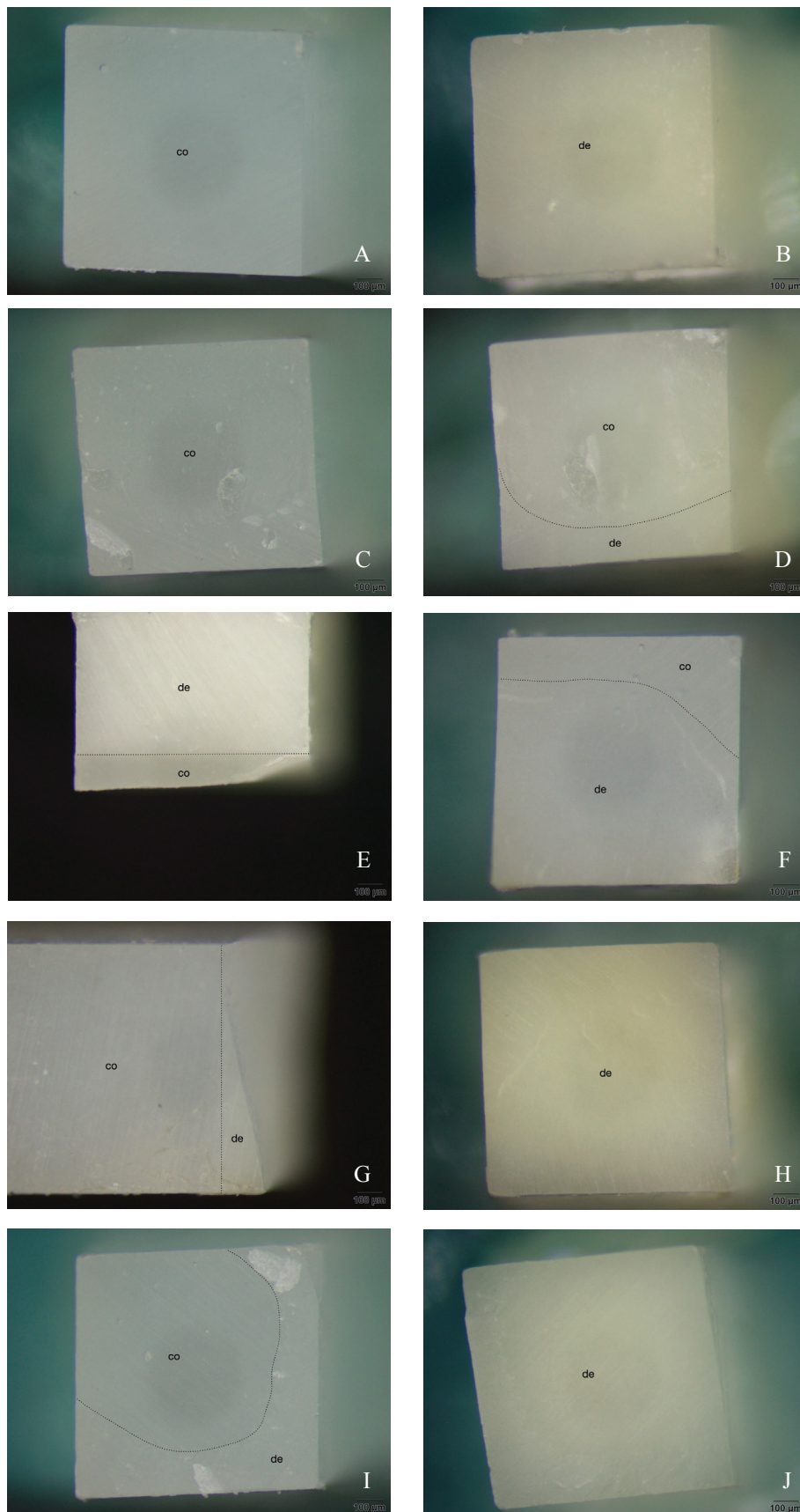


Figure 5: Representative stereomicroscope images of bond failure modes (original magnification $\times 40$). (A) and (B) AF (from group of SiSE's composite and dentin, respectively). (C), (D), (E) CFR (from group of SE's composite, dentin and lateral view of dentin, respectively). (F), (G), (H) CFD (from group of ER's composite, lateral view of composite and dentin, respectively). (I), (J) MF (from group of SiER's composite and dentin, respectively). co: composite; de: dentin; dotted line: resin-dentin interface

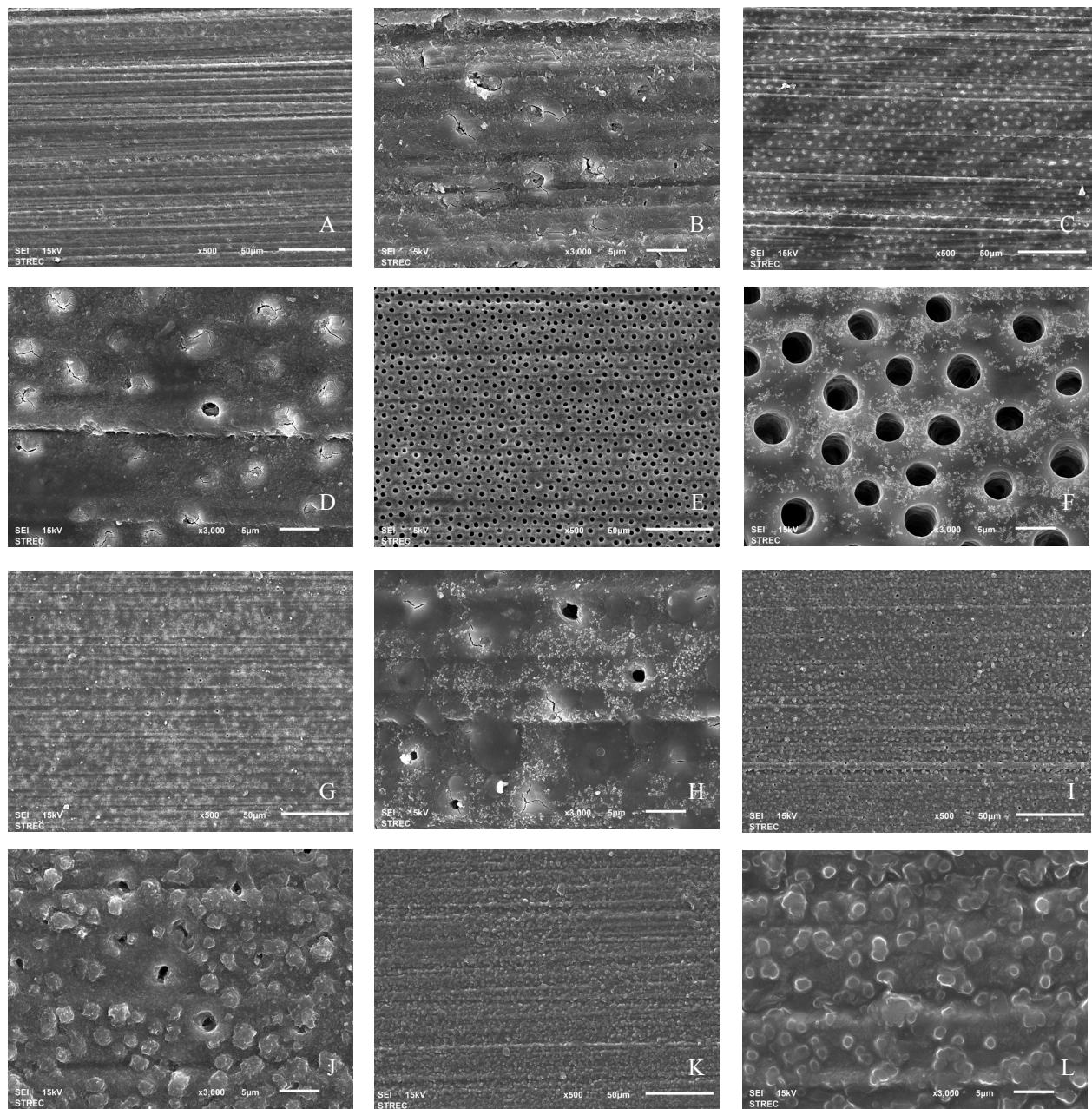


Figure 6: Representative SEM images of dentin surface with and without self-etch silane contamination; (A) and (B) Polished dentin with distill water; A smear layer was visible, and tubules were closed. (C) and (D) Polished dentin with silane contamination; The smear layer was superficially removed, but tubules were sealed. (E) and (F), Phosphoric acid-etched dentin; There was no smear layer visible, and tubules were open. (G) and (H) Silane-contaminated dentin with phosphoric acid etching; A smear layer was mostly visible, and only some tubules were open. (I) and (J) Dentin with self-etched primer; A smear layer was partly visible, and some tubules were partially open. (K) and (L) Silane-contaminated dentin with self-etched primer; occluded tubules and smear layer were clearly visible. ($\times 500$ and $\times 3000$ indicated the magnification)

Although the present study was designed the silane contamination before etching in SiER group, these two earlier research conclusions differ from ours could be since different silane agents were examined. The silane contained purely MPS was evaluated in two earlier investigations. Conversely, the present study investigated MEP containing a MPS and a new polyfluoride or TADF.⁽⁴⁾ These results were consistent with those of Kanzow *et al.*,⁽²¹⁾ who found the undefined precipitate formed by the polyfluorides in the MEP led to impaired resin infiltration and reduced bond strength. With MEP, the pretreatment of glass ceramic surfaces for the adhesive luting could be faster due to single application step and less harmful due to the absence of HF acid, and therefore seem to be an interesting option for glass ceramic repair. Regarding the intraoral repair of direct and indirect restorations, consistent protocols are lacking, and the literature presents various approaches.⁽²²⁾ According to Saracoglu *et al.*,⁽²³⁾ it was found that bonding to tooth tissues conditioned with HF acid gel resulted in a significant reduction in enamel and dentin bond strengths to resin composite, regardless of whether it was applied before or after phosphoric acid etching. However, the etch-and-rinse adhesive performed slightly better when HF acid was applied after phosphoric acid etching instead of before. In contrast, a study by Kanzow *et al.*,⁽²¹⁾ found a negative significant effect of bovine dentin contamination with HF acid or an MEP before applying universal adhesive in the self-etch mode. In the etch-and-rinse mode of universal adhesive application, the shear bond strength of bovine dentin contamination with HF acid or an MEP showed an insignificant decrease, regardless of whether it was contaminated before or after phosphoric acid etching.

In addition, Lührs *et al.*,⁽⁸⁾ investigated the impact of surface contamination on the dentin bond strength of universal adhesives using various etching modes during repair procedures involving HF acid, silane, or MEP. The study also investigated different etching modes before and after thermocycling. The findings indicated that dentin contamination with a silane primer containing 10-MDP before the application of a universal adhesive did not affect bond strength, regardless of the aging process. Compared to the control group, however, contamination with HF acid or an MEP leads to a significantly lower bond strength after aging, but there were no significant

differences in the immediate μ TBS.

On the other hand, in our study, immediate μ TBS of the SiER group was significantly reduced when MEP contamination before phosphoric acid etching. Similarly, in the SiSE group, immediate μ TBS was significantly decreased compared to the control SE group. These different results may be influenced by the different methodologies, such as specimen preparation, application sequence, and the type of dental adhesive used.

Additionally, the SiSE group exhibited the lowest performance among all the groups, resulting in an 85% AF. On the other hand, the ER group primarily showed CFR, suggesting that the bond strength between the resin and dentin might be stronger than the strength of the composite resin and/or dentin itself. The immediate μ TBS of the SiER group was insignificantly higher than that of the SE group. This finding suggests that the etch-and-rinse adhesive may be more suitable than the self-etch adhesive for self-etch silane contamination dentin.

