SRINAKHARINWIROT UNIVERSITY
DENTAL JOURNAL

วิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

ปีที่ 18 ฉบับที่ 1
2568

ISSN 2774-0811 (Online)

SRINAKHARINWIROT UNIVERSITY
DENTAL JOURNAL
วิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

สำนักงาน | **Office**

คณะทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ — Faculty of Dentistry, Srinakharinwirot University
สุขุมวิท 23 เขตวัฒนา กรุงเทพมหานคร 10110 — Sukhumvit 23, Wattana, Bangkok 10110 Thailand
โทรศัพท์ 02-649-5000 ต่อ 15140 — Tel. 02-649-5000 ext. 15140
จดหมายอิเล็กทรอนิกส์ swudentj@yahoo.com — E-mail : swudentj@yahoo.com
เว็บไซต์ http://ejournals.swu.ac.th/index.php/swudentj — Web site : http://ejournals.swu.ac.th/index.php/swudentj

กำหนดการออกวารสาร (ออนไลน์) ปีละ 2 ฉบับ ฉบับที่ 1 เดือนมกราคม-มิถุนายน ฉบับที่ 2 เดือนกรกฎาคม-ธันวาคม

Author Guidelines
Srinakharinwirot University Dental Journal (SWU Dent J)

About the Journal

Aims and Scope:
SWU Dent J is a dental journal of Srinakharinwirot University established since 2005. The online version has been published since 2022. There is a policy to support the dissemination of knowledge in academic presentation and research in dentistry and related fields which accept articles from both internal and external authors free of charge. SWU Dent J. publishes (Online) two issues per year, January-June and July-December.

Purpose of the Journal:
1. To publish the original research articles, literature reviews, case reports in oral sciences and other related areas.
2. To introduce new knowledge and observations related to the any aspects of oral and dental cares as well as other healthcare are especially encouraged.
3. To communicate academically between dentists and others in related areas.

SWU Dent J publishes the following types of articles:

1. **Original Articles** report results of original research in dental and oral sciences as well as other related fields (preclinical, clinical, or translational). The presented work must not be published elsewhere.
2. **Review Articles** describe and evaluate previously published materials in a given topic, diagnosis, or treatment in order to suggest new approaches or ideas.
3. **Case Reports** of rare or unusual cases, or treatment with good long-term follow-up information, particularly in areas in which good statistics on results of treatment are needed.
4. **Miscellaneous** Other manuscript that reports useful information could be written in a form of "Letter to editor" or "Brief communications".

Manuscript Submission:

All abstracts must be submitted in English. Manuscript written in Thai and/or Thai authors need to include abstract in both languages. Authors of non-native English speakers should consider their work reviewed by either a native English speaker or academic proofreading services prior to submission.

All submissions must include a cover letter signed by all authors. Cover letters should certify the research is original, not being simultaneously considered for publication elsewhere, and free of conflict of interest. The format of cover letter is available at the end of this document.

In addition, title page should be submitted. Title page includes title of the manuscript, name, degree and affiliation of all authors. Moreover, mailing address, telephone number and email address of corresponding author should be included.

Submission can be done online via SWU eJournals System (OSJ) at
http://ejournals.swu.ac.th/index.php/swudentj
or the authors can send the manuscript and related document to
email: swudentj@yahoo.com

Manuscript preparation:

Manuscripts must adhere to the following instructions or they will be rejected before undergoing peer review.

- Prepare the manuscript in Microsoft Word (docx) using Cordia new font 16. Leave a one-inch margin on all sides. Do not right justify.
- Manuscript must be done in plain and simple style format as possible.
- Number all manuscript pages consecutively in the upper right-hand corner (text and references, followed by illustrations on separate pages).
- Manuscript length (including all references, tables, figures) should be no more than 15 pages (standard A4 21.1 x 29.7 cm page size).
- All figures, illustrations, graphs, and tables must be provided in the text and should include title and foot note. In addition, explanation for abbreviation and symbols is required.
- Identifying teeth by their name, rather than a number or letter. Be consistent throughout the manuscript. If it is not possible to use the name, use FDI two-digit system after which their full names have been firstly mentioned in the text.
- Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.
- Abbreviation should be explained in the parenthesis when first used. Avoid using abbreviation in title and abstract.

Manuscript format:

Please order the manuscript as follows: Title, Abstract, Introduction, Materials and Methods, Results, Discussion, Conclusion, Acknowledgements, References, Start each section on a separate page.

Title page:

- The title page should contain:
  1. Title: a concise and informative title (do not include numbers, acronyms, abbreviations).
  2. Corresponding Author must provide: full name, complete address, phone, fax, and a valid e-mail address, which will be used for all communication with the journal.
  3. Author information including;
     3.1 Full name of all authors
     3.2 Complete mailing address for each author
     3.3 Degrees (e.g. DDS, PhD)
     3.4 Affiliation (e.g. Department of Stomatology, Faculty of Dentistry, Srinakharinwirot University)

Abstract:

- Up to 300 words.
- Original article : abstract Should include as follows: Objectives, Methods, Results, and Conclusions.

All abstracts must be submitted in English. Manuscript written in Thai need to include abstract in both languages.

Keywords: Up to 3-6 keywords should be provided, and selected from Medical Subject Headings (MeSH). Information on the selection of keywords: see http://www.nlm.nih.gov/mesh/MBrowser.html.

Introduction:

- Briefly explain the existing knowledge, and demonstrate the problems leading to the main objectives of the research.

Materials and methods:

- Describe the procedures in detail.
- If human subjects or related specimens (including teeth, saliva, tissues) have been involved in the research, please state evidence of IRB or local ethical committee approval, including an obtaining of informed consent. When laboratory animals are used, inform the level of institutional review allowing for human practices, as appropriated.
- Provide names and sources of all instruments or commercial products e.g.
  - Instrument e.g. Universal Testing machine (Shimadzu, EZtest, Japan)
  - Commercial product e.g. Polyether (Impregum, 3MESPE, USA)
- Statistics used in the manuscript should be clearly demonstrated.

Results:

- Report data directly with complete statistical analysis.
- Always describe statistic values with standard errors or standard deviations, including particular degree of probability level e.g. $p = 0.026$ or $p < 0.05$
- Tables and figures should be numbered in the order in which they are described and cited in the text.

Discussion:

- Carefully explain and evaluate data.
- Compare with other findings particularly what causes the difference.
- Suggest directions for future research.

Conclusion (if included):

- State shortly the most important finding of the research.
- Do not repeat Results or Discussion.

Acknowledgements (if applicable):

- Inform all assistances from individuals as appropriated.
- Report all sources of grant and other support for the project.

References:

- All reference must be written in English.
- Citations should be numbered consecutively in parentheses as they appear in the text and they should present after punctuation e.g. text (1,4-7,9,10).
- Examples of reference citation formats:

- Journal article:

Aroonrerk N, Pichyangkul S, Yongvanitchit K, Wisetchang M, Sa-Ard-Iam N, Sirisinha S, et al. Generation of gingival T cell lines/clones specific with *Porphyromonas gingivalis* pulsed dendritic cells from periodontitis patients. J Periodontal Res. 2003;38(3):262-8.

- Books/Chapter in a book:

Profit WR, Fields HW. Contemporary orthodontics. 3rd ed. St. Louis: Mosby; 2000.

Yamada KM. Fibronectic and other cell interactive glycoproteins. In: Hay ED, editor. Cell biology of extra-cellular matrix. 2nd ed. New York: Plenum Press; 1991. p.111-46.

- Dissertation:

Kerdmanee K. *In Vitro* Study of Er,Cr:YSGG Laser in Adjunct to Ultrasonic Root Debridement on The Attachment of Human Periodontal Ligament Fibroblasts [Master thesis, M.S. (Periodontology)]. Bangkok: Srinakharinwirot University; 2015.

- Journal article on the Internet (Article with digital object identifier)

Lam-ubol A, Rungsiyanont S, Vacharotayangul P, Sappayatosok K, Chankanka O. Oral manifestations, salivary flow rates and Candida species in Thai HIV-infected patients. J Clin Exp Dent. 2019;11(2):e138-45. doi: 10.4317/jced.55384.

- Database on the Internet

National Statistical Office. The 2013 survey on health and welfare [Internet]. Bangkok: Ministry of Information and Communication Technology; 2017 [cited 2017 March]. Available from: URL: http://service.nso.go.th/nso/nsopublish/themes/files/healthy/healthRep56.pdf. (in Thai).

- Scientific or technical report (Issued by performing agency)

Chaiwerawattana A, Laowahutanont P, editors. Hospital based cancer registry annual Report 2013. Bangkok: Information and Technology Division; 2015. ISBN 978-616-11-2581-3. Sponsored by the National Cancer Institute.

The reference style of the journal follows the format suggested by "International Committee of Medical Journal Editors Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References (http://www.nlm.nih.gov/bsd/uniform_requirements.html)"

Abbreviations of journal names should flow the forms in Index Medicus, National Library of Medicine (NLM)

Tables and Figures:

Tables

- Table design and layout must be plain and simple as possible
- All tables must be inserted in the main manuscript.
- Use explanatory footnotes if possible. In the table or title, indicate the order of footnotes with lower-case letters in superscript. If needed in footnotes, cite the short form of references in parentheses.

Figures

- All figures must be inserted in the main manuscript.
- Clearly numbered files containing figures to indicate their placement in the text
- Illustrations, graphs and figures must be provided as TIFF or JPEG files (8.9 x 16 cm) with a minimum resolution of 600 dpi.
- Radiographic images should be prepared by taking a photo in black and white from original films, or using digital imaging when applicable. Put magnification markers directly on the micrographs.

Peer Review process:

All submissions will be subjected to **double blind peer-review by at least 3 independent reviewers from different institutions which are the experts in the field.** The article will be accepted for publication when at least 2 reviewers agree. Please note that the authors agree to transfer copyright to SWU Dent J on submission, if the manuscript is accepted for publication. Attempts to reproduce parts of the article may be done with written permission from SWU Dent J.

Address for Correspondences:

Mrs. Kanokporn Sukyanan
Faculty of Dentistry, Srinakharinwirot University,
114, Sukhumwit 23, Wattana, Bangkok 10110, Thailand.
Telephone: (662) 6495000 ext 15140, Fax. (662) 6641882,
E-mail: swudentj@yahoo.com

Conflicts of Interest:

Authors must state all possible conflicts of interest in the manuscript, including financial, consultant, institutional and other relationships that might lead to bias or a conflict of interest. If there is no conflict of interest, this should also be explicitly stated as none declared. All sources of funding should be acknowledged in the manuscript. All relevant conflicts of interest and sources of funding should be included on the title page of the manuscript with the heading “Conflicts of Interest and Source of Funding”.

Ethical and Legal Considerations and Copyright Transfer:

A submitted manuscript must be an original contribution not previously published (except as an abstract or a preliminary report), must not be under consideration for publication elsewhere, and, if accepted, must not be published elsewhere in similar form, in any language, without the consent of SWU Dent J. Each person listed as an author is expected to have participated in the study to a significant extent. All authors must sign and submit the copyright transfer letter together with journal submission.

Notice: Author guidelines is available at the journal website http://ejournals.swu.ac.th/index.php/swudentj.

The cover letter format

Publication Ethics SWU Dent J.

บทบรรณาธิการ

สวัสดีท่านผู้อ่านทุกท่านครับ สำหรับวิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ ฉบับที่ 1 ประจำปี พ.ศ.2568 ทางกองบรรณาธิการฯ ได้คัดสรรผลงานทางวิชาการที่หลากหลายและมีคุณภาพมาเผยแพร่เช่นเดิมครับ สำหรับเล่มนี้ประกอบไปด้วยบทวิทยาการจำนวน 9 บทความ ครอบคลุมสาขาทันตกรรมชุมชนทันตกรรมสำหรับเด็ก ชีววิทยาช่องปาก ทันตกรรมรากเทียม และวิทยาเอนโดดอนต์

ทั้งนี้ช่วงปลายปี พ.ศ. 2567 ที่ผ่านมา คณะทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ เป็นเจ้าภาพในการจัดงานประชุมขององค์กรผู้บริหารคณะทันตแพทยศาสตร์แห่งประเทศไทย (อ.บ.ท.ท.) ครั้งที่ 21 ระหว่างวันที่ 13–15 พฤศจิกายน พ.ศ.2567 ณ อำเภออัมพวา จังหวัดสมุทรสงคราม และเปิดโอกาสให้ผลงานทางวิชาการที่ผ่านการคัดเลือกให้นำเสนอในงานประชุมดังกล่าว สามารถเลือกเผยแพร่ผลงานลงเล่ม ว.ทันต.มศว ปีที่ 18 ฉบับที่ 1 พ.ศ. 2568 ได้ โดยต้องผ่านกระบวนการคัดเลือกและพิจารณาผลงานทางวิชาการตามมาตรฐานการพิจารณบทความของ SWU Dent J.

ดังนั้นบทความทั้งหมดที่ลงตีพิมพ์ในวารสารฉบับนี้ จึงเป็นบทความซึ่งส่งมาร่วมนำเสนอในงานประชุมดังกล่าว บทความทั้งหมดที่ตีพิมพ์ในเล่มนี้ ผ่านการนำเสนอผลงานในรูปแบบ Oral Presentation หรือ Poster Presentation ในสถานที่จัดงานโดยไม่มีการบันทึกวีดิโอเผยแพร่ มีเพียงบทคัดย่อของบทความเท่านั้นที่ลงเผยแพร่ในเล่มสูจิบัตรและรายงานสืบเนื่องการประชุมวิชาการของงานประชุมฯ

เริ่มตั้งแต่เล่มนี้เป็นต้นไป ทางกองบรรณาธิการได้มีการปรับคณะทำงานเล็กน้อย รวมทั้งเพิ่มเติมกองบรรณาธิการรุ่นใหม่ไฟแรงมาเสริมการทำงาน พร้อมทั้งมีการปรับการจัดรูปแบบตีพิมพ์ให้เป็นไปตามสากลนิยมมากขึ้น และเรายังคงยึดมั่นพัฒนาคุณภาพวารสารฯ เพื่อเป็นช่องทางในการเผยแพร่ ส่งเสริมสนับสนุนงานวิจัยและวิชาการและเป็นสื่อสัมพันธ์ทางวิชาการในสาขาทันตแพทยศาสตร์และสาขาวิชาการที่เกี่ยวข้อง สำหรับรายละเอียดท่านผู้อ่านสามารถดูข้อมูลได้ตั้งแต่เล่มนี้เป็นต้นไปครับ

ในฐานะตัวแทนของกองบรรณาธิการฯ ผมขอขอบพระคุณผู้นิพนธ์ทุกท่านที่ได้มอบความไว้วางใจให้วิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ เป็นช่องทางในการเผยแพร่ผลงานทางวิชาการ และผมขอขอบพระคุณผู้ทรงคุณวุฒิทุกท่านที่ได้อุทิศและทุ่มเทแรงกายใจในการพิจารณาบทความทางวิชาการให้ถูกต้องและเป็นไปตามมาตรฐานครับ ซึ่งความพยายามมุ่งมั่นรักษาคุณภาพมาอย่างต่อเนื่อง จึงทำให้ SWU Dent J. ได้รับการรับรองคุณภาพวารสารวิชาการ “กลุ่มที่ 1 และ ACI” ตามประกาศผลการประเมินคุณภาพวารสารวิชาการที่อยู่ในฐานข้อมูลศูนย์ดัชนีการอ้างอิงวารสารไทย THAI-JOURNAL CITATION INDEX (TCI) รอบที่ 5 (รับรองคุณภาพวารสารเป็นเวลา 5 ปี ระหว่าง พ.ศ. 2568-2572)

และวิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ ยังคงเปิดรับบทความทางวิชาการประเภทต่าง ๆ ทั้งบทความวิจัย บทความปริทัศน์ และรายงานผู้ป่วยที่น่าสนใจทั้งภาษาไทยและภาษาอังกฤษตลอดทั้งปีครับ จึงขอเชิญชวนผู้นิพนธ์ทุกท่านส่งบทความทางวิชาการด้านทันตแพทยศาสตร์และสาขาที่เกี่ยวข้อง ตามช่องทางที่ได้แนะนำไว้ทางหน้าเว็บไซต์ออนไลน์ของวิทยาสารฯ สุดท้ายนี้ผมขออำนวยพรให้กองบรรณาธิการทุกท่าน ท่านผู้ทรงคุณวุฒิ ผู้นิพนธ์และผู้อ่านทุกท่าน มีแต่ความสุขกายสุขใจ ปราศจากโรคภัยไข้เจ็บ และคิดสมหวังในทุก ๆ สิ่งตามที่ท่านปรารถนาตลอดทั้งปีนี้ครับ สวัสดีปีใหม่ พ.ศ. 2568 อีกครั้งครับ

ผศ.ดร.ทพ.ชัชพันธุ์ อุดมพัฒนากร
บรรณาธิการวิทยาสารทันตแพทยศาสตร์
มหาวิทยาลัยศรีนครินทรวิโรฒ

SRINAKHARINWIROT UNIVERSITY
DENTAL JOURNAL
วิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

Table of Contents

Vol.18 No.1
2025

SRINAKHARINWIROT UNIVERSITY
DENTAL JOURNAL
วิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

Table of Contents

Vol.18 No.1
2025

Survival After 3-Year of Partial Pulpotomy Using Bioactive Cements as Pulp Capping Materials in Adult's Permanent Teeth with Carious Pulp Exposure

Thanwarat Thonthanan[1] Warit Powcharoen[2] Dujrudee Chinwong[3] Patchanee Chuveera[4*]

Abstract

Objective: The present study aimed to explore the survival rate after 3 years of partial pulpotomy in adult permanent teeth with carious pulp exposure, using ProRoot MTA® and Biodentine™.

Materials and Methods: This study is a follow-up to a previously reported 1-year outcome of a randomized clinical trial investigating the non-inferiority of Biodentine compared to MTA for partial pulpotomy in adults' permanent teeth with carious pulp exposure (Thai Clinical Trials Registry: TCTR20171228003). All 58 participants whose teeth were treated for at least 3 years were contacted for follow-up, which included clinical examinations, radiographic evaluations, and a patient satisfaction survey.

Results: Forty-eight patients (82.8% recall rate) were followed up for an average of 40.95 ± 4.24 months. The overall cumulative pulp survival rate was 78.7%, with 87.4% for ProRoot MTA® and 70.7% for Biodentine™, showing no significant difference between the materials. The highest frequency of failure occurred between 24 and 35 months. Discoloration of teeth was observed in both groups, 42.1% (8/19) in teeth treated with ProRoot MTA® and 10% (2/20) in teeth treated with Biodentine™, showing a statistically significant difference ($p = 0.031$). Acceptable restorations were present in 74.4% of patients, and most patients were very satisfied with the treatment.

Conclusion: The cumulative pulp survival rate for adult permanent teeth with carious pulp exposure was 78.7% after at least 3 years of partial pulpotomy, with no significant difference between ProRoot MTA® and Biodentine™. Since treatment failures were most frequent between 24 and 35 months, clinical and radiographic follow-ups are recommended for at least 3 years. For patients with significant tooth structure loss who have not received full coverage restoration, annual follow-ups are advised to assess restoration quality.

Keywords: Biodentine™, Cohort study, Mineral trioxide aggregates, Partial pulpotomy, Survival analysis

Received Date: Jun 24, 2024
Revised Date: Aug 30, 2024
Accepted Date: Oct 09, 2024

[1]Master degree student in General Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
[2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
[3]Department of Pharmaceutical care, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, 50200, Thailand.
*[4]Department of Family and Community Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand. (*corresponding author)*

Introduction

Currently, vital pulp therapy has gained interest and support due to increasing empirical evidence (1,2). Partial pulpotomy, a method of vital pulp therapy, involves the removal of a small portion of the vital coronal pulp as a means of preserving the remaining coronal and radicular pulp tissues (3). This is coupled with the application of biocompatible materials over the pulp tissue to promote healing and coronal seal.

Biological rationales for partial pulpotomy procedure in treating carious pulp exposure have been underpinned by histo-bacteriological studies (4,5) that pulp tissue affected by caries is usually limited in the area close to exposure site and removing the inflamed portion appeared to sufficiently encourage self-healing.

Calcium silicate cements (CSCs) are increasingly used in vital pulp therapy (VPT) procedures. CSCs include materials like tricalcium silicates, dicalcium silicates, hydraulic calcium silicate cements, and “bioceramics” or “bioactive” cements. Mineral trioxide aggregate (MTA) is the first and well-established CSCs that is widely used and extensively studied. Systematic reviews and meta-analyses have shown that MTA as a capping material in partial pulpotomy has overall higher clinical success than calcium hydroxide (6). However, MTA has some clinical limitations, such as a long setting time, tooth discoloration, and high cost (7,8). Biodentine™ is a second-generation calcium silicate cement which compensates for some of the limitations of MTA by having a shorter setting time and a lower incidence of tooth discoloration (9,10). Nevertheless, studies comparing the success rates of partial pulpotomy using Biodentine™ versus MTA are still limited.

A recent systematic review (11) suggested that partial pulpotomy resulted in high success rates in treating cariously exposed permanent posterior teeth for up to 2 years, with limited studies on success rates over 2 years. Various factors have been proposed as potential prognostic factors on success rate of pulpotomy in permanent teeth. However a comprehensive review supported by best available evidence concluded that only caries depth, inflammatory status of the pulp, capping material, level of inflammatory pulpal-biomarkers and the final restoration integrity were influential factors while other factors such as age and gender did not impact significantly on pulpotomy outcome (12).

This study aimed to evaluate survival rate over 3 years of partial pulpotomy treatment in mature permanent teeth with carious pulp exposure, comparing the use of two bioactive cements: Biodentine™ and ProRoot MTA® as capping materials. This study was a follow-up analysis of patients from the previously reported clinical trial (Thai Clinical Trials Registry: TCTR20171228003) by Suwannaphrom et al (13) which reported 1-year treatment outcomes. Additional objective was to evaluate patient satisfaction with the partial pulpotomy treatment, providing insights into patient perspectives and overall acceptance of the procedure.

Survival analysis was advantageous for dealing with incomplete data where the event of interest has not occurred for some subjects during the study period (censored data). Moreover, it focuses on the time until the occurrence of an event (Time-to-Event analysis), providing insights into the timing and risk factors associated with the event. The findings from this study were

expected to provide valuable insights into the survival rates of partial pulpotomy treatments, guiding the appropriate follow-up intervals and care.

Materials and methods

Study design

The present study was designed as a follow-up to the previously reported 1-year outcome of a randomized clinical trial investigating the non-inferiority of Biodentine™ compared to MTA for partial pulpotomy in adult's permanent teeth with carious pulp exposure (Thai Clinical Trials Registry: TCTR20171228003)(13). All 58 trial participants (ProRoot MTA® = 29, Biodentine™ = 29) whose teeth had received treatment for at least 3 years were contacted for recall. These participants had premolars or molars with deep caries extending more than two-thirds of the dentin thickness, both with and without clinical symptoms, and responded to pulp sensibility tests. After completed caries removal under the rubber dam isolation, the caries had reached the pulp, which was still vital. The tissue was excised from the superficial level of the pulp to the remaining pulp tissue, which appeared relatively dense. The pulp chamber was irrigated with 2.5% sodium hypochlorite. A cotton pellet soaked in sodium hypochlorite was applied to achieve hemostasis and hemostasis could be achieved within 10 minutes (13). The follow-up included clinical examinations, radiographic evaluations, and a patient satisfaction survey. The present study has received ethical approval from the Human Research Ethics Committee of the Faculty of Dentistry, Chiang Mai University (Approval No. 25/2564, dated May 24, 2021).

Inclusion Criteria

1. Participants of the trial "Partial Pulpotomy Treatment in Permanent Teeth with Cariously Exposed Pulp in Adult Patients: A Non-inferiority Randomized Controlled Trial Comparing Two Calcium Silicate-based Cements" (Study ID: TCTR20171228003) who received treatment at the Comprehensive Dental Clinic, Faculty of Dentistry, Chiang Mai University, from May 2017 to October 2018.

2. Patient consent to participate in the follow-up research.

Exclusion Criteria

1. Patients who cannot be contacted.
2. Patients who refuse to participate in the follow up.

Follow-up Procedures

Patients who attended the follow-up received an information sheet detailing the study and an informed consent form. The follow-up included clinical examinations, radiographic examination, and a patient satisfaction survey.

Clinical examination procedures

First, the presence of the treated tooth in the oral cavity was verified. In cases where the tooth was missing, the reasons for extraction and any associated symptoms were reviewed and documented. Patients were asked about any current symptoms and the functionality of the treated tooth. Pulp sensibility was evaluated using cold tests (Endo-Ice®, Coltene/Whaledent, USA) and electric pulp tests (EPT) (Vitality Scanner™, Kerr, USA). Percussion and palpation

tests were performed to detect any tenderness or abnormal responses. Tooth mobility and periodontal status were examined. Tooth discoloration was evaluated visually by comparing it with the adjacent normal teeth. The condition of the restorative material was assessed using the Modified USPHS criteria(14). Intraoral photographs were taken with a digital camera (EOS 700D, Canon Inc., Japan) to document the condition of the restorative material and any observed tooth discoloration. If any tooth exhibited signs of pulp inflammation or necrosis, patients were informed about the available treatment options and referred for appropriate care. Additionally, if the restorative material was found to be defective, patients received a new dental filling.

Radiographic examination and assessment procedures

The parallel periapical radiographs of the treated teeth were taken using digital imaging plate no.2 (Durr Dental Imaging, Germany) with a film holder (XCP®, Densply, USA). Assessments focused on: Periapical Index (PAI) score (15), widening of the periodontal ligament space, presence or absence of the lamina dura, presence of apical radiolucencies, root resorption, pulp chamber and canal calcification, pulp canal obliterations, and formation of dentine bridges. A certified endodontist (Diplomate, Thai Board of Endodontics) who was blinded to the treatment information performed radiographic evaluations. The intra-examiner calibration process involved the assessing a set of 20 periapical radiographs twice, with a two-week interval of readings. At least 0.8 Cohen Kappa statistic was required.

Patient satisfaction survey

The survey questionnaire employed a 7-point scale ranging from “very satisfied” to “strongly dissatisfied” responses on: the symptoms of the treated tooth, the functionality of the treated tooth, the satisfaction on the condition of the restorative material, the aesthetics, and the treatment and follow-up care procedure.

Data for Survival Analysis

To perform a survival analysis using Kaplan-Meier statistic, first the event and censor date of each treated tooth must be defined. Then the survival time were calculated from the treatment date to the event or censoring date, measured in months. The characteristic of event and censor were that:

1. Event date: the date when clinical failure was documented in dental records or when the patient reported receiving treatment elsewhere. An event occurred in cases where:

1.1 The treated tooth had clinical failures, which determined by presence of pain, no response to sensibility tests, swelling, or sinus tract opening, or

1.2 The treated tooth underwent tooth extraction or root canal treatment.

2. Censor date: the last follow-up date of patient who did not experience the event. Censoring occurred in cases where:

2.1 The treated tooth remained in the oral cavity, responded to vitality tests, and functioned normally upon follow-up date, or

2.2 The patient was lost to follow-up and could not be tracked.

Statistical Analysis

Kaplan-Meier survival analysis was used to evaluate the pulp survival probability at the end of the study. The pulp survival probabilities of the ProRoot MTA® group and Biodentine™ group were compared using the log-rank test. Tooth discoloration rates and the success rates of restoration of each group were compared using the Chi-square test. Patient satisfactions were analyzed using descriptive statistics. The significance level was set at 5%, and the analyses were conducted using SPSS 20.0 statistical software (SPSS Inc., 2011, Chicago, IL).

Results

The intra-examiner's Cohen Kappa reliability of the radiographic evaluator was 0.90. Demographic and preoperative characteristics of the participants who attended the present follow-up was shown in Table 1. No significant differences were observed among these variables.

Out of the 58 patients, 48 patients (82.8%) attended the follow-up. Among these, 39 patients showed pulp survival of the treated teeth (19 with ProRoot MTA® and 20 with Biodentine™). Nine patients underwent tooth extraction or root canal treatment (3 with ProRoot MTA® and 6 with Biodentine™). Ten patients (17.2%) were lost to follow-up (7with ProRoot MTA® and 3 with Biodentine™) (Fig. 1).

Table 1. Demographic, preoperative and intra-operative characteristics of the samples who attended the present follow-up (tooth is unit of analysis).

Factors	Total	ProRoot MTA®	Biodentine™	p-value
n	48	22	26	-
Sex, % (n/N)				
Male	52.1 (25/48)	54.5 (12/22)	50.0 (13/26)	0.753a
Female	47.9 (23/48)	45.5 (10/22)	50.0 (13/26)	
Age (year)				
Range	18–56	19-56	18-42	0.959¥
Mean ± SD	26.77 ± 9.60	28.18 ± 11.76	25.58 ± 7.33	
Pre-operative symptoms, % (n/N)				
Initial pulpitis	8.3 (4/48)	13.6 (3/22)	3.8 (1/26)	0.540§
Mild pulpitis	64.6 (31/48)	59.1 (13/22)	69.2 (18/26)	
Moderate pulpitis	27.1 (13/48)	27.3 (6/22)	26.9 (7/26)	
Pre-operative PAI score, % (n/N)				
PAI score of 1	27.1 (13/48)	27.3 (6/22)	26.9 (7/26)	0.555a
PAI score of 2	56.2 (27/48)	50.0 (11/22)	61.5 (16/26)	
PAI score of 3	16.7 (8/48)	22.7 (5/22)	11.5 (3/26)	
Caries site, % (n/N)				
Occlusal/buccal/lingual	14.6 (7/48)	13.6 (3/22)	15.4 (4/26)	1.000§
Proximal	85.4 (41/48)	86.4 (19/22)	84.6 (22/26)	
Time to control bleeding (mins), % (n/N)				
0 - 5	83.3 (40/48)	95.5 (21/22)	73.1 (19/26)	0.055§
> 5 - 10	16.7 (8/48)	4.5 (1/22)	26.9 (7/26)	
Exposure size (mm.), % (n/N)				
< 2	72.9 (35/48)	77.3 (17/22)	69.2 (18/26)	0.532a
=, > 2	27.1 (13/48)	22.7 (5/22)	30.8 (8/26)	

SD, standard deviation; a Chi-square test; § Fisher's exact Test; ¥ Mann-Whitney Test.

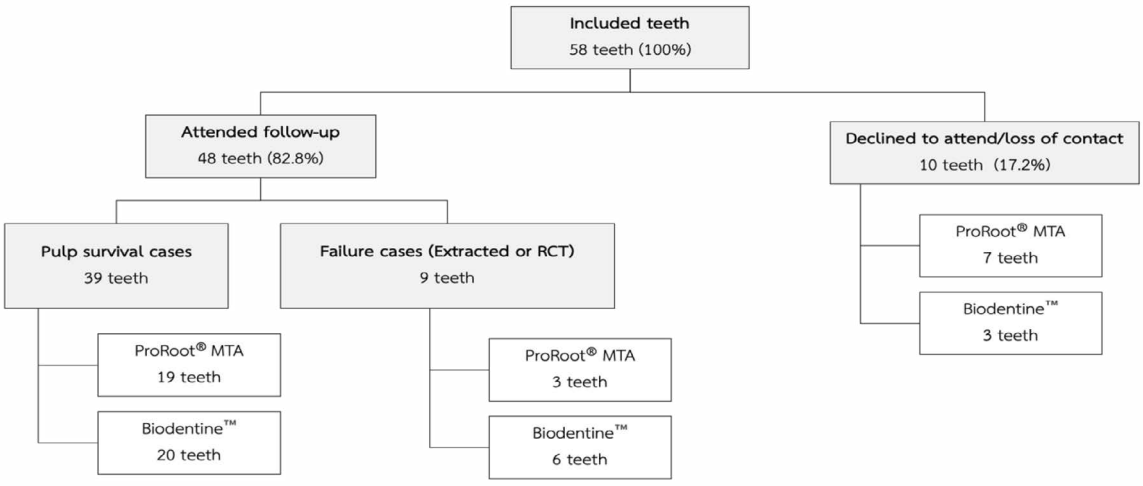

Fig.1 Flow chart showing patient follow-up.

The average follow-up period was 40.95 ± 4.24 months. The estimated cumulative pulp survival rate in the first 12 months was 98.2% and decreased to 78.7% at the end of the study. The highest frequency of failures occurred between 24 and 35 months (4 teeth) (Table 2). The overall mean survival time is 46.52 ± 1.43 months. The median survival time cannot be determined because the cumulative survival throughout the study period was greater than 50% (Table 3).

Table 2. Survival table.

Follow-up time (months)	Number of teeth at beginning	Number of survival teeth	Number of teeth that had failure	Number of teeth withdrawing (censor/loss of follow-up)	Estimated cumulative survival probability % (Standard error)
0-11	58	57	1	4	98.2 (1.76)
12-23	53	51	2	5	94.3 (3.18)
24-35	46	42	4	1	86.0 (4.91)
36-47	41	39	2	35	78.7 (6.68)
48-51	4	4	0	4	78.7 (6.68)

Table 3. Mean and median survival time.

Group	Mean			Median		
	Estimate	Std.Error	95% CI	Estimate	Std.Error	95% CI
ProRoot MTA®	45.38	2.117	41.232–49.530	-	-	-
Biodentine™	46.03	1.880	42.347–49.716	-	-	-
Overall	46.52	1.434	43.710–49.331	-	-	-

From the Kaplan-Meier survival curve (Fig. 2), a cumulative pulp survival rate at the end of the study (51 months) was 87.4% for ProRoot MTA® group and 70.7% for Biodentine™ group. The log-rank test showed no significant difference between the groups (p = 0.416).

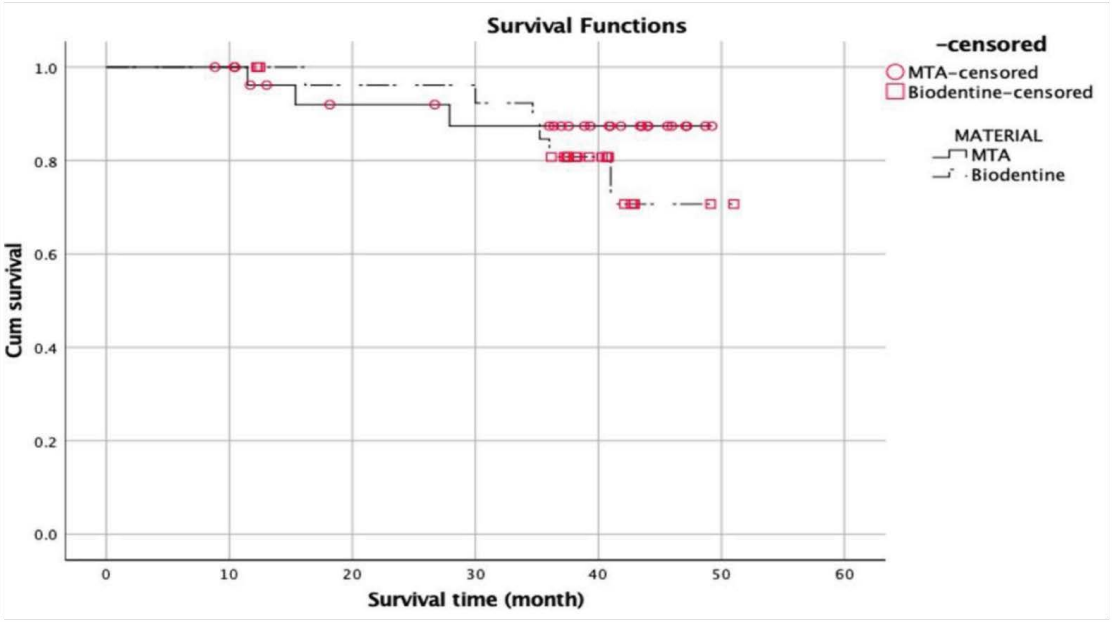

Fig.2 Kaplan-Meier survival curve shown the cumulative pulp survival probabilities of ProRoot MTA® and Biodentine™ group.

The descriptive radiographic results showed that, among the 39 patients, no internal or external root resorption was observed. Diffusion calcification in pulp chamber was found in 26 teeth (10 with ProRoot MTA® and 16 with Biodentine™). Diffusion calcification in root canal was found in 11 teeth (3 with ProRoot MTA® and 8 with Biodentine™). Pulp canal obliteration after treatment was found in 8 teeth (3 with ProRoot MTA® and 5 with Biodentine™). Reparative dentin formation was detected in 31 teeth (14 with ProRoot MTA® and 17 with Biodentine™). PAI score of 1 was observed in 33 teeth (15 with ProRoot MTA® and 18 with Biodentine™). PAI score of 2 was observed in 5 teeth (4 with ProRoot MTA® and 1 with Biodentine™). PAI score of 3 was observed in 1 tooth with a positive pulp sensibility test (Biodentine™ group) (table 4).