The results of SEM are shown in Figure 6 with magnifications of $\times 500$ and $\times 3000$. The controlled polished dentin with distill water revealed a distinct smear layer with closed tubules (Figure 6A, 6B). Despite the self-etch silane contamination, the polished dentin appeared to superficially remove the smear layer, allowing for more visible dentinal tubule openings. However, the tubules remained sealed with precipitates (Figure 6C, 6D). These findings align with the previous studies.^(8,21) It is possible that an interaction between the fluorides present in the self-etch silane and the dentin surface at a molecular level, similar to the process observed with HF acid, could explain the decrease in μ TBS.⁽⁸⁾

It is known that HF acid creates dense amorphous fluoride precipitates on top of the tooth surface, which leads to the sealing of dentin and the closure of dentinal tubule openings. This process inhibits phosphoric acid etching and the infiltration of resin adhesive. However, there is a lack of other studies in the literature that describe the interaction between ammonium polyfluoride primers and dentin. Further research is needed to clarify this phenomenon. Dentin that had been etched with phosphoric acid showed apparent tubules and the complete removal of the smear layer (Figure 6E, 6F). On the other hand, dentin contaminated with self-etch silane, followed by phosphoric acid etching, exhibited a smear layer and only some open tubules (Figure 6G, 6H). This consistent with

the results published by Kanzow *et al.*,⁽²¹⁾. The MEP precipitation partially occluded the dentinal tubules, but it appeared to be less severe compared to HF acid contamination. Furthermore, the sequencing of phosphoric acid etching suggested that performing the etching step prior to self-etch silane contamination rather than after would result in less severe surface precipitates. This outcome is similar to dentin contamination caused by HF acid, which results in thick, amorphous fluoride precipitates. Furthermore, these precipitates cannot be effectively removed by further phosphoric acid etching.⁽²¹⁾

Dentin treated with acidic primer exhibited a smear layer that was partially visible and partially open tubules (Figure 6I, 6J), which was consistent with the previous study.⁽²⁴⁾ In contrast, self-etch silane contaminated dentin, followed by self-etched primer, revealed occluded tubules and a clearly visible smear layer (Figure 6K, 6L). This result also corresponds to another previous study.⁽²¹⁾ Although SE (Clearfil SE Bond) and MEP contain a methacrylated phosphoric acid ester such as 10-MDP.⁽⁴⁾ As 10-MDP also bonds to residual calcium ions, the chemical bonding process may be inhibited by the reaction mechanism triggered by the application of MEP. Nevertheless, it was still unclear how the MEP precipitation hampered the MDP-10 interaction and also self-etch adhesive bonding process. It may require further research to identify the molecular interaction.

In this study, only immediate μ TBS in dentin was investigated. However, the immediate enamel μ TBS and the aged μ TBS can be used for further investigation in future studies. Moreover, Fourier transform infrared spectroscopy (FTIR) can be analyzed to examine the alterations to resin monomer, silane, and collagen composition on the dentin surfaces. In the SEM observation, the specimens can be sectioned perpendicular to the resin-dentin interface to determine the thickness of the hybrid layer.

Conclusions

Based on the provided context, the *in vitro* study found that self-etch silane cross-contamination on dentin negatively impacted the μ TBS of both etch-and-rinse and self-etch adhesive systems. However, the study suggests that the etch-and-rinse adhesive system may be more effective in mitigating the effects of dentin contamination compared to the self-etch adhesive system.

Conflicts of interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, or company that is presented in this article.

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The Comparative Study of the Mouse Osteoblast Response to Two Different Platelet-rich Fibrins with Low Speed Centrifugation Concept

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Abstract

Objectives: To compare the response of mouse osteoblast (MC3T3-E1 cells) to advanced platelet-rich fibrin (APRF) and advanced platelet-rich fibrin plus (APRF+).

Methods: Blood was collected from eight volunteers, 25 - 38 years of age (four males and four females) to prepare platelet concentrates APRF (1,300 rpm, 14 minutes), APRF+ (1,300 rpm, 8 minutes). The exudates were collected from both platelet concentrates at day 1, 3, 7 and 14. The level of TGF- β 1 from exudates were quantified using an ELISA. MC3T3-E1 cells were cultured with the exudates. The cultured cells were tested with MTT assays, alkaline phosphatase (ALP) staining and mineralization, which were analyzed on day 7 and again on day 14.

Results: APRF and APRF+ continuously released TGF- β 1 during 14-days period. Only exudates collected at day 1 showed significantly difference of ALP staining between APRF and APRF+ group on day 14 of observation. On day 7 of mineralization assays, cells treated with exudates from APRF+ collected at day 14 resulted in the highest level of mineralization within APRF+ group. On day 14 of mineralization assays, cells treated with exudates from APRF collected at day 7 showed the highest level of mineralization within APRF group.

Conclusions: APRF+ released TGF- β 1 at day 14 significantly higher than day 1. On day 14 of mineralization, cells treated with exudates from APRF+ collected at day 14 showed significantly higher mineralization than APRF collected at the same time point.

Keywords: advanced platelet-rich fibrin, advanced platelet-rich fibrin plus, low speed centrifugation concept, mineralization, platelet concentrates

Introduction

Periodontal diseases are the consequences of inflammatory process that affect the supporting structures of the teeth. Periodontal diseases initiate by the buildup of dental plaque and microorganisms which begin as gingivitis and establish the local inflammation on gingiva. Periodontitis occurs when untreated gingivitis proceeds the destruction to gingiva, alveolar bone and periodontal ligament that results in a periodontal pocket which finally cause tooth loss. The goal of treatment is effectively control the inflammation, inhibit disease progression and maintain periodontal status in a healthy state. Periodontal regeneration is an absolute goal in the treatment of periodontal disease, aim to restore both hard and soft tissues to their original state or as closely as possible.⁽¹⁾ The major objectives of most biomaterial studies are focused on promoting wound healing and tissue reaction, while supporting the natural healing in defective areas.⁽²⁾ The study of biomaterials which support wound healing, and its regenerative abilities are based on the principle that closely mimic the natural mechanism. The earlier study using platelet concentrate concept started in 1970⁽³⁾ with fibrin glue usage. Then the application of platelet rich-plasma (PRP) in the treatment of periodontal disease is becoming widely used.⁽⁴⁾ PRP has been utilized by both oral surgeons and periodontists to demonstrate its regenerative properties. The disadvantage of PRP is its containing anticoagulant which can interfere the healing process and increase risk of life-threatening coagulopathies.⁽⁵⁾ Platelet rich-fibrin (PRF) is the second generation of platelet concentrates⁽⁶⁾, prepared from centrifuged blood. This technique does not require any anticoagulants or other agents, and it is convenience and inexpensive to perform. PRF was derived from the natural polymerization occurred during centrifugation. Slow release of growth factors including transforming growth factor β 1 (TGF- β 1), platelet derived growth factor AB (PDGF-AB) and vascular endothelial growth factor (VEGF) were observed in PRF investigations. Dohan *et al.*,⁽⁷⁾ demonstrated that PRF had a sustainably slow release of key growth factors at least 7 days. PRF has been used in various treatment procedures i.e. maxillary sinus lift⁽⁸⁾, regeneration of intrabony periodontal defect⁽⁹⁾, treatment of gingival recession.⁽¹⁰⁾ Recently, investigators have modified centrifugation protocol to increase the growth factor of platelet and leukocyte in the PRF-based matrix.⁽¹¹⁾ The modified preparation was based on a low speed

centrifugation concept (LSCC), described by applying a reduction in relative centrifugation force (RCF)⁽¹²⁾, resulting in different clot formation and distribution of cells called advanced-PRF (APRF), the study revealed a more porous structure compared to PRF and increase in total leukocyte numbers was observed.⁽¹¹⁾ Based on LSCC, the modification of centrifugation time would affect the structure and growth factor releasing characteristics, introducing a new PRF-based matrix called advanced-PRF+ (APRF+; 1,300 rpm; 8 minutes).^(12,13) Both of APRF and APRF+ showed a gradual but significant release of TGF- β 1, platelet derived growth factor AA (PDGF-AA), platelet derived growth factor BB (PDGF-BB), PDGF-AB and VEGF.^(12,13) APRF+ displayed the dispersed homogeneously of platelets all over the clot⁽¹²⁾ and demonstrated the highest value of TGF- β 1 at day 1, 3, 10 of observation when compared to PRF and APRF.⁽¹³⁾ Furthermore, various PRF-matrices exhibited good biocompatibility *in vitro* and also shown that they were able to produce a 3-fold increase in collagen synthesis of human fibroblast cells.⁽¹³⁾

Therefore, the centrifugal force and time in different preparative protocols may cause a various response of cells, which then affects the wound healing process. At present, there are limited studies which compare the level of growth factors, cell biocompatibility between APRF and APRF+. In addition, it remains unknown the effect of APRF and APRF+ on osteoblast cell.

In theory, less centrifugation time would allow for the increased collection of cells and subsequent growth factors in a fibrin clot. We then evaluated the level of growth factor (TGF- β 1) in the APRF+ and APRF groups, comparing the response of MC3T3-E1 cells by determining cell viability, differentiation, and mineralization. Therefore, the aim of this study was to determine *in vitro* effect of APRF, APRF+ on mouse osteoblast (MC3T3-E1 cells), which have been prepared by using a low speed centrifugation concept (LSCC).

Materials and Methods

Ethical approval

This study was approved prior to the data collection by the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University, Thailand (NO.74/2020). The study details were explained to all subjects and

informed consent was obtained before participation.

Platelet concentrates preparation

Sample size was calculated by using G Power program from previous study.⁽¹³⁾ Inclusion criteria of blood donors included 1. age 25-40 years 2. healthy 3. denies drug allergy 4. no history of anticoagulant usage. Exclusion criteria of blood donors included 1. smoking 2. pregnancy. The peripheral blood was drawn from eight healthy volunteers (four males and four females) with a mean age of 30.13 years (ranging from 25-38 years of age). Informed consent was obtained from each donor who participated in this study. The venous blood was collected in 10-ml sterile tube (A-PRF, Zhejiang Gongdong Medical Technology Co, Ltd, Zhejiang, China) and centrifuged using centrifugation machine (DUO centrifuge, Process for PRF, Nice, France). The preparation was performed according to following protocols:

APRF: 10 ml; 1300 rpm; 14 minutes

APRF+: 10 ml; 1300 rpm; 8 minutes

The clots were carefully removed and separated from the red blood cell layer. Individual platelet concentrates were collected and placed on separated well on 6-well plates. Five milliliters of α -modified Eagle medium (α -MEM; Gibco, Grant Island, NY, USA) was added to APRF and APRF+, and then incubated at 37°C in 5% CO₂ for 14 days. At each time point (day 1, 3, 7 and 14), all exudates were collected and replaced with 5 ml of the culture media. The exudates were stored at -80°C. Each collected exudate from APRF and APRF+ was defined as 100% exudates. For the cell culture experiments, exudates were diluted with α -MEM to get 20% exudates concentration.

Measurement of TGF- β 1 level

To determine the level of TGF- β 1 released from APRF and APRF+ at day 1, 3, 7 and 14. At the desired time intervals, TGF- β 1 from the exudate was quantified using an ELISA assays according to the manufacturer's instruction (R&D systems, Minneapolis, MN, USA). Absorbance was measured using a microplate reader (Tecan Microplate Reader, Grödig, Austria) at a wavelength of 450 nm and the process were repeated.

Cell culture

MC3T3-E1 cells were cultured with α -MEM containing 10% fetal bovine serum (Invitrogen, Waltham, MA, USA) and 1% penicillin-streptomycin (Invitrogen, Waltham, MA, USA) at 37°C in 5% CO₂. For osteogenic experiment, the media used containing 5 mM of β -glycerophosphate (Sigma-Aldrich, St. Louis, MO, USA) and 25 mg/ml of ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA). MC3T3-E1 cells were seeded at a density of 2×10^3 cells/well in 96-well plates for MTT assays and mineralization assays, and at a density of 1.2×10^4 cells/well in 24-well plates for analysis of alkaline phosphatase (ALP) staining. Each experiment was performed on day 7 and day 14 of the cell culture procedure.

Cell viability

Cell viability was determined using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) (BioChemica, Darmstadt, Germany) assay. Ten microliters of 5 mg/ml MTT was added to the cell cultures. The plates were incubated at 37°C in 5% CO₂ for 4 hours. Then the media was removed and 100 μ l of dimethyl sulfoxide was added to dissolve the formazan crystals for 15 minutes. Absorbance was measured by using a microplate reader at 595 nm wavelength. The mean values of the triplicated experiments were determined.

Measurement of alkaline phosphatase staining

The ALP staining was performed by fixing experimented cells for 30 minutes with a 4% paraformaldehyde then 500 μ l of CHAP buffer (100 mM Tris pH 9.5, 100 mM NaCl and 50 mM MgCl₂ in distilled water) was added and incubated in the dark room at room temperature for 30 minutes. Then the CHAP buffer was removed and cells were stained using a 0.5 ml BCIP/NBT solution (ROCHE, Basel, Switzerland) in distilled water at room temperature for 30 minutes in the dark room. Each well was washed with PBS twice. The ratio of positive surface area for each well was captured using stereoscopic microscope and calculated using ImageJ program (Figure 1).

Measurement of mineralization

The mineralization of the MC3T3-E1 cells was determined by fixing treated cells for 30 minutes with a 4% paraformaldehyde. Then the cells were stained with

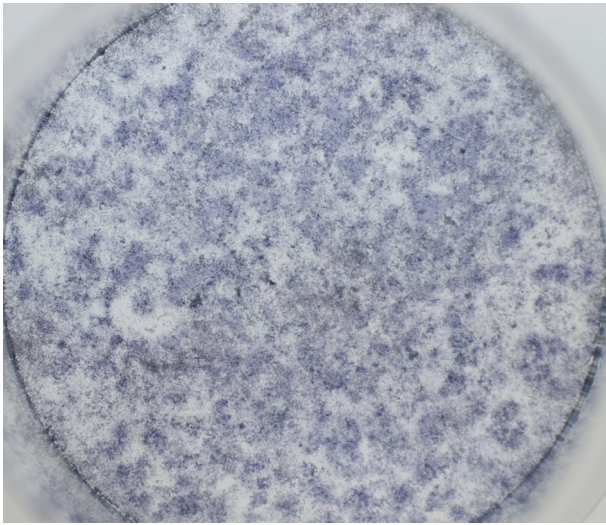


Figure 1: ALP staining on 24-well plate which captured by stereoscopic microscope

100 μ l of 2% alizarin red solution (Sigma-Aldrich, St. Louis, MO, USA), pH 4.1-4.3 at room temperature for 45 minutes, and reaction was stopped with 500 μ l of PBS. For quantification of mineralization, 10% cetylpyridinium chloride was then added and incubated at 37°C in 5% CO₂ for 1 hour. The absorbance was measured at a 550 nm wavelength. The mean values of the triplicated experiments were determined.

Statistical analysis

SPSS program version 25 (IBM, Chicago, IL, USA) was used. The mean and standard deviation was analyzed for levels of TGF- β 1, cell viability, ratio of positive surface area for ALP staining and mineralization of each interested time point using pair t-test, one-way ANOVA and one-way repeated ANOVA ($\alpha=0.05$). For all analyses, statistical significances were accepted at p values of <0.05 .

Results

Level of TGF- β 1 in APRF and APRF+ at each time points

There were no statistical significant differences comparing TGF- β 1 levels between APRF and APRF+ at each time interval. In the APRF+ group, TGF- β 1 level at day 14 were significantly higher than day 1 ($p<0.05$). Conversely, there were no statistically significant differences shown in APRF group at each time interval. (Figure 2)

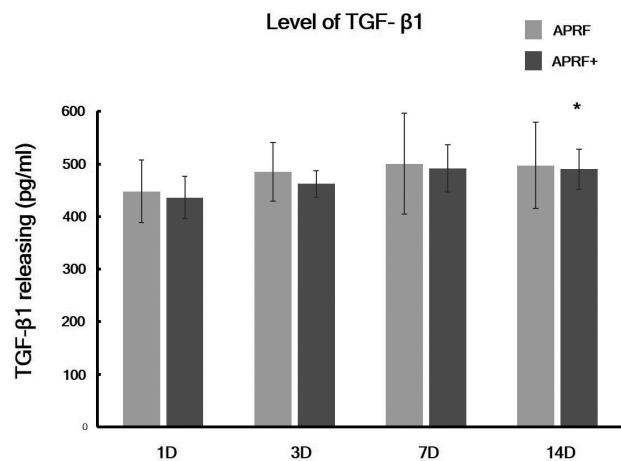


Figure 2: The levels of TGF- β 1 release from APRF, APRF+ during each experiment time. Exudates collected at day 1 (1D), day 3 (3D), day 7 (7D), day 14 (14D). Statistical analysis of $*p<0.05$, compared with APRF+ at day 1

Cell viability

MTT assay was performed to test for cell viability. On day 7, cells that were treated with exudates collected at day 7 and day 14 of APRF and APRF+ exhibited statistically significant differences in cell viability ($p<0.05$). Cells that were treated with exudates from APRF+ collected at day 1 resulted in higher cell viability than that treated with exudates from APRF ($p<0.05$). Cells that were treated with exudates from APRF and APRF+ collected at day 3, 7, 14 showed statistically significant differences when compared to the control group ($p<0.05$). For MC3T3-E1 cells that were cultured for 14 days, only exudates from APRF and APRF+ collected at day 1 showed statistically significant difference ($p<0.05$). Moreover, cells that were treated with exudates from APRF collected at day 1, 3, 14 and APRF+ at day 1, 3 showed statistically significant differences in cell viability when compare to the control group ($p<0.05$). (Figure 3)

Alkaline phosphatase staining

ALP staining of cells were performed after cells were cultured for 7 days. There were no statistically significant differences of ALP staining in cell cultured from exudates of both APRF and APRF+ at all tested time intervals. Following 14 days of mineralization, only cell treated with exudate collected from APRF+ at day 1 showed significantly higher expression of ALP staining than that cultured with exudate from APRF collected at the same day ($p<0.05$). (Figure 4)

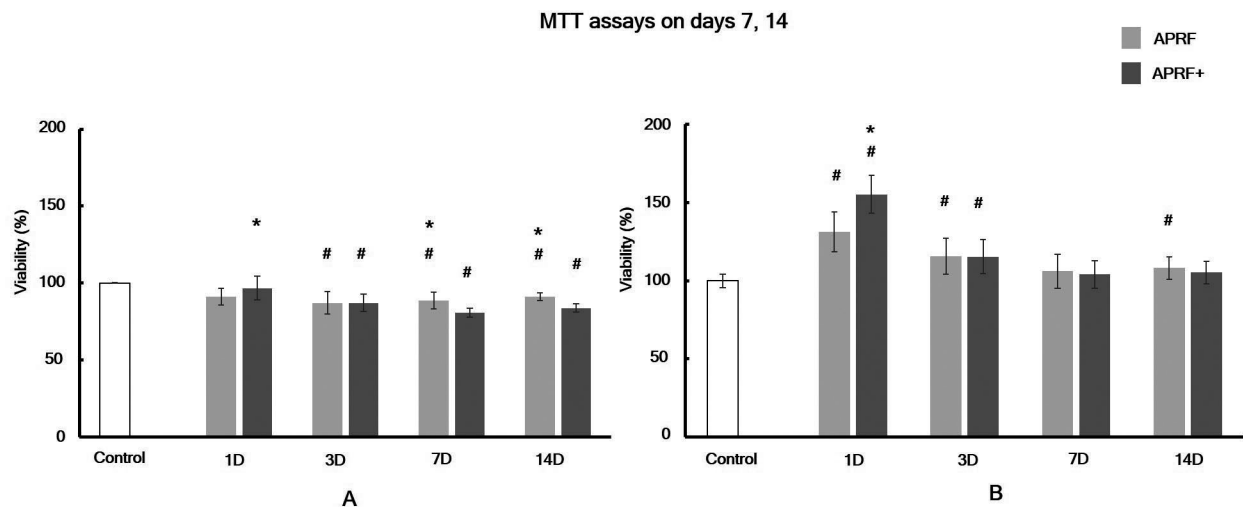


Figure 3: MC3T3-E1 cells viability, incubated with exudates of APRF or APRF+ collected at different time intervals (from day1- day 14). (A) MTT assays on day 7. (B) MTT assays on day 14. Exudates collected at day 1 (1D), day 3 (3D), day 7 (7D), day 14 (14D). Statistical analysis of $*p<0.05$, compared between APRF and APRF+ group. $\#p<0.05$, compared with control group

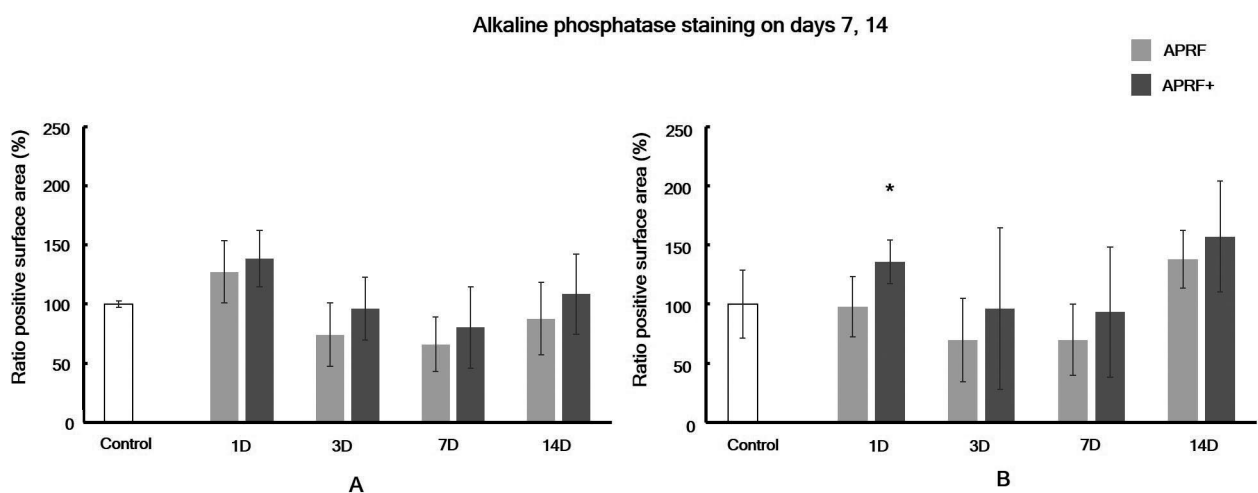


Figure 4: ALP staining of MC3T3-E1 cells, incubated with exudates of APRF or APRF+ collected at different time intervals (from day1- day 14). (A) Ratio of the positive surface area on day 7. (B) Ratio of the positive surface area on day 14. Exudates collected at day 1 (1D), day 3 (3D), day 7 (7D), day 14 (14D). Statistical analysis of $*p<0.05$, compared between APRF and APRF+ group

Mineralization

The effects of APRF and APRF+ on mineralization of MC3T3-E1 cells were shown. (Figure 5) On day 7 of mineralization assay, cells treated with exudate from APRF+ collected at day 14 showed the highest mineralization when compared to those treated with exudates from other time intervals in APRF+ group ($p<0.05$). Cells treated with exudate from APRF+ collected at day 7 reached significantly higher mineralization than cell treated with exudate from APRF+ collected at day 1 ($p<0.05$). When comparing between APRF and APRF+ groups, only cells cultured with exudate from APRF collected at day 1

resulted in a higher mineralization than that cultured with exudate from APRF+ ($p<0.05$).