Table 4. Radiographic appearance of the treated teeth according to pulp capping material group.

Radiographic aspects	ProRoot MTA® (teeth, N = 19)	Biodentine™ (teeth, N = 20)
Internal root resorption	0	0
External root resorption	0	0
Diffusion calcification in pulp chamber	10	16
Diffusion calcification in root canal	3	8
Pulp canal obliterations	3	5
Detectable dentine bridge formation	14	17
PAI score of 1	15	18
PAI score of 2	4	1
PAI score of 3	0	1

Discoloration of teeth was observed in both groups, 42.1% (8/19) in teeth treated with ProRoot MTA® and 10% (2/20) in teeth treated with Biodentine™, with a statistically significant difference between the group ($p = 0.031$) (Fig. 3).

The acceptable restorations rate was 74.4%. Losing of anatomical form and the recurrence of caries were found as the main reasons of unacceptable condition. Of the 10 teeth with unacceptable restorations, 5 with ProRoot MTA® and 5 with Biodentine™, showing no significant difference between the groups ($p = 0.925$). Reparative dentin formation was observed during the replacement of unacceptable restorations in all cases.

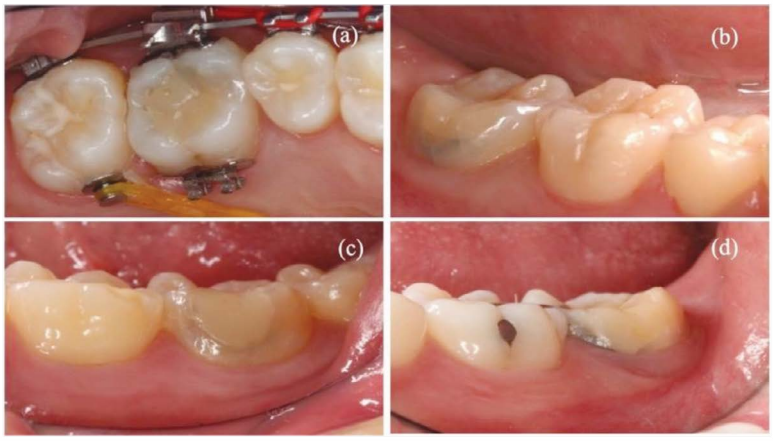

Fig.3 Representative cases of tooth discoloration after partial pulpotomy. (a) Tooth 16 with ProRoot MTA® at 36-months follow-up. (b) Tooth 47 with ProRoot MTA® at 41 months-months follow-up. (c) Tooth 37 with Biodentine™ at 40-months follow-up. (d) Tooth 37 with Biodentine™ at 36-months follow-up.

Cumulatively, 9 teeth failed after treatment, 3 were treated with ProRoot MTA® and 6 were treated with Biodentine™. The earliest failure (ProRoot MTA® group) occurred at 11.5 months post-treatment, the patient exhibited an abscess and non-responsive sensibility test, resulting in tooth extraction. The latest failure (Biodentine™ group) occurred at 41 months post-treatment, the patient reported the dislodgement of restoration, followed by spontaneous pain, resulting in tooth extraction. Details of all failed teeth are shown in Table 5 (Table 5).

The results of satisfaction survey were favorable as presented in Table 6 (Table 6).

Table 5. Characteristic of the failed cases.

ID	Tooth	Pulp dressing material	Age	Pre-op PAI score	Preoperative symptom	Time at failure (month)	Conditions	Treatment
3	46	Biodentine™	31	2	moderate pulpitis	36	crown-root fracture	extraction
6	17	Biodentine™	36	2	initial pulpitis	16.2	spontaneous pain	RCT
10	47	Biodentine™	28	2	moderate pulpitis	35.2	crown fracture	RCT, post and core with crown
28	16	Biodentine™	21	2	moderate pulpitis	41	dislodgement of restoration and spontaneous pain	extraction
38	37	Biodentine™	22	2	mild pulpitis	30	dislodgement of restoration and spontaneous pain	RCT
40	36	ProRoot MTA®	35	2	moderate pulpitis	27.9	spontaneous pain	extraction
43	36	Biodentine™	21	2	mild pulpitis	34.7	spontaneous pain	RCT
45	36	ProRoot MTA®	20	3	mild pulpitis	15.4	secondary caries causing asymptomatic apical periodontitis	RCT
55	48	ProRoot MTA®	51	2	mild pulpitis	11.5	negative to sensibility test, sinus tract	extraction

Table 6. Patient's satisfaction level on various aspects of treatment.

Satisfaction level	Very satisfied %(n/N)	Satisfied %(n/N)	Somewhat satisfied %(n/N)	Neutral %(n/N)	Somewhat dissatisfied %(n/N)	Dissatisfied %(n/N)	Strongly dissatisfied %(n/N)
Post-operative symptoms	46.1 (18/39)	46.1 (18/39)	7.7 (3/39)	0	0	0	0
Functionality of the tooth after treatment	71.8 (28/39)	17.9 (7/39)	10.2 (4/39)	0	0	0	0
Restoration on the tooth	64.1 (25/39)	17.9 (7/39)	7.7 (3/39)	10.2 (4/39)	0	0	0
Aesthetic of the tooth	48.7 (19/39)	33.3 (13/39)	10.2 (4/39)	7.7 (3/39)	0	0	0
Treatment procedure	71.8 (28/39)	17.9 (7/39)	10.2 (4/39)	0	0	0	0
Follow-up care	71.8 (28/39)	12.8 (5/39)	10.2 (4/39)	5.1 (2/39)	0	0	0

Discussion

This study found a cumulative pulp survival rate of 70.7% in the Biodentine™ group, similar to the prospective study by Tan et al. (16), which reported a success rate of 72.7% in adult permanent teeth with mild or no symptoms treated with partial pulpotomy using Biodentine™ over an average follow-up period of 3 years. For the ProRoot MTA® group, a cumulative pulp survival rate of 87.4% was observed, aligning closely with the findings of Taha and Khazali (17), who reported a success rates of 85% of ProRoot MTA®, and Tzanetakis et al (18), who reported a success rates of 89.2% of MTA Angelus. Both studies were randomized clinical trials on permanent teeth with closed apices and carious pulp exposure, with an average follow-up period of 2 years. The survival criteria used in the present study was focused on the retain of tooth with pulp response as indicated by pulp sensibility testing, showing "procedural survival", regardless of radiographic outcome. However, trials reported success rate usually determined success using both success on clinical and radiographic outcome(17,19,20).

The present study found no statistically significant difference in pulp survival between the ProRoot MTA® and Biodentine™ group. This aligned with Uesrichai et al (19), who also found no significant difference in the success rates of partial pulpotomy in young cariously exposed permanent teeth with either ProRoot MTA® or Biodentine™ at average 32-month follow-up period. Similarly, a systematic review and meta-analysis by Sabeti et al (21) reported no significant

difference in the success rates of various vital pulp therapies using MTA or calcium silicate-based materials such as Biodentine™. This lack of significant difference is likely due to the ability of calcium silicate-based cements to induce reparative dentin formation at the site of pulp exposure (22).

Although no significant difference was observed between Biodentine™ and ProRoot MTA®, it is notable that the success rates for Biodentine™ treated teeth were slightly lower than those for MTA treated teeth in some studies (19,23,24). The possible explanation could be related to the procedure and material itself, for instance, Nekoofar et al (25) investigated the shear bond strength of Biodentine™ and various resin materials at different setting times. They found that the shear bond strength of RMGIC (resin-modified glass ionomer cement) to Biodentine™ increased when RMGIC was placed 1 week after setting compared to its placement after 12 minutes. In this single-visit treatment study, RMGIC was applied over Biodentine™ after a 12-minute setting time, which might have resulted in reduced adhesion between the two materials, potentially affecting the overall success rate.

Treated teeth in both groups exhibited tooth discoloration, with a significantly higher incidence in the ProRoot MTA® group. This discoloration was believed to result from the oxidation of bismuth oxide, a radiopacifier in MTA, which contributes to tooth discoloration (26). This observation aligned with the study by Uesrichai et al. (19) which reported a perceptible grey discoloration for 80% in ProRoot MTA® treated teeth and 27% in Biodentine™ treated teeth at average 32 months follow-up period. However, the discoloration rate observed in this study was lower. In contrast, the initial study by Suwannaphrom et al (13) found no tooth discoloration in the Biodentine™ group after a 1-year follow-up. However, in this study, which followed up for at least 3 years, tooth discoloration was observed in the Biodentine™group, suggesting that Biodentine™ may cause discoloration over longer periods. This was supported by the study by Shokouhinejad et al (27), which indicated that the incidence of tooth discoloration increased with time in an in vitro setting comparing various calcium silicate-based materials. Taha et al (28) also reported an increased discoloration over time in clinical trials of full pulpotomy in adult permanent teeth using MTA, TotalFill, and Biodentine™ as capping materials, the highest rate of discoloration was observed in MTA group. In addition to the oxidation process of bismuth oxide, which was believed to cause tooth discoloration, laboratory studies have identified blood contamination as another factor contributing to tooth discoloration (27,29,30). Therefore, it was possible that the discoloration observed in the Biodentine™ group might have cause by blood contamination from the pulp tissue during the treatment.

Pulp inflammation, secondary caries, and fractures of the restorative material or the tooth itself were reasons of failures in this study, leading to tooth extraction or root canal treatment. Due to the limitations in identifying the causes of failure, which mostly reported by patients, it was difficult to diagnose the true condition of the pulp before failure. This aligned with other studies (17,20,31) that classified teeth requiring further treatment, such as root canal therapy or tooth extraction, as failures.

Failure of the coronal seal led to bacterial infiltration into the dentinal tubules, potentially causing recurrent pulp inflammation. Studies have shown that a good coronal seal significantly enhances the success of root canal treatments (32,33). In this study, teeth with fractured restorations or recurrent caries, whose pulp still survived for more than 3 years, exhibited reparative dentin formation. This was observed during the replacement of the old restorations (Fig.4). A study found that the presence of reparative dentin on radiographs significantly increased the success rate (34). From the radiographic analysis in this study, reparative dentine formation was observed in 31 (14 in the ProRoot MTA® group and 17 in the Biodentine™ group) out of 39 surviving teeth, which was considered a high level. Therefore, the detection of reparative dentine formation after treatment may serve as an indicator of a favorable prognosis.

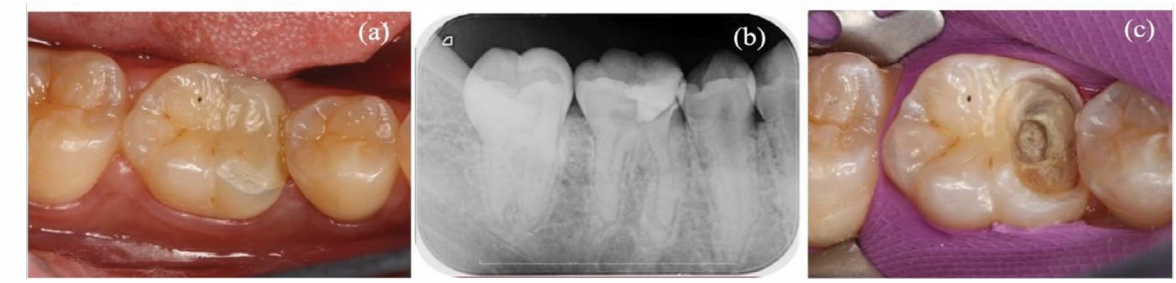

Fig.4 Representative case of the reparative dentine formation in a tooth with restorative failure but pulp survival. (a) Tooth 46 with marginal leakage and secondary caries (ProRoot MTA® group). (b) A 36-month follow-up radiograph. (c) The reparative dentine was seen after removal of defective restoration and caries.

Currently, there are no definitive guidelines for the optimal duration to assess the success of partial pulpotomy. Nosrat and Nosrat (35) suggested that reparative dentin formation occurred within 3 months post-treatment in all samples, supporting a 3-month post-treatment as a suitable timeframe for initial success evaluation. Elmsmari et al (11) found no significant difference in success rates at 6 months, 1 year, and 2 years post-treatment, suggesting 6 months as a reasonable assessment period. Matsuo et al (36) reported no difference in success rates at 3 months and 18 months following direct pulp capping, supporting a 3-month evaluation period. However, for a longer term follow-up, Awawdeh et al (37) suggested that 3 years might insufficient for fully assess the success rates of vital pulp therapy with MTA and Biodentine™ as success rates tend to decrease over time. This finding aligned with the present study, which failure frequency increased continuously at 2-3 years.

The survival rate of teeth restored with composite resin after root canal treatment were reported to be significantly decreased around 2 years (38), particularly when multiple tooth walls were lost, which further reduced the survival rate against fractures (39). The present study, most of teeth had lost occlusal and proximal surfaces

due to caries and were restored with composite resin. Survival analysis indicated that failures increased after 12 months, with the highest frequency of failures occurring between 24 and 35 months. Therefore, it is recommended to provide full-coverage restorations as soon as possible or after a 1-year follow-up to minimize the risk of fractures and ensure a good coronal seal.

Conclusion

The pulp survival of adult permanent teeth with carious pulp exposure after at least 3 years of partial pulpotomy was relatively high, with the overall pulp survival rate of 78.7%. There was no significant difference in survival between the use of ProRoot MTA® and Biodentine™ as pulp capping materials. Tooth discoloration was more prevalent in ProRoot MTA® group compared to Biodentine™ group, with a statistically significant difference. The majority of patients were very satisfied with the treatment. Full - coverage restorations should be provided as soon as possible, or no later than a 1-year follow-up, since the highest frequency of treatment failures occurred between 24 and 35 months.

Study Limitations

Radiographic Evaluation: This study assessed the radiographic outcome using periapical radiographs, which are two-dimensional images. These images often show overlapping of the tooth and root structures from the buccal and lingual sides, as well as the restorative materials, which are typically large. Consequently, it was sometimes challenging to assess internal root canal features such as dentin bridge formation or canal obliteration accurately.

Tooth Discoloration Assessment: Tooth discoloration was evaluated visually by comparing the treated tooth with adjacent teeth. Human visual assessment is inherently limited and may underestimate the extent of discoloration. Standardized measurement tools should be employed for more accurate and reliable assessments of tooth color changes.

Patient Follow-Up: Some patients were lost to follow-up due to relocation or inability to contact them. In long-term follow-up studies, the rate of patient retention may decrease, affecting the overall evaluation of treatment outcomes.

Identifying the causes of failure: Some failures were tracked by dental record or reported by patients, making it difficult to diagnose the true condition of the pulp survival before failure.

Acknowledgements

We would like to express our gratitude to all the staff in the General Dentistry Department for their assistance and support in completing this research. We also extend our heartfelt thanks to all the research volunteers for dedicating their valuable time to participate in this study.

Funding

This study was supported by Faculty of Dentistry, Chiang Mai University.

Ethics declarations

Ethics approval and consent to participate Ethical approval was taken from the Human Research Ethics Committee of the Faculty of Dentistry, Chiang Mai University (Approval No. 25/2564, dated May 24, 2021).

Competing interests

The authors declare no competing interests.

References

1. Duncan HF, Galler KM, Tomson PL, Simon S, El-Karim I, Kundzina R, et al. European Society of Endodontology position statement: Management of deep caries and the exposed pulp. Int Endod J. 2019;52(7):923-34.

2. Craig SH, George B, Johnah CG, Ronald RL, Ove AP, Nikita BR, et al. AAE Position Statement on Vital Pulp Therapy. J Endod. 2021; 47(9):1340-4.

3. Glossary of Endodontics Terms [Internet]. Chicago: American Association of Endodontists; 2020 [cited 2023 17 May]. Available from: https://www.aae.org/specialty/clinical-resources/glossary-endodontic-terms/.

4. Ricucci D, Loghin S, Siqueira JF, Jr. Correlation between clinical and histologic pulp diagnoses. J Endod. 2014;40(12):1932-9.

5. Ricucci D, Siqueira JF Jr., Li Y, Tay FR. Vital pulp therapy: histopathology and histobacteriology-based guidelines to treat teeth with deep caries and pulp exposure. J Dent. 2019;86:41-52. doi: 10.1016/j.jdent.2019.05.022.

6. Li Y, Sui B, Dahl C, Bergeron B, Shipman P, Niu L, et al. Pulpotomy for carious pulp exposures in permanent teeth: a systematic review and meta-analysis. J Dent. 2019;84:1-8. doi: 10.1016/j.jdent.2019.03.010.

7. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review--part II: leakage and biocompatibility investigations. J Endod. 2010;36(2):190-202.

8. Caicedo R, Gettleman L. Physical properties of MTA. In: Torabinejad M, editor. Mineral trioxide aggregate: properties and clinical applications. Ames, Iowa, USA John Wiley & Sons; 2014. p. 37-70.

9. Vallés M, Roig M, Duran-Sindreu F, Martínez S, Mercadé M. Color stability of teeth restored with Biodentine: a 6-month in vitro study. J Endod. 2015;41(7):1157-60.

10. Parinyaprom N, Nirunsittirat A, Chuveera P, Na Lampang S, Srisuwan T, Sastraruji T, et al. Outcomes of direct pulp capping by using either ProRoot mineral trioxide aggregate or Biodentine in permanent teeth with carious pulp exposure in 6- to 18-year-old patients: a randomized controlled trial. J Endod. 2018;44(3):341-8.

11. Elmsmari F, Ruiz XF, Miró Q, Feijoo-Pato N, Durán-Sindreu F, Olivieri JG. Outcome of partial pulpotomy in cariously exposed posterior permanent teeth: a systematic review and meta-analysis. J Endod. 2019;45(11):1296-306.

12. Duncan HF, El-Karim I, Dummer PMH, Whitworth J, Nagendrababu V. Factors that influence the outcome of pulpotomy in permanent teeth. Int Endod J. 2023;56(Suppl 2):62-81.

13. Suwannaphrom N, Nirunsittirat A, Srisuwan T, Chompu-inwai P, Louwakul P, Chattipakorn S, et al. A non-inferiority randomized control trial comparing two calcium silicate-based cements as pulp capping material for partial pulpotomy in permanent teeth with cariously exposed pulp in adult patients. Khon Kaen Dent J. 2022;25(2):14-27.

14. Van Dijken JWV. A clinical evaluation of anterior conventional, microfllller, and hybrid composite resin fillings: a 6-year follow-up study. Acta Odontol Scand. 1986;44(6):357-67.

15. Orstavik D, Kerekes K, Eriksen HM. The periapical index: a scoring system for radiographic assessment of apical periodontitis. Endod Dent Traumatol. 1986;2(1):20-34.

16. Tan SY, Yu VSH, Lim KC, Tan BCK, Neo CLJ, Shen L, et al. Long-term pulpal and restorative outcomes of pulpotomy in mature permanent teeth. J Endod. 2020;46(3):383-90.

17. Taha NA, Khazali MA. Partial pulpotomy in mature permanent teeth with clinical signs indicative of irreversible pulpitis: a randomized clinical trial. J Endod. 2017;43(9):1417-21.

18. Tzanetakis GN, Koletsi D, Georgopoulou M. Treatment outcome of partial pulpotomy using two different calcium silicate materials in mature permanent teeth with symptoms of irreversible pulpitis: A randomized clinical trial. Int Endod J. 2023;56(10):1178-96.

19. Uesrichai N, Nirunsittirat A, Chuveera P, Srisuwan T, Sastraruji T, Chompu-Inwai P. Partial pulpotomy with two bioactive cements in permanent teeth of 6- to 18-year-old patients with signs and symptoms indicative of irreversible pulpitis: a noninferiority randomized controlled trial. Int Endod J. 2019;52(6):749-59.

20. Jassal A, Nawal RR, Yadav S, Talwar S, Yadav S, Duncan HF. Outcome of partial and full pulpotomy in cariously exposed mature molars with symptoms indicative of irreversible pulpitis: A randomized controlled trial. Int Endod J. 2023; 56(3):331-44.

21. Sabeti M, Huang Y, Chung YJ, Azarpazhooh A. Prognosis of vital pulp therapy on permanent dentition: a systematic review and meta-analysis of randomized controlled trials. J Endod. 2021;47(11):1683-95.

22. Laurent P, Camps J, About I. Biodentine induces TGF-β1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012;45(5):439-48.

23. Peskersoy C, Lukarcanin J, Turkun M. Efficacy of different calcium silicate materials as pulp-capping agents: Randomized clinical trial. J Dent Sci. 2021;16(2):723-31.

24. Taha NA, Al-Rawash MH, Imran ZA. Outcome of full pulpotomy in mature permanent molars using 3 calcium silicate-based materials: A parallel, double blind, randomized controlled trial. Int Endod J. 2022;55(5):416-29.

25. Nekoofar MH, Motevasselian F, Mirzaei M, Yassini E, Pouyanfar H, Dummer PM. The Micro-Shear Bond Strength of Various Resinous Restorative Materials to Aged Biodentine. Iran Endod J. 2018;13(3):356-61.

26. Możyńska J, Metlerski M, Lipski M, Nowicka A. Tooth discoloration induced by different calcium silicate-based cements: a systematic review of in vitro studies. J Endod. 2017;43(10):1593-601.

27. Shokouhinejad N, Nekoofar MH, Pirmoazen S, Shamshiri AR, Dummer PM. Evaluation and Comparison of Occurrence of Tooth Discoloration after the Application of Various Calcium Silicate-based Cements: An Ex Vivo Study. J Endod. 2016; 42(1):140-4.

28. Taha NA, Hamdan AM, Al-Hiyasat AS. Coronal discoloration induced by calcium silicate-based cements used in full pulpotomy in mature permanent molars: a randomized clinical trial. Clin Oral Investig. 2022;27(4):1723-30.

29. Felman D, Parashos P. Coronal tooth discoloration and white mineral trioxide aggregate. J Endod. 2013;39(4):484-7.

30. Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G. Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J. 2012;45(10):942-9.

31. Taha NA, Abdelkhader SZ. Outcome of full pulpotomy using Biodentine in adult patients with symptoms indicative of irreversible pulpitis. Int Endod J. 2018;51(8):819-28.

32. Gillen BM, Looney SW, Gu LS, Loushine BA, Weller RN, Loushine RJ, et al. Impact of the quality of coronal restoration versus the quality of root canal fillings on success of root canal treatment: a systematic review and meta-analysis. J Endod. 2011;37(7): 895-902.

33. Aquilino SA, Caplan DJ. Relationship between crown placement and the survival of endodontically treated teeth. J Prosthet Dent. 2002;87(3):256-63.

34. Kunert GG, Kunert IR, da Costa Filho LC, de Figueiredo JAP. Permanent teeth pulpotomy survival analysis: retrospective follow-up. J Dent. 2015;43(9):1125-31.

35. Nosrat IV, Nosrat CA. Reparative hard tissue formation following calcium hydroxide application after partial pulpotomy in cariously exposed pulps of permanent teeth. Int Endod J. 1998;31(3):221-6.

36. Matsuo T, Nakanishi T, Shimizu H, Ebisu S. A clinical study of direct pulp capping applied to carious-exposed pulps. J Endod. 1996; 22(10):551-6.

37. Awawdeh L, Al-Qudah A, Hamouri H, Chakra RJ. Outcomes of Vital Pulp Therapy Using Mineral Trioxide Aggregate or Biodentine: A Prospective Randomized Clinical Trial. J Endod. 2018;44(11):1603-9.

38. Jirathanyanatt T, Suksaphar W, Banomyong D, Ngoenwiwatkul Y. Endodontically treated posterior teeth restored with or without crown restorations: A 5-year retrospective study of survival rates from fracture. J Investig Clin Dent. 2019;10(4):e12426. doi: 10.1111/jicd.12426.

39. Dammaschke T, Nykiel K, Sagheri D, Schäfer E. Influence of coronal restorations on the fracture resistance of root canal-treated premolar and molar teeth: a retrospective study. Aust Endod J. 2013;39(2):48-56.

Corresponding author:

Asst.Prof.Dr. Patchanee Chuveera

Faculty of Dentistry, Chiang Mai University, 239, Suthep Sub-district, Muang District, Chiang Mai 50200, Thailand.

Tel: (668) 5716 2130

E-mail: patchanee.ch@cmu.ac.th

The Study of Satisfaction on Chewing Jelly with Coconut Oil for Oral Moisturization in the Elderly: A Pilot study

Panurak Kaewnoi[1] Serena S. Sakoolnamarka[2*] Sorasun Rungsiyanont[3]

Abstract

Dry mouth problems are common among the elderly, and many products have been developed to relieve and treat this issue.

Objectives: This research aimed to develop clinical innovations for saliva stimulation and oral moisturizing in the elderly using chewing jelly containing coconut oil. A pilot study of its satisfaction was performed.

Materials and Methods: The study population was selected based on pre-defined inclusion criteria from elderly members of the Nong Sadao Elderly Club, Suphan-Buri. Participants were aged 60 years and above and self-reported dry mouth conditions. Data were collected through interviews using satisfaction questionnaires that addressed appearance, mouthfeel, and oral moisturization after chewing two jelly formulas containing coconut oil. Descriptive statistics summarized basic data, such as mean and standard deviation. Differences in satisfaction across demographics were tested using the Mann-Whitney U Test, and differences between the original and herbal formula jellies were tested using the Wilcoxon Signed-Rank Test, with a confidence level of 95%.

Results: The study population consisted of 30 elderly individuals with an average age of 70.20 ± 3.70 years. Participants included 6 males (20%) and 24 females (80%). Most participants were satisfied with the innovation, rating the appearance of both formula jellies the highest, followed by oral moisture. The lowest score was for the mouthfeel. The group experiencing moderate to severe dry mouth rated the overall appeal of the herbal formulation significantly higher. The overall scores for appearance and moisturization satisfaction indicated a statistically significant difference, with the herbal formula scoring higher.

Conclusion: The chewing jelly showed high overall satisfaction. Overall, the herbal formula received higher score than the original formula.

Keywords: Elderly, Dry mouth, Xerostomia, Saliva Stimulation, Coconut Oil

Received Date: Jul 08, 2024
Revised Date: Sep 24, 2024
Accepted Date: Oct 09, 2024

[1]*Nong Sadao primary care hospital, Suphan Buri, 72130, Thailand.*
[2]*Department of Pediatric and Preventive Dentistry Faculty of Dentistry, Srinakharinwirot University, Bangkok, 10110, Thailand.*
[3]*Department of Oral Surgery and Oral Medicine, Faculty of Dentistry, Srinakharinwirot University, Bangkok, 10110, Thailand.*
*(*corresponding author)*

Introduction

Dry mouth is a common issue among the elderly, primarily due to decreased saliva secretion. This reduction can result from various factors, including the use of multiple medications and chronic conditions such as diabetes and rheumatoid arthritis. Several products have been developed to address and manage this problem. Dry mouth significantly increases the risk of developing oral diseases and greatly affects an individual's quality of life. It is influenced by factors such as reduced salivary gland function, underlying health conditions, medication side effects, and radiation therapy for head and neck cancers (1,2). The prevalence of dry mouth in the general population ranges from 10% to 46%, with a higher prevalence in females compared to males and a greater incidence in the elderly (3). Medical management of dry mouth involves several strategies tailored to its underlying cause. Adjusting medications that may cause dry mouth and using moisturizing products can help relieve dryness. These products include saliva substitutes, mouth rinses, and moisturizers in the form of sprays, lozenges, and gels. For patients with dry mouth resulting from radiation therapy or Sjögren's syndrome, medications such as pilocarpine may be prescribed to stimulate saliva production. (4,5).

Coconut oil has been noted for its benefits in oral health There is a significant statistical difference in plaque and gingival indices between individuals who use coconut oil and those who do not (6,7,8). The use of coconut oil as a treatment strategy for xerostomia post-HNC radiation is feasible, inexpensive, and safe (9). Coconut oil has properties that help increase moisture and has antimicrobial effects. Lauric acid, a component of coconut oil, plays an essential role in reducing bacterial accumulation in the mouth. Lauric acid can be converted into monolaurin, which effectively destroys the cell walls of bacteria, fungi, and viruses, thus helping to reduce bacterial accumulation in the mouth (10). Coconut oil may help form a protective layer in the oral cavity, which can alleviate the symptoms of dry mouth (11).

Chewing significantly increases saliva production, with the parotid glands contributing at least 50% of the total saliva during this process. In the unstimulated state, salivary secretion is primarily from the submandibular glands 60%, followed by the parotid glands 25%, sublingual glands(7-8%, and minor salivary glands 8%, which are crucial for mucin production (12,13). Some reports stated that mechanical stimulation through chewing gum greatly enhances saliva flow rate and output (14).

Herbal supplements, such as ginger and mint leaves, have anti-inflammatory properties (15,16). Additionally, chemical stimulation, such as taste, significantly boosts saliva production from both the parotid and submandibular glands through both taste and smell (17).

Therefore, this study aims to compare user satisfaction across different demographics and between the original and herbal formulas in elderly individuals aged 60 and above, residing in Suphan Buri Province.

Materials and Methods

This study received approval from the Human Ethics and Research Committee of Srinakharinwirot University, approval number SWUEC 153/2566E. The objective of this research was to develop an oral care product for the elderly in Thailand, utilizing accessible local plants

and health-beneficial natural resources. The product features a jelly encapsulating coconut oil, designed to stimulate saliva secretion and oral moisturization through chewing, thereby increasing mouth moisture and reducing dry mouth symptoms.

Sample Selection:

The sample size was based on the Nong Sadao Elderly Club's membership in Sam Chuk District, Suphan-Buri Province, totaling 150 people. Approximately 50-70 members who regularly participated in government-organized activities were considered.

Inclusion Criteria:

Participants had to self-report symptoms of dry mouth and low saliva. Symptoms were assessed using the Visual Analog Scale (VAS) (17), where dryness was rated from 0 (no dryness) to 10 (worst possible dryness). The classifications were as follows: Mild (1-3), Moderate (4-6), and Severe (7-10). The final study population consisted of 30 individuals who met the inclusion criteria. With an average age of 70.20 ± 3.70 years.

The Original Formula was designed to provide a baseline for comparison, while the Herbal Formula included additional herbal ingredients known for their potential benefits in stimulating saliva production and managing inflammation, such as ginger and mint leaves. Material used were:

- Coconut Oil: High-quality, food-grade coconut oil used for its moisturizing, antimicrobial properties and also contains beneficial substances such as Lauric acid.
- Jelly Powder: Standard commercially available jelly powder used for its gel-forming ability.
- Herbal Extract: Raw, natural herbal materials were used.

Ginger (Zingiber officinale): Fresh ginger roots were sourced from a reliable agricultural supplier known for organic and pesticide-free produce. The ginger was cleaned, peeled, and minced before being incorporated into the jelly.

Mint Leaves (Mentha spp.): Fresh mint leaves were collected from local pesticide-free produce. The leaves were washed and finely chopped before being added to the jelly mixture.
Production Process:

- Preparation of Coconut Oil: Poured 200 ml of cold-pressed coconut oil (Original Formula) and coconut oil with herbs (Herbal Formula) into 3.0 ml,1 cm diameter round jelly molds. Freeze until set.
- Preparation of Jelly Mixture: Mixed 2 tablespoons of ready-made jelly powder in 500 ml of water. Stirred until the powder dissolves, then simmered over low heat until boiling and removed from heat and let cool slightly.
- Coating Process: Dipped the set coconut oil into the warm jelly mixture and repeated the dipping process 2-3 times until the jelly set and was ready for use.
- Storage and Reliability of Chewable Jelly: Stored the jelly at approximately 4°C (39°F) to prevent melting and maintain quality in refrigerator or use an insulated cooler with ice

The appearance of the finished chewing jelly was characterized by a smooth, translucent white texture, with a slight tint imparted by the incorporated herbal ingredients. The jelly was formed into small, bite-sized pieces, each encapsulating coconut oil to deliver its beneficial properties effectively upon mastication.

Fig. 1 The Finished Chewing Jelly.

Data Collection

Thirty subjects participated in testing two different formulas of jelly. Each subject followed these steps for the test:

- Rinse their mouth with water for 30 seconds to remove food particles.
- Chew the jelly for 60 seconds until it breaks down.
- Spit out any remaining pieces and residue.

Afterward, they completed a satisfaction questionnaire using a Likert scale from 0 to 5. The interpretation of the mean score was as follows: Highest (above 4.51), High (3.51-4.50), Moderate (2.51-3.50), Low (1.51-2.50), and Lowest (less than 1.5). The cross-over study was performed to test for Formula 1 and Formula 2 were conducted with a one-week interval between them.

Data Analysis

The collected data included demographic information and satisfaction scores on various aspects such as appearance, mouthfeel, and the impact on oral moisture. Descriptive statistics were used to summarize the basic information, including mean scores, standard deviation, and frequency distribution. The Mann-Whitney U Test was employed to determine any differences in satisfaction between demographic characteristics and the original and herbal formula jellies. A confidence level of 95% (p-value < 0.05) was set to evaluate statistical significance.

We collected satisfaction survey results from the participants and calculated the mean and standard deviation of the satisfaction scores for each aspect of the jellies. Then, we created frequency distribution tables to organize the satisfaction scores for both the original and herbal formulas. To identify any differences in satisfaction between the two formulas, we performed the Wilcoxon Signed-Rank Test.

Results

The survey was conducted with 30 participants. The majority were female, with 24 women making up 80% of the group and 6 men making up the remaining 20%. Most participants were aged between 60 and 69 years, accounting for 27 people or 90% of the sample, while 3 people or 10% were aged between 70 and 79 years and average age of 70.2 ± 3.70 years

Regarding health conditions, 16 participants (53.30%) had chronic illnesses and required regular medication, whereas 14 participants (46.70%) did not have any chronic conditions. In terms of dry mouth or low saliva issues, 21 participants (70.00%) reported mild dryness and 9 participants (30.0%) had issues with moderate and severe dry mouth.

Concerning oral health, 22 participants (73.33%) did not use dentures and 8 participants (26.67%) had a combination of natural teeth and dentures that worked well.

Table 1. Demographic Information of Population.

Items	Number	Percentage
1. Gender		
Female	24	80.00
Male	6	20.00
total	**30**	**100.00**
2. Age		
60-69 years	27	90.00
70-79 years	3	10.00
Total	**30**	**100.00**
3. Chronic Conditions		
Yes	16	53.30
No	14	46.70
Total	**30**	**100.00**
4. Dry Mouth Issues		
Moderate to Severe Dryness	9	30.00
Mild Dryness	21	70.00
Total	**30**	**100.00**
5. Oral Health		
No dentures	22	73.33
With Dentures	8	26.67
Total	**30**	**100.00**

The original coconut oil formula was rated highly by participants, with an average score of 3.78 (SD = 0.16). The appearance received the highest average score, rated at 4.13 (SD = 0.27). This was followed by overall oral moisturization, with an average rating of 3.89 (SD = 0.36). the lowest average score was the mouthfeel, which was rated moderately at 3.32 (SD = 0.36), as shown in Table 2.

Table 2. Average Scores of the Original Coconut Oil Formula results. (Formula1).

Items	Satisfaction level		Interpret
	Mean	SD	
Appearance			
Size	4.51	0.57	Highest
Taste	4.12	0.51	High
Smell	3.89	0.46	High
Color	4.00	0.31	High
Overall appearance average	**4.13**	**0.27**	**High**
Mouthfeel			
Chewiness	2.60	0.56	Moderate
Viscosity	2.93	0.25	Moderate
Ease of swallowing	4.43	0.50	High
Overall Mouthfeel average	**3.32**	**0.36**	**Moderate**
Oral Moisturization:			
While chewing	2.90	0.31	Moderate
Jelly breaking	4.36	0.50	High
After swallowing	4.42	0.50	High
Overall oral moisturization average	**3.89**	**0.36**	**High**
Overall average	**3.78**	**0.16**	**High**

The herbal coconut oil formula was rated highly by participants, with an average score of 3.91 (SD = 0.17). The appearance of the jelly received the highest average score, rated at 4.44 (SD = 0.36). This was followed by overall oral moisturization, with an average rating of 3.97 (SD = 0.38). On the other hand, the aspect with the lowest average score was the mouthfeel, which was rated moderately at 3.32 (SD = 0.36), as shown in Table 3.

Table 3. Average Scores of the Herbal Coconut Oil Formula results. (Formula2)

Items	Satisfaction level		Interpret
	Mean	**SD**	
Appearance			
Size	4.52	0.57	Highest
Taste	4.44	0.50	High
Smell	4.58	0.49	Highest
Color	4.21	0.43	High
Overall appearance average	**4.44**	**0.36**	**High**
Mouthfeel			
Chewiness	2.60	0.56	Moderate
Viscosity	2.93	0.25	Moderate
Ease of swallowing	4.43	0.50	High
Overall mouthfeel average	**3.32**	**0.36**	**Moderate**
Oral Moisturization:			
While chewing	2.87	0.31	Moderate
Jelly breaking	4.49	0.51	High
After swallowing	4.53	0.51	Highest
Overall oral moisturization average	**3.97**	**0.38**	**High**
Overall average	**3.91**	**0.17**	**High**

In table 4, The study analyzed the relationship between basic demographic factors and satisfaction with coconut oil jelly innovations (both original and herbal formulas) using the Mann-Whitney U Test. Results showed higher mean satisfaction scores in the moderate to severe dryness group for both formulas, with scores of 0.13 for the original formula and 0.33 for the herbal formula. Statistically significant differences (p-value < 0.05) were found only in the overall appearance average score in the Dry Mouth Issues group, specifically in the moderate and severe dryness group for the herbal formula.

Table 4. Satisfaction of overall appearance average score Across Dry Mouth Issues in Original Formula and Herbal Formula.

Dry Mouth Issues	Number (percentage)	Appearance	
		Original Formula Mean (SD)	Herbal Formula Mean (SD)
Moderate to Severe Dryness	9 (30.00)	4.22 (0.28)	4.67 (0.28)
Mild Dryness	21 (70.00)	4.09 (0.26)	4.34 (0.36)
Mean Difference		0.13	0.33
Sig		0.32	0.02**

To test the difference in satisfaction between the original and herbal coconut oil jelly formulas, we used the Wilcoxon Signed-Rank Test with a confidence level of 95% (p-value < 0.05). The results showed that the p-values for the overall appearance average score, overall oral moisturization score, and overall satisfaction average score were all less than 0.05, indicating a statistically significant difference in satisfaction between the two formulas. Specifically, the herbal formula had higher mean scores in these categories, with p-values of 0.31, 0.08, and 0.13 for the respective measures. However, the p-value for the overall mouthfeel score was greater than 0.05, indicating no statistically significant difference in satisfaction between the two formulas, as shown in Table 5.

Table 5: Comparison of Satisfaction Levels Between Original and Herbal Coconut Oil Jelly with Statistical Significance (2-Tailed).

Items	Mean difference (Herbal-Original)	SD	Sig (2 tailed)
Overall appearance average score	0.31	0.26	0.00**
Overall mouthfeel average score	0.00	0.26	1.00
Overall oral moisturization average score	0.08	0.23	0.04*
Overall satisfaction average score	0.13	0.14	0.00**

Discussion

Coconut oil has properties that help increase moisture and have antimicrobial effects. (10) Additionally, chewing jelly containing coconut oil can immediately stimulate saliva secretion by activating natural saliva secretion mechanisms, resulting in increased moisture in the mouth and reduced dry mouth symptoms.(17) A study by Schimmel et al (2017) showed that chewing gum can help increase saliva production and reduce acidity in the mouths of the elderly . This supports the idea of using chewable products to stimulate saliva production, as explored in our current study. Chewing jelly with coconut oil can offer health benefits, but it's important to consider safety and potential side effects. Individuals with allergies to coconuts should avoid these products, as they can trigger allergic reactions. Swallowing coconut oil may lead to gastrointestinal issues such as bloating, diarrhea, or stomach discomfort, and it's generally advised to spit it out after use. (18)

However, our study found that satisfaction scores for both formulas were rated as moderate, with the lowest scores in chewiness (2.60, SD 0.56 for both). This suggests that the ready-made jelly powder used for encapsulation did not work as well as expected. Additionally, the jelly was not practical to keep at room temperature, which negatively affected user satisfaction regarding chewiness.

When comparing our innovative product to traditional oil pulling in terms of convenience and ease of use, the chewable product is small size makes it convenient for use in various settings. In contrast, oil pulling involves preparing the oil and swishing it in the mouth for 5-20 minutes (7,19) which is impractical in workplaces. This aligns with our study results, which show the highest satisfaction scores for the size of both formulas. The chewable product offers convenience, immediate saliva stimulation, and improved oral moisture, making it an alternative for elderly individuals.

The study found a statistically significant difference in satisfaction between the two formulas. Specifically, the herbal formula received higher mean scores in several categories. The p-values for overall appearance, oral moisturization, and overall satisfaction indicate a greater preference for the herbal formula. Additionally, the highest satisfaction scores were found for the scent of the herbal formulas.

Individuals with moderate to severe dry mouth issues expressed significantly higher satisfaction with the overall appearance of the herbal formulas compared to those with milder symptoms. These results suggest that users were more satisfied with the product when herbal features, especially scents, were included. This is particularly important for those experiencing more severe dryness.

Limitations of the Study:

Sample Size: The study involved only 30 participants, which may limit the generalizability of the results. Future studies should include a larger sample size to enhance the reliability and applicability of the findings.

Limited Budget: The constrained budget affected the ability to optimize the encapsulation method. Addressing this issue in future research could improve the formulation.

Product Storage: The coconut oil jelly has storage limitations, as it may melt at high temperatures. This could impact its practicality and usability in varying environmental conditions.

Practical Application: The study showed high satisfaction with the coconut oil jelly, but further research is needed to assess its long-term effects on the elderly. Future studies should focus on refining the formula beyond appearance and saliva stimulation, specifically improving chewiness and encapsulation. Comparing the new product with established treatments, such as oil pulling, and investigating the safety and efficacy of different application methods-rinse-and-spit versus rinse-and-swallow-will be valuable. Enhancing the production process will also improve the product's overall effectiveness and acceptability. Additionally, future research should include objective clinical measures, such as oral moisture levels and saliva flow rates, to provide a comprehensive understanding of the product's impact and validate its efficacy. Confirming the long-term safety and effectiveness of the product in increasing oral moisture and reducing dry mouth symptoms is essential.

Conclusion

The chewing jelly developed in this study demonstrated overall high satisfaction, particularly in terms of appearance, which was rated higher than oral moisturization. Individuals with moderate to severe dry mouth issues expressed significantly higher satisfaction with the overall appearance of the herbal formula compared to those with milder symptoms. This suggests that the features of the jelly, such as its appearance, taste, scent and color, are especially important for those experiencing more severe dryness.

Reference

1. Hopcraft MS, Tan C. Xerostomia: an update for clinicians. Aust Dent J. 2010;55(3):238–44.

2. Thomson WM, Poulton R, Broadbent JM, Al-Kubaisy S. Xerostomia and medications among 32-year-olds. Acta Odontol Scand. 2006; 64(4):249–254.

3. Han P, Suarez-Durall P, Mulligan R. Dry mouth: A critical topic for older adult patients. J Prosthodont Res. 2015;59(1):6–19.

4. Carpenter G, editor. Dry mouth: a clinical guide on causes, effects and treatments. Springer; 2015.

5. Wu AJ, Daniels TE. Dry mouth. In: Wallace DJ, editor. The Sjögren's Book. 5thed. New York: Oxford Academic; 2022.

6. Khijmatgar S, Reddy U, Hegde M, Del Fabbro M. Effects of coconut oil on oral health status of patients with poor oral hygiene: systematic review and meta-analysis. J Indian Oral Health Assoc. 2021;13(6). doi: 10.4103/jioh.jioh_58_21.

7. Woolley J, Gibbons T, Patel K, Sacco R. The effect of oil pulling with coconut oil to improve dental hygiene and oral health: a systematic review. Heliyon. 2020; 6(8):e04789. doi: 10.1016/j.heliyon.2020.e04789.

8. Peng TR, Cheng HY, Wu TW, Ng BK. Effectiveness of oil pulling for improving oral health: a meta-analysis. Healthcare (Basel). 2022 Oct 11;10(10):1991. doi: 10.3390/healthcare10101991.

9. Quimby AE, Hogan D, Khalil D, Hearn M, Nault C, Johnson-Obaseki S. Coconut oil as a novel approach to managing radiation-induced xerostomia: a primary feasibility study. Int J Otolaryngol. 2020;2020:8537643. doi: 10.1155/2020/8537643.

10. Dayrit FM. The properties of lauric acid and their significance in coconut oil. J Am Oil Chem Soc. 2015;92(1):1-15.

11. Kontogiannopoulos KN, Kapourani A, Gkougkourelas I, Anagnostaki ME, Tsalikis L, Assimopoulou AN, et al. A review of the role of natural products as treatment approaches for xerostomia. Pharmaceuticals (Basel). 2023;16(8): 1136. doi: 10.3390/ph16081136.

12. Edgar WM. Saliva: its secretion, composition and functions. Br Dent J. 1992; 172(8):305–12.

13. Dawes C, Wood CM. The contribution of oral minor mucous gland secretions to the volume of whole saliva in man. Arch Oral Biol. 1973;18(3):337–42.

14. Dawes C, Macpherson LM. Effects of nine different chewing-gums and lozenges on salivary flow rate and pH. Caries Res. 1992; 26(3):176-82.

15. Ali RA, Minarchick VC, Zahavi M, Rysenga CE, Sturm KA, Hoy CK, et al. Ginger intake suppresses neutrophil extracellular trap formation in autoimmune mice and healthy humans. JCI Insight. 2023;8(18):e172011. doi: 10.1172/jci.insight.172011.

16. Brown N, John JA, Shahidi F. Polyphenol composition and antioxidant potential of mint leaves. Food Prod Process Nutr. 2019;1:1. doi: 10.1186/s43014-019-0001-8.

17. Pedersen AML, Sørensen CE, Proctor GB, Carpenter GH. Salivary functions in mastication, taste and textural perception, swallowing and initial digestion. Oral Dis. 2018;24(8):1399–416.

18. Schimmel M, Katsoulis J, Genton L, Müller F. Masticatory function and nutrition in old age. Swiss Dent J. 2015;125(4):449-54.

19. Shanbhag VK. Oil pulling for maintaining oral hygiene-a review. J Tradit Complement Med. 2016;7(1):106-9.

Corresponding author:
Dr. Serena S. Sakoolnamarka
Department of Pedodontics and Preventive Dentistry Faculty of Dentistry, Wattana, Bangkok 10110, Thailand.
Tel: (668) 5159 1082
E-mail: serena@g.swu.ac.th

Human Osteoclasts Enhance Osteogenic Differentiation of Bone Stromal Cells from Mandibular Tori

Sumit Suamphan[1] Anupong Makeudom[1] Ekapong Dechtham[1] Piyanat Sangangam[1] Suttichai Krisanaprakornkit[1] Chidchanok Leethanakul[2*]

Abstract

Objective: To determine the effect of human osteoclasts on osteogenic differentiation of bone stromal cells via the receptor activator of nuclear factor kappa B (RANK)-RANK ligand (RANKL) reverse signaling.

Materials and Methods: Human peripheral blood mononuclear cells were cultured with stimulating factors until they became multinucleated mature osteoclasts. After being identified for the characteristics of mature osteoclasts, their conditioned medium (OC-CM) was collected. Bone stromal cells harvested from mandibular tori of four patients were treated with OC-CM prior to assessments of osteogenic gene expressions, differentiation, and biomineralization. Both the osteoprotegerin (OPG)-pretreated bone stromal cells and the conditioned medium from GW4869-treated mature osteoclasts (GW-OC-CM) were analyzed for suppression of osteogenic induction in order to investigate the inducible effect of OC-CM.

Results: The OC-CM significantly upregulated expressions of osteogenic genes and enhanced differentiation and biomineralization of bone stromal cells ($p < 0.05$). Pretreatment with OPG, a decoy receptor of RANKL, significantly reduced the inducible effects of OC-CM ($p < 0.05$). Similarly, the upregulated expressions and enhanced biomineralization were also significantly diminished by treatment with GW-OC-CM ($p < 0.05$).

Conclusion: Mature osteoclasts can induce osteogenic differentiation of bone stromal cells possibly via the RANK-RANKL reverse signaling.

Keywords: Bone Mineralization, Osteoblast, Osteoclast, Osteoprotegerin, RANK Ligand

Received Date: Jul 09 2024
Revised Date: Aug 30 2024
Accepted Date: Oct 09, 2024

[1]*School of Dentistry, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*Orthodontic Section, Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Songkhla 90112, Thailand. (*corresponding author)*

Introduction

In bone biology, receptor activator of nuclear factor kappa B (RANK) and its ligand (RANKL) are major regulatory molecules in bone resorption of periodontitis. RANKL released by osteoblasts directly binds to membrane-bound RANK on osteoclast precursors for osteoclastic differentiation and function. Osteoprotegerin (OPG), also secreted from osteoblasts, acts as a decoy receptor of RANKL, thereby functioning as an inhibitor of osteoclastogenesis. The equilibrium of the RANKL/RANK/OPG system regulates bone remodeling (1).

Several lines of evidence indicate that osteoclasts provide coupling signals for not just playing a role in bone resorption but also coordinating in bone formation by activation of membranous RANKL on osteoblast lineage cells (2-6). This mechanism is known as the RANK-RANKL reverse signaling (7), which stimulates differentiation of osteoblasts only through a paracrine effect, not via cell-to-cell contact (8). The key molecule for activation of osteoblastic function via RANKL is RANK, which has been previously shown to be present on the membrane of extracellular vesicles (EVs), which had been isolated from osteoclasts' conditioned medium (OC-CM) (9). Note that RANK-containing EVs are released from mature osteoclasts more than the immature ones, so the mature osteoclasts' EVs have been recognized as a potent intercellular mediator in bone biology (10). In addition, OPG, specifically binding to RANKL with high affinity, competitively blocks the inducible effect of RANK-containing EVs on osteogenic induction (2).

Although several previous studies have demonstrated osteoclast-induced osteogenic differentiation and biomineralization in mouse osteoblast cell lines (2,3), mesenchymal stem cells (4), and adipose tissue-derived mesenchymal stromal cells (5), none of these studies has investigated the inducible effects on primary bone stromal cells isolated from human mandibles. Therefore, this study aimed to determine the effect of human osteoclasts on osteogenic induction of bone stromal cells harvested from mandibular tori via the RANK-RANKL reverse signaling.

Materials and Methods

Culture of human bone stromal cells

All human cell protocols were approved by the Institutional Ethics Committee Board, Mae Fah Luang University (EC23097-22). Discarded bone specimens were obtained from four healthy patients (18-45 years old), who underwent surgical removal of their mandibular tori. Bone pieces were sequentially digested with 1 mg/ml of collagenase/dispase® (Roche, Basel, Switzerland), and the cell pellet was resuspended in complete medium, i.e., DMEM (Gibco, Gaithersburg, MD, USA), containing 10% fetal bovine serum (FBS; Gibco), 1% penicillin-streptomycin, and 1% amphotericin B (Gibco), and cultured in a humidified chamber with 5% CO_2 at 37^{o}C. The cells at the third to the fifth passages were used in subsequent experiments. To characterize their mesenchymal cell type, bone stromal cells were collected and stained with mesenchymal cell surface markers, including APC-conjugated anti-human CD73, Alexa Fluor®700-conjugated anti-human CD90, and APC/Fire™ 750-conjugated anti-human CD105 antibodies (BioLegend, San Diego, CA, USA). Expressions of these markers were analyzed by a flow cytometer (CytoFLEX SRT, BECKMAN COULTER, Brea, CA, USA). As a negative control, two markers for hematopoietic

stem cells, FITC-conjugated anti-human CD34 (Beckman Coulter Life Sciences, Marseille, France) and PC5-conjugated anti-human CD45 antibodies (Beckman Coulter Life Sciences) were utilized.

Culture of human mature osteoclasts

Peripheral blood mononuclear cells (PBMCs) were isolated from a 20-ml volume of peripheral blood from five healthy donors. The blood was diluted with phosphate-buffered saline (PBS) at 1:1 ratio, layered on top of the Ficoll® (Cytiva Sweden AB, Sweden) density gradient medium, and centrifugated at 400g for 30 minutes at room temperature without deceleration. PBMCs were collected from the buffy coat and resuspended in complete αMEM medium (Gibco), containing 10% FBS, and 1% penicillin-streptomycin at 2 x 10^7 cells per ml. On the following day, non-adherent cells were removed by thorough washing with PBS. To obtain mature osteoclasts, PBMCs were cultured for 9 days in the complete αMEM medium, supplemented with 15 ng/ml recombinant human macrophage-colony stimulating factor (M-CSF; Stemcell Technologies, Vancouver, Canada) and 30 ng/ml recombinant human RANKL (Stemcell Technologies) with medium replacement every 3 days. To characterize the multinucleated mature osteoclasts, they were stained with tartrate-resistant acid phosphatase (TRAcP; Sigma-Aldrich, Darmstadt, Germany), 20 nM Alexa Fluor® 488-conjugated phalloidin (Invitrogen, Eugene, Oregon, US), and 1 μM DAPI (Biotium, Inc., Hayward, CA, USA). Stained cells were visualized by a fluorescence microscope (ECLIPSE Ni-E, Nikon, Tokyo, Japan).

Preparation of conditioned medium from mature osteoclasts (OC-CM)

According to the protocol of Stessuk et al. (5), OC-CM from days 9, 12, 15, and 18 was collected, pooled, centrifuged at 2,000g for 10 minutes to remove cell debris, and then concentrated by twenty-fold using centrifugation at 4,000g with the Amicon Ultra Filter Unit (100kDa Merk Millipore Ltd., Tullagreen, Carrigtwohill, Co. Cork, Ireland) for 15 minutes at 4°C. To inhibit EVs' release, mature osteoclasts on day 9 were treated with 20 μM of GW4869 for 3 days, and the conditioned medium from GW4869-treated mature osteoclasts (GW-OC-CM) were collected from days 12, 15, and 18, and then concentrated by twenty-fold following the protocol, as described above.

Osteogenic differentiation

Human bone stromal cells were seeded at 5 x 10^5 cells per well in 6-well plates. At 70-80% cell confluence, the cells were treated with concentrated OC-CM at a ratio of 1:10 (v/v) in complete medium, supplemented with 10 mM β-glycerophosphate (Sigma-Aldrich, Darmstadt, Germany), or left untreated as a negative control. This ratio, taken from a previous study (11) and tested in our pilot study, was shown to enhance biomineralization (data not shown). As a positive control for osteogenic induction, the bone stromal cells were cultured in complete medium, supplemented with 50 mg/ml ascorbic acid (Sigma-Aldrich), 10 mM β-glycerophosphate, and 100 nM dexamethasone (Sigma-Aldrich). To inhibit the RANK-RANKL signaling, the bone stromal cells were pre-treated with 1 ng/ml of recombinant human OPG (Stemcell

Technologies) for one hour before OC-CM treatment. Culture medium with or without OC-CM or OPG was refreshed every 3 days. To investigate the role of EVs from osteoclasts, the bone stromal cells were treated with concentrated GW-OC-CM at the same ratio of 1:10 (v/v) and analyzed for osteogenic gene expressions and biomineralization in comparison with those treated with OC-CM.

Analyses of osteogenic differentiation, biomineralization, and gene expressions

Alkaline phosphatase (ALP) staining (Abcam, Cambridge, UK) was performed on day 7, while biomineralization was examined by Alizarin red staining (Sigma-Aldrich) and von Kossa staining (Abcam) on day 14. ALP and von Kossa staining intensities were analyzed by ImageJ program (National Institutes of Health, Bethesda, MD, USA). The Alizarin red staining dye was dissolved in 10% cetylpyridinium chloride solution (Sigma-Aldrich), and its amounts were quantified by absorbance at a 570-nm wavelength.

Expressions of *RUNX2,* Osterix *(OSX),* and Collagen type I alpha 1 *(COL1A1)* mRNA were determined at 24 h by reverse transcription (RT)-quantitative polymerase chain reaction (qPCR). In brief, total RNA was extracted and quantified by NanoDrop One (Thermo Fisher Scientific, Carlsbad, CA, USA). Complementary DNA (cDNA) was synthesized from a 200ng amount of each RNA sample using the High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific). PCR was performed using CFX Opus 96 Real-Time PCR System (Bio-Rad Laboratories, Inc, Berkeley, CA, USA). The sequences of oligonucleotide primers are summarized in Table 1.

Table 1. Oligonucleotide primers used in this study, (F = Forward, R = Reverse).

Gene		Primer sequences
GAPDH	F	5'-TCA TGG GTG TGA ACC ATG AGA A-3'
	R	5'-GGC ATG GAC TGT GGT CAT GAG-3'
RUNX2	F	5'-ATG ATG ACA CTG CCA CCT CTG A-3'
	R	5'-GGC TGG ATA GTG CAT TCG TG-3'
OSX	F	5'-GCC AGA AGC TGT GAA ACC TC-3'
	R	5'-GCT GCA AGC TCT CCA TAA CC-3'
COL1A1	F	5'-GTG CTA AAG GTG CCA ATG GT-3'
	R	5'-ACC AGG TTC ACC GCT GTT AC-3'

Statistical analysis

All experiments were performed in triplicate. Shapiro-Wilk test was used to check the normality of data distribution. One-way analysis of variance followed by Student–Newman–Keuls post hoc test was used to determine the significant difference between groups at $p < 0.05$. The statistical analysis was performed using SPSS software version 26.0 for Windows.

Results

Characterization of human bone stromal cells and mature osteoclasts

The harvested bone stromal cells showed a spindle-shaped morphology (Fig 1A), were negative for expression of the hematopoietic cell marker, CD34 (Fig 1B) or CD45 (Fig 1C), but were positive for expressions of mesenchymal cell markers, CD73 (Fig 1D), CD90 (Fig 1E), and CD105 (Fig 1F). The mature osteoclasts were positive for TRAcP staining (Fig 1G), contained multiple nuclei (Fig 1H), and showed actin ring formation by immunofluorescence (Fig 1I). Treatment of mature osteoclasts with 20 μM of GW4869 for 3 days showed entrapped vesicles within the cells (Fig 1J).

Inhibition of enhanced osteogenic differentiation by pretreatment of bone stromal cells with osteoprotegerin or by treatment of mature osteoclasts with GW4869

Treatment of the bone stromal cells with OC-CM significantly increased ALP staining and biomineralization by Alizarin red and von Kossa staining, compared with a negative untreated control ($p < 0.05$; Fig 2A-D). Enhanced ALP staining and biomineralization were significantly decreased by pretreatment of bone stromal cells with OPG ($p < 0.05$; Fig 2A-D). Likewise, the degrees of *RUNX2, OSX,* and *COL1A1* mRNA expressions were upregulated by OC-CM treatment ($p < 0.05$; Fig 2E-G), whereas these upregulated expressions were significantly inhibited by pretreatment of bone stromal cells with OPG ($p < 0.05$; Fig 2E-G).

Moreover, the enhanced staining intensities of ALP, Alizarin red, and von Kossa were significantly decreased by treatment of bone stromal cells with GW-OC-CM ($p < 0.05$; Fig 3A-D). The upregulated expression of *RUNX2* and *OSX* were significantly inhibited by treatment of bone stromal cells with GW-OC-CM ($p < 0.05$; Fig 3E-G). As a positive control for osteogenic induction, increased ALP and biomineralization as well as upregulated osteogenic gene expressions were observed in bone stromal cells treated with osteogenic medium (Fig 2,3).

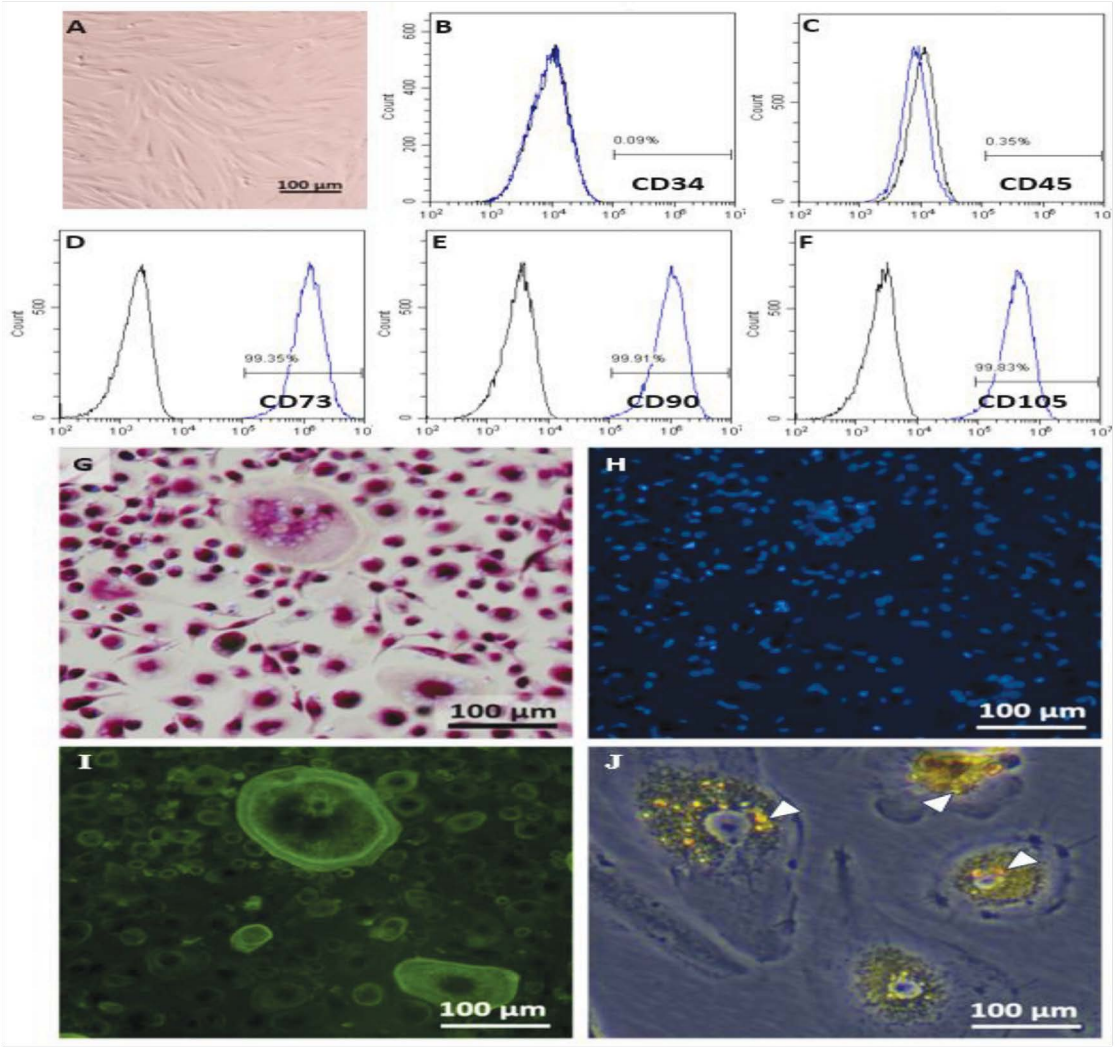

Fig.1 Characterization of bone stromal cells (A-F) and multinucleated mature osteoclasts that were positive for TRAcP staining (G), contained multiple nuclei (H), and showed actin ring formation by immunofluorescence (I). By inverted phase-contrast microscopy, treatment of mature osteoclasts with 20 µM of GW4869 for 3 days showed entrapped vesicles within the cells (arrowheads in J).

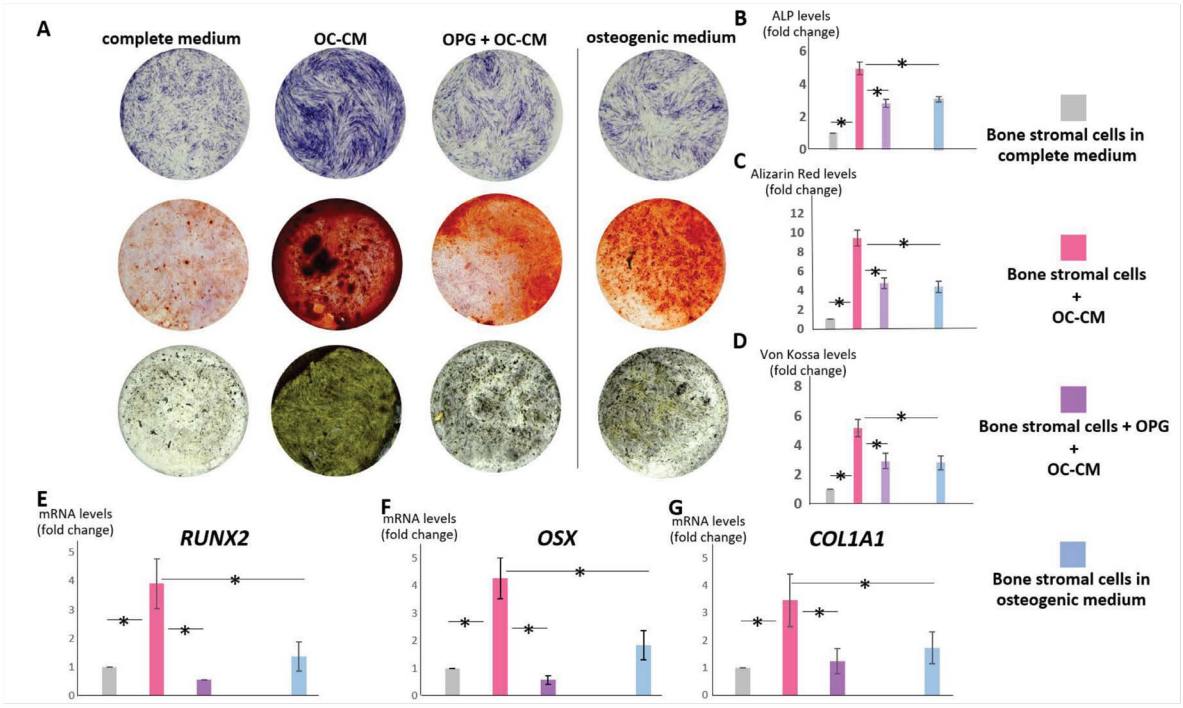

Fig.2 Enhanced ALP staining and biomineralization staining (A-D), and expressions of osteogenic genes, *RUNX2* (E), *OSX* (F), and *COL1A1* (G), of bone stromal cells by treatment with conditioned medium of mature osteoclasts (OC-CM) were significantly inhibited by pretreatment of bone stromal cells with osteoprotegerin (OPG).
Error bars in B-G = standard deviation; *p < 0.05.

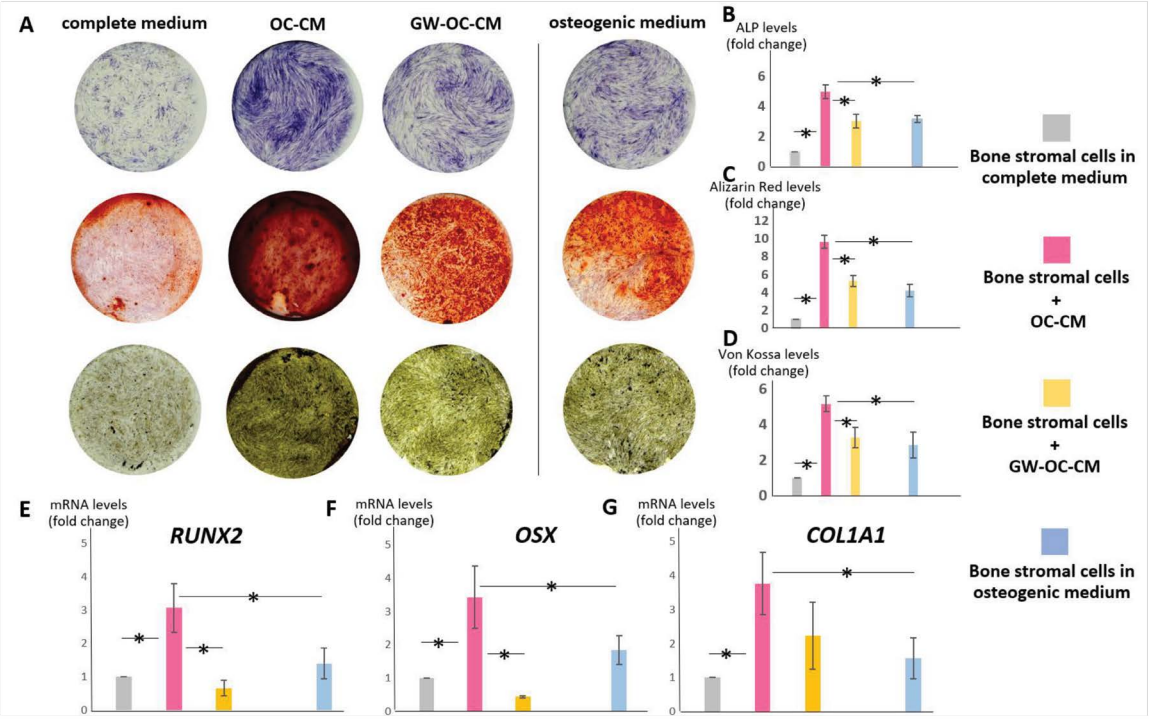

Fig 3. Enhanced ALP staining and biomineralization staining (A-D), and expressions of osteogenic genes, *RUNX2* (E), *OSX* (F), and *COL1A1* (G), of bone stromal cells by treatment with conditioned medium of mature osteoclasts (OC-CM) were inhibited by treatment with conditioned medium of mature osteoclasts treated with GW4869 (GW-OC-CM). Error bars in B-G = standard deviation; *p < 0.05.

Discussion

The current study demonstrated that mature osteoclasts, derived from human PBMCs, released mediators into their culture medium, which influenced the osteogenic differentiation of primary bone stromal cells harvested from human mandibular tori, as evidenced by increased ALP staining and biomineralization upon Alizarin red staining and von Kossa staining as well as upregulation of *RUNX2, OSX,* and *COL1A1* mRNA expressions. These results are consistent with the findings from previous studies (4-6), in which enhanced osteogenic differentiation of mesenchymal stem cells was observed. However, to further verify changes in the degrees of mRNA expressions as aforementioned, analysis of protein expressions for *RUNX2, OSX,* and *COL1A1* will be required in a future study, as with previous studies (12,13). Pretreatment of the bone stromal cells with OPG significantly decreased this osteogenic differentiation, implying that the binding between OPG and RANKL on the membrane of bone stromal cells prevents the RANK-RANKL reverse signaling from mature osteoclasts, indicating that RANKL present on the membrane of bone stromal cells plays a crucial role in this signaling pathway.

Furthermore, the significance of the RANK-RANKL reverse signaling from mature osteoclasts is verified by treatment of mature osteoclasts with GW4869, an inhibitor of EV release. The entrapment of EVs within mature osteoclasts implied that EVs' release was prohibited by treatment with this inhibitor (Fig 1J). This treatment resulted in significant suppression of ALP staining, biomineralization, and mRNA expressions of *RUNX2* and *OSX* in the bone stromal cells, corresponding with the depletion of EVs from OC-CM that significantly reduced their ability to induce osteogenic differentiation (6). EVs released into the culture medium may thus be a critical messenger for cell-to-cell communication. Indeed, it was confirmed by this study that a substantial part of the osteogenic inductive mechanism from OC-CM was mediated by EVs. However, the upregulated mRNA expression of *COL1A1* was not significantly inhibited by treatment with GW-OC-CM. This may be explained by the inducible effect of other mediators, found within OC-CM but not present on EVs' membrane, which can also exert the osteo-inductive action. Particularly, a previous study (14) has shown an ability of apoptotic bodies released from osteoclasts to activate the RANK-RANKL reverse signaling, as with EVs. In that study, even though 68% of the induced apoptotic osteoclasts are demonstrated to release the apoptotic bodies, a few non-induced live cells (4%) can still release these bodies. Therefore, it is of interest to further investigate the role of apoptotic bodies released from mature osteoclasts in upregulation of *COL1A1* gene expression.

Under an appropriate culture condition, bone marrow–derived mesenchymal cells are multipotent, meaning that they can give rise to various mesodermal cell types, including adipocytes, chondrocytes, and osteoblasts (15). The ability of osteoclasts-derived EVs has been shown, with an emphasis on RANK as a key molecule to enhance osteogenic induction of osteoblastic cell lines (2,3), human mesenchymal cells (4), and adipose tissue-derived mesenchymal cells (5). Besides RANK-containing EVs, microRNAs (miRNAs) in EVs are essential for regulating diverse osteoblastic functions in osteogenesis (16). For example, miR-324 in osteoclasts' EVs promotes the osteogenic differentiation of mesenchymal stem cells (17), whereas osteoclasts' EVs, containing miR-214-3p, inhibit osteoblastic bone formation (18). Particularly, miR-214-3p has been shown to suppress osteogenic differentiation of myoblast cells by targeting *OSX* (19). Consequently, it is worthwhile to explore the combined effects of both RANK and miRNAs, which are associated with osteoclasts' EVs, on regulation of osteogenic differentiation. Note that the bone stromal cells of the osteoblast lineage vary in their differentiation stages and osteogenic potencies, which result in differences in bone cell stemness, proliferation, differentiation, and bone homeostasis (20). For instance, the bone stromal cells harvested from cancellous bone of human mandibular tori are mostly osteoblast progenitors, which are by default limited to differentiation into osteoblasts (21).