On day 14, comparing cells treated within APRF and APRF+ group, cells treated with exudate from APRF collected at day 7 showed highest mineralization than those treated with exudates from other time interval in APRF group ($p<0.05$), cells treated with exudate from APRF+ collected at day 14 resulted in higher mineralization than those treated with exudates collected from day 1 and 3 ($p<0.05$). Cells treated with exudate from APRF collected at day 7 showed significantly higher mineralization than that treated with APRF+ at the same time point

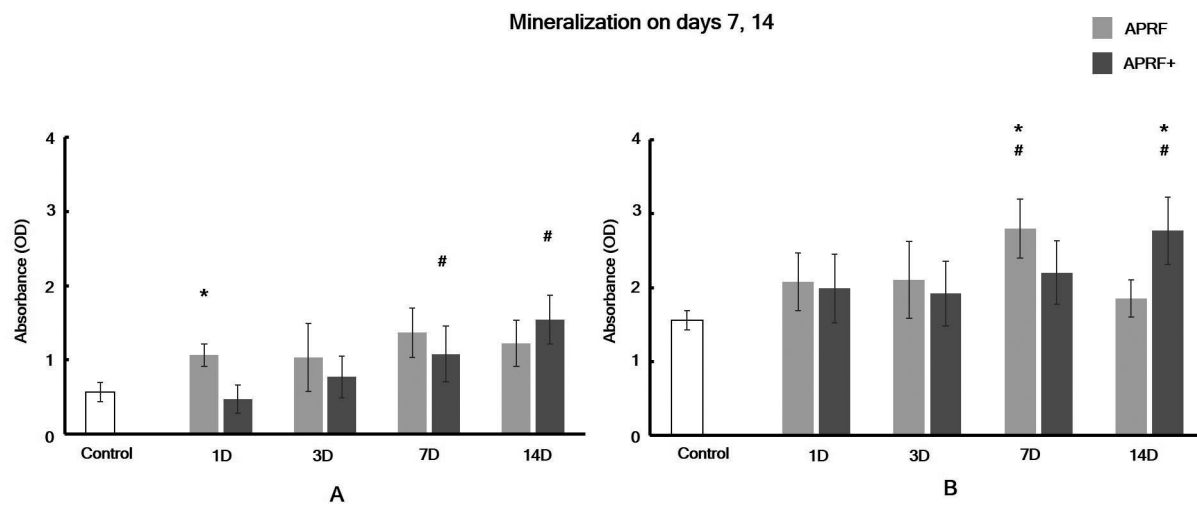


Figure 5: Mineralization of MC3T3-E1 cells, incubated with exudates of APRF or APRF+ collected at different time points (from day 1- day 14). (A) Mineralization on day 7. (B) Mineralization on day 14. Exudates collected at day 1 (1D), day 3 (3D), day 7 (7D), day 14 (14D). Statistical analysis of * $p < 0.05$, compared between APRF and APRF+ group. # $p < 0.05$, compared within APRF and APRF+ group

($p < 0.05$). Conversely, cells treated with exudates collected at day 14, APRF+ group resulted in more mineralization than APRF group ($p < 0.05$).

Discussion

PRF was prepared without addition of thrombin, many investigations showed that PRF slowly release the growth factors for at least 7 days.⁽⁷⁾ The development of the low speed centrifugation concept has been initiated to improve the capability of PRF.⁽¹¹⁻¹³⁾ In this concept, less centrifugation time increases cell numbers and growth factors in the PRF matrix. TGF- β 1 plays a key role during bone formation, contributes to the chemotaxis and mitogenesis of the osteoblast. This growth factor is crucial in the osteoblast's deposition of mineralized tissue.⁽¹⁴⁾ In this study, APRF+ showed a gradual increase of TGF- β 1, while a steady release of character was shown in APRF group (Figure 2). When comparing APRF and APRF+, no statistically significant differences of TGF- β 1 levels were found in every experimental time interval. The similar result was observed in the study by Bagdadi *et al.*,⁽¹²⁾ that reported no difference between TGF- β 1 release from APRF+ and APRF. Notably, only VEGF showed a higher release on day 7, and higher level in the accumulated release on day 10 for APRF+ compared with APRF and PRF. On contrary Kobayashi *et al.*,⁽¹³⁾ revealed that APRF+ released the highest value of TGF- β 1 at day 1, 3 and 10 when compared to PRF and APRF. Kobayashi *et al.*,⁽¹⁴⁾ placed the sample of platelet concentrates into

a shaking incubator at 37°C to allow the growth factor release into the culture media that was different than the methods done by Bagdadi *et al.*,⁽¹²⁾ and this study. This difference may be affect the outcome in the release of TGF- β 1. Thus, the method that was employed to detect growth factor was very specific in each study. In addition, the mechanism of growth factor release in PRF depends on leukocyte, platelet, and its structure.⁽¹⁵⁾ Dohan *et al.*,⁽¹⁶⁾ revealed approximately 97% of the platelets and 50% of the leukocytes were entrapped in the PRF clot. From this study showed that the level of TGF- β 1 in APRF+ group collected at day 14 statistically significant difference from day 1, this result may cause by the reducing centrifugation time that collected percentage of cells within the APRF+ clot more than APRF clot. The histologic features, compositions and quantity of cells in APRF and APRF+ should be investigated in further study. Additionally, the unique profile of PRF matrix may be influenced by the individual growth factor binding affinity to the fibrin matrix. VEGF had a high affinity binding to the fibrin, so this growth factor was found to be sustainably released during the study period.⁽¹⁷⁾ From this point, VEGF released from Bagdadi *et al.*,⁽¹²⁾ showed a significant increase on day 7. Conversely, EGF reached its highest level on day 1, due to low binding affinity of EGF to the fibrin.⁽¹⁸⁾ Further studies of growth factors that play a role in bone and soft tissue regeneration, such as EGF, PDGF⁽¹⁹⁾ and VEGF⁽²⁰⁾ should be inspected.

The MC3T3-E1 cells showed a reduced viability on day 7 and day 14 when incubated with the exudates of APRF+ and APRF from day 3, 7 and 14. This finding is similar to the result of Kermani *et al.*,⁽²¹⁾ The study was done using mouse dental pulp stem cells (DPSCs) that showed a reduction in cell viability during the differentiation of cells in osteoblastic induction state. Suggesting that the MC3T3-E1 cells decrease the ability to proliferate during differentiation state.

From the result of cell viability on day 14, exudates from both APRF and APRF+ collected at day 1 (Figure 3B) showed statistically significant differences from the control group. We hypothesized that the exudates collected at earlier time point (1D) might have a greater effect on cell proliferation than cell differentiation. During differentiation of osteoblast cells, cells always express and produce ALP.⁽²²⁾ MC3T3-E1 cells incubated with exudates from APRF and APRF+ did not show any statistically significant differences in ALP production when compared to the control group. The outcome was similar to the study by He *et al.*,⁽²³⁾ that showed no significant difference in the level of ALP of rat osteoblasts when cultured with PRP and PRF. Therefore, the future study should include the cell proliferation assays and ALP activity of osteoblast to elucidate the effect of APRF, APRF+.

Finally, the different mineralization patterns (Figure 5B) probably caused by the clot of APRF+ providing additional protection of growth factors and living cells from proteolytic degradation.⁽²⁴⁾ From the results of our study, the use of a low speed centrifugation concept that modified the centrifugation time affect the release of growth factor, viability and mineralization of MC3T3-E1 cells. The clinical applications from this study should be limited because of the study was an *in vitro* system. Future research comparing APRF and APRF+ in clinical scenarios should be established.

Conclusions

APRF and APRF+ having been prepared by using a low speed centrifugation concept were found to released TGF- β 1 during 14 days of the experiment period. APRF+ demonstrated release TGF- β 1 gradually and statistically significant difference from day 1 to day 14. On day 14 of mineralization, MC3T3-E1 cells treated with exudates from APRF collected at day 7 showed the highest mineralization among APRF group. Besides, exudates from

APRF+ collected at day 14 showed significantly higher mineralization than APRF.

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Conflicts of interest

The authors declare no conflicts of interest.

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Comparison of the Shear Bond Strength of Compomer Bonding on Different Enamel Surface Preparations

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Abstract

Objectives: To compare the effects of etching time and bonding agent application on the shear bond strength of compomer bonding in orthodontic bite raising.

Methods: Seventy-five sectioned crown of maxillary premolar teeth were embedded in acrylic rings. The samples were divided into 5 groups according to enamel surface preparation before applying Ultra Band-Lok[®] (Reliance Orthodontic Products). Group 1: without surface preparation, Group 2: etched with 37% phosphoric acid (Kerr Gel Etchant, Kerr[®]) for 15 seconds, Group 3: etched with 37% phosphoric acid for 15 seconds, then apply bonding (OptiBond[™] FL), Group 4: etched with 37% phosphoric acid for 30 seconds and Group 5: etched with 37% phosphoric acid for 30 seconds, then apply bonding. All samples were put through the thermocycling procedure and then shear bond strength was tested using the Universal Testing Machine. The mean and standard deviation of shear bond strength were statistically analyzed with two-way ANOVA and the enamel surface was observed by scanning electron microscope at 10,000x magnifications.

Result: In Group 1, all Ultra Band-Lok[®] dislodged from the enamel surface during the thermocycling process. Consequently, shear bond strength testing could not be conducted for Group 1. The mean shear bond strength of Groups 2-5 were 19.80±7.06, 18.97±4.60, 18.04±5.09 and 16.80±5.47 MPa respectively. The mean shear bond strength of each group was not statistically significant difference ($p=0.887$).

Conclusions: Varying enamel etching times (15 and 30 seconds) did not affect the compomer shear bond strength. Furthermore, the application of a bonding agent during tooth surface preparation did not significantly improve the bond strength between the compomer and the tooth surface.

Keywords: bite raising, compomer, shear bond strength, surface preparation

Introduction

In the field of orthodontics, bite raising is commonly practiced to correct deep bite, open bite, scissor bite, and crossbite. The bite-raising technique involves using a temporary instrument or material to create an artificial surface, facilitating contact between the teeth of opposing arches for occlusion. Full closure of the jaws is prevented either anteriorly or posteriorly. Bite planes are primarily categorized into removable and fixed bite planes, which can be placed in either the anterior or posterior section of the mouth.⁽¹⁾

Initially, the removable plates of the bite plane were crafted to fit the patient's palate fully. Over time, designs have become more compact and practical, allowing for easy attachment to the teeth. This development ensured patient comfort and encouraged compliance.⁽²⁾ The benefits of removable bite planes include ease of cleaning and removal, vertical and horizontal anchorage due to palatal coverage, effective reduction of overbite in growing children, and the ability to transfer forces to the blocks of teeth.⁽³⁾ Removable bite planes depend greatly on patient cooperation and must be adjusted frequently to accommodate orthodontic tooth movements. They are easily lost or broken, and there is a risk that the patient may swallow them.⁽⁴⁾ This appliance may also promote plaque accumulation, leading to poor oral hygiene and an increased risk of dental caries and *Candida* infection. Additionally, they may affect speech, and their fabrication requires more time in the laboratory and expense.^(5,6)

The metal bite turbo, a fixed anterior bite plane, was first introduced by Joe Mayes in 1994 as an alternative to removable acrylic bite plates; it uses a simple lingual bracket modification.⁽⁷⁾ Fixed metal bite turbos demonstrated greater muscle deprogramming qualities than acrylic biting planes and gained widespread use due to their simplicity, hygiene, solidity, and compatibility with oral hygiene procedures.^(7,8) However, the difficulty of application stemming from anatomical differences in the palatal surfaces of the teeth was a significant drawback of these devices.⁽⁷⁾

Advances in restorative materials and the drawbacks of fixed metal bite turbos have led to the exploration of fixed bite planes made from various non-metal restorative materials, including acrylic gel, resin composite, flowable resin composite, glass ionomer cement and compomer.⁽⁴⁾ Ultra Band-Lok[®], a compomer or polyacid modified resin

composite adhesive, is one of the most popular materials. Compomer materials combine the advantages of glass ionomer cement and resin composite, featuring physical properties that closely resemble those of resin composite.⁽⁹⁾ Several studies found that compomer exhibits high bond strength, compressive strength, flexural strength, and fracture hardness.⁽⁹⁻¹²⁾ However, Ultra Band-Lok[®] is subject to a clinical issue: If it slips out of the tooth, there can be negative consequences for the treatment, such as breaking bonded brackets, which can slow the teeth-moving process.⁽¹⁾

The bond strength between the enamel and compomer must withstand the stresses occurring in the oral environment. The compomer must be removable without leaving any residue or harming the enamel. Typically, tooth surface preparation, whether physical or chemical, influences the strength of the material's bond to the tooth surface. Mechanical methods, such as acid etching and sandblasting, as well as chemical techniques involving bonding agents, contribute to creating a strong bond between the tooth and the restorative material. A previous study evaluated the microtensile bond strength between human dentin and the compomer base material.⁽¹³⁾ Nevertheless, research on the shear bond strength of Ultra Band-Lok[®] bonded to human enamel surface preparations, whether chemical or physical, has not been reported. The aim of this study was to compare the effects of etching time and bonding agent application on the shear bond strength of compomer, with specific attention given to Ultra Band-Lok[®].