In summary, our findings demonstrate the involvement of RANKL on human bone stromal cells' membrane and RANK in osteoclast conditioned medium for the reverse signaling of regulatory osteogenic differentiation of human bone stromal cells.

Acknowledgements

Financial support from Intramural Endowment fund, Mae Fah Luang University, to S.S. and S.K. is gratefully acknowledged.

References

1. Yasuda H. Discovery of the RANKL/ RANK/OPG system. J Bone Miner Metab. 2021; 39(1):2-11.

2. Ikebuchi Y, Aoki S, Honma M, Hayashi M, Sugamori Y, Khan M, et al. Coupling of bone resorption and formation by RANKL reverse signalling. Nature. 2018;561(7722):195-200.

3. Zhang S, Wang X, Li G, Chong Y, Zhang J, Guo X, et al. Osteoclast regulation of osteoblasts via RANK-RANKL reverse signal transduction in vitro. Mol Med Rep. 2017;16(4): 3994-4000.

4. Pederson L, Ruan M, Westendorf JJ, Khosla S, Oursler MJ. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. Proc Natl Acad Sci U S A. 2008;105(52):20764-9.

5. Stessuk T, Husch J, Hermens IAT, Hofmann S, van den Beucken JJJP. Osteogenic differentiation driven by osteoclasts and macrophages. J Immunol Regene Med. 2021;12: 100044. doi: 10.1016/j.regen.2021.100044.

6. Liang M, Yin X, Zhang S, Ai H, Luo F, Xu J, et al. Osteoclast-derived small extracellular vesicles induce osteogenic differentiation via inhibiting ARHGAP1. Mol ther Nucleic acids. 2021;23:1191-203. doi: 10.1016/j.omtn.2021.01.031.

7. Honma M. The potential of RANKL reverse signaling as a novel pharmacological target. Nihon Yakurigaku Zasshi. 2023;158(3):253-7.

8. Andersen TL, Abdelgawad ME, Kristensen HB, Hauge EM, Rolighed L, Bollerslev J, et al. Understanding coupling between bone resorption and formation: are reversal cells the missing link? Am J Pathol. 2013;183(1):235-46.

9. Huynh N, VonMoss L, Smith D, Rahman I, Felemban MF, Zuo J, et al. Characterization of Regulatory Extracellular Vesicles from Osteoclasts. J Dent Res. 2016;95(6):673-9

10. Liu M, Sun Y, Zhang Q. Emerging Role of Extracellular Vesicles in Bone Remodeling. J Dent Res. 2018;97(8):859-68.

11. Matsuoka K, Park KA, Ito M, Ikeda K, Takeshita S. Osteoclast-derived complement component 3a stimulates osteoblast differentiation. J Bone Miner Res. 2014;29(7):1522-30.

12. Wang N, Wang H, Chen J, Wang F, Wang S, Zhou Q, et al. ACY-1215, a HDAC6 inhibitor, decreases the dexamethasone-induced suppression of osteogenesis in MC3T3-E1 cells. Mol Med Rep. 2020;22(3):2451-9.

13. Liu M, Xu Z. Berberine promotes the proliferation and osteogenic differentiation of alveolar osteoblasts through regulating the expression of mir-214. Pharmacology. 2021;106 (1-2):70-8.

14. Ma Q, Liang M, Wu Y, Ding N, Duan L, Yu T, et al. Mature osteoclast-derived apoptotic bodies promote osteogenic differentiation via RANKL-mediated reverse signaling. J Biol Chem. 2019;294(29):11240-7.

15. Bhartiya D. Are Mesenchymal Cells Indeed Pluripotent Stem Cells or Just Stromal Cells? OCT-4 and VSELs Biology Has Led to Better Understanding. Stem Cells Int. 2013;2013: 547501. doi: 10.1155/2013/547501.

16. Loh HY, Norman BP, Lai KS, Cheng WH, Nik Abd Rahman NMA, Mohamed Alitheen NB, et al. Post-transcriptional regulatory crosstalk between microRNAs and canonical TGF-β/BMP signalling cascades on osteoblast lineage: a comprehensive review. Int J Mol Sci. 2023;24(7): 6423. doi: 10.3390/ijms24076423.

17. Liang M, Yin X, Zhang S, Ai H, Luo F, Xu J, et al. Osteoclast-derived small extracellular vesicles induce osteogenic differentiation via inhibiting ARHGAP1. Mol Ther Nucleic Acids. 2021;23:1191-203. doi: 10.1016/j.omtn.2021.01. 031.

18. Liu M, Sun Y, Zhang Q. Emerging role of extracellular vesicles in bone remodeling. J Dent Res. 2018;97(8):859-68.

19. Shi K, Lu J, Zhao Y, Wang L, Li J, Qi B, et al. MicroRNA-214 suppresses osteogenic differentiation of C2C12 myoblast cells by targeting Osterix. Bone. 2013;55(2):487-94.

20. Shen F, Huang X, He G, Shi Y. The emerging studies on mesenchymal progenitors in the long bone. Cell Biosci. 2023;13(1):105. doi: 10.1186/s13578-023-01039-x.

21. Aubin JE. Chapter 4 - Mesenchymal Stem Cells and Osteoblast Differentiation. In: Bilezikian JP, Raisz LG, Martin TJ, editors. Principles of Bone Biology. 3rded. San Diego: Academic Press; 2008. p. 85-107.

Corresponding author:
Prof. Chidchanok Leethanakul
Department of Preventive Dentistry, Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand.
Tel: (668) 1711 4040
E-mail: chidchanok.l@psu,ac.th

Effect of Saliva Storage Conditions on Bacterial DNA Quantification by Real-time Polymerase Chain Reaction

Jantipa Jobsri[1*] Rutchanoo Chansamart[2] Krittiphat Rungnapapaisarn[3] Wanida Yokliant[3] Donlayawan Tidpraman[3] Vijai Sensakul[3]

Abstract

Objective: This research aimed to study the effects of temperature and duration of saliva samples storage on quantification of bacterial DNA by Real-time polymerase chain reaction (Real-time PCR)

Materials and Methods: Human saliva samples were collected from 3 healthy volunteers, aliquoted into microcentrifuge tubes and stored in the following conditions; centrifuged immediately before storing at -80^{o}C, kept at room temperature, on ice, or dry ice for 6 hours, 24 hours or 120 hours. Genomic DNA was extracted for quantitative analysis of total bacteria by Real-time PCR.

Results: Absolute quantitative real-time PCR showed that concentrations of bacterial DNA from saliva samples stored at almost every condition which were stored at room temperature for 120 hours, on ice for every duration, or on dry ice for 6 or 24 hours were similar to that of the control, saliva centrifuged and frozen immediately after collection. However, bacterial DNA concentration from saliva stored at room temperature for 6 or 24 hours was significantly higher than that of the control. In contrast, the concentration of DNA from saliva stored on dry ice for 120 hours was significantly lower than that of the control.

Conclusion: Temperature and duration had effects on bacterial DNA quantity. Saliva storage at room temperature within 24 hours had increasing bacterial DNA as quantify by Real-time PCR. Saliva storage on ice and dry ice could maintain bacterial DNA similar to sample centrifugation immediately and then frozen at -80^{o}C. But long duration of storage, 120 hours, would decrease bacterial quantity.

Keywords: saliva sample storage, bacterial DNA, Real-time Polymerase Chain Reaction

Received Date: Aug 07, 2024
Revised Date: Oct 07, 2024
Accepted Date: Oct 09, 2024

[1]*Department of Oral Biology, Faculty of Dentistry, Naresuan University, Phitsanulok, 65000, Thailand.*
[2]*Department of Preventive Dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, 65000, Thailand.*
[3]*Faculty of Dentistry, Naresuan University, Phitsanulok, 65000, Thailand.*
*(*corresponding author)*

Introduction

Salivary bacterial DNA has shown potential in many applications. It can be used to identify saliva stain on subject for forensic investigation (1). It has potential to be diagnosis tools for many diseases (2). For examples, in pediatric ciliac disease, there was significant increase in the Bacteroidetes phylum, while the Actinobacteria phylum, *Rothia* genus and *R.aeria* showed a significant decrease (3). In oral squamous cell carcinoma (OSCC), *Actinobacteria, Fusobacterium, Moraxella, Bacillus,* and *Veillonella* species exhibited strong correlations with OSCC (4). Different in microbial DNA profiles provided information for further development of diagnosis tools.

In most studies, salivary DNA was extracted immediately (1) or froze for a period of time before extraction (4,5). But in research studies required to collect saliva specimens in the fields or remote areas, the specimen storage in the freezer may not be available.

There are not many studies of effect of temperature and time used for saliva storage on DNA analysis and the data showed some controversial results. In human gene genotype analysis study, storage of saliva at room temperature up to 5 days before DNA extraction and genotypes analysis had no effect on the analysis (6).

In the study of impact of temperature and time storage on the microbial detection of saliva mixed with supragingival biofilm samples by Checkerboard DNA-DNA hybridization method, total bacterial count of sample stored at -20^{o}C for 2 weeks was higher than that of the sample processed immediately (7). While the samples kept at room temperature or 4^{o}C or -80^{o}C show similar count to the control. The author showed that storage for 12 months reduced the bacterial counts detected by this method.

Storage of saliva samples at -20^{o}C for 24 hours or 36 months before DNA extraction, followed by quantitative real-time PCR analysis showed no significant different of bacterial DNA quantity when the DNA was extracted using QIAamp DNA Blood Mini Kit (8). However, when using phenol-chloroform extraction, DNA quantity of the 36 months sample was lower.

In bacterial DNA semi-quantitation by conventional PCR, saliva sample that was pelleted and kept for 1 week at -70^{o}C before DNA extraction provided glutamine-fructose-6-phosphate transaminase 1 (GFPT1) PCR band quantity more than that of sample that was stored at 4^{o}C or -70^{o}C for 1 week before pellet formation and DNA extraction (9).

Freezing of saliva samples maintain the sample integrity, but in some studies, such as in remote areas, freezer may not be available. Storage of saliva samples on ice and dry ice, materials provide low temperature that are easy to obtain from markets, have not been studied. We designed to examine the effect on bacterial DNA quantity of saliva samples storage at room temperature or on ice or on dry ice. Time of storage was 6 hours or 24 hours or 120 hours (5 days) which is the time used for delivery of samples to laboratory in different situations. Microbial DNA quantities in the samples were analyzed by real-time quantitative PCR as it is one of the reliable DNA quantification techniques.

Materials and Methods

Saliva samples collection and storage conditions

This project was approved by Naresuan University Institutional Review Board, IRB No. 183/57. Three healthy volunteers with no dental cavities, no oral mucosal lesions, and no antibiotic treatments within 3 months were included for saliva samples collections. DMFT index, plaque index and gingival index values of all volunteers were examined. The volunteers were instructed not to drink or eat for 2 hours before saliva collections. The volunteers chewed paraffin for 5 minutes and then 5 ml of stimulated saliva samples were collected from right buccal vestibule using sterile syringe. One hundred and fifty microliters of saliva sample were aliquoted into 1.5 ml microcentrifuge tubes and stored in the following conditions; 1) centrifuged immediately at 14100 x g for 10 min at 4^{o}C and the pellet was stored at -80^{o}C for 5 days before DNA extraction, 2) the saliva samples were stored at room temperature (25^{o}C) for 6, 24, or 120 hours before DNA extraction, 3) the saliva samples were stored on ice for 6, 24, or 120 hours before DNA extraction, and 4) the saliva samples were stored on dry ice for 6, 24, or 120 hours before DNA extraction. For storage on ice or dry ice, the samples were placed in a box containing ice or dry ice and the box was sealed with sealing tape. Melted ice or evaporated dry ice was replaced with new ice or dry ice every 6 hours.

Bacterial genomic DNA extraction

Bacterial genomic DNA from saliva samples were extracted using PrestoTM Mini gDNA Bacteria Kit (Geneaid Biotech Ltd., Taiwan). The saliva pellet was mixed with 200 µl of Gram+ buffer containing 4 mg/ml of Lysozyme and incubated at 37^{o}C for 30 minutes. The mixture was added with 20 µl of Proteinase K and incubated at 60oC for 10 minutes. Bacteria were further lysed by addition of 200 µl of GB buffer containing 50 mg/ml of RNase A and incubation at 70^{o}C for 10 minutes. The bacterial lysate was added with 200 µl of absolute ethanol and the mixture was placed in the GD column. The column was washed with 400 µl of W1 buffer and further washed with 600 µl of Wash buffer. Bacterial DNA was eluted with 100 µl of Elution buffer. Bacterial DNA concentration was determined using NanoDrop^{TM} 200c UV-Vis spectrophotometer (Thermo ScientificTM, Thermo Fisher Scientific, USA).

Real-time PCR for Quantitation of bacterial DNA in saliva samples

Real-time PCR was performed with Prbac1 (5' ACTACGTGCCAGCAGCC 3') and Prbac2 (5' GGACTACCAGGGTATCTAATCC 3') primers for 16s rRNA gene of all bacteria (10). The PCR product sizes were ranged from 296 to 300 bp. *S. mutans* DNA, 0.0005–50 ng/ml, was used to create a standard graph of DNA concentration. Ten nanograms of salivary bacterial DNA was mixed with LightCycler® 480 II SYBR Green I Master mix (Roche Applied Science, Germany), primers, and water. The PCR mixture was amplified and analyzed using LightCycler® 480 II Instrument (Roche Applied Science, Germany) with Absolute Quantification program (LightCycler® 480 II Software, Version 1.5).

Statistical analysis

Concentrations of the extracted DNA or the DNA concentrations quantified by real-time PCR of each storage condition were tested for normality of data distribution by Shapiro-Wilk test. Concentration of extracted DNA data from

every storage condition passed normality test. Difference between groups was analyzed by one-way ANOVA and found that there was no statistically difference ($p = 0.44$).

Data of DNA concentration quantified by real-time PCR from every storage condition passed normality test, except data of storage on ice for 120 hours group. Difference between groups was analyzed by Kruskal-Wallis test and found that there was statistically difference ($p = 0.0036$). Difference between 2 normal distribution data groups was further analyzed by unpaired t test, while difference between normal distribution data group and non-normal distribution data group was analyzed by Mann-Whitney test. All statistical analysis was performed using GraphPad Prism software version 10.3.1.

Results

DMFT index mean value of three healthy volunteers, aged between 22 to 24 years old, was 8.67 +/- 2.08 (Table 1). Plaque index and gingival index mean values were 73.51 +/- 7.44 and 1.28 +/- 0.23, respectively.

Table 1. DMFT, plaque, and gingival index values of three volunteers.

	DMFT index	plaque index	Gingival index
Volunteer 1	11	65.17	1.29
Volunteer 2	8	75.89	1.05
Volunteer 3	8	79.46	1.5
Mean +/- SD	8.67 +/- 2.08	73.51+/- 7.44	1.28 +/- 0.23

Genomic DNA from saliva samples stored at different conditions were extracted and concentration of the DNA samples were measured using NanoDrop 200c UV-Vis spectrophotometer. Concentration of bacterial DNA extracted from saliva stored at different conditions was similar ($p = 0.44$) as shown in figure 1.

The extracted genomic DNA was subjected to analyze by absolute quantitative real-time PCR using primers for 16s rRNA gene of all bacteria (10). The quantification analysis showed that concentration of bacterial DNA from saliva stored at room temperature for 6 or 24 hours was significantly higher than that of DNA extracted from the control, the saliva sample that was centrifuged and frozen immediately after collection (figure 2). In contrast, the concentration of bacterial DNA from saliva stored on dry ice for 120 hours was significantly lower than that of the control. In other storage conditions, storage at room temperature for 120 hours, on ice for every duration, or on dry ice for 6 or 24 hours, the concentration of extracted DNA was similar to that of the control. Even though the storage of saliva sample on ice for 120 hours seemed to show lower concentration of bacterial DNA but it was not statistically significant.

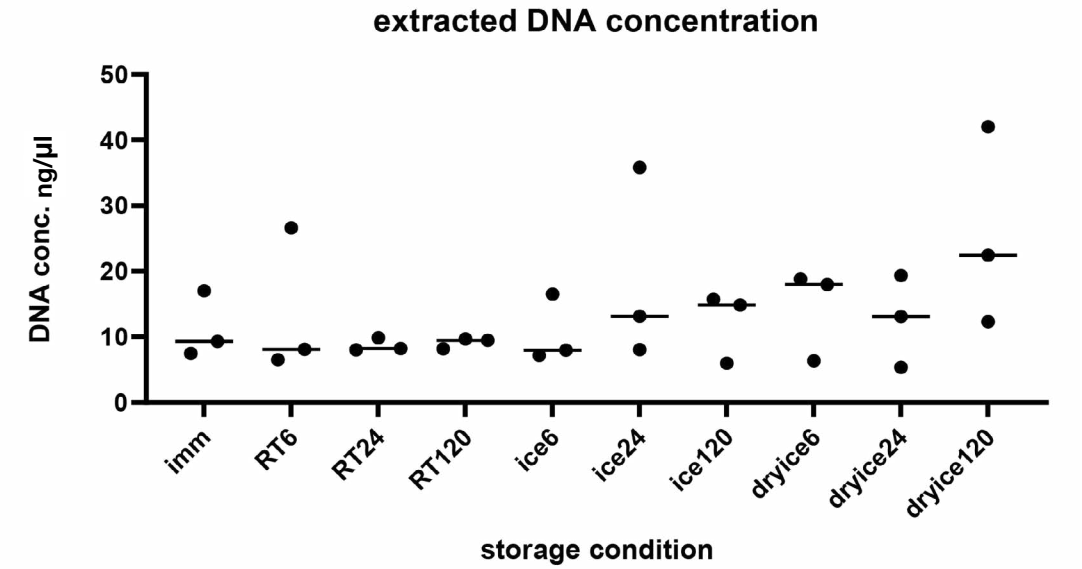

Fig.1 Concentration of genomic DNA extracted from saliva stored at different temperature and duration, Bacterial genomic DNA was extracted from saliva sample stored at -80°C after immediately centrifuged (imm), stored at room temperature for 6, 24, or 120 hours (RT6, RT24, or RT120), stored on ice for 6, 24, or 120 hours (ice6, ice24, or ice120), or store on dry ice for 6, 24, or 120 hours (dryice6, dryice24, or dryice120). Each dot represents extracted DNA concentration in ng/µl of each volunteer.

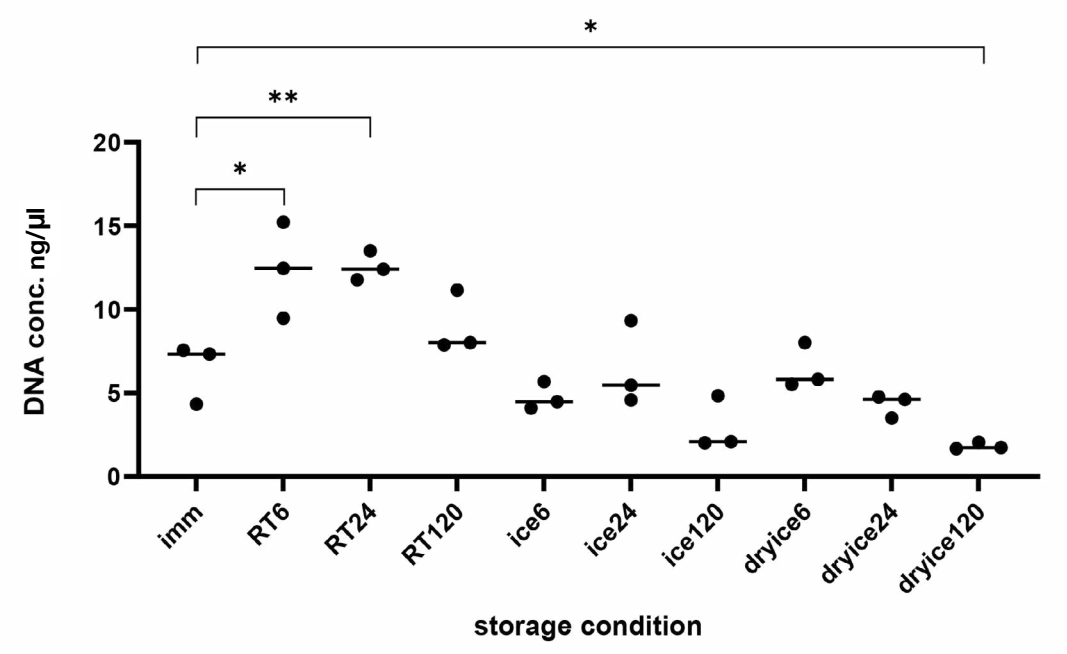

Fig. 2 Concentration of bacterial DNA quantified by real-time PCR, Bacterial genomic DNA extracted from saliva sample stored -80oC after immediately centrifuged (imm), stored at room temperature for 6, 24, or 120 hours (RT6, RT24, or RT120), stored on ice for 6, 24, or 120 hours (ice6, ice24, or ice120), or store on dry ice for 6, 24, or 120 hours (dryice6, dryice24, or dryice120) was quantified by absolute quantitative real-time PCR using 16s rRNA gene primers for total bacteria. Each dot represents DNA concentration in ng/µl from each volunteer. *indicates that p-value is lower than 0.05; **indicates that p-value is lower than 0.01.

Discussion

Saliva samples stored at room temperature for 6 or 24 hours appeared to contain more bacterial DNA than that of the sample pelleted and frozen immediately at -80°C. This might be resulted from bacterial growth using nutrients present in saliva (11-13). The increased bacteria would die as the nutrient was exhausted and bacterial waste was produced. Since bacterial DNA would be destroyed by bacterial and salivary DNase (14,15), bacterial DNA quantity at 120 hours was reduced to the level similar to the control.

Storage of saliva on ice up to 120 hours or on dry ice, about -80°C, for 6 or 24 hours had no effect on bacterial DNA quantity. These results were similar to the study that stored saliva at -20°C for up to 36 months before DNA extraction and followed by quantitative real-time PCR (8). However, storage on dry ice for 120 hours the DNA quantity was reduced significantly. This result was similar to the study that analyzed salivary DNA with conventional PCR (9). But it was different to the study that quantify bacterial count by Checkerboard DNA-DNA hybridization method (7). This might be a result of different quantitation techniques used in each study.

From this study, storage of saliva on ice for 120 hours seemed to reduce bacterial DNA concentration but it was not statistically significant. Study with more saliva sample number is needed to confirm this result.

Certainly, storage on ice or dry ice for up to 24 hours could maintain quantity of bacterial genomic DNA. Therefore, saliva samples collected for bacterial DNA quantitative analysis could be kept on ice or dry ice for transportation from a research field to a laboratory within 24 hours. Storage on ice or dry ice for a longer period of time might affect DNA quantity. The effect of saliva storage on ice or dry ice for 2 to 4 days should be studied. If storage within 4 days doesn't affect DNA quantity, transportation of saliva samples from the study field that is far away from the laboratory is possible.

Conclusion

As quantify by Real-time PCR, saliva storage at room temperature at 6 or 24 hours had increasing bacterial DNA. Saliva storage on ice and dry ice could maintain bacterial DNA similar to centrifugation immediately and then frozen at -80°C. However, storage on dry ice for 120 hours reduced bacterial DNA quantity.

Acknowledgement

This work was partly supported by a grant from Faculty of Dentistry, Naresuan University.

References

1. Jung JY, Yoon HK, An S, Lee JW, Ahn ER, Kim YJ, et al. Rapid oral bacteria detection based on real-time PCR for the forensic identification of saliva. Sci Rep. 2018;8(1):10852. doi: 10.1038/s41598-018-29264-2.

2. Shinde DB, Mahore JG, Giram PS, Singh SL, Sharda A, Choyan D, et al. Microbiota of Saliva: A Non-invasive Diagnostic Tool. Indian J Microbiol. 2024;64(2):328-42.

3. Noruzpour A, Gholam-Mostafaei FS, Looha MA, Dabiri H, Ahmadipour S, Rouhani P, et al. Assessment of salivary microbiota profile as a potential diagnostic tool for pediatric celiac disease. Sci Rep. 2024;14(1):16712. doi: 10.1038/s41598-024-67677-4.

4. Zhou X, Hao Y, Peng X, Li B, Han Q, Ren B, et al. The Clinical Potential of Oral Microbiota as a Screening Tool for Oral Squamous Cell Carcinomas. Front Cell Infect Microbiol. 2021;11: 728933. doi: 10.3389/fcimb.2021.728933.

5. Lim Y, Totsika M, Morrison M, Punyadeera C. The saliva microbiome profiles are minimally affected by collection method or DNA extraction protocols. Sci Rep. 2017;7(1):8523. doi:10.1038/s41598-017-07885-3.

6. Nemoda Z, Horvat-Gordon M, Fortunato CK, Beltzer EK, Scholl JL, Granger DA. Assessing genetic polymorphisms using DNA extracted from cells present in saliva samples. BMC Med Res Methodol. 2011;11:170. doi: 10.1186/1471-2288-11-170.

7. do Nascimento C, dos Santos JN, Pedrazzi V, Pita MS, Monesi N, Ribeiro RF, et al. Impact of temperature and time storage on the microbial detection of oral samples by Checkerboard DNA-DNA hybridization method. Arch Oral Biol. 2014;59(1):12-21.

8. Durdiakova J, Kamodyova N, Ostatnikova D, Vlkova B, Celec P. Comparison of different collection procedures and two methods for DNA isolation from saliva. Clin Chem Lab Med. 2012; 50(4):643-7.

9. Ng DP, Koh D, Choo SG, Ng V, Fu Q. Effect of storage conditions on the extraction of PCR-quality genomic DNA from saliva. Clin Chim Acta. 2004;343(1-2):191-4.

10. Hata S, Hata H, Miyasawa-Hori H, Kudo A, Mayanagi H. Quantitative detection of Streptococcus mutans in the dental plaque of Japanese preschool children by real-time PCR. Lett Appl Microbiol. 2006;42(2):127-31.

11. Bradshaw DJ, Homer KA, Marsh PD, Beighton D. Metabolic cooperation in oral microbial communities during growth on mucin. Microbiology. 1994;140(12):3407-12.

12. Wickström C, Herzberg MC, Beighton D, Svensäter G. Proteolytic degradation of human salivary MUC5B by dental biofilms. Microbiology. 2009;155(9):2866-72.

13. Byers HL, Tarelli E, Homer KA, Beighton D.Isolation and characterisation of sialidase from a strain of Streptococcus oralis. J Med Microbiol. 2000;49(3):235-44.

14. Palmer LJ, Chapple ILC, Wright HJ, Roberts A, Cooper PR. Extracellular deoxyribonuclease production by periodontal bacteria. J Periodontal Res. 2012;47(4):439-45.

15. Konečná B, Gaál Kovalčíková A, Pančíková A, Novák B, Kovaľová E, Celec P, et al. Salivary Extracellular DNA and DNase Activity in Periodontitis. Appl. Sci. 2020;10(21):7490. doi. org/10.3390/app10217490.

Corresponding author:
Asst.Prof.Dr. Jantipa Jobsri
Faculty of Dentistry, Naresuan University,
Phitsanulok, 65000, Thailand.
Tel: (66) 5596 6100
E-mail: jantipaj@nu.ac.th

Effect of Building Orientation on Marginal Gap and Internal Fit of The Implant-Supported 3D-Printed Provisional Crown

Atittaya Chaowthawee[1] Patcharanun Chaiamornsup[1] Pornpot Jiangkongkho[1] Yosnarong Sirimethawong[1*]

Abstract

Background: With the increasing popularity of immediate implant placement and restoration, precise fabrication methods for provisional restorations have become crucial since it helps preserving the soft tissue around the implant. 3D-printed PMMA provides cost-effective alternatives despite concerns about polymerization shrinkage. Previous studies have shown that building orientation for 3D-printed methods can affect the marginal gap and internal fit of the restoration. This study aims to examine the effect of building orientation on the marginal gap and internal fit of implant-supported provisional prostheses.

Materials and Methods: The implant-supported provisional restoration of the right maxillary central incisor was designed by complete digital workflow using 3shape software. The virtual implant crowns were fabricated with 3D-printer (DLP technology) in three different building orientations (0°, 45°, and 90°) with 10 samples per group. The samples underwent post-processing methods according to the manufacturer's recommendation. Marginal gap and internal fit examination were conducted using a digital silicone replica technique and superimposition method. After digitizing the silicone replica into .stl file, the gap distances were measured at sixteen reference points for each sample using Artec Studio 18 software. The degree of discrepancy was reported in color mapping. The measurements were statistically analyzed using One-way ANOVA .

Results: Statistical analysis revealed significant differences in marginal, cervical, and axial gap across the three groups ($p < 0.05$) except the occlusal gap. The 45° group had a significantly smaller marginal gap (19.1 ± 6.98 μm) than the 0° group (29.78 ± 10.63 μm) and the 90° group (35.68 ± 18.37 μm). Color mapping indicated thicker cement space around the margin of the Ti-base abutment in the 0° and 90° groups compared to the 45° group.

Conclusion: Building orientation in 3D printing significantly affects the marginal gap and internal fit of implant-supported provisional restorations. The 45° orientation produced the best results among the three groups.

Keywords: implant, provisional restoration, 3D printing, marginal gap

Received Date: Jul 30, 2024
Revised Date: Sep 26, 2024
Accepted Date: Oct 09, 2024

[1]*Department of Restorative dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, 65000, Thailand.*
*(*corresponding author)*

Introduction

Dental implants have gained remarkable popularity for dental substitution. Their distinguished features, such as exceptional strength, reliable retention, stability, and the capacity to support surrounding structures, not only ensure comfort but also contribute to the preservation of alveolar bone. Moreover, their esthetic resemblance to natural teeth enhances patient satisfaction (1). Capitalizing on these advantages, patients now hold higher expectations for swift and uncomplicated surgical protocols. Immediate implant placement simultaneous with restoration has now become a widely accepted protocol, particularly in areas with esthetic significance. This technique involves attaching a prosthetic crown or bridge attached to the dental implant shortly after its surgical placement. This approach offers several benefits in terms of both functional and esthetic outcomes. It provides patients with a continuous dental structure without the need to endure an edentulous phase and less additional surgical procedure from the second stage surgery. Additionally, an immediate restoration aids in the preservation of soft tissue architecture, preventing gingival recession and shaping the soft tissue to achieve a proper emergence profile around the implant (2).

The use of static computer-assisted implant surgery (sCAIS) is widely used in recent implant placement protocols. This technology significantly enhances the accuracy of surgical protocols, even in cases involving immediate implant placement accompanied by the immediate loading of provisional restorations. Consequently, the design and production of a well-crafted provisional restoration can be accomplished prior to the actual surgical appointment (3). Such provisional restorations, when employed in conjunction with immediate implant placement, not only offer patients a temporary substitute for their extracted teeth but also play a crucial role in facilitating soft tissue healing. Through proper restoration contouring, they have the potential to avert gingival recession post-extraction and aid in shaping the soft tissue to achieve a proper emergence profile (4-6). In this scenario, the utilization of immediate provisional restorations becomes even more significant, given their prolonged usage throughout the healing period. Thus, provisional restorations should possess an optimal level of strength, reliable dimensional stability, and a satisfactory esthetic appearance.

Traditionally, auto-polymerized polymethyl methacrylate (PMMA) has been the material of choice for crafting provisional restorations (7). However, this material does come with certain limitations, including exothermic reactions, polymerization shrinkage, and an unpleasant odor (8-9). The advent of computer-aided design and computer-aided manufacturing (CAD/CAM) technology has revolutionized the field of restorative dentistry, offering enhanced precision and accuracy in the fabrication of dental prostheses.

Provisional restorative materials like auto-polymerized polymethyl methacrylate (PMMA) and bis-acryl resin have been widely used for decades. However, auto-polymerized PMMA can lead to exothermic reactions, polymerization shrinkage, and unpleasant odors, while bis-acryl resin, despite offering good strength and high esthetics, typically requires direct fabrication techniques that demand more clinical chair time and greater clinician skill to achieve flawless outcome(7-9). The advent of computer-aided

design and computer-aided manufacturing (CAD/CAM) technology has revolutionized restorative dentistry, offering enhanced precision and accuracy in the fabrication of dental prostheses, with the added benefit of allowing restorations to be prepared in advance of surgery. Although several studies have compared the mechanical and physical properties of conventional PMMA, bis-acryl resin, and 3D-printed resin, the findings remain controversial, indicating the need for further investigation (10).

There are two approaches for producing provisional prosthesis from CAD/CAM PMMA: by milling or 3D-printing. A milled PMMA undergoes complete polymerization under optimal conditions, resulting in reduced residual porosity and monomer content. Thus, milled PMMA presents better mechanical properties compared to conventional PMMA (10-12). Although milled PMMA has greater strength and dimensional stability, it involves higher costs in terms of both material and milling tools. On the other hand, 3D-printed or additive manufacturing is a process that builds objects layer-by-layer. Therefore, 3D-printing generates less waste and proves to be a more cost-effective option. However, a primary concern associated with 3D-printed PMMA is polymerization shrinkage during manufacturing process (13-14). This shrinkage can lead to dimensional alterations, potentially affecting the precision of the restoration's margin and resulting in discrepancies in both the marginal area and the internal gap fit.

The marginal gap and internal fit are two important factors for the long-term success and durability of a fixed prosthesis. Poor marginal fit and clinical unacceptable gap of the fixed prosthesis can lead to cement dissolution, plaque accumulation and microleakage which compromise the surrounding periodontium and may consequences to a marginal bone loss (15). Earlier research indicates that the marginal gap of 3D-printed provisional restorations typically falls within the range of 56–212 μm (14,16-17). Since the properties of printed PMMA are influenced by various factors, including parameter adjustments derived from CAD design, building orientation, as well as the specific post-rinsing and post-curing protocols applied.

Previous studies have shown that building orientation had an influence on marginal gap and internal fit of the tooth-supported 3D-printed provisional tooth-supported crown and bridge (18-19). However, the study about building orientation on marginal gap and internal fit of 3D-printed provisional restoration on implant abutment is insufficient and needs for a further study.

Therefore, this study aims to examine the effect of building orientation on marginal gap and internal fit of the implant-supported provisional prosthesis. The goal is to enhance the precision in fabrication process of provisional implant crowns, ultimately contributing to more favorable treatment outcomes.

Materials and Method

An implant fixture analog (RC analog; Straumann, Basel, Switzerland) was centralized and fixed within 3D-printed acrylic resin block (P Pro Master Model Grey, Straumann, Basel, Switzerland) size 20 x 20 x 20 mm designed from CAD software (3D builder; Microsoft, Redmond, WA, USA). The block was designed with lines across the upper surface to serve as a reference for gap measurement.

An implant scan body was inserted into the implant analog, and then the scan body and the resin base were scanned using an intraoral scanner (Trios3; 3Shape, Copenhagen, Denmark). Computer-aided design (CAD) software (Dental System, 3Shape, Copenhagen, Denmark) was utilized to design the provisional restoration for the right maxillary central incisors through a complete digital workflow. The cement space for the provisional restoration was set at 40 μm. Subsequently, the virtual provisional crown was saved as an STL file for further processing.

The virtual crown STL file was assigned into 3 groups according to different building orientation angles. In this context, 0 degrees indicate that the incisal edge of the implant crown was oriented toward the build platform. (Figure 1A)

- Group 1: 3D-printed crown with build orientation 0 degree
- Group 2 : 3D-printed crown with build orientation 45 degree
- Group 3 : 3D-printed crown with build orientation 90 degree

All samples were produced by DLP printer (Straumann® P30; Straumann®, Basel, Switzerland) with printable methacrylate-based resin (P-Pro Crown & Bridge; Straumann®, Basel, Switzerland) with a curing wavelength of 385 nm and the layer thickness of 100 μm. After 3D printing, all specimens underwent the manufacturer's recommended post-processing steps. Upon completion of printing, the samples were removed from the platform and individually wrapped in tissue paper. To eliminate excess uncured resin, the samples were centrifuged in a laboratory centrifuge (EBA 20; Hettich, Tuttlingen, Germany) at 1500 rpm for 2 minutes

Following this, each sample received a secondary exposure using a flash-light curing machine (Straumann® P cure; Straumann®, Basel, Switzerland). Subsequently, the samples were rinsed with isopropanol alcohol using an automated machine (Straumann® P wash; Straumann®, Basel, Switzerland). Then, left to air dry completely for a minimum of 30 minutes. To standardize the procedures, 3D printer, automated curing and washing machine will be calibrated and set the time and temperature according to the manufacturer's recommendations. All procedures will be performed by a single trained researcher.

For marginal gap and internal fit measurement, the digital silicone replica technique adapted from Zeller et al. (20) was used. First, the Ti-base abutment (Straumann Variobase®, Straumann®, Basel, Switzerland) was sandblasted with 50 um Al_2O_3 to reduce reflectivity and need for a coating spray. Then the Ti-base abutment will be scanned as a 'abutment scan' using laboratory scanner (E4, 3Shape, Copenhagen, Denmark). After that, polyvinyl siloxane (Aquasil Ultra, Dentsply Sirona, North Carolina, USA) was loaded into the sample and seated onto the Ti-base abutment. A pressure of approximately 50 N was applied by a specimen positioner (Instron, Massachusetts, USA) and maintained for 5 minutes according to the setting time from the manufacturer's instructions. Then, the crown was removed from the abutment, leaving the PVS replica adhered to the Ti-base abutment. The Ti-base abutment with the PVS replica was scanned using the laboratory scanner (E4, 3Shape, Copenhagen, Denmark). Before repeating all steps for each sample, the silicone replica was removed completely using a dental laboratory steam cleaner to prevent silicone remnants from the previous sample.

All 50 Ti-base abutments with PVS replica scans and reference abutment scans were exported as STL files and imported into engineering software (Artec Studio 18; Artec 3D, Senningerberg, Luxembourg) to analyze the marginal gap and internal fit. The abutment scan and the Ti-base with PVS replica scan were aligned using the "best-fit alignment" function. Once the reference scan and the sample scan were aligned, the scans were sectioned along the labiolingual and mesiodistal directions using a line on the resin block as a reference line.

The discrepancy between the two scans was evaluated at four specific points, covering all four aspects: labial, lingual, mesial, and distal. This evaluation summarized the findings across 16 points for each sample, focusing on two key measurements: (1) the marginal gap (the perpendicular distance of the cement space at the margin of the abutment) and (2) the internal gap, which was averaged from three areas: (a) the cervical gap (1 mm medial to the margin of the abutment), (b) the middle of the engagement area of the abutment, and (c) the middle of the flat surface on the upper half of the abutment. (Figure1B,1C,1D)

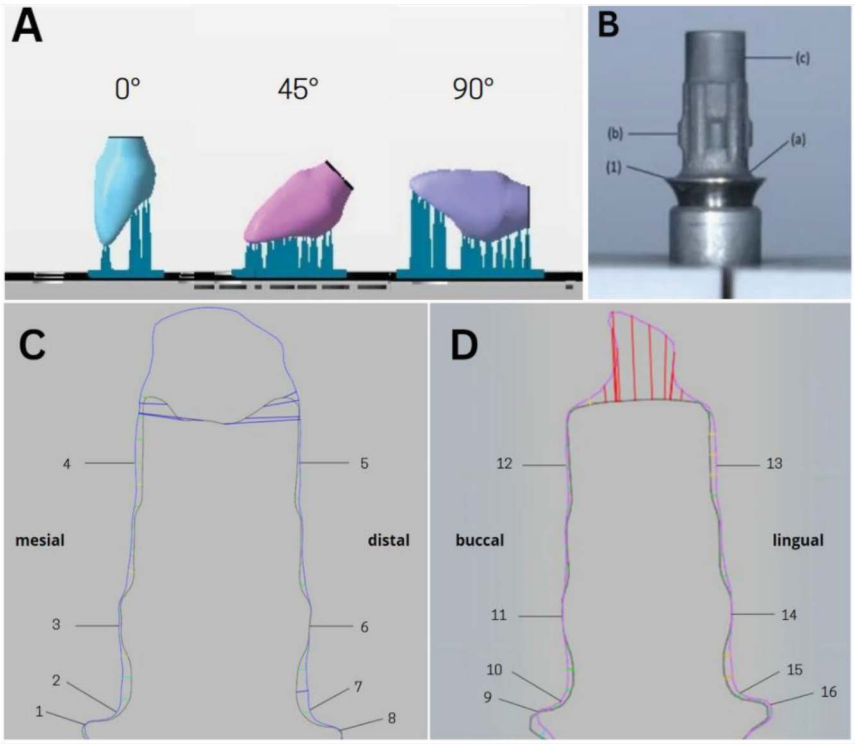

Fig.1 (A) the provisional crowns were built in 3 different orientation (0°, 45°, and 90°) and (B) the four specific points where the gap were measured covering all four aspects of the abutment; (1)marginal gap, (a)cervical gap, (b)axial gap and (c)occlusal gap. (C) and (D) all 16 points measured for gap investigation in each specimen.

Following gap measurement, the sections below the finish line of the abutment, including the base and other components, were deleted. This process resulted in a Ti-base abutment with a silicone replica, which was transformed into a point discrepancy grading represented by color. The program applied a color spectrum ranging from blue to red. Yellow to red hues indicated that the PVS scan had a positive value compared to the Ti-base reference scan, while light blue to navy colors indicated a negative value from the Ti-base reference scan.

The measurement results were statistically analyzed with one-way ANOVA using SPSS for Windows (SPSS v17.0, IBM, Chicago, IL, USA). To further explore any significant differences, multiple comparisons were performed using the Tukey post-hoc test. The chosen level of significance for these analyses was set at $\alpha = 0.05$.

Results

The measurement results of the marginal and internal gaps for different locations (marginal, cervical, axial, and occlusal gaps) and aspects (labial, lingual, mesial, and distal) of implant-supported prostheses at orientations of 0°, 45°, and 90° are presented in Figure 2. One-way ANOVA indicated that building orientation had a significant influence on marginal gap, cervical gap, and axial gap except the occlusal gap.

The Marginal gaps showed significant variation across orientations, especially in the labial and lingual aspects. The mean circumferential of marginal gap of the 45° group had a significantly smaller marginal gap (19.1 ± 6.98 μm) than the 0° group (29.78 ± 10.63 μm)($p = 0.002$) and the 90° group (35.68 ± 18.37 μm) ($p < 0.001$). The post-hoc test suggested that the marginal gap of the labial aspect of the 45° (14.50 ± 8.00 μm) significantly lower than the 0° (32.30 ± 11.42 μm) and 90° (36.90 ± 18.78 μm) orientations group ($p = 0.002$). And the marginal gap of the lingual aspect of the 45° group (19.5 ± 6.35 μm) is significantly smaller than the 90° group (33.70 ± 16.72 μm) with p-value 0.037. The same trends were observed for the mesial and distal aspects regardless of statistical significance.

For the internal fit of the prostheses, Cervical gaps were smaller in 0° group and larger in the 90° orientation for all aspects. The mesial aspect at 90° showed the largest cervical gap with a mean of 112.90 ± 28.31 μm, compared to 55.70 ± 16.41 μm at 0° and 73.70 ± 23.60 μm at 45° ($p < 0.001$). Axial gaps showed significant differences primarily in the lingual aspect where the mean gap is 39.20 ± 16.14 μm, considerably larger than at 0° (17.50 ± 8.36 μm) and 45° (15.50 ± 4.93 μm), with a p-value < 0.001. In contrast, occlusal gaps exhibited no significant differences across orientations. These results suggest that the orientation significantly affects the gap measurements, with the 90° orientation generally presenting the largest gaps, particularly in cervical and marginal locations.

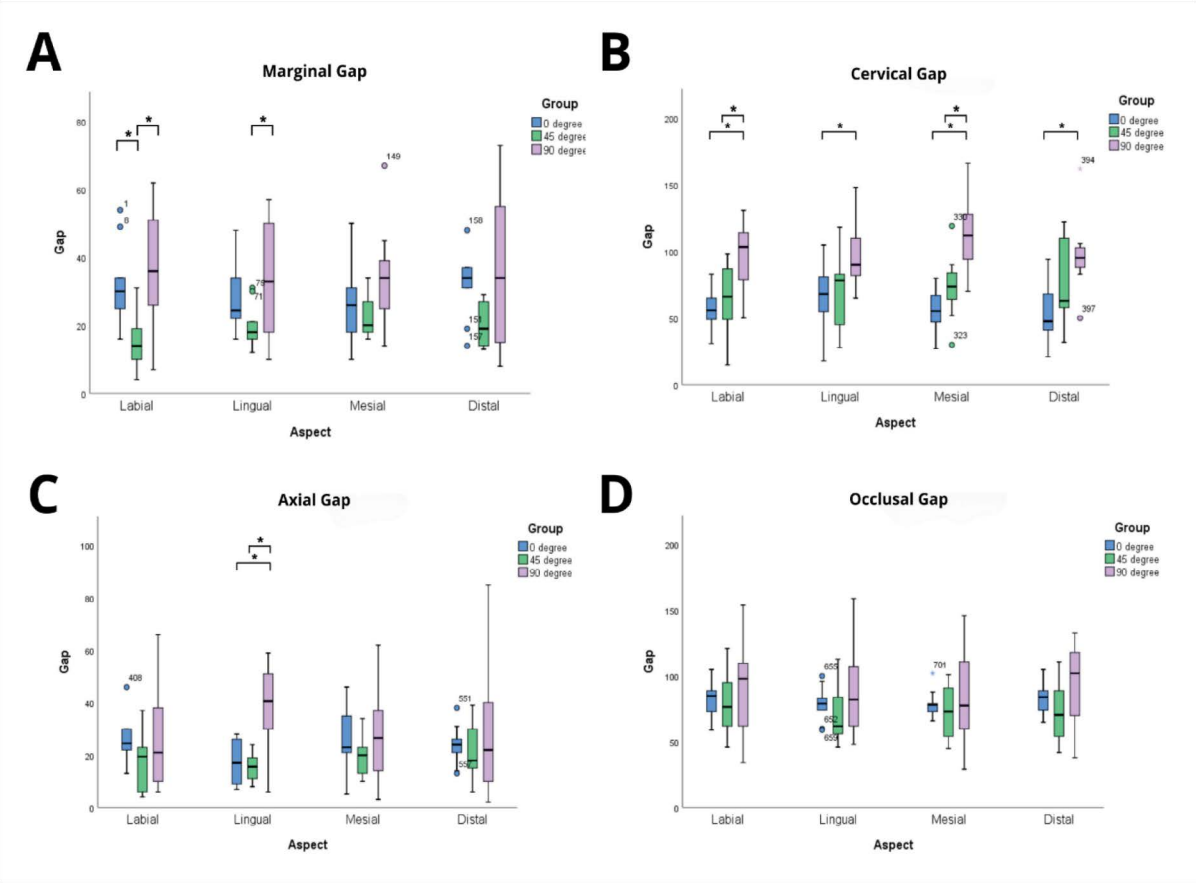

Fig.2 box plot graph of the gap measurement for different locations. (A) marginal gap, (B) cervical gap, (C) axial gap, and (D) occlusal gaps in four aspects (labial, lingual, mesial, and distal) of implant-supported prostheses at orientations of 0°, 45°, and 90°.
***indicate statistical significance between groups.**

The software displayed the deviation of the specimen and reference files in a color range from blue to red. Since the PVS substitutes for the cement space, only positive deviations, shown in green to red hues, were demonstrated. The color mapping results showed larger cement gaps, indicated in yellow, at the marginal area of the abutment in the 0° and 90° groups compared to the 45° group. Additionally, the overall cement space in the 90° group was larger than in the other groups. (Figure 3)

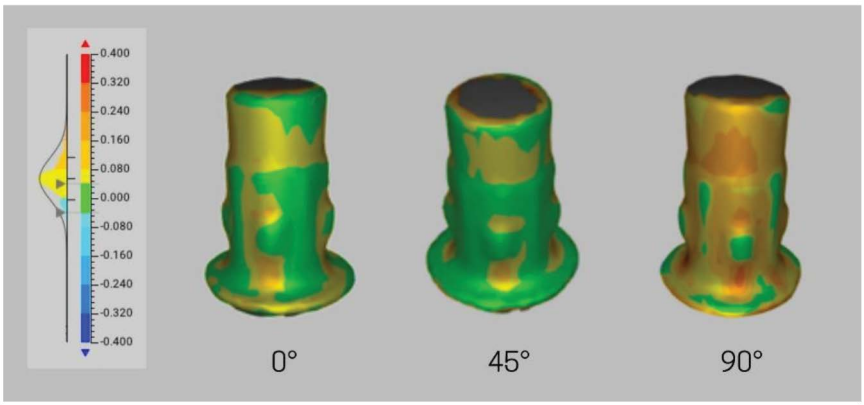

Fig.3 color map of the PVS replica substitute for the cement space in each group. The discrepancy of samples and reference file were demonstrated in color, ranging from green to red, indicating small to large gaps.

Discussion

This study aimed to evaluate the marginal gap and internal fit of the implant-supported provisional crown produced by 3D-printed technology in various building orientations. The results showed that the building orientation had an influence on marginal gap and internal fit of the 3D-printed implant-supported provisional crown especially at marginal and cervical area.

A range of marginal gaps in 3D-printed provisional restorations using DLP technology has been documented in prior research. Ryu et al. reported gaps for 3D-printed crowns between 58-113 μm (19), while Park et al. observed 52-61 μm for implant-supported crowns (21). Although Farag et al. reported a higher fit of marginal gap of the provisional crown printed by SLA printer than those by DLP printer (22). Yet, the results showed that the marginal gap of provisional crown from SLA printer was range between 40-72 μm. This study found marginal gaps ranging from 19-35 μm, which is notably lower than previously reported figures. This may be because this study implied the whole digital workflow and design the provisional crown from the abutment library provided in the CAD software, which can reduce the error of the scanning process of the abutment. McLean and von Fraunhofer analyzed the fit of fixed restorations over five years and concluded that a gap under 80 μm is clinically acceptable (23). Consequently, the marginal gaps identified in this study fall within acceptable clinical parameters.

In this study, the 45° group was found had the lowest marginal gap coincided with the results from Park et al. who studied 3D-printed implant-supported 3-unit bridge in 5 build angles (0°, 30°, 45°, 60°, 90°) and found a significant difference in the marginal and internal fit, in which the optimal build angles were 45° and 60° (24). Similarly, Osman et al. evaluated the accuracy of 3D-printed provisional crowns at various angles, discovering that 135° (corresponding to 45° in this study) and 210° showed the lowest discrepancies (25). However, Chaiamornsup et al. reported significant larger marginal discrepancies

in 3-unit fixed partial denture casting patterns printed at a 45° orientation than those in 0° and 30° (18). These differing results may be attributed to variations in prosthesis type and design, including tooth-supported versus implant-supported restorations, the number of crowns involved and cement gap configuration in CAD software.

Several factors elucidate why building orientation influences accuracy, resulting in varying marginal gaps and internal fits of 3D-printed restorations. In this study, the cervical gap was increased in more tilted building orientation in all aspects. This may have occurred because the DLP printer cures the resin layer by layer, alterations in building orientation affect the shape of each cured resin layer. In this study, the specimen featured a cylindrical space in the center for the abutment and the screw access channel. During printing, the shape of the central hole of uncured resin varied across the three groups. In 0° group, this hole is nearly circular; however, it became increasingly elliptical with greater tilt degree and eventually nearly rectangular at the 90° group. This variation resulted in the mesial and distal aspects of the crown being cured separately. Such changes in shape influence the form and degree of polymerization shrinkage, as well as the direction of the shrinkage (19,24). When a polymer layer is repeatedly photopolymerized from one side, the previously cured material contracts to release internal stress, while the newly cured material shrinks within the boundaries of the previous layer (26). This may result in larger gap in 90° group especially at the cervical area and in lingual aspect of the axial area because it is the site where support structure is attached.

Modifications in building orientation also alter the area where the supporting structure attaches to the prosthesis. Since the 90° group had a support structure attached near the margin of the prothesis more than the other groups, this may lead to distortion of the crown margin (19) during removal and post-curing process resulting in larger marginal gap of the implant crown printed in 90° than those in 0° and 45°. (Figure 1)

Unkovskiy et al. investigated the accuracy of rectangular bars printed in various building orientations, finding that Z-axis accuracy decreases as specimen length increases (27). This decreased accuracy in the Z-axis may contribute to the inaccuracy of the margin of the 0° group in this study. Additionally, the 0° group exhibited the lowest cervical gap, potentially leading to an improper seat of the crown on the abutment. These factors together might account for the larger marginal gap in the 0° group compared to the 45° group. Although the low cervical gap in the 0° group could theoretically be compensated for by increasing the cement space setting in CAD software, doing so could result in an improper fit and reduced stability of the entire restoration.

The findings of this study indicated that a 45° building orientation yields the smallest marginal gap and best internal fit. However, these outcomes may not be applicable to all DLP printer configurations. Additionally, various factors, such as the design of the prosthesis, number of the teeth involved, the type of printing material, and post-processing methods, can affect the results. Recognized limitations of this study include the restricted generalizability due to variations in printer specifications and materials.

Conclusion

The 45° orientation demonstrated the smallest marginal gap and superior internal fit, indicating it as the optimal angle for achieving high precision. Therefore, this technique is recommended for implant-supported provisional restorations, particularly in immediate implant placements within esthetic zones, where enhanced precision and tissue compatibility are crucial for promoting soft tissue healing and ensuring favorable clinical outcomes.

Conflict of interest

Non declared.

References

1. Misch, CE. Dental Implant Prosthetics. 2nd ed. Amsterdam: Elsevier; 2014.

2. Javaid MA, Khurshid Z, Zafar MS, Najeeb S. Immediate Implants: Clinical Guidelines for Esthetic Outcomes. Dent J (Basel). 2016;4(2): 21. doi: 10.3390/dj4020021.

3. Abduo J, Lau D. Seating accuracy of implant immediate provisional prostheses fabricated by digital workflow prior to implant placement by fully guided static computer-assisted implant surgery: An in vitro study. Clin Oral Implants Res. 2021;32(5):608-18.

4. Amato F, Amato G, Polara G, Spedicato GA. Guided Tissue Preservation: Clinical Application of a New Provisional Restoration Design to Preserve Soft Tissue Contours for Single-Tooth Immediate Implant Restorations in the Esthetic Area. Int J Periodontics Restorative Dent. 2020;40(6):869-79.

5. González-Martín O, Lee E, Weisgold A, Veltri M, Su H. Contour Management of Implant Restorations for Optimal Emergence Profiles: Guidelines for Immediate and Delayed Provisional Restorations. Int J Periodontics Restorative Dent. 2020;40(1):61-70.

6. Wang WCW, Hafez TH, Almufleh AS, Ochoa-Durand D, Manasse M, Froum SJ, et al. A Guideline on Provisional Restorations for Patients Undergoing Implant Treatment. J Oral Bio. 2015; 2(2):1-7

7. Burns DR, Beck DA, Nelson SK; Committee on Research in Fixed Prosthodontics of the Academy of Fixed Prosthodontics. A review of selected dental literature on contemporary provisional fixed prosthodontic treatment: report of the Committee on Research in Fixed Prosthodontics of the Academy of Fixed Prosthodontics. J Prosthet Dent. 2003;90(5):474-97.

8. Gratton DG, Aquilino SA. Interim restorations. Dent Clin North Am. 2004;48(2):487-97.

9. Tom T, Uthappa MA, Sunny K, Begum F, Nautiyal MC, Tamore S. Provisional restorations: An overview of materials used. J Adv Clin Res Insights. 2016;3(6):212-4.

10. Al-Humood H, Alfaraj A, Yang CC, Levon J, Chu TG, Lin WS. Marginal Fit, Mechanical Properties, and Esthetic Outcomes of CAD/CAM Interim Fixed Dental Prostheses (FDPs): A Systematic Review. Materials (Basel). 2023;16(5): 1996. doi: 10.3390/ma16051996.

11. Igreţ A, Rotar RN, Ille C, Topală F, Jivănescu A. Marginal fit of milled versus different 3D-printed materials for provisional fixed dental prostheses: an in vitro comparative study. Med Pharm Rep. 2023;96(3):298-304.

12. Suwannasing A, Chaoklaiwong B, Banthitkhunanon P. Materials for provisional crowns, CAD/CAM polymethyl methacrylate: literature review. CM Dent J. 2020;41(2):25-35.

13. Ishida Y, Miyasaka T. Dimensional accuracy of dental casting patterns created by 3D printers. Dent Mater J. 2016;35(2):250-6.

14. Wu J, Xie H, Sadr A, Chung KH. Evaluation of Internal Fit and Marginal Adaptation of Provisional Crowns Fabricated with Three Different Techniques. Sensors (Basel). 2021;21(3): 740. doi: 10.3390/s21030740.

15. Srimaneepong V, Heboyan A, Zafar MS, Khurshid Z, Marya A, Fernandes GVO, et al. Fixed Prosthetic Restorations and Periodontal Health: A Narrative Review. J Funct Biomater. 2022;13(1):15. doi: 10.3390/jfb13010015.

16. Deeb LA, Ahdal KA, Alotaibi GN, Alshehri A, Alotaibi B, Alabdulwahab F, et al. Marginal integrity, internal adaptation and compressive strength of 3D printed, computer aided design and computer aided manufacture and conventional interim fixed partial dentures. J Biomater Tissue Eng. 2019;9(12):1745-50.

17. Aldahian N, Khan R, Mustafa M, Vohra F, Alrahlah A. Influence of Conventional, CAD-CAM, and 3D Printing Fabrication Techniques on the Marginal Integrity and Surface Roughness and Wear of Interim Crowns. Appl Sci. 2021;11(19): 8964. doi.org/10.3390/app11198964.

18. Chaiamornsup P, Iwasaki N, Tsuchida Y, Takahashi H. Effects of build orientation on adaptation of casting patterns for three-unit partial fixed dental prostheses fabricated by using digital light projection. J Prosthet Dent. 2022;128(5):1047-54.

19. Ryu JE, Kim YL, Kong HJ, Chang HS, Jung JH. Marginal and internal fit of 3D printed provisional crowns according to build directions. J Adv Prosthodont. 2020;12(4):225-32.

20. Zeller S, Guichet D, Kontogiorgos E, Nagy WW. Accuracy of three digital workflows for implant abutment and crown fabrication using a digital measuring technique. J Prosthet Dent. 2019;121(2):276-84.

21. Park JY, Jeong ID, Lee JJ, Bae SY, Kim JH, Kim WC. In vitro assessment of the marginal and internal fits of interim implant restorations fabricated with different methods. J Prosthet Dent. 2016;116(4):536-42.

22. Farag E, Sabet A, Ebeid K, El Sergany O. Build angle effect on 3D-printed dental crowns marginal fit using digital light-processing and stereo-lithography technology: an in vitro study. BMC Oral Health. 2024;24(1):73. doi:10.1186/ s12903-024-03851-4.

23. McLean JW, von Fraunhofer JA. The estimation of cement film thickness by an in vivo technique. Br Dent J. 1971;131(3):107-11.

24. Park GS, Kim SK, Heo SJ, Koak JY, Seo DG. Effects of Printing Parameters on the Fit of Implant-Supported 3D Printing Resin Prosthetics. Materials (Basel). 2019;12(16):2533. doi:10.3390/ma12162533.

25. Osman RB, Alharbi N, Wismeijer D. Build Angle: Does It Influence the Accuracy of 3D-Printed Dental Restorations Using Digital Light-Processing Technology?. Int J Prosthodont. 2017;30(2):182-8.

26. Zhao Z, Wu J, Mu X, Chen H, Qi HJ, Fang D. Origami by frontal photopolymerization. Sci Adv. 2017;3(4):e1602326. doi: 10.1126/sciadv. 1602326.

27. Unkovskiy A, Bui PH, Schille C, Geis-Gerstorfer J, Huettig F, Spintzyk S. Objects build orientation, positioning, and curing influence dimensional accuracy and flexural properties of stereolithographically printed resin. Dent Mater. 2018;34(12):e324-e333.

Corresponding author:
Dr. Yosnarong Sirimethawong
Department of Restorative dentistry, Faculty of Dentistry, Naresuan University, Phitsanulok, 65000, Thailand.
Tel: (668) 9891 7112
E-mail: yosnarong@gmail.com

Knowledge and Attitudes Towards Emergency Management of Traumatic Dental Injuries Among Elementary School Teachers in Sakaeo, Thailand

Kanchana Hemantakul[1] Praewpat Pachimsawat[1] Pornpoj Fuangtharnthip[1*]

Abstract

Objective: This study aimed to evaluate the knowledge and attitudes of elementary school teachers towards the emergency management of traumatic dental injuries (TDIs) and to identify their associated factors.

Materials and Methods: A cross-sectional study was conducted using a structured self-administered questionnaire distributed to 385 elementary school teachers across 31 schools in Sakaeo province. The questionnaire assessed demographic characteristics, knowledge, and attitudes regarding the emergency management of TDIs. A validity test evaluated the questionnaire through content validity, and a reliability test through Cronbach's alpha. Knowledge was evaluated through two scenario cases involving fractured teeth and avulsion. Descriptive analysis and Pearson Chi-square tests were employed to analyze the data and examine the association between knowledge and teachers' characteristics at a significance level of 0.05.

Results: The study revealed significant gaps in knowledge, particularly regarding the evaluation period for neurological symptoms (14.0% correct responses) and ideal replantation time (31.9%). Conversely, a majority of teachers correctly answered the questions on permanent tooth identification (74.5%) and on-site management of fractured teeth (70.9%). Regarding attitudes, a substantial proportion of teachers (86.0%) acknowledged their responsibility to assist students in dental emergencies, and most recognized the importance of permanent teeth for a high quality of life (96.6%). However, only 22.3% of teachers expressed confidence in managing TDIs. No association of overall knowledge was found with teachers' characteristics.

Conclusions: Our study indicates that while elementary school teachers in Sakaeo province have insufficient knowledge about managing TDIs, their attitudes towards handling such emergencies are generally positive. This suggests a need for targeted training programs to enhance teachers' knowledge and confidence in managing TDIs effectively.

Keywords: Traumatic dental injuries, knowledge, attitudes, school teacher

Received Date: Jul 26, 2024
Revised Date: Sep 20, 2024
Accepted Date: Oct 09, 2024

[1]*Department of Advanced General Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, 10400, Thailand.*
*(*corresponding author)*

Introduction

An oral injury is defined as an injury to the teeth or the hard and soft tissues inside or around the mouth (1). Traumatic dental injuries (TDIs) constitute 85% of oral injuries and are regarded as a public health issue due to their prevalence and negative impact on quality of life. TDIs are a significant childhood oral health issue, causing considerable discomfort and tooth loss. They can range from minor enamel chips to severe damage of supporting structures, or tooth loss (2). The most common cause of TDIs is falls, particularly in children whose neuromuscular systems are not fully developed (3).

Children aged 6 to 12 are especially prone to TDIs due to their active involvement in sports and physical activities at school (3). Common complications of TDIs include crown or root fractures, luxation injuries, avulsion, and damage to alveolar bone, soft tissue, gingiva, and dental pulp. These injuries often result in significant pain, discomfort, and a course of cosmetic, functional, psychological, and social issues, adversely affecting the patient's quality of life (3). Teachers play a crucial role in managing severe oral injuries as they are often in close contact with students. In cases of dental trauma, teachers can provide first aid by knowing the proper procedures and referring students to dental professionals (4). However, numerous studies have revealed significant deficiencies in teachers' knowledge of TDIs (5). A study in Brazil found that teachers lacked exceptional understanding regarding TDIs and often relied on incorrect information and practices (6). Similarly, a study by Nirwan M. et al. in South Jaipur, India, reported that 46% of elementary school teachers had inadequate knowledge of TDIs, while only 7% had adequate knowledge (7). Inappropriate initial treatment increases the probability of persisting problems and reduces the probability of the teeth remaining vital, causing distress to the damaged child and his/her parents in the form of unnecessary suffering and cost (8).

In Thailand, Malikaew et al. reported in 2006 that 35.0% of children experienced TDIs, with a higher prevalence among children from disadvantaged households and those with less educated parents (9). A 2022 study in Thailand found that teachers lacked the necessary knowledge and were not adequately prepared to handle dental injuries (10). Despite the high prevalence of dental trauma among Thai children, public awareness remains insufficient, and there are limited studies on TDIs in Thai elementary schools.

Sakaeo province, located approximately 237 kilometers from Bangkok, is one of Thailand's eastern provinces with a socio-economic status slightly below average. This makes Sakaeo a representative area for assessing the reach of public health education programs. School teachers in rural areas like Sakaeo province likely lack sufficient understanding of TDI management.

This study aimed to evaluate the knowledge and attitudes of Thai elementary school teachers regarding TDIs in Sakaeo province. Additionally, the study explored factors associated with the teachers' knowledge.

Materials and methods

A cross-sectional survey using a structured self-administered questionnaire was conducted to collect information on the management of TDIs regarding the knowledge and attitudes of elementary school teachers in Sakaeo province, Thailand. The study received approval from the Human Research Ethics Committee of the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board (COA.No.MU-DT/PY-IRM 2023/073.2311).

A sample size of 385 teachers was calculated using the Yamane formula (11) and based on all registered teachers (N = 3140) in Sakaeo province, which has 278 elementary schools. A proportional stratified random sampling technique was applied to recruit subjects from 31 elementary schools across seven districts in Sakaeo province.

Data collection was performed using a self-administered questionnaire comprising three sections: demographic data, ten questions on knowledge, and ten questions on attitudes. The questionnaire was modified from a previous study, and the content validity of each question was assessed using the Item-Objective Congruence (IOC) scores. Additionally, the reliability of the questionnaire was determined by calculating Cronbach's alpha, resulting in a value of 0.84. The knowledge questions were based on two scenario cases concerning fractured and avulsed teeth, with each question having only one correct answer, except for the question on storage media, which had three correct answers. The attitude questions were presented on a five-point Likert scale. The questionnaire was initially assessed by a group of 20 elementary school teachers for clarity and subsequently validated using Cronbach's alpha, which yielded a value of 0.84. During the survey, questionnaires were distributed directly to the respondents at their schools by the researchers, with the permission of the school and the written consent of the participants.

During data analysis, the sum of the correct answers was calculated to determine the knowledge scores. A score of "1" was assigned for each correct answer, while a score of "0" was given for wrong or "do not know" answers. For attitude scores, responses of "strongly agree" and "agree" were scored as "1" (considered "agree"), whereas responses of "uncertain," "disagree," and "strongly disagree" were scored as "0" (considered "disagree"). Descriptive analysis and Pearson Chi-square tests were used to evaluate knowledge and attitude levels and to identify factors associated with knowledge, using SPSS version 18 (IBM, Armonk, NY, USA).

Results

A total of 385 teachers from 31 elementary schools responded to the questionnaire. The personal and professional profiles of the teachers are summarized in Figure 1. The majority of respondents were female (78.4%), below the age of 40 (63.9%), had more than 5 years of teaching experience (51.7%), and held a bachelor's degree (84.2%). Almost 80% were employed by public schools and had never received special training on dental emergencies.

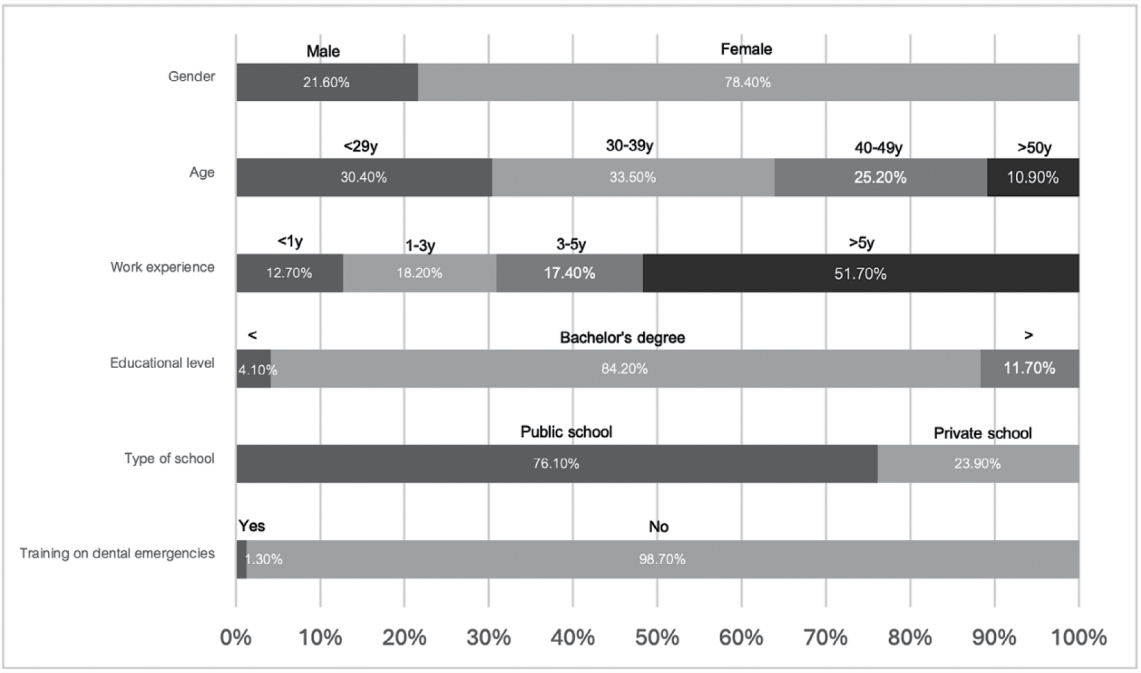

Fig.1 Demographic characteristics of 385 school teachers.

The assessment of the teachers' knowledge of the management of TDIs is shown in Table 1. Regarding knowledge on neurological impacts, 34% of school teachers responded correctly to the initial evaluation of neurological effects after TDIs, with only 14% answering correctly on the duration of the neurological symptoms.

Table 1. Assessment of teachers' knowledge of TDI management (n = 385), (10,12,13,14,15).

Case I "A 9-year-old child was hit by a ball in physical education class, breaking his top front teeth and injuring his face, teeth, and head."		
Topics	**Responses (n)**	**%**
Knowledge on neurological impact		
1. How should you take care of your students FIRST?		
☐ Find out what happened, when it happened, and where it happened.	53	13.8
☐ **Evaluate any parts of the head that may be affected.**	**131**	**34**
☐ Examine the teeth that most likely got impacted.	32	8.3
☐ Find out about any facial or dental pain.	158	41
☐ Do not know.	11	2.9
2. For students who suffered from significant impacts to the head, face, and teeth in an accident, how long is the follow-up period to assess their brain symptoms?		
☐ 15 minutes.	133	34.5
☐ 2-3 hours.	124	32.2
☐ **2-3 days.**	**54**	**14**
☐ 2-3 weeks.	74	19.2
Knowledge on emergency management in fractured teeth		
3. What should you do if so much blood flows that the situation in the mouth is invisible?		
☐ Contact his/her parents to get the student to the hospital promptly.	126	32.7
☐ Rinse his/her mouth out with normal saline solution to clean it.	178	46.2
☐ **Try to stop the bleeding by applying manual pressure. Determine the source of the persistent hemorrhaging.**	**60**	**15.6**
☐ Do not know.	21	5.5
4. If the student's front tooth is split in half due to an accident and is extremely sensitive to air blow, how should you give support and help to him/her?		
☐ No emergency treatment is required. Wait until the symptoms subside on their own.	13	3.4
☐ **Avoid biting against hard objects, or drinking ice-cold water, and then promptly visit a dentist.**	**273**	**70.9**
☐ Inform the parents so they can take their child to the dentist after the class finishes.	76	19.7
☐ Do not know.	23	6

Table 1. (Continued)

Topics	Responses (n)	%
5. If you find a fragment of the broken tooth, what additional care should you provide before referring him/her to the dentist?		
☐ Clean the tooth fragment properly. Replace and fix it on to the damaged tooth. Ask the students to hold it until he/she can visit the dentist.	45	11.7
☐ **Clean the tooth fragment properly and store it in a box or wrapper until being used.**	**175**	**45.5**
☐ Do not try to wash or clean the tooth fragment but store it in normal saline solution immediately.	58	15.1
☐ Do not try to wash or clean the tooth fragments but store it in milk or wrap it with gauze.	38	9.9
☐ Do not know.	68	17.7
Knowledge on the set of dentitions		
6. Which tooth is the broken upper front tooth?		
☐ **Permanent tooth.**	**287**	**74.5**
☐ Primary tooth.	63	16.4
☐ Do not know.	35	9.1
Case II “A 12-year-old child was hit, resulting in an avulsed tooth with bleeding but no significant pain.”		
Knowledge on emergency management for an avulsed tooth		
1. If you face a student with an avulsed tooth, how should you manage the situation?		
☐ There is no reason to search for an avulsed tooth because it is of no benefit for further treatment.	148	38.4
☐ The tooth can be held at any part of the tooth.	50	13
☐ **Hold it at the crown part. Avoid touching the root surface.**	**179**	**46.5**
☐ Hold it at the root part. Avoid touching the tooth crown.	8	2.1
2. If the avulsed tooth is found dirty on the ground, how should you manage the avulsed tooth?		
☐ **Wash the tooth gently with water or saline solution and replant it back to the socket.**	**171**	**44.4**
☐ Scrub the tooth with a sponge and soap, and replace it back to the socket.	18	4.7
☐ Replace it back to the socket immediately without cleaning.	2	0.5
☐ No need to use the avulsed tooth for treatment.	123	31.9
☐ Do not know.	71	18.4

Table 1. (Continued)

Knowledge on storage media		
3. What is the best storage media for keeping the avulsed tooth if it cannot be replaced back to the socket on the site?		
☐ Water.	112	29.1
☐ **Milk.**	**64**	**16.6**
☐ **Saliva.**	**2**	**0.5**
☐ Alcohol.	15	3.9
☐ **Normal saline solution.**	**124**	**32.2**
☐ Do not know.	68	17.7
Knowledge on replantation time		
4. When is the appropriate time to replace the avulsed tooth back to the socket?		
☐ **Immediately after the accident.**	**123**	**31.9**
☐ Any time on the same day.	25	6.5
☐ 30 minutes after the bleeding stops.	98	25.5
☐ Do not know.	136	35.3

Bold letters indicate “correct answers”.