Materials and Methods

This experimental study compared the shear bond strength of compomer attachment to enamel surfaces with different preparation techniques. The study received ethical approval from the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University, Thailand (No. 38/2022).

Teeth preparation

The sample size was determined from a previous study.⁽¹³⁾ The G*power program was used to calculate the sample size based on an effect size of 1.46. Using the 2-tailed test, α error = 0.05 and power = 80.0%, the total calculated sample size was 9 for each group.^(14,15) In this study, the sample size was 15 per group. Seventy-five

extracted upper premolar teeth were selected with the criteria that the teeth did not have the following defect conditions: caries, enamel hypoplasia, fluorosis, enamel cracks, and history of bracket bonding and restorations. All teeth were stored in a 0.1% aqueous solution of thymol for no longer than 6 months. Tooth specimens were prepared by sectioning with carborundum discs 3 mm apical to the cemento-enamel junction (Figure 1A). Each tooth sample was embedded in a molded acrylic block (made from self-curing acrylic resin and cylindrical polyvinylchloride rings with a 15 mm diameter and a height of 10 mm, exposing the buccal side of the crown to the superficial surface (Figure 1B, 1C). For controlled curvature of the tooth surface, the buccal surface of each crown was flattened and polished using a specimen grinding machine (MoPao 160E Metallographic, Jinan Hensgrand Instrument, Jinan, China) with wet sandpaper under water cooling for 20 seconds for each grit from 200 to 600 grit. The prepared tooth surface of each sample is 4 mm in diameter and was localized on the enamel surface only. Each tooth surface was observed by stereomicroscope (SZX7 & SZ2-ILST LED illuminator stand & E-330, Olympus, Tokyo, Japan) with a magnification of 20× to confirm that the prepared area was enamel without dentin.

Bonding Process

The ground enamel surfaces of each sample were polished with superfine pumice and water for 10 seconds, rinsed with water spray, and dried by an air jet for 10 seconds. The samples were randomly divided into 5 groups of 15 samples. Each group underwent different bonding methods on the prepared tooth surface, as follows. Group

1: Apply Ultra Band-Lok[®] (Reliance Orthodontic Products, Inc. West Thorndale Ave, IL, USA) directly to tooth surface; Group 2: Prepare tooth surface by etching with 37% phosphoric acid (Kerr Gel Etchant, Kerr[®], Kloten, Switzerland) for 15 seconds, then rinsing with water spray and drying by air jet for 10 seconds before applying Ultra Band-Lok[®]; up 3: Prepare the surface as for Group 2, then apply the bonding agent (OptiBond[™] FL adhesive, Kerr[®], Kloten, Switzerland) with a microbrush, and light cure for 10 seconds before applying Ultra Band-Lok[®]; Group 4: Prepare tooth surface by etching with 37% phosphoric acid for 30 seconds then rinsing with water spray and drying by air jet for 10 seconds before applying Ultra Band-Lok[®]; and Group 5: Prepare the surface as in Group 4, then apply the bonding agent (OptiBond[™] FL) with a microbrush, and light cure for 10 seconds before applying Ultra Band-Lok[®].

For each Ultra Band-Lok[®] application, a tubelike thermoplastic template with a diameter of 3 mm and a height of 3 mm was used to transfer and control the amount of Ultra Band-Lok[®] material applied to the tooth surface. All samples were light-cured for 5 seconds on each of the four sides of the sample surface using a high-power light-emitting diode curing unit (Mini LEDTM, Satelec[®] Acteon Group, Merignac, France) with a light intensity of 1,250 mW/cm². The distance between the Ultra Band-Lok[®] and the light tip is 4 mm, perpendicular to the Ultra Band-Lok[®] surfaces.

Thermocycling Process

All samples were incubated in distilled water for 24 hours at 37°C in a water bath (Model WNB-14, Mem-

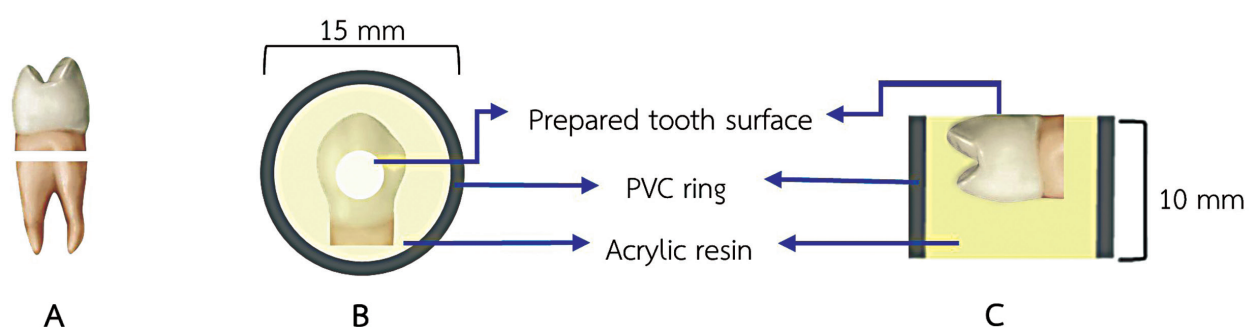


Figure 1: (A) Sectioning of tooth specimen 3 mm apical to the cemento-enamel junction
(B) Tooth embedded in polyvinylchloride ring and self-curing acrylic resin
(C) Buccal side of crown exposed to the superficial surface

mert Corporation, Germany). A thermocycling procedure with a thermocycling machine (Model TC 301 with cold and hot water baths, models CWB332R and HWB332R, Medical and Environment Equipment Research Laboratory, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand) was used to perform 5,000 cycles at 5°C and 55°C for 30 seconds per bath with a transfer time of 10 seconds.

Shear bond strength testing

Using a holder, each sample was clamped onto a universal testing machine (Instron® Model 5566, Instron Universal Testing Machine Calibration Laboratory, Norwood, Massachusetts, USA). Subsequently, a load cell of 1 kilo-Newton with the knife edge head was pressed to the junction between the compomer and enamel surface (Figure 2) with a speed of 0.5 mm/min until the Ultra Band-Lok® broke away from the enamel tooth surface. The data were analyzed with Bluehill software, CAT No. 2603-080 (Bluehill Software Company, Whitstable, Kent, UK). The load needed for debonding the Ultra Band-Lok® cylinders was expressed in Newton/millimeter² (N/mm²). This value was converted to megapascal (MPa), and then descriptive statistics were calculated.

Assessment of the adhesive remnant on enamel surface after shear bond strength testing

The tested specimens were examined under a stereomicroscope at 20× magnification to determine the amount of residual adhesive on the enamel surface. The adhesive

remnant index (ARI) scores modified from Artun and Bergland⁽¹⁶⁾ were recorded with the following scores: 1, Cohesive failure in enamel; 2, Mixed failure: Adhesive failure and cohesive failure in enamel; 3, Adhesive failure between compomer and enamel; 4, Cohesive failure in compomer with all of compomer remaining on the enamel; 5, Mixed failure, adhesive failure and cohesive failure in compomer with more than half of the compomer remaining on the enamel; 6, Mixed failure, adhesive failure and cohesive failure in compomer with less than half of the compomer remaining on the enamel; and 7, Mixed failure, a combination of adhesive and cohesive failures in enamel and compomer.

Selected surfaces of each group were also examined under a scanning electron microscope (TESCAN, VEGA3, Czech Republic) at 10,000× magnification to observe the enamel surface after shear bond strength testing.

Results

In Group 1, the Ultra Band-Lok® dislodged from the enamel surface during the thermocycling process in every sample. Therefore, shear bond strength testing could not be performed on any of the samples in Group 1. The mean shear bond strength values for the other experimental groups are as follows: Group 2: 19.80±7.06 MPa, Group 3: 18.97±4.60 MPa, Group 4: 18.04±5.09 MPa and Group 5: 16.80±5.47 MPa (Table 1). The statistical analyses of the mean shear bond strength of each method of surface preparation using two-way ANOVA analysis revealed no significant differences between the mean shear bond strengths of these groups ($p=0.887$).

The results of the mode of failure are presented in Table 2. In Group 1, all specimens exhibited adhesive failure. As for Groups 2, 3, 4, and 5, all specimens demonstrated mixed failures, comprising adhesive and cohesive failures in the compomer, with over half of the compomer remaining on the enamel.

SEM observations

Scanning electron micrographs of the enamel surface before shear bond strength testing are shown in Figure 3. The enamel surface etched with 37% phosphoric acid for 15 seconds shows dissolved enamel prisms. The demineralized enamel presents significant honeycomb appearance on the surface (Figure 3A). Whereas the enamel surface

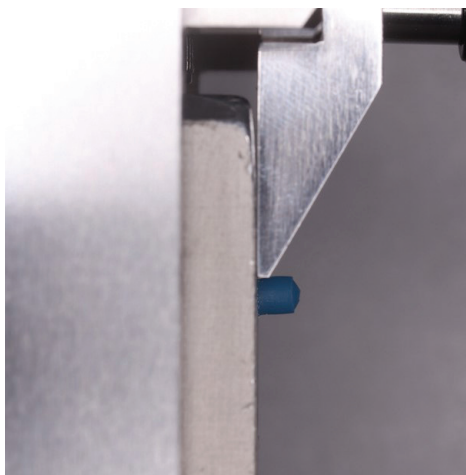


Figure 2: Knife edge head of universal testing machine pressing on the junction between the compomer and the enamel surface

Table 1: The mean and standard deviations (SD) of shear bond strengths of each experimental group

Group	Enamel surface preparation	N	Shear bond strength (MPa)			p-value
			Mean \pm SD	Min	Max	
1	No etching and no bonding	15	N/A	N/A	N/A	N/A
2	Etching 15 s.	15	19.80 \pm 7.06	8.31	32.93	0.887
3	Etching 15 s. + bonding	15	18.97 \pm 4.60	12.74	26.94	
4	Etching 30 s.	15	18.04 \pm 5.09	10.64	24.84	
5	Etching 30 s. + bonding	15	16.80 \pm 5.47	8.83	28.19	

N/A: Not applicable.

Table 2: The ARI score of specimens in each group

Group	Enamel surface preparation	ARI						
		1	2	3	4	5	6	7
1	No etching and bonding			15				
2	Etching 15 s.					15		
3	Etching 15 s. + bonding					15		
4	Etching 30 s.					15		
5	Etching 30 s. + bonding					15		

etched with 37% phosphoric acid for 30 seconds shows more dissolved enamel prism cores and peripheries. The demineralized enamel presents more significant honeycomb appearance on the surface (Figure 3B).

Scanning electron micrographs of enamel surface after shear bond strength testing are shown in Figure 4. In Groups 2 and 4, most of the enamel surfaces are covered by the Ultra Band-Lok[®]. Group 2 shows the enamel surface generally covered with Ultra Band-Lok[®] (Figure 4A). In Group 4, Ultra Band-Lok[®] covers the enamel surface and shows a honeycomb appearance (Figure 4C). Whereas Groups 3 and 5, which used a bonding agent, the enamel surface is covered by bonding filler and Ultra Band-Lok[®]. In Group 3, Ultra Band-Lok[®] and bonding filler cover the surface in generalized pattern (Figure 4B). While in Group 5, Ultra Band-Lok[®] and bonding filler cover the enamel surface, which also shows a honeycomb appearance (Figure 4D).

Discussions

This study evaluated the shear bond strength of compomers, specifically Ultra Band-Lok[®], when applied to different enamel surface preparations. It examined the effects of various etching times and bonding methods on the bond strength of the compomer materials.

A compomer, or polyacid-modified resin composite, contains some of the same components as resin composite and glass ionomers cement.⁽¹⁷⁾ Several studies found that compomers have significantly lower flexural modulus of elasticity, compressive strength, flexural strength, fracture toughness, and hardness than resin composite.⁽¹⁸⁻²⁰⁾ Compared to glass ionomers, compomers have higher bond strength, compressive strength, flexural strength, and fracture hardness, but lower wear rates and fluoride release levels.⁽²⁰⁻²³⁾ Compomer is effective in a wide range of applications, including Class I, Class II, and Class V restorations, as well as fissure sealants.⁽²⁴⁾ Overall, clinical findings indicate that compomers work well and are well-suited for their recommended applications in dental repair.