In terms of emergency management of fractured teeth, 15.6% and 45.5% knew how to manage the bleeding and the tooth fragment, respectively. However, a high number of respondents (70.9%) correctly answered how to help their students before meeting with a dentist, and 74.5% had knowledge of permanent tooth identification. Regarding the management of avulsed teeth and appropriate storage media, almost half of the respondents answered correctly. Conversely, most respondents answered incorrectly or admitted having no idea about the appropriate replantation time for avulsed teeth.

Examination of the association between knowledge topics and teachers’ characteristics revealed that no association was found between overall knowledge score and any of the teachers’ characteristics. However, some characteristics, such as age, work experience, and type of school, were associated with specific knowledge topics on the management of TDIs (Table 2). The teacher’s age was significantly associated with knowledge scores on permanent tooth identification, storage media, and replantation time ($p = 0.019$, 0.002, and 0.006, respectively). Work experience was associated with knowledge on replantation time ($p = 0.003$). Additionally, the type of school was associated with knowledge regarding the neurological impact, permanent tooth identification, and emergency management of fractured teeth.

Table 2. Association between knowledge of TDI management and teachers' characteristics.

Variables	n	Number of respondents with correct answers (%)						Overall knowledge score
		Knowledge on neurological impact	Knowledge on emergency managemen in fractured teeth	Knowledge on teeth the set of dentitions	Knowledge on emergency management in avulsed teeth	Knowledge on storage media	Knowledge on replantation time	Mean ± SD
Gender								
Male	83	36(43.3)	80(90.4)	66(79.5)	57(68.7)	42(50.6)	28(33.7)	4.43 ± 1.55
Female	302	127(42.1)	248(82.1)	221(73.2)	201(66.6)	148(49.0)	95(31.5)	4.18 ± 1.73
p-value		0.829	0.070	0.240	0.716	0.797	0.693	0.231
Age								
< 29	117	49(41.9)	98(83.8)	77(65.8)	78(66.7)	73(62.4)	26(22.2)	4.19 ± 1.66
30-39	129	44(34.1)	107(82.9)	95(73.6)	81(67.4)	51(39.5)	52(40.3)	4.12 ± 1.72
40-49	97	49(50.5)	82(84.5)	79(81.4)	64(66.0)	43(44.3)	36(37.1)	4.42 ± 1.61
> 50	42	21(50.0)	36(85.7)	36(85.7)	29(69.0)	23(54.8)	9(21.4)	4.31 ± 1.92
p-value		0.064	0.974	0.019*	0.986	0.002*	0.006*	0.574
Work experience								
< 1	49	23(46.9)	43(87.8)	31(63.3)	36(73.5)	31(63.3)	6(12.2)	4.20 ± 1.57
1-3	70	21(30.0)	56(80.0)	53(75.7)	48(68.6)	39(55.7)	23(32.9)	4.11 ± 1.65
3-5	67	26(38.8)	54(80.6)	46(68.7)	38(56.7)	33(49.3)	30(44.8)	4.18 ± 1.71
> 5	199	93(46.7)	170(85.4)	157(78.9)	136(68.3)	87(43.7)	64(32.2)	4.31 ± 1.74
p-value		0.082	0.530	0.088	0.224	0.059	0.003*	0.850
Education level								
<Bachelor's degree	16	9(56.3)	15(93.8)	12(75.0)	7(43.8)	9(56.3)	5(31.3)	4.56 ± 1.59
Bachelor's degree	324	138(42.6)	268(82.7)	242(74.7)	220(67.9)	156(48.1)	108(33.3)	4.23 ± 1.74
>Bachelor's degree	45	16(35.6)	40(88.9)	33(73.3)	31(68.9)	25(55.6)	10(22.2)	4.13 ± 1.39
p-value		0.346	0.314	0.980	0.128	0.553	0.325	0.684
Type of school								
Public	293	136(46.4)	240(81.9)	209(71.3)	207(70.6)	145(49.5)	95(32.4)	4.24 ± 1.84
Private	92	27(29.3)	83(90.2)	78(84.8)	51(55.4)	45(48.9)	28(30.4)	4.24 ± 1.09
p-value		0.004*	0.059	0.010*	0.007*	0.923	0.721	0.982

* indicates "statistical significance".

The results of the attitude assessment are shown in Table 3. The majority of teachers responded positively to most of the positive statements, except that concerning their confidence in managing TDIs (g), and disagreed with the negative statements (c and f). Specifically, 86.0% agreed that it is their responsibility to help students in dental emergencies. According to 79.0% of the teachers, dental incidents were a common occurrence in schools.

Table 3. Assessment of teachers' attitudes towards TDI management.

		n	%
(a) You think that teachers have a role and responsibility in helping students with dental accidents.	**Agree**	**331**	**86.0**
	Disagree	54	14.0
(b) You agree that dental accidents occur frequently in schools.	**Agree**	**304**	**79.0**
	Disagree	81	21.0
(c) You think that an avulsed tooth should not be replaced back to the socket but had better be treated by the professional at the site.	Agree	169	43.9
	Disagree	**216**	**56.1**
(d) You agree that basic dental accident management training should be provided.	**Agree**	**351**	**91.2**
	Disagree	34	8.8
(e) You think a dental accident is an urgent situation that requires immediate proper first aid.	**Agree**	**344**	**89.4**
	Disagree	41	10.6
(f) You believe that a delay in TDI management at the site does not have much impact on treatment success.	Agree	90	23.4
	Disagree	**295**	**76.6**
(g) You are confident that a school teacher like you can manage the dental emergency.	Agree	86	22.3
	Disagree	**299**	**77.7**
(h) You agree that wearing a sport guard should be mandatory for school sports.	**Agree**	**220**	**57.1**
	Disagree	165	42.9
(i) You agree that tetanus vaccinations are necessary for TDIs in some cases.	**Agree**	**267**	**69.4**
	Disagree	118	30.6
(j) You agree that permanent teeth are crucial for quality of life.	**Agree**	**372**	**96.6**
	Disagree	13	3.4

Bold letters indicate "most responses".

Negative statements are shown in shaded boxes.

A high percentage of respondents (96.6%) strongly supported the belief that permanent teeth are essential for a high quality of life, that dental accidents require immediate proper first aid without delay, and that tetanus vaccination should be considered. However, only 22.3% of respondents reported sufficient confidence in their ability to manage dental emergencies. Accordingly, over 90% of respondents agreed that teachers should receive training in dental injury management.

Discussion

The prognosis of TDIs depends on appropriate emergency management and early professional treatment. Previous research has found that school teachers frequently encounter dental injuries in children at school and consider knowledge of TDI management crucial (16).

In our study, only about one-third recognized that trauma might impact the head, and just 14% knew they should follow up with children 2-3 days after the incident, which is interpreted as most school teachers showing insufficient knowledge regarding the evaluation of the neurological effects following TDIs. According to a study in the United Kingdom, a force strong enough to fracture, intrude, or avulse a tooth in a child is strong enough to cause cervical spine or intracranial damage, potentially leading to physical disability, seizure disorders, and developmental delays in children (17).

However, it is encouraging that 74.5% of respondents could recognize whether a damaged front tooth in a 9-year-old child is primary or permanent, which is critical in determining the emergency treatment and prognosis of the tooth. This finding aligns with a study in Karnataka, India, where 72.4% of teachers could distinguish between deciduous and permanent teeth (18).

Our study found that most teachers had a satisfactory understanding of emergency management for fractured teeth: 45.5% recognized the importance of preserving and taking the tooth fragment to the dentist. This contrasts with a study conducted in South Jaipur in 2016, where only 1.4% of participants considered it essential to save the tooth fragment for treatment (7). The availability of public health information in Thailand and the educational level of Thai school teachers (95.9% with a bachelor's degree or higher) may have contributed to greater awareness of this issue compared to those in the study conducted in India.

The prognosis of avulsed teeth depends on immediate replantation in the alveolar socket or storage in an appropriate medium and rapid transportation to a dentist (19). Unexpectedly, a significant percentage of teachers (38.4%) believed there was no reason to search for an avulsed tooth as it was of no benefit for further treatment. Moreover, only 31.9% knew the appropriate time to replace the avulsed tooth back to the socket. These findings were consistent with research by Prasanna et al., who found that only 23% of teachers had knowledge of managing tooth avulsion injuries (20). Most previous research indicated that teachers recommended referring patients with avulsed tooth injuries to a dentist without taking any initial action. Additionally, our study found that about half of the respondents provided inaccurate answers or admitted no knowledge regarding the appropriate storage medium for the avulsed tooth. Similar to the

South Jaipur study, only 20.4% of participants could correctly select a suitable storage medium for tooth avulsion (7). It is well-accepted that avoiding drying time (less than 30 minutes) and storing in a suitable medium can facilitate the survival of the periodontal ligament cells, resulting in a high success rate for tooth avulsion treatment (21,22).

The study found no association between overall knowledge scores and school teachers' characteristics. However, specific characteristics, such as age, work experience, and type of school, were associated with particular knowledge topics on the management of TDIs. For example, age was significantly associated with knowledge scores on permanent tooth identification, storage media, and replantation time ($p = 0.019$, 0.002, and 0.006, respectively). Work experience was associated with knowledge on replantation time ($p = 0.003$). Additionally, the type of school was associated with knowledge regarding the neurological impact, permanent tooth identification, and emergency management of fractured teeth. These findings are helpful for designing focused TDI management programs.

Regarding attitudes towards TDI management, 86.0% believed they had a role and responsibility in helping students with dental accidents. This positive attitude could promote effective TDI management in schools. Contrarily, a survey from Lithuania found that most respondents mistakenly believed that only professionals could provide effective emergency management in cases of TDI (23). In the present study, 77.7% of teachers did not have enough confidence in TDI management, while 89.4% considered dental accidents urgent situations requiring immediate proper first aid. Moreover, over 90% of teachers were enthusiastic about receiving further training in dental injury management. Teachers at elementary schools in Sakaeo province demonstrated a positive attitude towards TDI management.

A study from Brazil reported that the teachers who received prior knowledge were more likely to actively search for a tooth fragment in the event of a crown fracture and for a lost tooth in the case of avulsion (24). Similarly, a study from South India found that teachers' confidence in administering first aid was significantly associated with prior training (25). Emergency management training programs should incorporate TDI management to provide teachers with the necessary skills to effectively respond to such situations. This would promote knowledge regarding preventative measures and the use of appropriate safety gear, such as properly fitted mouthguards, face cages, and helmets during sports and leisure activities that provide a possible risk of unexpected facial impact (26).

Our study found that school teachers in rural areas of Thailand like Sakaeo province understand the importance of first aid in TDIs and are willing to support school policies or public health prevention programs on this issue.

Although this study successfully achieved its intended objectives, the results are based on a restricted sample size. Further studies to evaluate the knowledge and attitudes towards emergency management of TDIs in other regions would provide a broader perspective. The present study was limited by its focus only on school teachers. Future research should aim to evaluate the understanding of TDI management among

the general public, parents, and medical professionals. This would aid in identifying any gaps in knowledge within society. Additionally, a study in Brazil revealed an association between a teacher's dental trauma experience and their knowledge of TDI management. Therefore, further research is necessary to clarify the extent of teachers' experiences in this area (27).

Conclusion

Our study indicates that elementary school teachers in Sakaeo province have insufficient knowledge about the management of TDIs, while their attitudes are generally positive. This study suggests that an educational program that focuses on specific topics related to TDI management should be a mandatory component of the public policy curriculum for elementary school teachers. The study further asserts that such a curriculum would be beneficial and useful.

Acknowledgments

We owe gratitude to all staff from the Khlonghad Hospital for their kind coordination with the elementary schools. We are thankful to Associate Professor Dr. Chulaluk Komoltri for her statistical advice and to Assistant Professor Dr. Ariya Chantaramanee for providing valuable suggestions for discussion.

References

1. Glendor U, Halling A, Andersson L, Eilert-Petersson E. Incidence of traumatic tooth injuries in children and adolescents in the county of Västmanland, Sweden. Swed Dent J. 1996;20(1-2):15-28.

2. Singh G, Garg S, Damle SG, Dhindsa A, Kaur A, Singla S. A study of sports related occurrence of traumatic orodental injuries and associated risk factors in high school students in north India. Asian J Sports Med. 2014;5(3): e22766. doi: 10.5812/asjsm.22766.

3. Razeghi S, Mohebbi SZ, Gholami M, Mashayekhi M, Maraghehpour B, Rahnama E. Effect of two educational interventions on primary school teachers' knowledge and self-reported practice regarding emergency management of traumatic dental injuries. BMC Oral Health. 2019; 19(1):130. doi: 10.1186/s12903-019-0823-4.

4. Dauparė S, Narbutaitė J. Primary school teachers' knowledge and attitude regarding traumatic dental injuries. J Indian Soc Pedod Prev Dent. 2020;38(3):216-21.

5. Siddiqui AA, Alhobeira HA, Altamimi YS, Al-Amer NS, Alsaleh MK, Mirza AJ. Dental trauma: School teachers' understanding of handling the situation. Int J Contemp Med Res. 2017;4(2):512-4.

6. Pacheco LF, Filho PFG, Letra A, Menezes R, Villoria GEM, Ferreira SM. Evaluation of the knowledge of the treatment of avulsions in elementary school teachers in Rio de Janeiro, Brazil. Dent Traumatol. 2003;19(2):76-8.

7. Nirwan M, Syed AA, Chaturvedi S, Goenka P, Sharma S. Awareness in primary school teachers regarding traumatic dental injuries in children and their emergency management: a survey in South Jaipur. Int J Clin Pediatr Dent. 2016;9(1):62-6.

8. Dorney B. Inappropriate treatment of traumatic dental injuries. Australian Endodontic Journal. 1999;25(2):76-8.

9. Malikaew P, Watt RG, Sheiham A. Prevalence and factors associated with traumatic dental injuries (TDI) to anterior teeth of 11-13 year old Thai children. Community Dent Health. 2006;23(4):222-7.

10. Fuangtharnthip P, Sakulsak K, Raksatcha N, Visanuyothin N, Menasuta T, Khongpreecha T. Readiness for emergency dental trauma in secondary schools in Bangkok. J Jpn Assoc Dent Traumatol. 2022;18(1)(45):45-50.

11. Uakarn C, Chaokromthong K, Sintao N. Sample size estimation using Yamane and Cochran and Krejcie and Morgan and Green formulas and Cohen statistical power analysis by G* power and comparisons. Apheit Int J. 2021;10(2):76-88.

12. Bayram M, Koruyucu M, Seymen F. Assessment of knowledge among public and private elementary school teachers in dental trauma management. J Dent Res. 2017;5(1):9-15.

13. Jokic NI, Kristic J, Cicvaric O, Simunovic-Erpusina M, Stanfel D, Bakarcic D. Preschool teachers' knowledge and attitudes about dental trauma in Rijeka, Croatia: a cross-sectional study. Journal of Oral Research. 2021;10(4):1-7.

14. Almulhim B. Knowledge and Awareness of School Teachers Regarding Emergency Management of Tooth Avulsion in the Kingdom of Saudi Arabia: A Cross-Sectional Study. The Open Dentistry Journal. 2022;16(1). doi: 10.2174/18742106-v16-e2112231.

15. Kara S, Crosswell H, Forch K, Cavadino A, McGeown J, Fulcher M. Less than half of patients recover within 2 weeks of injury after a sports-related mild traumatic brain injury: a 2-year prospective study. Clinical Journal of Sport Medicine. 2020;30(2):96-101.

16. Arikan V, Sönmez H. Knowledge level of primary school teachers regarding traumatic dental injuries and their emergency management before and after receiving an informative leaflet. Dent Traumatol. 2012;28(2):101-7.

17. Davis MJ, Vogel L. Neurological assessment of the child with head trauma. ASDC J Dent Child. 1995;62(2):93-6.

18. Bhandary S, Shetty S. Knowledge of physical education teachers regarding dental trauma and its management in Karnataka. Int J Res Dent. 2014;4(1):20-31.

19. Day P, Gregg T. Treatment of avulsed permanent teeth in children - UK National Clinical Guidelines in Paediatric Dentistry, British Society of Paediatric Dentistry. BSPD Clinical Guidelines. 2012.

20. Prasanna S, Giriraju A, Narayan NL. Knowledge and attitude of primary school teachers toward tooth avulsion and dental first aid in Davangere city: a cross-sectional survey. Inter J Clin Pediatr Dent. 2011;4(3):203-6.

21. De Brier N, O D, Borra V, Singletary EM, Zideman DA, De Buck EB, Jason C Berry DC, Carlson JN. Storage of an avulsed tooth prior to replantation: A systematic review and meta-analysis. Dent Traumatol. 2020;36(5):453-76.

22. Osmanovic A, Halilovic S, Kurtovic-Kozaric A, Hadziabdic N. Evaluation of periodontal ligament cell viability in different storage media based on human PDL cell culture experiments—A systematic review. Dent Traumatol. 2018;34(6):384-93.

23. Antipovienė A, Narbutaitė J, Virtanen JI. Traumatic dental injuries, treatment, and complications in children and adolescents: a register-based study. Eur J Dent. 2021;15(3):557-62.

24. Kneitz FB, Scalioni FAR, Tavares LCD, Campos MJdS, Carrada CF, Machado FC. Elementary school teachers' knowledge and attitudes toward emergency management of traumatic dental injuries. Braz Oral Res. 2023;37: e073. doi: 10.1590/1807-3107bor-2023.

25. Joseph N, Narayanan T, Bin Zakaria S S, Venugopal Abhishek N, Belayutham L, Mihiraa Subramanian A, Gopakumar KG. Awareness, attitudes and practices of first aid among school teachers in Mangalore, South India. J Prim Health Care. 2015;7(4):274-81.

26. Supraja KK, Poorni S, Suryalakshmi V, Duraivel D, Srinivasan MR. Knowledge, attitude, and practice of Chennai school teachers on traumatic dental injuries management–A cross-sectional study. Journal of Conservative Dentistry. 2021;24(4):364-8.

27. Antunes LAA, Rodrigues AS, Martins AMdC, Cardoso ES, Homsi N, Antunes LS. Traumatic dental injury in permanent teeth: knowledge and management in a group of Brazilian school teachers. Dental traumatology. 2016;32(4):269-73.

Corresponding author:
Assoc.Prof.Dr. Pornpoj Fuangtharnthip
Faculty of Dentistry, Mahidol University, 6 Yothi Road, Ratchathewi, Bangkok, 10400, Thailand.
Tel: (662) 200 7853
E-mail: pornpoj.fun@mahidol.ac.th

The Remineralization Effect of Theobromine and CPP-ACP on Enamel of Primary Molars

Krairavee Theeranut[1] Varisara Sirimaharaj[2*] Sithichai Wanachantarak[3]

Abstract

Background: Studies have investigated remineralization process utilizing non-fluoridated agents, however the efficacy of theobromine and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) remain relatively scarce, especially in primary teeth.

Objective: This study aimed to investigate the remineralization effects of theobromine and CPP-ACP on enamel of primary molars under carious simulation condition.

Methods: Extracted forty-five primary molars were collected. The crown was divided into 4 pieces. Three samples from each crown were randomly assigned to one of three treatment groups: deionized water (control), theobromine, and CPP-ACP for microhardness test and another sample was randomly used for ultrastructure and Energy-Dispersive X-ray Spectroscopy (EDS) evaluation. Microhardness testing was applied before and after pH cycling for all three groups (n = 15 each). Ultrastructure and EDS analyzed were performed on samples from four groups: untreated, deionized water, theobromine, and CPP-ACP (n = 10 each). The 7 days pH cycling process was employed to simulate a caries model. Data were described and compared statistically.

Result: After pH cycling, the microhardness of enamel decreased in all 3 groups. The theobromine group did not exhibit significant changes after pH cycling (220.72 ± 24.75 VHN from 275.84 ± 25.34) compared to deionized water (163.21 ± 28.08 from 275.18 ± 27.71 VHN) and CPP-ACP (184.74 ± 44.55 from 277.29 ± 28.48 VHN) groups. Theobromine and CPP-ACP groups showed mineral precipitation covered demineralized enamel surface, as well as deionized water group. The Wt.% of Ca and P elements in theobromine group (Ca 39.54 ± 4.11 and P 18.38 ± 1.61) were not significantly different from untreated enamel (43.33 ± 5.48 and 19.66 ± 1.70) while CPP-ACP (Ca 36.37 ± 3.77 and P 17.13 ± 1.71) and deionized water groups (Ca 36.88 ± 4.67 and P 17.24 ± 2.10) showed a significant reduction ($p < 0.05$).

Conclusion: Under the carious simulation conditions, theobromine might induce remineralization on demineralized enamel by increased Ca and P elements concentration and improved the microhardness while CPP-ACP did not show significant change from the deionized water group.

Keywords: Theobromine, CPP-ACP, enamel surface microhardness, SEM, EDS, primary molars

Received Date: Aug 13, 2024
Revised Date: Oct 18, 2024
Accepted Date: Oct 24, 2024

[1]Candidate of master's degree Program in Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
[2]Department of Orthodontics and Pediatric Dentistry, Faculty of Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
[3]Department of Oral Biology and Diagnostic Sciences, Faculty of Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
*(*corresponding author)*

Introduction

Dental caries is a multifactorial disease caused by an imbalance between demineralization and remineralization processes affecting enamel and dentin (1). The elements of crystal hydroxyapatite (HA), including calcium, phosphate play an important role in regulating demineralization and remineralization processes (2). When the pH of the oral environment drops below 5.5, demineralization occurs, resulting in a reduction in enamel hardness (3). The remineralization can effectively arrest the progression of active early-stage (non-cavitated) caries when the pH increased.

Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has the capacity to promote the remineralization of subsurface enamel lesions and inhibit demineralization (4). The ability of CPP is to bind and stabilize calcium and phosphate in an amorphous form through a cluster of sequence phosphoryl residues in addition to its ability to adhere to dental biofilm and tooth enamel (4). CPP-ACP enhances enamel microhardness by creating a state of supersaturation with calcium and phosphate ions (5). However, CPP-ACP has a significant drawback, it has been reported to potentially cause anaphylactic reactions in some patients (6).

The natural substances might also be used as alternative approaches for remineralization due to their relative safety and affordability (3). Theobromine, an acrid alkaloid derived from the cacao plant, may contribute to future dental health, Theobromine is available in form of crystallized, white in appearance, odourless and hydrophilic powder (7). Previous research has established that theobromine solution showed an efficacy on strengthen enamel and prevent cavities (8). Application of theobromine enhances the microhardness of dental enamel that underwent demineralization comparable to those achieved with fluoride (9). Theobromine which also found in chocolate demonstrates superior remineralization and enamel hardening properties compared to fluoride when tested on human-extracted molar enamel (10) and as evidenced by the results of EDS (11). The application of 200 mg/L theobromine gel on either bovine incisor crown (3) or human premolar (12) has been shown to improve enamel hardness. Theobromine has been employed as a food ingredient, a pharmaceutical agent/product in various medical and dental applications. Examples of theobromine used in dentistry include its application in toothpaste formulations (Theodent^{TM}) Theobromine-containing dentifrice, Theodent^{TM}, also demonstrated a remineralization (13). Nassar et al (2021) (14) suggested that combining theobromine with fluoride did not enhance mineral uptake.

Studies by Ten cate et al (15) had shown that after demineralization the enamel surface hardness could be improved by remineralization during pH cycling. However, it is important to note that none of the studies compared theobromine with CPP-ACP in the primary teeth. Therefore, the aims of this study were to investigate the remineralization effects of theobromine and CPP-ACP on surface microhardness, ultrastructural changes, and EDS analysis of the enamel of primary molars subjected to 7 days of pH cycling.

Materials and Methods

The experimental protocol, including the use of human tissue, was approved by the Human Experimentation Committee of the Faculty of Dentistry, Chiang Mai University (No.15/2022). Forty-five extracted primary molar teeth were

collected from healthy children, aged 6 to 12 years at the Pedodontics Clinic, Faculty of Dentistry, Chiang Mai University, Thailand, with parental consent. The teeth were extracted due to prolonged retention or an inability to undergo pulpal treatment and had intact buccal and lingual surface. The samples were cleaned and stored in a 0.1% (w/v) thymol solution at 4°C until use.

The sample size calculation was calculated using the following formula:

$$n = \frac{\left(z_{\frac{\alpha}{2}} + z_{\beta}\right)^2 \left(\sigma_1^2 + \sigma_2^2\right)}{e^2}$$

Where 95% confidence interval, 80% power, 0.9 as a margin of error, standard deviation 1 = 0.4, and standard deviation 2 = 0.7. A sample size of 6 samples was indicated as the minimum to reveal statistical significance among groups. The sample size calculation was based on previous studies (3,16,17). A total of 45 primary molar teeth, 15 per group (for microhardness test), and 10 per group (for ultrastructure and EDS test) were utilized.

After the roots were cut off at the CEJ level, the crowns were sectioned into four pieces by cutting along the mesiodistal and buccolingual directions using a cylinder diamond bur (Intensive®, Swiss Dental Product, Switzerland) mounted on high-speed airotor handpiece under water cooling. One section from each tooth specimen (totally 45 samples) was used for the microhardness test. Forty sections were randomly selected from the 45 tooth samples and examined under scanning electron microscope (SEM : VEGA3, Tescan, Kohoutovice, Czech Republic) to assess ultrastructural changes, and EDS (Ultim Max 40, Oxford Instruments, High Wycombe, UK) analysis was performed (Fig.1).

For microhardness test, the 45 samples were randomly divided into 3 groups (n = 15 each) for testing 3 solutions: deionized water, Theobromine and CPP-ACP. The Vickers hardness tester (STARTECH SMV-1000, Guiyang Sunproc International Trade Co., Ltd., Guiyang, China) was used to measure the microhardness of the enamel surface before and after pH cycling. The red nail varnish (Maybelline New York, Thailand) was applied to the enamel surface to define a testing area and prevent excessive polishing. Monitoring the nail varnish allowed us to ensure that an area of 1 × 1 mm^2 of enamel was exposed. When the nail varnish was removed to achieve the desired area, polishing was promptly ceased.

Position the enamel surface, with the nail varnish coating facing downward in PVC circular block (20 mm in diameter, 4 mm height), and embedded in the clear liquid epoxy resin (Rungart, Thailand). After the epoxy resin cured, polished the nail varnish coated enamel surface with 800-grit silicon carbide abrasive paper until an approximately 1 x 1 mm^2 flat enamel was exposed. Further polishing was performed with 1000, 1500, and 2000 grit papers for an additional 5 seconds each under running water to achieve a smooth enamel surface. Additionally, before testing the Vicker surface microhardness, all specimens were examined under the light microscope at 40x magnification to confirm the condition of the enamel surface.

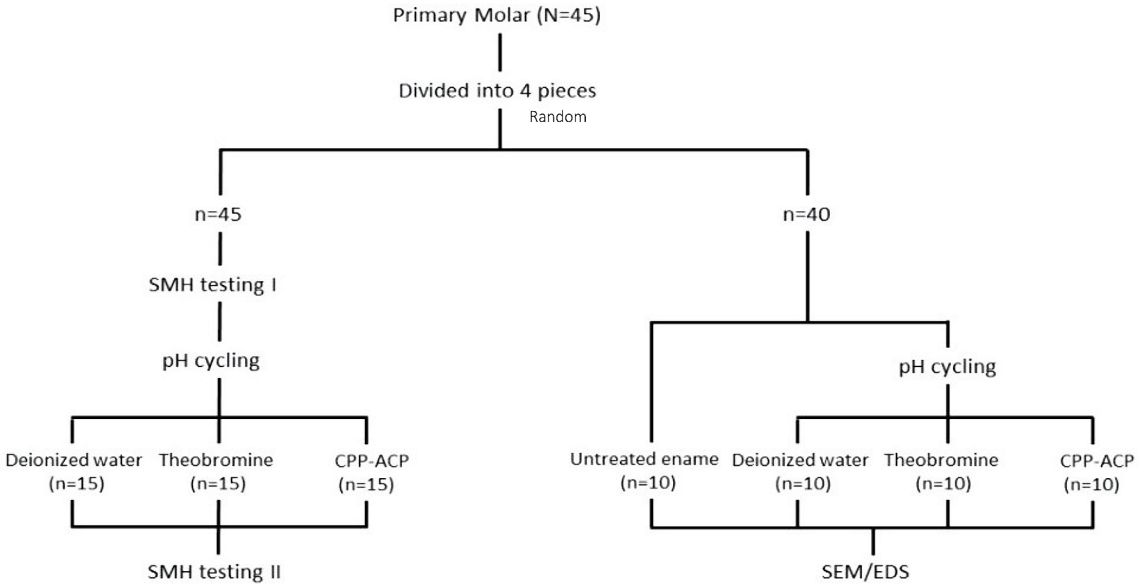

Fig.1 Forty-five primary molars were divided into 4 pieces. One piece from each tooth was randomly allocated into 3 groups for surface microhardness test (SMH). Another piece from each tooth was divided into 4 groups for SEM and EDS analysis.

A Vickers diamond indenter was positioned on the polished flat enamel surface (1 x 1 mm^2) under a x40 objective lens and applied a 100 grams load for 15 seconds. Five indentations were placed randomly along occluso-gingival direction with approximately 100 microns apart. The locations of the indentations varied between pre and post-treatment measurements, and consistently within the designated 1 × 1 mm^2 flat surface. Any samples showing cracks after indentation were excluded from the analysis. The Vickers hardness number (VHN) was calculated from each indentation by using formular:

$$VHN = 1.8544 \frac{F}{D^2}$$

Whereas F in the formular stands for the load in kilogram force (kgf), while D represents the mean of 2 diagonals lengths measured in millimeters (mm) using built-in scaled in microscope.

After microhardness test, the specimens were processed through an artificial caries model utilizing a slightly modified pH cycling method from Featherstone et al (1986) (18). The demineralized and remineralized conditions were cycling for 7 days. For one cycle which involved 24 hour, the specimen was immersed in 0.5 ml of demineralization solution composed of 2.0 mmol/L (0.437 g/L) calcium nitrate ($Ca(NO_3)_2.4H_2O$), 2.0 mmol/L (0.2722 g/L) potassium phosphate (KH_2PO_4), and 0.075 mol/L (4.3 ml) acetic acid (CH_3COOH) at pH 4.8 (pH adjusted with NaOH) slightly modified from Ten Cate J and Duijsters P (19) for 4 hours. The specimen was then immersed in 5 ml of either distilled water (Group A), 5 ml of 200mg/L of theobromine (3,7-Dimethylxanthine: (Sigma Aldrich, Hamburg, Germany) (Group B) or applied onto the test surface with a single fold (approximately 0.01 g) of CPP-ACP paste (GC Tooth Mousse Plus® contains CPP-ACP RECALDENTTM, GC America,

USA) (Group C) for 5 minutes (according to the manufacturer instruction). Finally, all specimen immersed in the 5 ml artificial saliva contained 1.5 mmol/L (0.3542 g/L) Calcium nitrate $Ca(NO_3)_2.4H_2O$, 0.9 mmol/L (0.1224 g/L) Potassium phosphate (KH_2PO_4), 150 mmol/L (9.692 g/L) potassium chloride (KCl) and 20 mmol/L (4.28 g/L) cacodylate buffer (NaCacodylate) at a pH of 7.2 (pH adjusted with citric acid) for 20 hours at 37°C. Before and after immersing the specimen in each solution, it was rinsed with deionized water for 10 seconds each time. All solutions used in the pH-cycling process were freshly prepared and replaced daily to ensure consistency and accuracy throughout the experiment. After finished 7 days pH cycling, the specimens underwent microhardness testing once again.

For ultrastructure and EDS analysis, the 40 samples were randomly divided into 4 groups (n = 10 each): a negative control (untreated group) and three test groups (deionized water as a positive control for pH cycling, Theobromine and CPP-ACP).

The specimens in the deionized water, theobromine and CPP-ACP groups were undergoing 7 days pH cycling of demineralization and remineralization similar to the microhardness test specimens described above. These specimens, along with the untreated enamel group serving as the negative control, were prepared for examination SEM EDS. At the end of the pH cycling, each specimen was rinsed in deionized water for 10 seconds. Then, each specimen which processed for SEM was placed in a screw cap containing deionized water and cleaned in an ultrasonic cleaner for 10 minutes to eliminate any debris from the tooth surface. Each specimen was dried by storing it in a desiccated jar with silica gel at room temperature for 3 days and then gold-coated using a gold sputtering machine (SPI-Module^{TM} Sputter Coater, West Chester, Pennsylvania, USA). The specimens were examined at x10,000 and x30,000 magnification. Nine major elements: Calcium (Ca), Phosphorus (P), Carbon (C), Sodium (Na), Manganese (Mg), Fluorine (F), Chlorine (Cl), Oxygen (O) and Nitrogen (N) as well as the proportion of Ca and P, were measured using EDS as weight percent (Wt. %).

The mean microhardness of the three groups at baseline and after treatment was compared by two-way repeated ANOVA and multiple comparison tests. The ultrastructure morphologies of enamel surfaces for all four groups were descriptively compared at x10,000 and x30,000 magnification, while the weight percentage (Wt. %) of major elements was quantitatively compared using one-way ANOVA. The p value less than 0.05 was considered statistically significant.

Results

After undergoing pH cycling for 7 days, the microhardness of all samples declined significantly. The deionized water groups showed the greatest reduction in microhardness, while the theobromine group showed the least reduction. When evaluating the ability to protect the enamel surfaces from demineralization from pH cycling, theobromine demonstrated a greater capacity to restore microhardness than CPP-ACP ($p < 0.05$). The data of microhardness was summarized in Table 1.

Table 1. Mean microhardness of the test groups at baseline (before treatment) and after 7 days pH cycling with remineralizing agents, analyzed by two-way repeated ANOVA using multiple comparison with Bonferroni test.

Group	Vickers hardness number (Mean ± SD)		
	Baseline	At day 7 post pH cycling	Mean difference
Deionized water	275.18 ± 27.71	163.21 ± 28.08	111.98 ± 39.07
Theobromine	275.84 ± 25.34	220.72 ± 24.75	55.12 ± 39.20
CPP-ACP	277.29 ± 28.48	184.74 ± 44.55	92.55 ± 64.68

In SEM images, untreated enamel showed a homogenous surface (Fig. 2A), while the enamel subjected to pH cycling and stored in deionized water showed enamel porosity, forming a fish scale like characteristic on the surface (Fig. 2B). Both the theobromine and CPP-ACP groups (Fig. 2C and 2D, respectively) showed evidence of remineralization, with deposition of amorphous calcium phosphate on demineralized enamel. At higher magnification of x30,000, there were fine granules were found on the surface treated with theobromine while larger granules of amorphous calcium phosphate coating on the surface treated with CPP-ACP. (Fig. 2E and 2F, respectively)

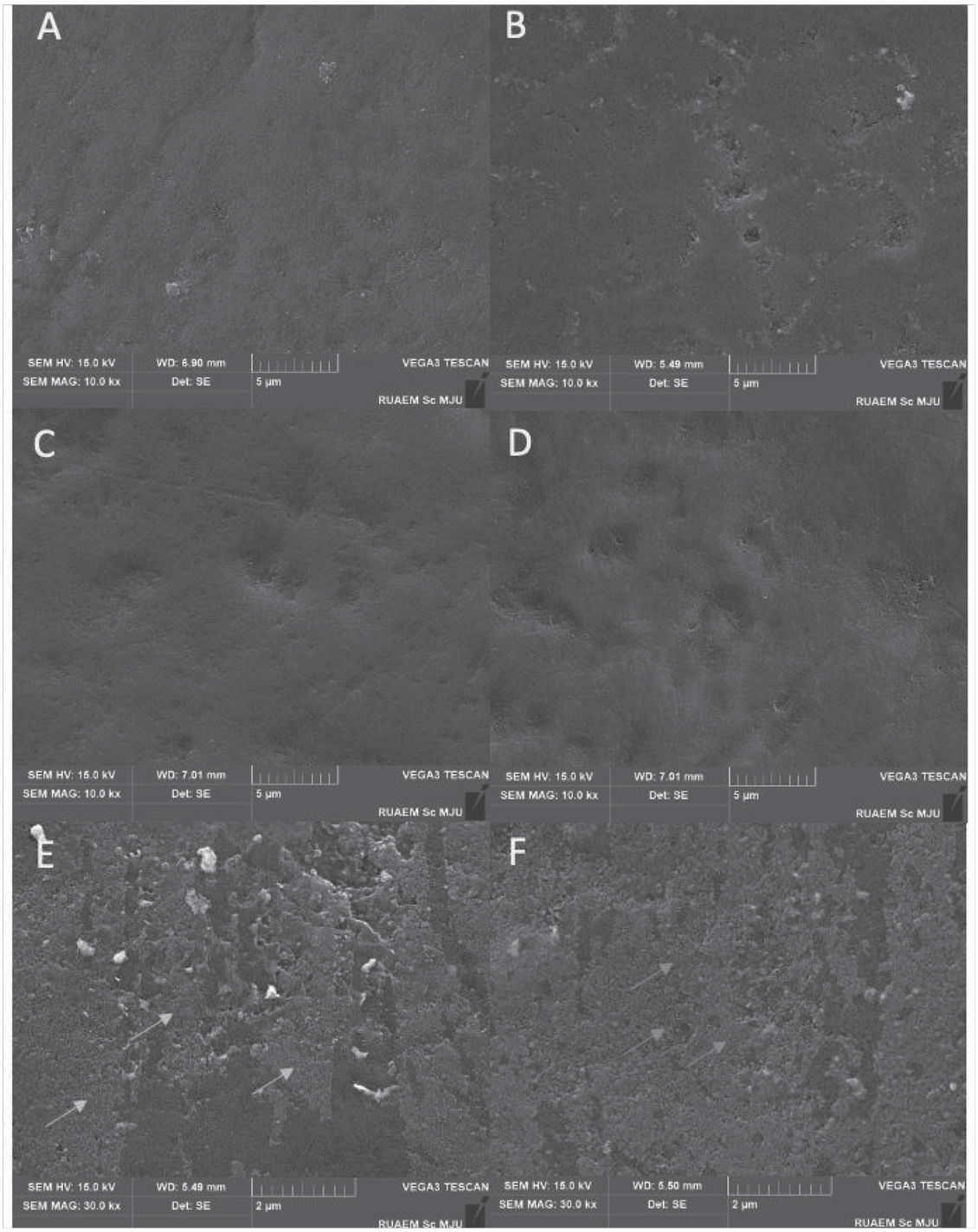

Fig.2 The SEM images of enamel surface taken at x10,000 magnification; untreated enamel (A),deionized water (B), Theobromine (C) and CPP-ACP (D). The SEM images of enamel surface taken at x30,000 magnification; Theobromine (E) with the yellow arrows to show finer granules, and CPP-ACP (F) with the orange arrows to show larger granules.

The results from the EDS analysis suggested that the weight percentages of calcium (Ca) and phosphorus (P) in the deionized water group which represented the enamel that underwent pH cycling was significantly reduced ($p < 0.05$) compared to the untreated enamel. Two remineralization groups showed different results: the theobromine group showed no significant difference from untreated enamel, but CPP-ACP group still showed a significant reduction in both elements compared to the untreated enamel ($p < 0.05$). Other elements were not significant different among groups. However, the Ca/P ratio did not show significant differences among the four groups (Table 2).

Table 2. EDS analysis of the two major elements (Ca and P) and Ca and P ratio of four groups presented in weight percent (Wt. %).

Elements	EDS analysis (Wt. %)			
	Untreated	Deionized water	Theobromine	CPP-ACP
Ca	43.33 ± 5.48	36.88 ± 4.67*	39.54 ± 4.11	36.37 ± 3.77*
P	19.66 ± 1.70	17.24 ± 2.10*	18.38 ± 1.61	17.13 ± 1.71*
Ca/P ratio	2.21 ± 0.11	2.14 ± 0.05	2.15 ± 0.05	2.12 ± 0.06
O	27.95 ± 5.75	32.65 ± 6.07	33.12 ± 6.21	34.69 ± 4.79
C	7.05 ± 1.73	10.84 ± 9.23	7.21 ± 2.10	9.37 ± 5.12
Na	0.39 ± 0.08	0.39 ± 0.08	0.39 ± 0.09	0.37 ± 0.07
Mg	0.17 ± 0.17	0.11 ± 0.12	0.17 ± 0.12	0.12 ± 0.15
F	0.39 ± 0.16	0.49 ± 0.24	0.48 ± 0.21	0.56 ± 0.23
Cl	0.55 ± 0.17	0.45 ± 0.10	0.44 ± 0.09	0.44 ± 0.05
N	0.13 ± 0.4	0.61 ± 0.15	0.00	0.3 ± 0.79

*Indicated significant different $p < 0.05$ from other groups.

Discussion

The pH cycling process involves repeatedly alternating demineralization and remineralization to simulate the progression of dental caries (17). The research by L.J. Wang et al. (2006) (20) demonstrated that the rate of demineralization in developing lesions varies with the direction and location of acid attacks, with primary enamel exhibiting a higher vulnerability to dissolution compared to permanent enamel. In this study, the demineralization and remineralization solutions were modified from Featherstone et al. (1986) (18) for use with primary teeth in order to reduce the rapid decalcification of dental hard tissue. Featherstone's pH-cycling model, commonly employed to induce artificial caries in permanent enamel, may have limitations when applied to primary teeth, which are more vulnerable to acid dissolution (20). The higher vulnerability of primary enamel to demineralization leads to increased lesion progression compared to permanent enamel (20,21). To address these challenges, a pilot

study was conducted to modify the pH conditions, adjusting the demineralized pH from 4.3 to 4.8 and the remineralized pH from 7.0 to 7.2. Additionally, the duration of acid challenges was reduced from 6 hours to 4 hours, and the cycle length shortened from 14 days to 7 days. Consistent with findings by Kargul et al (2012) (16) after primary tooth specimens were processed through 7 days of pH cycling, the microhardness of the enamel surface decreased, as shown in the deionized water group.

The use of both CPP-ACP and theobromine agents resulted in a lesser reduction in enamel hardness, indicating remineralization of artificial caries caused by pH cycling. Corresponding with Syafira et al (2013) and Amaechi et al (2013) (8, 10) studies, theobromine showed a smaller reduction in enamel microhardness compared to CPP-ACP, suggesting a higher remineralization potential. The application of theobromine gel significantly increased microhardness more than CPP-ACP in bovine teeth (3). Furthermore, Amaechi et al (2015) (22) suggested that theobromine enlarges hydroxyapatite crystal size, preventing dissolution and aiding in caries prevention. In contrast, Kargul et al (2012) (23) suggested that theobromine and CPP-ACP had equivalent remineralization effects and increased enamel surface microhardness in human third molar stored in a demineralized solution.

The SEM images of the deionized water group, serving as the control for pH cycling, showed fish scale characteristic as the mineral on the surface and enamel was dissolved, similar to the results from other studies (17,24). This is one of the three basic etching patterns for human enamel after exposure to acid (24). Both theobromine and CPP-ACP showed evidence of remineralization, characterized by the deposition of calcium phosphate on demineralized enamel and the disappearance of fish scale pattern. At higher magnification (x30,000), fine granules of amorphous calcium phosphate were presented in the theobromine group, whereas larger granules were found in CPP-ACP group. Similarly, SEM images from enamel treated with 200 mg/L theobromine solution for 5 minutes showed the highest quantity of globular precipitates on the surface (16).

The result from EDS analysis suggested that Ca and P elements levels in the theobromine-treated enamel were not significantly different from untreated enamel. In contrast, the Ca and P levels in the CPP-ACP treated enamel were significantly lower than in untreated enamel, suggesting that theobromine has higher remineralization potential than CPP-ACP. It possible that the amorphous calcium phosphate precipitation caused by CPP-ACP was dissolved during the demineralization process of pH cycling (25). Similar to the EDS study by Farhad et al (2021) (26), theobromine showed higher calcium deposition than artificial saliva and 0.05% sodium fluoride, while hydroxyapatite crystals produced by CPP-ACP are amorphous and do not generate HA crystals in various sizes and forms (3).

The small theobromine ($C_7H_8N_4O_2$) molecule can penetrate into the microchannels of hydroxyapatite crystals and bind to the crystals by replacing of crystalline ions (26). Its higher electronegativity attracts calcium and phosphate ions, forming of a novel hydroxyapatite crystal known as theobromine apatite [$Ca_{10}(PO_4)_6(OHC_7H_8N_4O_2)$] (26). This may clarify why calcium levels in the theobromine group are higher than those in the other treatment groups. This new apatite increased both crystallite size and crystallinity by the growing hydroxyapatite

in an apatite-forming system with a sufficient quantity of partially alkylated xanthine (10). Additionally, the new crystal apatite formed from theobromine is four times larger (2 μm) than normal hydroxyapatite crystals (0.5 μm) (22). Larger crystal sizes may result in a slower dissolution rate in acid compared to smaller crystal sizes. Smaller crystals require a much greater surface area for reactivity (28).

The application of CPP-ACP induces precipitation of various sizes and forms of amorphous calcium phosphate crystals ($Ca_9(PO_4)_6$) (29) on the enamel surface instead of HA crystal formation (3), which easily breaks down. After exposure to acid, H+ ions break down the amorphous calcium phosphate into calcium and phosphate ions ultimately forming CaHPO4 (30). This corresponds with the EDS analysis results, which showed that the Ca and P levels in the CPP-ACP group were significantly lower than those in the untreated enamel and theobromine groups, and the result from microhardness test of CPP-ACP group showed significant reduction compared to the untreated enamel group though less reduction than theobromine group.

According to a study from Karlinsey et al (2012) (31), an increase in enamel surface porosity is related to a reduction in enamel surface microhardness. Calcium and phosphate ions play an important role in stopping demineralization and promoting remineralization, thereby strengthening the crystal and restoring the microhardness of the structures (32). A naturally extracted substance such as theobromine may be used for remineralization by promoting the formation of larger hydroxyapatite crystals, especially in enamel, and retarding the progression of dental caries.

Theobromine is considered safe for use in young children due to its negligible negative effects and high safety dose response (27). Therefore, it is a suitable option for children who may be more prone to swallow toothpaste or other dental products. A limitation of our study is that the in vitro pH-cycling model does not fully replicate the oral conditions; thus, a clinical trial would be advisable for the future research.

Conclusion

This *in vitro* pH cycling model suggests that theobromine might induce some degree of remineralization by increasing microhardness and the concentration of calcium and phosphate on the surface of demineralized primary enamel. In contrast, CPP-ACP did not show significant changes compared to the deionized water group, which is consistent with the ultrastructural observations from SEM.

Acknowledgement

The authors sincerely appreciate the support from the Research Center at the Faculty of Dentistry, Chiang Mai University, Thailand, for their generous provision of scientific equipment and the expertise of a statistical consultant. We greatly appreciate the assistant from Advanced Scientific Instruments Unit, Faculty of Science, Chiang Mai University, Thailand, for providing Vicker Microhardness tester. We also acknowledge the invaluable guidance from the Science and Technology Service Center at the Faculty of Science, Maejo University, Thailand, which was instrumental in our SEM and EDS measurements.

Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References

1. Ritter AV, Eidson RS, Donovan TE. Dental caries: etiology, clinical characteristics, risk assessment, and management. Sturdevant's Art & Science of Operative Dentistry. 6thed. ELSEVIER: St. Louis; 2013.

2. Fejerskov O, Kidd E. Dental caries: the disease and its clinical management. 2nded. John Wiley & Sons; 2009.

3. Yuanita T, Zubaidah N, AR MI. Enamel hardness differences after topical application of theobromine gel and Casein Phosphopeptide-amorphous Calcium Phosphate. J Conserv Dent. 2020;10(1):5-8. doi: 10.20473/cdj.v10i1.2020.5-8.

4. Al-Batayneh O. The clinical applications of Tooth Mousse TM and CPP-ACP products in caries prevention: evidence-based recommendations. Smile Dent J. 2009;4(1):8-12.

5. Cochrane N, Cai F, Huq N, Burrow M, Reynolds E. New approaches to enhanced remineralization of tooth enamel. J Dent Res. 2010;89(11):1187-97.

6. Matsui T, Naito M, Kitamura K, Makino A, Takasato Y, Sugiura S, et al. Casein phosphopeptide in cow's milk is strongly allergenic. Authorea. 2021. doi: 10.22541/au.163252091.16716154/v1.

7. Fideles SOM, Ortiz AdC, Reis CHB, Buchaim DV, Buchaim RL. Biological properties and antimicrobial potential of cocoa and its effects on systemic and oral health. Nutrients. 2023;15(18):3927. doi: 10.3390/nu15183927.

8. Syafira G, Permatasari R, Wardani N. Theobromine effects on enamel surface micro-hardness: in vitro. J Dent Indones. 2013;19(2):32-6.

9. Silva AD, Gonçalves RDS, Catão MV. Theobromine for remineralization of white spot lesions on dental enamel: A systematic review and meta-analysis. Oper Dent. 2024;49(4):376-87.

10. Amaechi B, Porteous N, Ramalingam K, Mensinkai P, Vasquez RC, Sadeghpour A, et al. Remineralization of artificial enamel lesions by theobromine. Caries Res. 2013;47(5):399-405.

11. Mohamed M, Abdelhamid A, Mohammed S, Mahmoud A. Comparison between silver diamine fluoride and theobromine solutions in remineralization of demineralized human enamel [scanning electron microscope and edex study]. Egypt Dent J. 2023; 69:1893-904. doi: 10.21608/EDJ.2023.194928.2453.

12. Sharma D, Mandlik J, Singh S, Gujarathi N, Gir R, Sharma P. In vitro evaluation of effect of theobromine gel on surface hardness of demineralized enamel, at different time exposures. Eur Chem Bull. 2023;12(8):3051-61.

13. Samatha Y, Sai V, Pranitha K, Sridhar M, Kundeti S, Ramgopal A. Determining the efficacy of three potential remineralizing agents on artificial carious lesions. J Int Oral Health. 2020;14(1):1-5. doi: 10.5005/jp-journals-10062-0063.

14. Nassar HM, Lippert F. Artificial caries lesion characteristics after secondary demineralization with theobromine-containing protocol. Molecules. 2021;26(2):300. doi: http://dx.doi.org/10.3390/molecules26020300.

15. ten Cate JM, Arends J. Remineralization of artificial enamel lesions in vitro: II. Determination of activation energy and reaction order. Caries Res. 1978;12(4):213-22.

16. Kargul B, Özcan M, Peker S, Nakamoto T, Simmons WB, Falster AU. Evaluation of human enamel surfaces treated with theobromine: a pilot study. Oral Health Prev Dent. 2012;10(3):275-82.

17. Hussein S, El-Hadadd K. Comparison between solution and gel forms of theobromine and sodium fluoride in remineralization of the demineralized hman enamel (SEM and EDXA study). Egypt Dent J. 2018;64(3):2371-80.

18. Featherstone J, O'reilly M, Shariati M, Brugler S. Enhancement of remineralisation in vitro and in vivo. Factors relating to demineralisation and remineralisation of the teeth. Oxford: IRL Press; 1986. p. 23-34.

19. Ten Cate J, Duijsters P. Alternating demineralization and remineralization of artificial enamel lesions. Caries Res. 1982;16(3):201-10.

20. Wang LJ, Tang R, Bonstein T, Bush P, Nancollas GH. Enamel demineralization in primary and permanent teeth. J Dent Res. 2006;85(4):359-63.

21. Whittaker DK. Structural variations in the surface zone of human tooth enamel observed by scanning electron microscopy. Arch Oral Biol. 1982;27(5):383-92.

22. Amaechi B, Mathews S, Mensinkai P. Effect of theobromine-containing toothpaste on dentin tubule occlusion in situ. Clin Oral Investig. 2015;19(1):109–11. doi: 10.1007/s00784-014-1226-1.

23. Kargul B, Nakamoto T, Simmons W, Falster A. Remineralization potential of theobromine, APF Gel and CPP-ACP: Pilot Study. 2012: 6th Meeting of the Pan European Region in Helsinki, Finland. [cited 2024 July] Available from: https://iadr.abstractarchives.com/abstract/per12-168171/remineralization-potential-of-theobromine-apf-gel-and-cpp-acp-pilot-study.

24. Silverstone LM, Saxton CA, Dogon IL, Fejerskov O. Variation in the pattern of acid etching of human dental enamel examined by scanning electron microscopy. Caries Res. 1975; 9(5):373-87.

25. Tung MS. Amorphous Calcium Phosphates for Tooth. Compend Contin Educ Dent. 2004;25 (9 Suppl 1):9-13.

26. Farhad F, Kazemi S, Bijani A, Pasdar N. Efficacy of theobromine and sodium fluoride solutions for remineralization of initial enamel caries lesions. Front Dent. 2021;18(9):10. doi: 10.18502%2Ffid.v18i10.6134.

27. Nakamoto T, Falster AU, Simmons Jr WB. Theobromine: a safe and effective alternative for fluoride in dentifrices. J Caffeine Res. 2016; 6(1):1-9. doi: 10.1089/jcr.2015.0023.

28. Eanes ED. Enamel Apatite: Chemistry, Structure and Properties. J Dent Res. 1979;58 (SpecIssueB):829-36.doi:10.1177/00220345790580023501.

29. Zhao J, Yu L, Sun W-B, Yang X. First detection, characterization, and application of amorphous calcium phosphate in dentistry. J Dent Sci. 2012;7:316–23. doi: 10.1016/j.jds.2012.09.001.

30. Cross KJ, Huq NL, Reynolds EC. Casein phosphopeptide-amorphous calcium phosphate nanocomplexes: A structural model. Biochemistry. 2016;55(31):4316-25.

31. Karlinsey RL, Mackey AC, Blanken DD, Schwandt CS. Remineralization of eroded enamel lesions by simulated saliva in vitro. Open Dent J. 2012;6(1):170-6.

32. Featherstone JD. The continuum of dental caries--evidence for a dynamic disease process. J Dent Res. 2004;83 Spec No C:C39-42. doi: 10.1177/154405910408301S08.

Corresponding author:
Asst.Prof.Dr. Varisara Sirimaharaj
Division of Pediatric Dentistry, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand.
Tel: (665) 322 2844
E-mail: varisara.s@cmu.ac.th, krairavee13@gmail.com

The Accuracy of Single Tooth Mini Dental Implant Placement Using Computer Assisted Surgical Guide: A Randomized Clinical Trial Comparative Study

Voravan Vorasubin[1] Weerapan Aunmeungthong[1] Pathawee Khongkhunthian[1*]

Abstract

Background: This study aimed to assess the accuracy of mini dental implant placement using conventional surgical templates (CST) versus digital (computer assisted) surgical drill guides (SDG) for single tooth prosthesis.

Materials and methods: Sixteen participants recruited according to inclusion criteria. Twenty implants (16 participants) were randomly assigned to either the CST or SDG group. Mini dental implants were placed in the lower anterior and premolar area. The positioning of implants were analyzed and compared using Planmeca Romexis™.

Results: Statistical analysis using the Shapiro-Wilk test and independent sample T-test revealed significant differences between the two groups in 3 parameters out of 10 parameters: top horizontal deviation (CST; 1.43 ± 0.77 and SDG; 0.67 ± 0.3 mm), top global deviation (CST; 1.83 ± 0.85 and SDG; 0.82 ± 0.52 mm) and angular deviation (CST; 5.53 ± 3.14 and SDG; 1.36 ± 0.7 degrees) at $p < 0.05$.

Conclusion: CST and SDG are effective for placing single-tooth mini dental implants in limited-ridge spaces, especially in the anterior and premolar regions. Their versatility allows for adaptation to the specific circumstances of each case, ultimately enhancing clinical decision-making and patient outcomes.

Keywords: Surgical Drill Guide, Randomized Clinical Trial, Single-Tooth Dental Implant, Mini Dental Implants

Received Date: Jun 17, 2024
Revised Date: Nov 03, 2024
Accepted Date: Nov 11, 2024

[1]*Center of Excellence for Dental Implantology, Faculty of Dentistry, Chiang Mai University, Chiang Mai, 50200, Thailand. (*corresponding author)*

Introduction

Dental implants have emerged as a reliable treatment option for edentulous patients, significantly enhancing their quality of life over recent decades (1,2).

According to Glossary of Dental Implantology (3), implants with diameters ranging from 3.5 to 5 mm are considered standard. Those with diameters less than 3.75 mm are often referred to as 'small', 'narrow', or 'mini' implants, although there remains some ambiguity in their classification (4). However, there's no strict rule governing the terminology of dental implants, as long as authors clearly describe the implant sizes.

Mini dental implants were originally used in orthodontic treatments as the anchorage for tooth movement. They were also used for edentulous treatments, such as single tooth restoration and implant-assisted prostheses with high survival rate (5,6). Additionally, they are categorized into two types: single-piece and two-piece. Single-piece implant have garnered interest for their suitability in narrow-ridged spaces and their reduction of complex surgical procedures. With stable occlusion and good primary stability, mini dental implants placed in areas with sufficient bone width and height can facilitate immediate loading of prostheses, showing a high survival rate of 98% at the 1-year follow-up (7).

The flapless surgical technique has gained interested for implant placement, offering greater patient comfort and satisfaction with reduced post-operative complications (8). Although, designing surgical guides and prefabricating prostheses may prolong the preparation time, flapless surgery minimizes the chair time. Notably, there's no significant difference in survival rates, complications, or marginal bone level changes between flapless and open-flap surgeries over a mean follow-up period of 21.62 months (9).

Three-dimensional implant positioning (bucco-lingual, mesio-distal, and apico-coronal) plays an important role in treatment success. Proper positioning allows implants to mechanically adapt to the host bone until secondary stability is achieved. Computer-guided surgical procedures are considered advantageous, especially for deficit alveolar ridges prone to resorption. (10,11).

The Fourth ITI Consensus Conference (2008) (12) defined the two computer technological applications in surgical implant dentistry i.e., computer-guided (static) surgery and computer-navigated (dynamic) surgery. Computer-guided surgery, utilizing static surgical templates, reproduces virtual implant positions from CT data, making it practical in dental practice where available working space is limited (13).

The adoption of computer-guided surgery has enhanced patient satisfaction and treatment acceptance compared to conventional implant placement surgery. It facilitates effective surgical time management and reduces complication rates by providing precise virtual implant positioning (14). Studies have focused on the accuracy of computer-guided surgery (13,15-22), with favorable outcomes reported, particularly for standard-size implants (15,23-25).

However, concerns remain regarding its application to mini dental implants, given the limited research in this area. Until recently, there was no research focus on the accuracy of placing single-tooth dental mini implants using a digital guide.

The objective of this randomized clinical trial is to evaluate the accuracy of single-tooth implant positioning in limited-ridge spaces, comparing conventional surgical guides with tooth-borne computer-assisted surgical guides.

The null hypothesis for this study is that there is no difference in the accuracy of implant positioning between the control group and the experimental group.

Materials and Methods

Study Approval and Registration

This randomized clinical trial study received approval from the Human Experimentation Committee, Faculty of Dentistry, Chiang Mai University no.34/2562. The study adhered to the ethical principles outlined in the World Medical Association (WMA) Declaration of Helsinki. Informed consent was obtained from all participants prior to their involvement in the study and the CONSORT 2010 checklist for reporting randomized trials was followed.

Sample Size Calculation

Sample size was calculated using mean and standard deviation (SD) of the angular deviation at the implant apex based on the results of the pilot study. The significance level (α) was set at 0.05 and power of test (1- β) was set at 80%. By the use of G*power program for sample size calculating resulted in n = 6 implants each group or n = 12 implants in total. In this study we use n = 10 implants each group and n = 20 in total. All participants should meet the criteria listed in Table 1.

Table 1. Inclusion and exclusion criteria of this study.

Inclusion criteria	Exclusion criteria
1. Patients aged 20-65 years	1. Requirement for bone or soft tissue grafting at the time of implantation
2. The site of the study has Bone height ≥11 mm and Bone width between 5-7 mm	2. Sufficient bone width for conventional size dental implant placement
3. No contraindication for minor oral surgery	3. Uncontrolled systemic disease, ASA Class III
4. No smoking or smoke less than 10 cigarettes per day during past 5 years	4. Presence of periodontal disease or periapical lesions
5. No psychosis or psychiatric disorders	5. Alcoholism or drug abuse
6. No uncontrolled bleeding disorders	6. Implant that need submersion due to stability issue
7. Never received radiotherapy around head and neck regions	7. Pregnancy or positive to pregnancy test
8. Good oral hygiene with ability to maintain adequate conditions	8. Physical or mental disorders which would effect the ability to maintain good oral hygiene
9. No history of intravenous injection of bisphosphonate	9. Patients whom not able to provide informed consent
10. Participants must agree to undergo treatment and follow-up for at least one year.	10. Conditions that would prevent completion of study participation

Examination and Randomization

Participants underwent CBCT imaging using DentiiScan© (NECTEC, Thailand). The DICOM files were retrieved for later analysis. Each participant was assigned a unique number. A blinded investigator (S.A.) used computer software to randomly assign participants to one of two groups: Group 1 (control) using Conventional Surgical Templates (CST) and Group 2 (experimental) using Surgical Drill Guides (SDG). These assignments were kept confidential from the surgeon until the implant position planning was complete. The mini dental implants planned during this step were available in two sizes: 2.7 mm in diameter with a length of 12 mm, and 3.0 mm in diameter with a length of 10 mm.

Mini Dental implants placement procedure

Both groups followed identical initial procedures, beginning with the administration of 4% articaine with epinephrine 1:100,000 for local anesthesia to ensure patient comfort during the surgery. Following the administration of anesthesia, either a full-thickness flap or a flapless technique was employed. A full-thickness flap was indicated in cases where bone concavity or complex morphology is identified on pre-operative cone beam computed tomography (CBCT). In the absence of these conditions, the flapless technique was utilized.

Group 1: Conventional Surgical Template (CST)

The conventional surgical template (Fig.1a) was fitted to ensure proper alignment. A 2.0 mm diameter pilot bur was used, followed by sequentially larger implant drilling burs to prepare the implant site. From the pilot bur to final bur all as performed through the template. The dental mini implant (NOVEM®, Novem Innovations Co., Ltd., Thailand) was placed using a free-hand technique.

Group 2: Surgical Drill Guide (SDG)

The protocol for Group 2 was similar to that of Group 1, with the primary difference being the use of a surgical drill guide (Fig. 1b) instead of the conventional surgical template. The drill guide was used to assist in the drilling and placement of the implant.

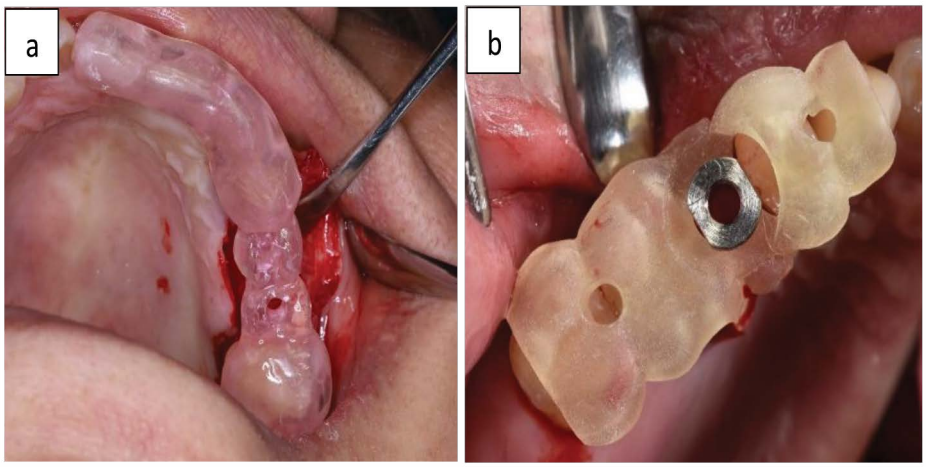

Fig.1 a: Conventional surgical template,
b: Surgical drill guide with metal collar.
Post-operative procedure.

A post-operative cone-beam computed tomography (CBCT) scan was conducted immediately after the surgery to analyze the implant position. Subsequently, the abutment, which is the same piece as the fixture, was prepared, and a temporary restoration was fabricated and fixed in place with temporary cement.

Discrepancy between planned and placed implant measurements

Discrepancies were measured from superimposed image between pre-operative CBCT and post-operative CBCT in millimeters for the nine parameters; Top horizontal deviation (TH), Top vertical deviation (TV), Top global deviation (TG), Coronal horizontal deviation (CH), Coronal vertical deviation (CV), Coronal global deviation (CG), Apical horizontal deviation (AH), Apical vertical deviation (AV), Apical global deviation (AG).

The deviation of the angle of the long axis between the planned and placed implants was also measured and recorded as Angular deviation (AD).

These parameters positions were shown in Fig. 2.

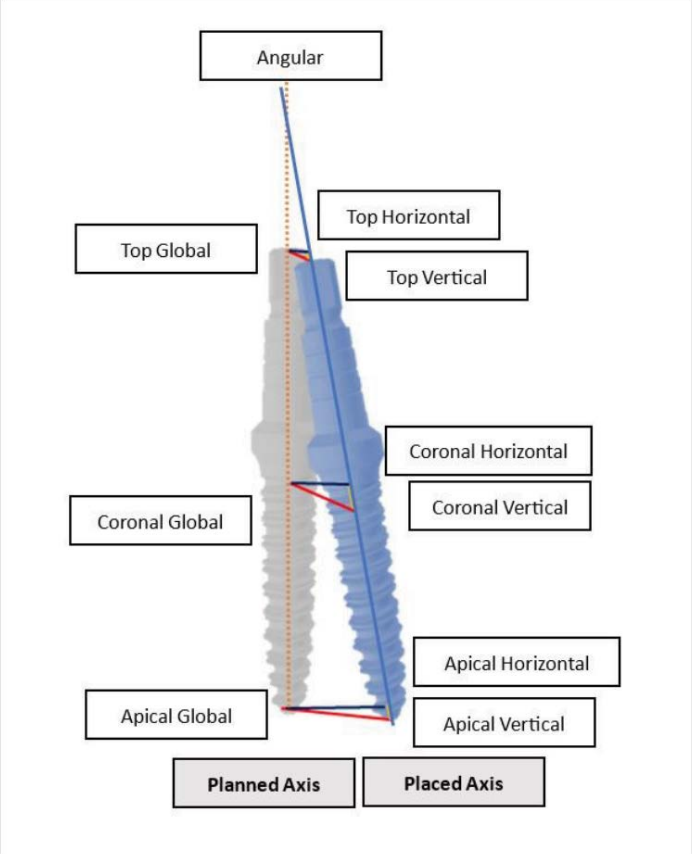

Fig.2 Evaluation parameters of planned and placed implant.

Data analysis and evaluation.

Alongside the completion of implant placement, the analysis of outcomes between the CST and SDG groups was performed by the same well-experienced surgeon and researcher of this study who hadn't been involved in randomization (P.V.). Self-calibration was performed using Cohen's kappa coefficient. Each patient's data was encoded by number ranging from No. 001 to No.020. The power of test was calculated by mean and SD of angular deviation resulted in 0.97 at 0.05 α probability level. Comparison between the planned and placed implant positions was determined in Planmeca Romexis™ software (Planmeca Co., Ltd., Helsinki, Finland) by means of pre and post-operative CBCT superimposition. All data parameters from both groups underwent thorough evaluation.

Statistical analysis

To assess the normal distribution of the data, the Shapiro-Wilk test was employed. Subsequently, statistical analysis was conducted using an independent sample T-test for comparison, utilizing SPSS 26 (IBM SPSS, USA). Statistical significance was defined as $p < 0.05$, indicating differences between groups.

Results

Participants were recruited at the Centre of Excellence for Dental Implantology, Faculty of Dentistry, Chiang Mai University. After evaluation of bone width and height using cone-beam computed tomography (CBCT), resulting in a final cohort of 16 individuals eligible for inclusion. These 16 participants underwent placement of 20 mini dental implants, with some individuals having the potential for placement in multiple areas.

The participants were randomly divided into two groups: the Conventional Surgical Template (CST) group and the Surgical Drill Guide (SDG) group. The CST group comprised 4 males and 4 females, while the SDG group consisted of 3 males and 4 females. The mean ages of the participants in each group were recorded (Table 2).

Table 2. Patient demographic data in each group.

		CST	SDG
Mean age		45.25 (29-66)	41.23 (24-61)
Gender			
	Male	4	3
	Female	4	4
Area			
	Anterior	5	3
	Premolar	5	7

The areas of interest for implant placement in this study were the lower anterior area and premolars area. The mini dental implant placement procedures were conducted over a period spanning from February 2020 to February 2023. All of the fabricated CSTs and SDGs met satisfactory stabilization and were fitted properly. There were no complications following the procedure and no implants were lost throughout the entire process. Then the evaluation process was executed as described in materials and methods (Fig.3)

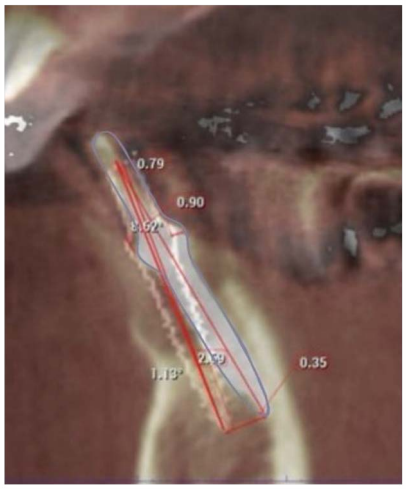

Fig.3 Evaluation of discrepancy between planned and placed implants using Planmeca Romexis™.

The results revealed that there were statistically significant differences of the discrepancy measurements ($p < 0.05$), between the CST and SDG groups, in 3 parameters including the top horizontal, top global and angular deviation as shown in Table 3.

Table 3. Discrepancy between planned and placed implants in two groups.

Parameters		Conventional surgical template (n = 10)	Surgical drill guide (n = 10)	p-value
		Mean ± SD (mm)	Mean ± SD (mm)	
Top	Horizontal	1.43 ± 0.77	0.67 ± 0.3	0.013*
	Vertical	0.93 ± 0.71	0.63 ± 0.59	0.319
	Global	1.83 ± 0.85	0.82 ± 0.52	0.005*
Coronal	Horizontal	1.09 ± 0.55	0.87 ± 0.41	0.326
	Vertical	0.99 ± 0.88	0.74 ± 0.56	0.463
	Global	1.57 ± 0.53	1.22 ± 0.48	0.142
Apical	Horizontal	1.37 ± 0.1	0.8 ± 0.4	0.12
	Vertical	1.29 ± 1.2	0.65 ± 0.57	0.15
	Global	1.75 ± 0.92	1.2 ± 0.46	0.12
Angular deviation		5.53 ± 3.14 degrees	1.36 ± 0.7 degrees	0.002*

Discussion

The present study aimed to compare the accuracy of single tooth implant positioning in narrow ridge spaces between conventional surgical guides and computer-assisted surgical guides. Through a randomized clinical trial design, we sought to contribute valuable insights into the efficacy of computer-assisted techniques in mini dental implant placement, thereby informing clinical practice and future research endeavors.

Our study identified top horizontal deviation, top global deviation and angular deviation as the most significant discrepancies, differing from previous findings of Ngamprasertkit et. al. highlighting only global deviation (26). The observed discrepancies may arise from the procedural nuances associated with mini dental implants. Unlike conventional methods that rely extensively on drilling burs to facilitate significant angle adjustments, the mini implant procedure entails a more conservative use of such tools, potentially limiting the extent of angle alterations. Additionally, the small size of the uppermost portion-specifically, the top of the abutment in the single-piece design of mini dental implants-presents challenges for visualization and precise placement during the surgical procedure. Further research on this topic may necessitate exploring additional possibilities.