In orthodontics, compomer is used for orthodontic band cement as well as to fabricate bite turbos.⁽²⁴⁾ These two applications require different preparation methods for the tooth surface. The procedure for band cementation using Ultra Band-Lok[®] involves prophylaxis of the tooth to be banded. Then, a bead of Ultra Band-Lok[®] is applied to the inner surface of the band. The band is placed on the tooth and seated into its ideal position, followed by a 30-second light cure. An etching procedure is only required in cases involving cementing high-stress banded appliances. For occlusal build-ups, the tooth is prophyl-

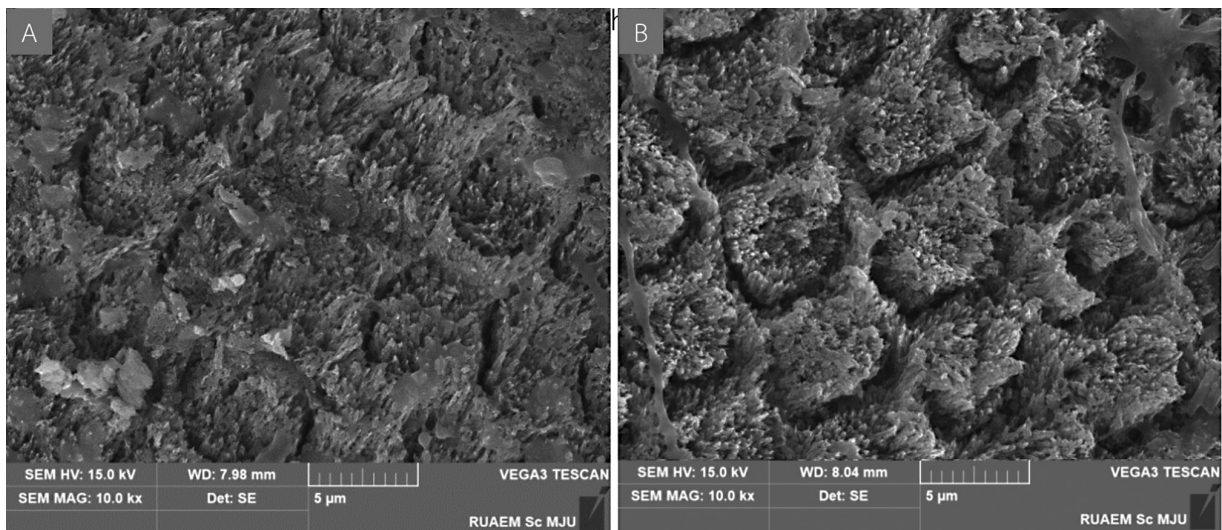


Figure 3: SEM images of enamel surface etched with 37% phosphoric acid for (A) 15 seconds and (B) 30 seconds at magnifications of 10,000×

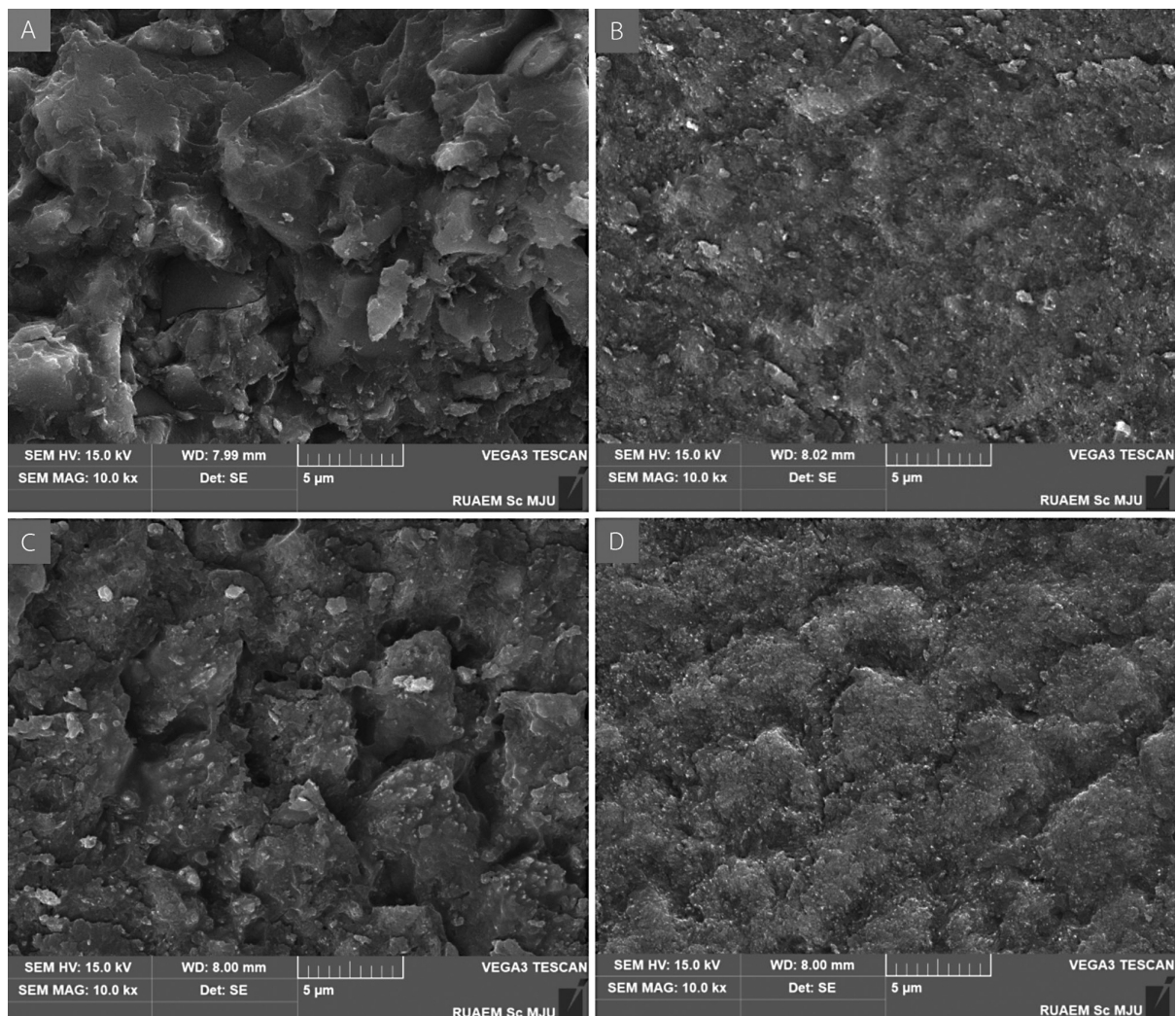


Figure 4: SEM images of debonded enamel surface at magnification of 10,000×: (A) Group 2 (15 s. etching): the enamel surface covered by Ultra Band-Lok® in a generalized pattern, (B) Group 3 (15 s. etching + bonding): the enamel surface covered by bonding filler and Ultra Band-Lok® in a generalized pattern, (C) Group 4 (30 s. etching): the enamel surface covered by Ultra Band-Lok® in a honeycomb appearance, and (D) Group 5 (30 s. etching + bonding): the enamel surface covered by bonding filler and Ultra Band-Lok® in a honeycomb appearance

laxed with pumice, rinsed, and the surface is dried. The occlusal surface is etched for 30 seconds, rinsed with water, and dried. Ultra Band-Lok[®] is applied to the occlusal surface in the required amount and shape, and finally, a 20-second light cure is administered.⁽²⁵⁾

Enamel etching is the crucial step for attachment of the compomer material. The effects of enamel etching include removing debris from enamel, creating an intricate, three-dimensional microtopography on the surface of the enamel, increasing the enamel surface area available for bonding, creating micropores where there is mechanical interlocking of the resin, and increasing the surface wettability by exposing more reactive surface layer.^(26,27) Furthermore, etching time is a critical factor of the bonding process, affecting both the quality of the bond between the dental materials and the enamel, as well as the surface condition of the enamel. When enamel is properly etched, a micro-rough surface is created that promotes the adhesion of dental materials.⁽²⁷⁾

Etch-and-rinse adhesive solutions have been successfully used on enamel and shown to be a long-lasting clinical method for adhesive restorative dentistry.⁽²⁸⁾ However, the etch-and-rinse adhesives that use 37% phosphoric acid cause enamel mineral loss of around 5 to 50 μm .⁽²⁶⁾ The amount of mineral loss depends on the concentration of phosphoric acid and the duration of the application.⁽²⁷⁾ Some research has recommended an optimal etching time of 15-30 seconds for reliable bonding for resin composite, ensuring the desired surface roughness.⁽²⁹⁾ The etching time directly influences the bond strength, with some scientific studies recommending a minimum of 15 seconds of 37% phosphoric acid etching for strong bonding with resin composite.^(26,30-32) Over-etching has been shown to cause enamel loss and weaken the tooth structure.⁽³³⁾ Some investigations on enamel pretreatment methods have demonstrated that the acid etching procedure achieves the strongest bond strength of compomer to human enamel while also significantly reducing the frequency of adhesive fractures, variability, and microleakage.⁽¹⁹⁻²¹⁾ In restorative work, etching for 15 seconds is sufficient for effectively bonding compomer to the enamel surface.⁽³⁴⁾ The manufacturer's instructions for using Ultra Band-Lok[®] for occlusal build-ups recommend etching the occlusal surface with an etchant for 30 seconds.⁽²⁵⁾ However, in this study, varied etching times of 15 and 30 seconds did not yield significantly different shear bond

strengths.

From this study, all of the Ultra Band-Lok[®] in Group 1 were dislodged from the enamel surface during the thermocycling process. The compomer cannot bond directly to the enamel surface when it does not have micromechanical surface interlocking from the etching process. Consequently, during the thermocycling process, all specimens experienced dislodgment due to the insufficient bond strength of the material. Thermocycling is a laboratory-based aging process for restorative materials that mimics the intraoral temperature. Rapid and frequent temperature changes during thermocycling can induce thermal stress in dental materials, potentially leading to bond degradation over time.⁽³⁵⁾ In a previous study, compomers were found to absorb water, significantly changing their mechanical properties when exposed to aqueous solutions. It has been observed that the mechanical characteristics of compomers are particularly sensitive to water storage.^(36,37) This susceptibility may arise from the higher organic matrix content of compomers, making them more prone to water absorption and subsequent surface disintegration in an aqueous environment.⁽³⁸⁾ Water may function as a plasticizer, weakening the covalent bonds, degrading components, and ultimately decreasing the strength of the material.⁽³⁹⁾

According to previous studies, compomer cannot sufficiently adhere to enamel and dentine, necessitating an additional bonding system.⁽⁴⁰⁻⁴²⁾ The use of bonding agents has been recommended to strengthen the bond of the compomer to the tooth.⁽⁴³⁾ The manufacturer advises using a bonding agent in conjunction with the compomer when it is used as a restorative material.⁽⁴⁴⁾ Because of the low viscosity property of the bonding agent, it rapidly wets and penetrates the microspaces in the dried, cleaned enamel, forming resin tags. Macrotags are the resin tags that develop in the spaces between enamel prisms. Microtags comprise the finer network of smaller tags that form across the end of each rod when individual hydroxyapatite crystals are dissolved. The fundamental process of enamel-resin adhesion is the development of resin micro- and macrotags within the enamel surface, which enhances the bond strength of the material.⁽⁴⁵⁾

However, the current study shows that the shear bond strength in the bonding groups (Groups 3 and 5), with mean shear bond strengths of 18.97 ± 4.60 and 16.80 ± 5.47 MPa, respectively, was not significantly different from the

group that did not use a bonding agent. Unlike the study by Prasansuttiporn in 2016, there was a considerable increase in the microtensile bond strength of the compomer base materials to human dentin in the groups restored with adhesive systems compared to those restored without adhesive systems.⁽¹³⁾ Due to the presence of a hybrid layer that formed from the interdiffusion of the low-viscosity monomers into the exposed collagen network and the intertubular dentin to form a micromechanical bond with dentin, the bond strength between the compomer and tooth surface increased. However, the previous research focused on the dentin surface, while the current study investigates the enamel surface.

When bonding an orthodontic bracket, the bond strength must be strong enough to endure the strains placed on it by the arch wires and the forces of mastication. Bond strength varies in each study. Reynolds has stated that for orthodontics attachment, a minimum resistance of 5.9–7.8 MPa is sufficient to withstand masticatory forces.⁽⁴⁶⁾ In contrast, Brantley and Eliades have surveyed orthodontic adhesive systems and found that shear bond strength can vary between 8 and 30 MPa.⁽⁴⁷⁾ The mean shear bond strengths for Groups 2, 3, 4, and 5 in this investigation were 19.80 ± 7.06 , 18.97 ± 4.60 , 18.04 ± 5.09 , and 16.80 ± 5.47 MPa, respectively, which fall within the range that can withstand the masticatory force. Therefore, the various surface preparation techniques used in this study can all be applied clinically for the bite-raising technique.

The ARI is widely employed as a method for evaluating the quality of the adhesion between the composite material and the tooth, as well as between the composite and the base of the bracket.^(48,49) In this study, Group 1 had ARI scores of 3 (adhesive failure between compomer and enamel), indicating that no adhesive remained on tooth surfaces. The ARI score of 5 (mixed failures with over half of the compomer remaining on the enamel) was the most prevalent in Groups 2–5. This could be clinically advantageous, as failures at the enamel–adhesive interface during a raised bite are less traumatic to the enamel surface. However, cleaning the teeth will probably be more difficult because some material remnants may still be attached to the enamel surface.^(50,51)

SEM images of the enamel etched with 37% phosphoric acid for 15 and 30 seconds showed a consistent honeycomb appearance; however, the groups that were etched for 30 seconds had greater height and deeper

prisms in the interrod and central regions than the groups that were etched for 15 seconds. On the microstructural level, enamel comprises enamel rods, which are tightly packed masses of organized hydroxyapatite crystallites.⁽⁵²⁾ The interfacial area between these rods with a width of approximately 1 μm , known as the interrod enamel, is rich in protein and results from the incoherence of combining crystals with different orientations.⁽⁵³⁾ Acidic etching of enamel, such as with phosphoric acid, selectively dissolves hydroxyapatite crystallites within each enamel rod and interrod.⁽⁵⁴⁾ In the interrod, where the organic matrix and water are primarily found, more corrosion occurs than in the rod area.⁽⁵²⁾

Following the shear bond strength testing, the surface of the groups etched for 30 seconds (Groups 4 and 5) exhibited a more honeycomb appearance than those etched for 15 seconds (Groups 2 and 3). Furthermore, the groups without a bonding agent (Groups 2 and 4) demonstrated Ultra Band-Lok[®] coverage on the enamel surface, while those with a bonding agent (Groups 3 and 5) exhibited coverage with bonding filler and Ultra Band-Lok[®]. In particular, the group in which the surface was etched for 30 seconds and used a bonding agent exhibited shallow interrod characteristics due to the low viscosity of the bonding agent, enabling it to penetrate the interrod space.⁽⁵⁵⁾ Whereas the viscosity of Ultra Band-Lok[®], with an average filler particle size ranging from 0.8 to 5.0 μm ,⁽⁵⁶⁾ may not thoroughly infiltrate the etched enamel structure, preventing the creation of stronger microlocking structures. Moreover, the degradation of the compomer due to water absorption during the thermocycling process further impacts its shear bond strength. Therefore, although SEM images of the groups etched for 15 and 30 seconds with and without using a bonding agent revealed different surface morphologies, shear bond strength values, and ARI showed no significant difference.

The factors for selecting the raise bite material are as follows: it must be hygienic, simple to apply, lessen interference with speech, biocompatible, be placed or removed quickly and painlessly without needing specific tools, and be accepted by the patient.⁽⁵⁷⁾ According to the results of this study, alternative approaches and materials to achieve enough bond strength between the tooth surface and compomer when used as a raised bite plane in orthodontic treatment procedures must be considered. The findings from this study indicate that varying etching times

of 15 and 30 seconds with and without a bonding agent on the enamel tooth surface did not result in significantly different shear bond strengths. Therefore, etching with 37% phosphoric acid for only 15 seconds without bonding achieves sufficient bond strength for clinical practice and facilitates the convenient fabrication of orthodontic bite raising.

Conclusions

The results obtained from this study indicate that varying the etching times of 15 and 30 seconds on the enamel surface did not yield significantly different shear bond strengths for the compomer material. Applying a bonding agent to the enamel surface does not substantially improve the bond strength between the compomer and the enamel surface.

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Conflicts of interest

The authors declare no conflicts of interest.

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Insurance Scheme: Inequality in Untreated Caries and Tooth Loss

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Abstract

Objectives: In Thailand, access to dental care services varies based on different insurance schemes. Our objective was to determine the association of untreated caries and tooth loss with insurance schemes among adult population in Thailand.

Methods: This is a cross-sectional study. Secondary data from oral examinations and a questionnaire administered during Thailand's National Oral Health Survey 2017 were analyzed. Untreated caries and tooth loss were used as continuous dependent variables. Based on the insurance schemes, participants were categorized into four groups: Universal Coverage Scheme (UCS), Civil Servant Medical Benefit Scheme (CSMBS), Social Security Scheme (SSS), and "others" (uninsured, do not use, do not know). Poisson regression with robust variance and sampling weights was used to calculate the ratio of means (RM), and 95% confidence interval (CI) was used for untreated caries and tooth loss, with adjustments for age, gender, and location.

Results: A total of 4,534 participants were included. The mean age and number of untreated caries and tooth loss were 39.6±2.9 years, 0.9±1.7 teeth, and 2.2±3.1 teeth, respectively. In covariate-adjusted models, participants under the UCS showed a significantly higher chance of untreated caries compared to those under the CSMBS (RM=1.23, 95% CI=1.04–1.45). Regarding tooth loss, participants under the others category had a substantially higher chance of tooth loss compared with those with the CSMBS. (RM=1.37, 95% CI=1.02–1.85).

Conclusions: Insurance schemes are predictors of untreated caries and tooth loss. Expansion of coverage of all insurance schemes to facilitate access to dental services is required.

Keywords: access to care, epidemiology, National Oral Health Survey, untreated caries

Introduction

Untreated caries and tooth loss cause pain, discomfort, impaired masticatory function, limited food intake, esthetic and psychosocial concerns, and poorer quality of life.⁽¹⁾ Untreated caries in permanent teeth is a global health challenge, affecting 3.5 billion people worldwide.⁽²⁻³⁾ Caries is one of the major causes of tooth loss. Among aging populations, although the age-standardized prevalence of tooth loss has reduced in recent decades, the number of people with tooth loss has increased.⁽³⁻⁶⁾ These prevalent oral diseases result in high direct and indirect costs.⁽⁷⁾

Despite the major health burden of oral diseases, oral health is constantly neglected in Universal Health Coverage (UHC).^(8,9) A study in the United States reported that, compared to other healthcare services, access to dental care was the most disrupted owing to the cost of treatment.⁽¹⁰⁾ Health insurance and education appeared to be the main contributors to oral health inequalities especially number of missing teeth.⁽¹¹⁻¹⁴⁾ The burden of UHC among low-income adults and adults with no private health insurance is high.⁽¹⁵⁾ In contrast, other studies reported that people who had dental insurance were more likely to have frequent dental checkups, a larger number of remaining natural teeth, and better oral health.⁽¹⁶⁾ Therefore, it has been recommended that UHC must include oral care. However, the impact of UHC on oral health is still unclear in countries with different backgrounds.

Thailand has a relatively wider insurance coverage for dental care and offers three public insurance schemes. To begin with, the Universal Coverage Scheme (UCS) insures more than 70% of the Thai population. The UCS is a tax-funded scheme that is free of charge and covers healthcare services (e.g., medical treatment, prescription drugs, and a part of dental treatment). Next, the Civil Servant Medical Benefit Scheme (CSMBS) covers around 9% of the Thai population.^(17,18) The CSMBS is a tax-funded scheme that includes government employees, pensioners, and their families. Some of the dental treatment costs are covered under the UCS and CSMBS. Lastly, the Social Security Scheme (SSS) covers those who work in the private sector. The SSS covers 16% of the population in Thailand. The SSS is a mixed-funding program that includes contributions from both employees and employers.^(17,18) Under the UCS and CSMBS, scaling, prophylaxis, restoration apart from esthetic or endodontic

treatments in the posterior teeth, and tooth extraction and surgical removal are free of charge. In the case of removable prosthetic treatment, renewal treatment is assured for 5 years after insertion. The SSS offers dental care for 900 TBH (28.74 USD) per year, which includes scaling, prophylaxis, restoration, and tooth extraction and surgical removal. If a treatment costs more than 900 TBH, the SSS payer is responsible for paying the dental facilities.

These insurance schemes are considered to affect the oral health of the population. Existing research pointed out that participants covered by the UCS appeared to use less dental care.⁽¹⁹⁾ In addition, adult participants covered by the UCS demonstrated a significantly higher prevalence of periodontal disease than those covered by the CSMBS.⁽²⁰⁾ Moreover, older participants (defined as those aged 60 years and older) insured with the CSMBS exhibited significantly higher dental utilization than those insured with the UCS.⁽²¹⁾

Therefore, we hypothesized that participants covered by the UCS would demonstrate a higher prevalence of untreated caries and tooth loss than others covered by the CSMBS. Hence, we aimed to investigate the association between insurance schemes and untreated caries and tooth loss among the adult population in Thailand.

Materials and Methods

Setting and participants

This study is an observational cross-sectional study. We utilized secondary data from the most recent Thailand's National Oral Health Survey (eighth TNOHS). TNOHS was conducted by the Bureau of Dental Health, Department of Health, Ministry of Public Health, Thailand. The oral health survey questionnaire and oral examination took place from June to September 2017, with the target population being the indexed age groups from 24 provinces in 13 health regions. The TNOHS involved a three-stage, stratified, random sampling method. Systematic and quota sampling was employed. Based on the guidelines by the World Health Organization (WHO)⁽²²⁾, the target age groups included: preschool children (3 and 5 years of age), teenagers (12 and 15 years of age), middle-aged adults (35-44 years of age), older adults (60-74 years of age), and late older adults (80-85 years of age). The detailed methods involved in the TNOHS have been described elsewhere.⁽²³⁾ The dental caries

prevalence in each age group was determined from the seventh TNOHS, a relative d of 10-15%, a 95% confidence interval, and a design effect of 2 were used to compute the sample size within each allocated area. The calculated sample size was 3,715 adults. Due to the possibility of subject absence or loss of data, the sample size was increased by 10%, resulting in a sample size of 4,128. However, the present study used data from the eighth TNOHS; thus, the data of 4,683 adults were used. The written informed consent was obtained from all adults (between 35 and 44 years of age) before the questionnaire survey began, followed by an oral examination. Reliability and validity of the data were crucial; therefore, intra-examiner and inter-examiner reproducibility was evaluated with 19 trained dentists who practiced under standardized conditions in the calibration stage, as advised by the WHO.⁽²²⁾ The Kappa score for caries was 0.78-0.87, indicating a substantial agreement level, and that for the periodontal status was 0.46-0.78, indicating a moderate agreement level.

Dependent variables

As dependent variables, we used the numbers of untreated caries (decayed teeth [DT]) and lost teeth, evaluated through an oral examination at the eighth TNOHS. We defined untreated caries as “teeth with an unmistakable coronal cavity at the dentine level, a root cavity in the cementum that feels soft or leathery to probing, or temporary or permanent restorations with a caries lesion”.⁽²⁴⁾ When evaluating the numbers of untreated caries and lost teeth, we omitted the third molar, and all the results were based on a maximum of 28 teeth. Data of people marked with codes 9 and X were excluded (tooth excluded or not present) from our study.

Independent variables and covariates

We inquired about 10 different insurance plans and integrated the responses into four categories: UCS, CSMBBS, SSS, and others. The entire population of Thailand is covered by insurance. Therefore, individuals who responded to the inquiry about the use of insurance with “uninsured,” “do not use,” and “do not know” were grouped together as “others.”

Determinants of socioeconomic status included educational and income levels. The participants had to indicate their highest level of education. The result grouped them as follows: lowest (≤ 6 years), moderate

(7-9 years), high (10-12 years), and highest (≥ 13 years). Moreover, the participants had to indicate their individual monthly income in USD where the exchange rate used was 1 USD = 31.31 THB. There were four income categories as follows: lowest (≤ 159.69 USD), moderate (159.72-479.08 USD), high (479.11-958.16 USD), and highest (≥ 958.19 USD).

As for covariates, we used age group (“35-39 years” and “40-44 years”) and gender (“men” and “women”). Residential areas were also used as a demographic covariate. The residential locations were divided into “rural” and “urban” areas based on where the participants lived.

Statistical analysis

For the descriptive analysis, we used the prevalence of untreated caries, the presence of < 24 teeth⁽²⁵⁾, and the mean of untreated caries (DT) and tooth loss (number of lost teeth) because the numbers of untreated caries and lost teeth were skewed. In covariate-adjusted models, we calculated the ratio of means (RM) and 95% confidence interval (CI) through Poisson regression with robust variance and sampling weights^(26,27) for the following variables: insurance schemes, educational level, and income level. First, univariate analyses were performed. Second, the covariates; age, gender, and residential location were included in the models for insurance, education, and income. Subsequently, all the independent variables and covariates were included in a final model. STATA[®] 15.0 (Stata Corporation, College Station, TX, USA) was used for all the statistical analyses.

Ethical approval

This study protocol was reviewed and exempted by the Human Research Ethics Committee of the Department of Health, Ministry of Public Health, Thailand (No. 353; extended no. RF 13-01-353).

Results

After excluding all the participants with missing data (N=149), the final analysis included 4,534 (2,194 male and 2,340 female) participants (response rate, 93.2%). The mean age and numbers of untreated caries and lost teeth were 39.6 ± 2.9 years, 0.9 ± 1.7 teeth, and 2.2 ± 3.1 teeth, respectively. Table 1 presents the distribution of untreated caries and presence of < 24 teeth among the participants.

Women who lived in rural areas, participants who had the lowest educational and income levels, and participants under the UCS had a higher prevalence of untreated caries. Moreover, men who lived in rural areas, participants with a moderate educational level, participants with the lowest income level, and participants with insurance schemes categorized as “others” had a higher chance of having < 24 teeth.

Table 2 presents the relationship between insurance schemes and untreated caries. In the covariate-adjusted models, participants under the UCS had a significantly higher chance of untreated caries (RM=1.23, 95% CI=1.04-1.45) compared with those under the CSMBS. After all the variables were simultaneously adjusted, the significance of the association disappeared. Regarding the relationship between insurance schemes and tooth loss, in the covariate-adjusted models, the participants with insurance schemes categorized as “others” had a significantly higher chance of tooth loss (RM=1.33, 95% CI=1.01-1.76) compared with those with the CSMBS. A significant association was also observed in the fully

adjusted model (RM=1.37, 95% CI=1.02-1.85). In the case of both untreated caries and tooth loss, there were no significant associations with educational and income levels.

Discussions

This study identified the association between insurance schemes, untreated caries, and tooth loss. Participants under the UCS and “others” category (uninsured, do not use, do not know) who were 35-44 years old had a significantly higher chance of untreated caries and tooth loss, respectively, compared to CSMBS, even after adjusting for relevant confounding variables.

From an economic perspective, previous studies also reported that insurance schemes were related to oral health inequalities. A study from Korea reported that a government policy for expanding the coverage of dental health insurance reduced the inequality in unmet dental needs due to treatment costs.⁽²⁸⁾ This can be explained by the observation that the number of dental visits increases when the costs of dental services are lower. In Japan, the

Table 1: Prevalence of untreated caries (DT > 0) and the presence of < 24 teeth among adults aged 35-44 years old in Thailand

Variables		Total (%) N = 4534	% DT > 0	Mean of DT	% Number of teeth < 24	Mean of tooth loss
Age	35-39 years old	2245 (49.5)	36.0	0.86 (1.7)	14.4	2.11 (3.0)
	40-44 years old	2289 (50.5)	37.3	0.87 (1.7)	15.6	2.25 (3.1)
Gender	Male	2194 (48.4)	35.9	0.86 (1.7)	15.3	2.22 (3.0)
	Female	2340 (51.6)	37.4	0.87 (1.7)	14.8	2.15 (3.1)
Location	Urban	2260 (49.8)	34.8	0.82 (1.7)	13.7	2.11 (2.9)
	Rural	2274 (50.2)	38.5	0.91 (1.7)	16.4	2.25 (3.2)
Educational level*	Highest (≥ 13 years)	1363 (30.1)	35.5	0.84 (1.7)	13.1	2.11 (2.9)
	High (10-12 years)	1344 (29.6)	35.9	0.86 (1.8)	14.9	2.18 (3.2)
	Moderate (7-9 years)	647 (14.3)	36.6	0.87 (1.6)	17.8	2.26 (3.2)
	Lowest (≤ 6 years)	1180 (26.0)	38.9	0.89 (1.7)	15.9	2.21 (3.0)
Income level	Highest (≥ 958.19 USD)	357 (7.9)	36.7	0.85 (1.7)	15.7	2.12 (2.8)
	High (479.11-958.16 USD)	1067 (23.5)	34.6	0.78 (1.7)	12.5	2.13 (3.0)
	Moderate (159.72-479.08 USD)	2238 (49.4)	36.6	0.90 (1.8)	15.6	2.20 (3.1)
	Lowest (≤ 159.69 USD)	872 (19.2)	39.2	0.89 (1.6)	16.4	2.20 (3.1)
Insurance	Civil Servant Medical Benefit Scheme (CSMBS)	934 (20.6)	33.3	0.73 (1.5)	12.4	2.04 (2.7)
	Social Security Scheme (SSS)	1359 (30.0)	35.8	0.88 (1.8)	14.3	2.11 (3.0)
	Universal Coverage Scheme (UCS)	2150 (47.4)	38.6	0.92 (1.8)	16.1	2.26 (3.2)
	Others (uninsured, do not use, do not know)	91 (2.0)	38.5	0.75 (1.2)	26.4	2.73 (3.3)

*determined by years of education attainment: lowest (elementary school), moderate (junior high school), high (high school), and highest (bachelor degree or more).

Table 2: Association of educational level, income, and insurance scheme with untreated caries (DT) and tooth loss (number of lost teeth) determined by Poisson regression among the adults aged 35-44 years old in Thailand

Variables	Untreated caries (DT)					Tooth loss				
	Univariate RM	95% CI	Covariate adjusted RM**	95% CI	Fully adjusted RM***	Univariate RM	95% CI	Covariate adjusted RM**	95% CI	Fully adjusted RM***
Educational level *										
Highest (≥ 13 years)	Ref.		Ref.		Ref.	Ref.		Ref.		Ref.
High (10-12 years)	1.04	0.89-1.21	1.03	0.88-1.20	0.92	0.76-1.11	0.89-1.11	0.98	0.88-1.10	0.96
Moderate (7-9 years)	1.07	0.89-1.28	1.05	0.87-1.27	0.91	0.72-1.14	0.92-1.20	1.04	0.91-1.19	1.00
Lowest (≤ 6 years)	1.10	0.95-1.29	1.09	0.93-1.28	0.93	0.75-1.15	0.93-1.16	1.02	0.91-1.14	0.97
Income (per month)										
Highest (≥ 958.19 USD)	Ref.		Ref.		Ref.	Ref.		Ref.		Ref.
High (479.11-958.16 USD)	0.91	0.72-1.15	0.91	0.72-1.16	0.91	0.71-1.15	0.89-1.23	1.05	0.89-1.24	1.05
Moderate (159.72-479.08 USD)	1.07	0.87-1.33	1.07	0.86-1.32	1.00	0.77-1.31	0.90-1.22	1.05	0.90-1.22	1.01
Lowest (≤ 159.69 USD)	1.08	0.86-1.35	1.06	0.84-1.33	0.98	0.73-1.31	0.88-1.23	1.04	0.87-1.23	0.97
Insurance										
Civil Servant Medical Benefit Scheme (CSMBS)	Ref.		Ref.		Ref.	Ref.		Ref.		Ref.
Social Security Scheme (SSS)	1.18	0.99-1.41	1.17	0.98-1.40	1.17	0.93-1.48	0.92-1.16	1.03	0.91-1.16	1.06
Universal Coverage Scheme (USC)	1.24	1.06-1.46	1.23	1.04-1.45	1.24	0.97-1.59	0.98-1.21	1.08	0.97-1.20	1.13
Others (uninsured, do not use, do not know)	1.07	0.74-1.55	1.07	0.74-1.55	1.08	0.73-1.60	1.01-1.76	1.33	1.01-1.76	1.37

DT = decayed teeth, RM = ratio of means, CI = confidence interval, Ref. = Reference

*determined by years of education attainment: lowest (elementary school), moderate (junior high school), high (high school), and highest (bachelor degree or more).

** Educational level, income, and insurance were separately included in the models with adjustments for age, gender, and residential location.

*** All independent variables and covariates were included in the model.

reduction in co-payments for dental care has increased the number of dental visits.⁽²⁹⁾ Another previous study demonstrated that children who were enrolled in insurance programs were more likely to use dental services.⁽³⁰⁾ From a global perspective, lower-income countries have lower oral healthcare coverage and higher socioeconomic inequality than higher-income countries.⁽³¹⁾

Time is also a barrier to accessing dental care. Studies from Germany and Australia reported that waiting in long queues and requiring longer appointment durations were obstacles to accessing healthcare services.^(32,33) In our study, participants under the UCS had a higher risk of having untreated caries compared to those under the CSMBS. An explanation for this could be that only CSMBS approves dental visits outside of office hours. However, neither UCS nor CSMBS covers dental care in private dental clinics, unlike SSS. Generally, the waiting time in private dental clinics is shorter than that in public dental clinics. Dental utilization under the UCS was lower than that under the other insurance plans, even though it allowed for cost-free access to dental treatment. The UCS could be that individuals can only access dental treatment in public dental care facilities during office hours. Under the UCS, long waiting times for dental appointments are considered a barrier to using public dental services.

Thus, the present study also confirmed that accessibility related to time is also an important factor affecting dental visits. Therefore, the expansion of insurance coverage to include treatment in private dental clinics could improve access to dental care in Thailand.

These findings have important implications for policymaking related to insurance and universal healthcare. The proceedings of the 74th session of the World Health Assembly (2021) and of other sessions highlighted the importance of UHC to tackle untreated dental conditions.⁽³⁴⁾ Lower utilization of dental treatments, as well as the lack of a public oral health policy and financial support from the government, have been linked to poor oral health.⁽³⁵⁾ Thailand has already made strides towards aligning dental care with UHC initiatives by including a minimum benefit package for prevention programs, such as the early detection of oral diseases, during regular dental checkups. However, dental care costs for treating oral diseases are not covered by all insurance schemes. To reduce the level of inequality in accessing dental care, UHC must include oral health care services, such as

cost-effective, minimally invasive intervention.⁽³⁶⁾ The result in this study clearly shows that, in comparison to the reference group, the others group had a significantly higher chance of tooth loss. Despite the small sample size, there might be a population-wide impact if this group isn't encouraged to start utilizing their insurance. The remaining teeth were removed before proper treatment was given. Moreover, during the coronavirus disease (COVID-19) pandemic, a comprehensive digital oral health program should have been developed as part of public health policies. Digital oral health provides an opportunity to enhance healthy behavior and to reduce common risk factors and threats related to oral diseases and other non-communicable diseases, which could contribute to the reduction of oral health inequalities.⁽³⁷⁾

The key advantage of our study was the fact that it followed a large epidemiological survey that took into account the Thai adult population and offered data regarding the insurance schemes, untreated caries, and tooth loss. The limitation of our study was that it was a cross-sectional study; therefore, a true cause-and-effect relationship between the independent variables and outcomes could not have been established. To identify causal relationships, longitudinal studies are necessary. Conducting an in-depth qualitative study of individuals who do not use insurance or are unfamiliar with the insurance are necessary to obtain suggestions for further public policy development.

Conclusions

Participants under the UCS and those who were uninsured, paid privately, or did not know about insurance had a significantly higher risk of untreated caries and tooth loss. In other words, untreated caries and tooth loss may be predictable by insurance schemes. Reorientated oral health services, both public and private partnerships, and greater access to dental health services in all insurance schemes are necessary and required in Thailand.

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Conflicts of Interest

The authors declare no conflict of interest.

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