The observed discrepancies may arise from the presence of adjacent teeth, as well as the quadrant and specific location of the implant site. Notably, the number of missing teeth did not appear to influence the outcomes (27). In our study, the limited types of teeth examined made it challenging to determine whether tooth

location affects accuracy. Similarly, there is currently no established relationship among the flap or flapless surgery techniques regarding their accuracy. Continued investigation into both issues is warranted.

In terms of prosthesis procedure, the angulation of the implant showed a significant effect on linear displacement of impressions when its larger than 25 degrees (28). However, there is no known direct connection with the survival rate or functionality of dental implant.

The successful integration of mini dental implants hinges significantly upon the surgeon's manual dexterity and clinical acumen. This stands in contrast to the guided implant insertion technique (26), which afford greater predictability through meticulous preoperative planning. This disparity underscores the critical role of surgical technique in influencing procedural outcomes.

Clinically, despite the observed discrepancies, the use of CST provided no different clinical outcomes compared to SDG. Immediately following the procedure, there were no complications or negative feedback from participants in either group. This suggests the capability of both techniques, followed by the potential for broader adoption of computer-assisted techniques in mini dental implant placement. While computer-guided surgery has traditionally been associated with standard-sized implants, our study suggests its feasibility and efficacy in the context of mini dental implants. This expansion of the scope of computer-assisted implantology offers clinicians greater versatility in treatment planning and execution. Although the differences in parameters between the two groups were not substantial, the use of SDG may be superior to CST in terms of ease of use during surgical implant placement. Novice surgeons can more readily adhere to the steps for implant placement, thereby facilitating the efficient execution of the procedure within an appropriate chair time.

Meanwhile, several limitations should be acknowledged. Firstly, the relatively small sample size in this study may limit the generalizability of the findings. Future research with larger cohorts is warranted to validate the outcomes and explore innovative strategies for participant recruitment and refine implant placement protocols to enhance accuracy and predictability.

Additionally, our study focused solely on implant positioning accuracy and did not assess other crucial clinical parameters such as peri-implant soft tissue health, patient satisfaction, or long-term implant survival rates. Future investigations should incorporate comprehensive outcome measures to provide a more holistic evaluation of treatment success and patient outcomes.

Overall, our study sheds light on the nuances of surgical template fabrication and implant placement procedures, highlighting the importance of precise techniques and the potential impact on treatment outcomes.

Conclusion

In conclusion, CST and SDG demonstrate effective applicability for the placement of single-tooth mini dental implants in limited-ridge spaces, particularly within the anterior and premolar regions. This versatility offers a broader array of techniques that can be adapted to the specific circumstances of each mini dental implantation case, enhancing clinical decision-making and improving patient outcomes.

Acknowledgements

The authors express special thanks to Chiang Mai University officers who provided resources and accommodated in the research, Dr.Thanapat Sastraruji for statistical analysis consultant. This research was supported and funded by Chiang Mai University, Chiang Mai, Thailand.

References

1. Buser D SL, De Bruyn H. Modern implant dentistry based on osseointegration: 50 years of progress, current trends and open questions. Periodontology 2000. 2016;73(1):7-21.

2. Block MS. Dental implants: the last 100 years. J Oral Maxillofac Surg. 2018;76(1):11-26.

3. Khalid Almas FJ, Steph Smith. Glossary of Dental Implantology. Wiley-Blackwell; 2018.

4. Al-Johany SS, Al Amri MD, Alsaeed S, Alalola B. Dental implant length and diameter: a proposed classification scheme. J Prosthodont. 2017;26(3):252-60.

5. Aunmeungtong W, Kumchai T, Strietzel FP, Reichart PA, Khongkhunthian P. Comparative clinical study of conventional dental implants and mini dental implants for mandibular overdentures: a randomized clinical trial. Clin Implant Dent Relat Res. 2017;19(2):328-40.

6. Threeburuth W, Aunmeungtong W, Khongkhunthian P. Comparison of immediate-load mini dental implants and conventional-size dental implants to retain mandibular Kennedy class I removable partial dentures: a randomized clinical trial. Clin Implant Dent Relat Res. 2018; 20(5):785-92.

7. Zembic A, Johannesen LH, Schou S, Malo P, Reichert T, Farella M, et al. Immediately restored one-piece single-tooth implants with reduced diameter: one-year results of a multi-center study. Clin Oral Implants Res. 2012;23(1): 49-54.

8. Brodala N. Flapless Surgery and Its Effect on Dental Implant Outcomes. Int J Oral Maxillofac Implants. 2009:24(Suppl):118-25.

9. Lemos CAA, Verri FR, Cruz RS, Gomes JML, Dos Santos DM, Goiato MC, et al. Comparison between flapless and open-flap implant placement: a systematic review and meta-analysis. Int J Oral Maxillofac Surg. 2020;49(9):1220-31.

10. Javed F, Ahmed HB, Crespi R, Romanos GE. Role of primary stability for successful osseointegration of dental implants: Factors of influence and evaluation. Interv Med Appl Sci. 2013;5(4):162-7.

11. Buser D, Martin W, Belser UC. Optimizing esthetics for implant restorations in the anterior maxilla: Anatomic and surgical considerations. Int J Oral Maxillofac Implants. 2004;19 (Suppl): 43-61.

12. Proceedings of the 4th International Team for Implantology (ITI) Consensus Conference, August 2008, Stuttgart, Germany. Int J Oral Maxillofac Implants. 2009;24 Suppl:7-278.

13. Younes F, Cosyn J, De Bruyckere T, Cleymaet R, Bouckaert E, Eghbali A. A randomized controlled study on the accuracy of free-handed, pilot-drill guided and fully guided implant surgery in partially edentulous patients. J Clin Periodontol. 2018;45(6):721-32.

14. Al Yafi F, Camenisch B, Al-Sabbagh M. Is digital guided implant surgery accurate and reliable?. Dent Clin North Am. 2019;63(3):381-97.

15. Bover-Ramos F, Vina-Almunia J, Cervera-Ballester J, Penarrocha-Diago M, Garcia-Mira B. Accuracy of implant placement with computer-guided surgery: a systematic review and meta-analysis comparing cadaver, clinical, and in vitro studies. Int J Oral Maxillofac Implants. 2018; 33(1):101-15.

16. Chai J, Liu X, Schweyen R, Setz J, Pan S, Liu J, et al. Accuracy of implant surgical guides fabricated using computer numerical control milling for edentulous jaws: a pilot clinical trial. BMC Oral Health. 2020;20(1):288. doi: 10.1186/s12903-020-01283-4.

17. Colombo M, Mangano C, Mijiritsky E, Krebs M, Hauschild U, Fortin T. Clinical applications and effectiveness of guided implant surgery: a critical review based on randomized controlled trials. BMC Oral Health. 2017;17(1):150. doi: 10.1186/s12903-017-0441-y.

18. Cristache CM, Gurbanescu S. Accuracy evaluation of a stereolithographic surgical template for dental implant insertion using 3d superimposition protocol. Int J Dent. 2017;2017:4292081. doi: 10.1155/2017/4292081.

19. Derksen W, Wismeijer D, Flugge T, Hassan B, Tahmaseb A. The accuracy of computer-guided implant surgery with tooth-supported, digitally designed drill guides based on CBCT and intraoral scanning. a prospective cohort study. Clin Oral Implants Res. 2019;30(10):1005-15.

20. Fang Y, An X, Jeong SM, Choi BH. Accuracy of computer-guided implant placement in anterior regions. J Prosthet Dent. 2019;121(5): 836-42.

21. El Kholy K, Lazarin R, Janner SFM, Faerber K, Buser R, Buser D. Influence of surgical guide support and implant site location on accuracy of static Computer-Assisted Implant Surgery. Clin Oral Implants Res. 2019;30(11):1067-75.

22. Miller RJ, Bier J. Surgical navigation in oral implantology. Implant Dent. 2006;15(1):41-7.

23. Hultin M, Svensson KG, Trulsson M. Clinical advantages of computer-guided implant placement: a systematic review. Clin Oral Implants Res. 2012;23(Suppl 6):124-35.

24. Gargallo-Albiol J, Barootchi S, Salomo-Coll O, Wang HL. Advantages and disadvantages of implant navigation surgery. a systematic review. Ann Anat. 2019;225:1-10. doi: 10.1016/j.aanat. 2019.04.005.

25. Unsal GS TI, Lakhia S. Advantages and limitations of implant surgery with CAD/CAM surgical guides: a literature review. J Clin Exp Dent. 2020;12(4):409-17.

26. Ngamprasertkit C, Aunmeungthong W, Khongkhunthian P. The implant position accuracy between using only surgical drill guide and surgical drill guide with implant guide in fully digital workflow: a randomized clinical trial. Oral Maxillofac Surg. 2022;26(2):229-37.

27. Tang T, Huang Z, Liao L, Gu X, Zhang J, Zhang X. Factors that Influence Direction Deviation in Freehand Implant Placement. J Prosthodont. 2019;28(5):511-8.

28. Mir Mohammad Rezaei S, Geramipanah F, Kamali H, Sadighpour L, Payaminia L. Effect of Arch Size and Implant Angulations on the Accuracy of Implant Impressions. Eur J Prosthodont Restor Dent. 2021;29(4):218-22.

Corresponding author:
Prof.Dr.med.dent. Pathawee Khongkhunthian
Center of Excellence for Dental Implantology,
Faculty of Dentistry, Chiang Mai University,
Chiang Mai, Suthep 50200, A. Muang, Thailand
50200
Tel: (665) 394 4484
E-mail: pathaweek@gmail.com

Fabrication and Clinical Evaluation of a Novel 3D printed Hydroxyapatite/Polycaprolactone Composite (Novel 3DP HA/PCL) for Maxillary Sinus Augmentation: A Preliminary Study

Poommarin Thammanoonkul[1] Suwit Limpattamapanee[2] Faungchat Thammarakcharoen[3] Jintamai Suwanprateeb[3,4] Borvornwut Buranawat[5*]

Abstract

Objective: To fabricate a 3DP hydroxyapatite/polycaprolactone composite graft material and assess its clinical efficacy in maxillary sinus augmentation through a preliminary assessment of bone density changes over a one-year period, compared to deproteinized bovine bone mineral graft.

Methods: A 3DP hydroxyapatite/polycaprolactone (HA/PCL) composite was fabricated using 3D printing of calcium sulfate-based material, followed by phase transformation and PCL infiltration. The composite was characterized through SEM, XRD, micro-CT, and compression testing. In a clinical study, 3DP HA/PCL composite material was compared with deproteinized bovine bone graft in sinus augmentation procedures. Cone-beam computed tomography (CBCT) was used to measure bone density at baseline, 6 months, and 1-year post-operation.

Results: SEM and micro-CT analyses revealed that the 3DP HA/PCL composite exhibited a highly porous, three-dimensional architecture with HA crystals combined with PCL. The microstructure was characterized by a mixture of spherical and irregular-shaped particles with 60.67% porosity. Compression testing demonstrated that the 3DP HA/PCL composite granules had a compressive load resistance of 7.55 ± 1.71 N. The calculated compressive strength of the granule was approximately 2.4 MPa. CBCT analysis of bone density changes revealed distinct patterns between the two groups. Significant differences in graft bone density were observed in the control group at all time points ($P < 0.05$) , while the 3DP HA/PCL group demonstrated no significant changes ($P < 0.3831$). However, between 6 months to 1 year, the 3DP HA/PCL group exhibited an increased bone density gain trend similar to the rate observed in the xenograft group. At 1 year, the increase in bone density from T1 to T3 was significant in both the control and test groups., These findings indicate that while 3DP HA/PCL grafts initially increase bone density more slowly than xenografts, they demonstrate a more pronounced gain in the later phase compared to the early phase.

Conclusion: Based on the promising preliminary results from the sinus augmentation study, 3DP HA/PCL composite demonstrates potential as an alternative bone graft material to deproteinized bovine bone mineral.

Keywords: Polycaprolactone, Augment bone graft, Hydroxyapatite, Bone density

Received Date: Aug 05, 2024
Revised Date: Oct 24, 2024
Accepted Date: Nov 11, 2024

[1]*Division of Periodontics and Implant Dentistry, Faculty of Dentistry, Thammasat University, Pathum Thani, 12120, Thailand.*
[2]*Nakhon Ratchasima Provincial Public Health Office, Nakhon Ratchasima, 30280, Thailand.*
[3]*Biofunctional Materials and Devices Research Group, National Metal and Materials Technology Center, National Science and Technology Development Agency, Pathum Thani, 12120, Thailand.*
[4]*Center of Excellence in Computational Mechanics and Medical Engineering, Thammasat University, Pathum Thani,12120, Thailand.*
[5]*Department of Implantology, Faculty of Dentistry, Thammasat University, Pathum Thani, 12120, Thailand.*
*(*corresponding author)*

Introduction

Dental implants have emerged as an effective treatment for patients with partial or complete edentulism. However, tooth loss triggers both horizontal and vertical bone resorption. The alveolar ridge volume decreases by 25% within the first year post-extraction, while its width diminishes by 40-60% over the initial three years (1,2). This significant bone loss poses challenges for implant placement, often necessitating bone augmentation procedures. The posterior maxilla, particularly in the premolar-molar region, presents unique challenges for dental implant placement. This area is characterized by low bone density, typically classified as D3-D4 type (150-850 Hounsfield Units) according to Misch's 1988 classification (3). Such bone quality features thin porous cortical bone and fine trabeculae, which can compromise implant stability. Furthermore, the natural pneumatization of the maxillary sinus often results in limited vertical bone height, further complicating implant procedures in this region. To address insufficient bone volume in the posterior atrophic maxilla, various surgical techniques have been developed for maxillary sinus augmentation. Lateral sinus augmentation is indicated when the residual alveolar ridge height is less than 4 mm. This technique has demonstrated superior predictability and reliability for bone augmentation in the posterior maxilla. Extensive literatures support its efficacy and long-term success in preparing sites for dental implant placement, with high reported implant survival rates (4,5). The maxillary sinus, however, exhibits significant anatomical variability. Sinus septa, for instance, are present in 25-31.7% of maxillary sinuses. Additionally, the proximity of posterior superior alveolar arteries to the surgical site can pose challenges. These anatomical variations may lead to potential complications during sinus augmentation procedures, necessitating careful preoperative assessment and surgical planning (6). Cone-beam computed tomography (CBCT) analysis provides crucial information that helps reduce complications and optimize treatment plans for both sinus augmentation and implant placement. This imaging modality enables precise evaluation of anatomical variations and vital structures, enhancing surgical predictability and safety. Bone grafting materials play a crucial role in maxillary sinus augmentation procedures, significantly enhancing outcomes compared to augmentation without grafts (7). In the absence of bone grafts, the elevated sinus membrane may collapse, resulting in insufficient bone formation and compromised implant stability. Grafting materials serve multiple functions: they maintain the elevated space and provide a scaffold for new bone growth.

A variety of options are available, including autografts, allografts, xenografts, and alloplasts. While autogenous bone is considered the gold standard for reconstruction due to its osteogenic properties, it is associated with a higher resorption rate. In recent years, xenografts have gained popularity in maxillary sinus augmentation, particularly deproteinized bovine bone (8,9). These materials exhibit excellent osteoconductive properties and a slow resorption rate, effectively maintaining space and volume until new bone formation occurs. However, the search for ideal grafting materials in maxillary sinus augmentation continues, driving ongoing research and development in this field.

In addition to allografts and xenografts, alloplastic or synthetic bone grafts have gained considerable popularity in the field of bone augmentation. These synthetic bone grafts, derived from biomaterials, offer an alternative to human or animal-derived products. Hydroxyapatite (10), a key component in many alloplasts, has been utilized in bone repair for years due to its similarity to the inorganic component of natural bone. As a bioceramic material, hydroxyapatite exhibits several advantageous properties: biocompatibility, bioactivity, non-immunogenicity, and excellent osteoconductivity. However, hydroxyapatite's brittleness and limited mechanical strength may make it unsuitable for use in load-bearing area (11-13).

Several studies have investigated the addition of biodegradable polymers, such as polycaprolactone (PCL), to hydroxyapatite (HA) to improve the mechanical properties of bone substitute materials, resulting in HA/PCL composites. (14,15) Polycaprolactone (PCL) exhibits exceptional toughness and biocompatibility (16). It degrades more slowly and has higher fracture energy compared to other biodegradable polymers, while producing less acidic and less toxic degradation products (17). The 3DP HA/PCL composite leverages the advantages of both materials. The HA component provides osteoconductive properties to support bone ingrowth, while the PCL phase improves the mechanical integrity of the graft (15).

The search for optimal grafting materials for maxillary sinus augmentation remains an active area of research. To address this need, a 3D printed hydroxyapatite/polycaprolactone (3DP HA/PCL) composite material has been developed. (18) This 3DP HA/PCL composite has undergone both in vitro (19) and in vivo (20) testing to assess its biofunction, biosafety, and biocompatibility.

This study has two primary objectives, to comprehensively characterize the 3DP HA/PCL composite material and to evaluate its clinical efficacy in maxillary sinus augmentation compared to the widely used deproteinized bovine bone mineral (DBBM) graft. This comparison will be conducted through a preliminary assessment of bone density changes over a one-year period, providing insights into the material's performance in a clinical setting.

Materials and Methods

The National Metal and Materials Technology Center (MTEC) manufactured a custom 3DP HA/PCL granules using a low-temperature technique (21) with the advantage of the bioresorption ability of HA, allowing for simultaneous bone formation (22,23) and enhancing the mechanical property by infiltration with the biodegradable synthetic polymer (15,21).

These 3DP HA/PCL granules were fabricated through a multi-step process. Initially, a powder-based binder jetting three-dimensional printing machine (Projet160, 3D Systems, USA) was used to fabricate structures using calcium sulfate-based powder (Visijet PXL core, 3D Systems, USA). These three dimensional printed specimens then underwent a phase transformation to 3D printed hydroxyapatite (3DP HA) by soaking in 1 M disodium hydrogen phosphate solution (Sigma Aldrich, USA) at 100°C for 48 hours. Following this transformation, the specimens were thoroughly cleaned overnight in deionized water and subsequently dried at 80°C for 4 hours.

To produce the final 3DP HA/PCL composite, the 3DP HA structures were impregnated with polycaprolactone. This was achieved by soaking the 3DP HA in a 50% w/w solution of polycaprolactone (Sigma Aldrich, Mn~10,000) in N-methylpyrrolidone (TSquare Synergy (Thailand) Co., Ltd, Thailand) at 50°C for 15 minutes.

The infiltrated samples were then transferred into the bottle which contained a 9:1 ratio of N-methylpyrrolidone and deionized water and the bottle was shaken continuously for 45 seconds. The specimens were taken out, cleaned in deionized water for 24 hours and dried at room temperature for 48 hours. Then packed in the glass vial and sterilizable pouch and sterilized by ethylene oxide gas. (Figure 1)

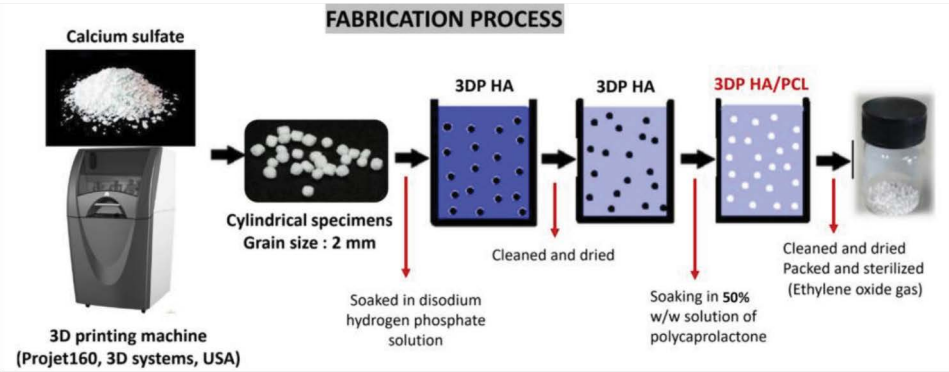

Fig.1 Schematic of the fabrication process for 3D printed hydroxyapatite/polycaprolactone (3DP HA/PCL) composite material. The process begins with the generation of hydroxyapatite (HA) particles, which are then soaked in a polycaprolactone (PCL) solution.

Characterization of the 3DP HA/PCL composite material

The physicochemical properties of the 3DP HA/PCL composite material were comprehensively characterized using a multi-modal approach. X-ray powder diffraction (XRD) analysis was performed using a TTRAX III (Rigaku, USA) with Cu source Kα line focused radiation (λ = 0.15406 nm) operating at 300 mA and 50 kV to elucidate the crystalline structure of the composite. Surface topography was examined using scanning electron microscopy (SEM) with a JCM 6000 (JEOL, Tokyo, Japan). This technique provided high-resolution images of the material's surface features and morphology.

To obtain a three-dimensional representation of the composite's internal structure, microCT scanning was employed using a Skyscan 1275 system (Bruker micro-CT, Kontich, Belgium). The accompanying Skyscan 1275 control software was utilized for image acquisition and subsequent 3D data analysis. This non-destructive imaging technique allowed for detailed visualization and quantification of the material's spatial characteristics.

Compression load resistance of individual granules was performed by using a universal testing machine (Instron 55R4502, Instron, USA) at the crosshead speed of 1 mm/min at 23 ± 2°C

and 50 ± 5 % RH. The maximum load before breakage was recorded and ten replicates were done.

Clinical study

This preliminary study was a prospective single-blinded randomized controlled clinical trial to evaluate the clinical efficacy of the 3DP HA/PCL composite material (Test group: 2.0 mm, MTEC, NSTDA, Thailand) compared to commercial xenograft (Control group: Straumann® Xenograft, 1.0-2.0 mm, Institut Straumann AG, Switzerland) for maxillary sinus augmentation. The study follows CONSORT and Helsinki guidelines, was approved by Thammasat University's Human Research Ethics Committee (COA number 053/2564), and registered on the Thai Clinical Trials Registry (TCTR20210622003)

This study enrolled 22 patients with 24 maxillary sinuses requiring lateral sinus augmentation for 1-2 dental implants, with residual alveolar bone height ≤ 4 mm in upper premolar-molar area, observed on CBCT image. Patients were aged 18-70 years and classified as ASA 1-2. All participants understood the protocols and signed informed consent before enrollment. None had a history of allergy or hypersensitivity to study materials or previous sinus pathology. Exclusion criteria included smoking over 10 cigarettes per day, medical conditions affecting bone and soft tissue healing (e.g., bone disease, osteoporosis, uncontrolled diabetes), and inability to take impressions (both conventional and digital) or CBCT. Sample size is calculated from the equation considering a 20% drop out rate. (24) The randomization and allocation are assigned to the control/test group by a sequentially numbered, sealed envelope protocol. The surgeon opened the sealed envelope before graft placement.

Surgical Procedure

All procedures were performed under local anesthesia using 4% articaine with epinephrine 1:100,000. A mucoperiosteal trapezoidal flap was raised, initiated by a crestal incision followed by two vertical releasing incisions. The flap was gently elevated from the native bone tissue to allow complete visualization of the defect and surrounding bone. Osteotomies on the lateral wall of the maxillary sinuses were performed using the DASK kit (Dentium, Korea). Subsequently, the sinus membrane was elevated, and bone grafting material was packed at the base of the sinus according to the allocated group. Valsalva maneuvers were conducted throughout all stages of sinus floor elevation to ensure membrane integrity.

Following graft placement, the lateral window access was covered with a resorbable collagen membrane (Lyoplant®, Aesculap, USA). Wound closure was achieved using VICRYL® 4-0 and Nylon ETHILON® 5-0 sutures.

Radiographic protocol

Cone-beam computed tomography (CBCT) imaging was performed with a 3D Accuitomo 170 scanner (J. Morita Manufacturing Corp) and taken immediately after maxillary sinus augmentation (T1), 6 months after maxillary sinus augmentation and before implant placement (T2) and 1 year after maxillary sinus augmentation and after prosthesis loading (T3). CBCT images were processed in DICOM file and three-dimensionally (3-D) reconstructed using software (i-Dixel One Volume Viewer, version 2.8.0, J. Morita Manufacturing Corp.) to evaluate bone density.

Clinical and radiographic evaluation

All patients were evaluated by a single examiner one day after sinus augmentation surgery, followed by a two-week post-operative check-up. Subsequently, regular follow-up examinations were conducted at three, six, and twelve months post-surgery to monitor healing progress and assess long-term outcomes.

Bone density, expressed in Hounsfield Units (HU), was measured using the initial floor of maxillary sinus prior to augmentation as a baseline reference. Measurements were taken at two vertical levels: 3 mm and 5 mm superior to this baseline, along the implant axis. At each level, four measurement points were established around the implant, resulting in a total of eight measurement points. These points were determined by extending horizontal lines perpendicular to the implant axis, 3 mm to each side.

The measurement points were designated as follows:

- At 3 mm above baseline: HU_{B3} (buccal), HU_{Pa3} (palatal), HU_{M3} (mesial), HU_{D3} (distal)
- At 5 mm above baseline: HU_{B5} (buccal), HU_{Pa5} (palatal), HU_{M5} (mesial), HU_{D5} (distal)

Radiographic assessments were performed at three time points (T1, T2, T3) for each patient (Figure 2). The mean values of these measurements (HUAv) were used for statistical analysis.

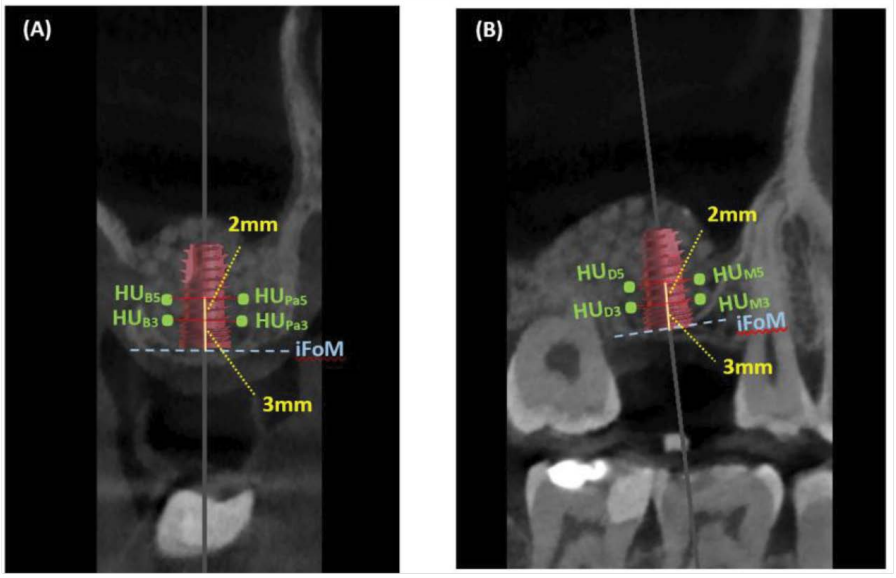

Fig.2 Quantitative analysis of grafted bone density using CBCT data. (A) Bucco-palatal view: This image shows the measurement points for grafted bone density at the buccal and palatal aspects. Measurements were taken at level 3 mm (HUB3, HUPa3) and 5 mm (HUB5, HUPa5) superior to the initial floor of the maxillary sinus (iFoM). The iFoM represents the baseline sinus floor position at T0, prior to augmentation. (B) Mesio-distal view: This image demonstrates the measurement points for grafted bone density at the mesial and distal aspects. Similarly, measurements were taken at level 3 mm (HUM3, HUD3) and 5 mm (HUM5, HUD5) superior to the initial floor of the maxillary sinus.

Statistical Analysis

All data were analyzed using descriptive statistics with GraphPad Prism 10.2.2. The significance level was set at $\alpha = 0.05$. Results are presented as mean ± standard deviation (SD). Given the normal distribution of data, unpaired t-tests were used to compare mean bone density at each time point (T1, T2, T3) between test and control groups. Changes in mean grafted bone density over time within each group were assessed using ANOVA.

Results

Characterizations of 3DP HA/PCL composite

X-ray diffraction (XRD) analysis confirmed the successful incorporation of polycaprolactone (PCL) into the hydroxyapatite (HA) to produce 3DP HA/PCL composite (Figure 3A). The XRD pattern exhibited a combination of characteristic peaks for both PCL and HA. Distinctive PCL peaks were observed at 2θ values of 15.3°, 21.4°, and 23.8°. Concurrently, the presence of HA was evidenced by peaks at 2θ values of 25.8°, 31.6°, 32.14°, 32.8°, and 34.01°. This composite diffraction pattern demonstrates the coexistence of both materials within the fabricated composite, validating the effectiveness of the impregnation process.

Scanning electron microscope (SEM) image displays the microstructure of 3DP HA/PCL composite bone graft material at 50x, 100x and 500x magnification (Figures 3C,3D,3E). The micrograph reveals a complex, three-dimensional architecture composed of particles varying in size and morphology in which the HA crystals were coated or infiltrated with PCL. Particles range from sub-micron to several micrometers in diameter, exhibiting both spherical and irregular shapes. This heterogeneous particle distribution creates a highly porous structure with interconnected spaces, crucial for cellular infiltration and vascularization in bone regeneration processes.

Mechanical properties : The compressive load resistance of the 3DP HA/PCL composite granules was 7.55 ± 1.71 N. The cross-sectional area at the largest diametral area was considered, the calculated compressive strength of the granule was approximately 2.4 MPa.

MicroCT analysis : The microtomographic reconstructed image is shown in figure 3B followed by microCT structural analysis values in table 1. The pore structure of a 3DP HA/PCL composite revealed good interconnectivity from the top to bottom of the construct with total porosity of 60.67%.

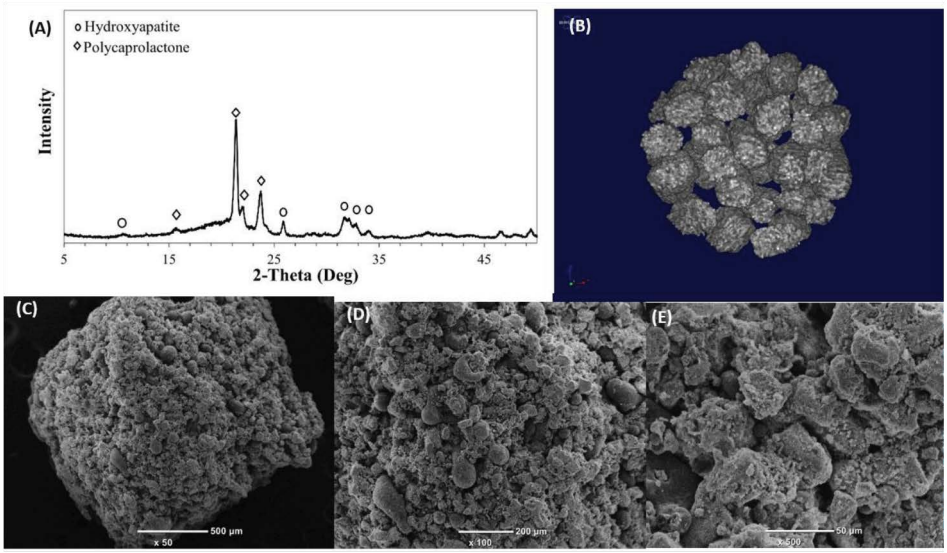

Fig.3 Characteristics of 3DP HA/PCL composite (A) XRD pattern showing characteristic peaks of both PCL and HA, confirming successful composite formation. (B) MicroCT 3D reconstructed images revealing scaffold microstructure and porosity distribution. (C-E) SEM micrographs at 50x, 100x, and 500x magnifications (scale bars: 500 μm, 200 μm, 50 μm) displaying scaffold surface topography and pore network.

Table 1. MicroCT structure analysis of the 3DP HA/PCL composite.

Description	Abbreviation	Value	Unit
Structure thickness	St.Th	0.083	mm
Structure separation	St.Sp	0.102	mm
Number of objects Obj	N	47	
Number of closed pores	Po.N(cl)	4	
Volume of closed pores	Po.V(cl)	0.00008	mm^3
Surface of closed pores	Po.S(cl)	0.015	mm^2
Closed porosity	Po	0.048	%
Open porosity	Po(op)	60.652	%
Total volume of pore space	Po.V(tot)	0.276	mm^3
Total porosity	Po(tot)	60.67	%
Connectivity	Conn	118	

Clinical evaluation:

Nineteen patients (21 maxillary sinuses) were included in this study from December 2022 to January 2024, with three patients (3 maxillary sinuses) being terminated due to lost to follow-up. Patients were randomized into control (n = 10) and test (n = 11) groups (Table 2). All patients showed normal soft tissue and bone healing without complications such as pain, inflammation, infection, sinusitis, or other maxillary sinus pathologies throughout the follow-up period. The dental implant was successfully placed in all cases.

Table 2. Patients Demographic data and Complication.

	Control group Xenograft(10)	Test group 3DP HAPCL(11)	Total (n = 21)
Age	59 ± 8.756	52.36 ±14.22	55.52 ± 12.13
Gender			
Male	3(30)	6(54.55)	9(42.86)
Female	7(70)	5(45.45)	12(57.14)
Tooth type			
Premolar	0(0)	2(18.19)	2(9.52)
Molar	10(100)	9(81.81)	19(90.48)
Complication			
Membrane perforation	1(10)	1(9.09)	2(9.52)

No significant difference in mean Hounsfield Units (HU) was observed between the control and test groups immediately after sinus augmentation (T1) (1059 ± 104.9 HU vs. 978.1 ± 118.8 HU, $p = 0.1155$). However, significant differences emerged at 6 months (T2) and 12 months (T3) post-augmentation. The control group showed mean HU values of 1303 ± 213 at T2 and 1523 ± 265.7 at T3, while the test group had 1048 ± 130.5 at T2 and 1184 ± 121.6 at T3. (Table 3)

Significant differences of graft bone density in the control group across all time points (T1, T2, T3) were seen ($p < 0.05$). In contrast, the test group showed significant differences only between T1 and T3 ($p < 0.0009$) and between T2 and T3 ($p < 0.0324$). The percentage analysis of grafted bone density showed increasing values overtime for both control and test groups. The percentage differences between consecutive time points (T1-T2, T2-T3) and overall change (T1-T3) are shown in Table 4

Table 3. Mean grafted bone density between the control and test groups at different time points.

	Control Group (Xenograft)	Test Group (3DP HA/PCL)	P value
T1	1059 ± 104.9^{abc}	978.1 ± 118.8^{ac}	0.1155
T2	1303 ± 213.8^{abc}	1048 ± 130.5^{bc}	0.0035*
T3	1523 ± 265.7^{abc}	1184 ± 121.6^{ca}	0.0011*

Note: Mean(HU) ± standard deviation values

Abbreviations:

- Xenograft (Straumann xenograft)
- 3DP HA/PCL (polycaprolactone impregnated 3D printed hydroxyapatite)
- T1 (CBCT at immediate sinus augmentation), T2 (CBCT 6 months after sinus augmentation),
- T3 (CBCT 12 months after sinus augmentation)
- *p-values (statistically significant at the level of $p < 0.05$) with unpaired t-test for differences in HU values at each timepoint between Xenograft and 3DP HA/PCL groups.
- abc p-values (statistically significant at the level of $p < 0.05$) with ANOVA for differences within each group between T1, T2 and T3.

Table 4. Percentage increase of grafted bone density overtime between the control and test groups at different time points.

	Control Group (Xenograft)	Test Group (3DP HA/PCL)
T1-2%	23%	7%
T2-3%	17%	13%
T1-3%	44%	21%

Abbreviations:

T1-2% Percentage increase of early grafted bone density changes

T2-3% Percentage increase of late grafted bone density changes

T1-3% Percentage increase of overall grafted bone density changes

Discussion

Recently, advancements in 3D printing technology combined with low-temperature phase transformation techniques have led to the development of 3DP hydroxyapatite (3DP HA) bone substitutes. These substitutes exhibit unique properties such as nanostructure, low crystallinity, resorbability, and high wicking ability, contrasting with the typically high-temperature sintered HA (21,25,26). Preclinical and clinical studies have demonstrated promising results for using this 3DP HA as a bone graft in socket preservation and bone block grafts for implant sites. (27,28) However, the mechanical strength of 3DP HA remains relatively low, potentially limiting its ability to withstand stress in certain applications. This limitation is associated with its high porosity due to calcium phosphate crystal precipitation during processing, which may present handling challenges in specific procedures (21,25,26). To address this drawback, a polycaprolactone (PCL) infiltrated 3DP HA composite (HA/PCL) was developed to enhance the toughness and strength of the material while maintaining its biocompatibility and bioactivity (18). PCL, a biodegradable polyester, was chosen as the infiltrant phase due to its favorable mechanical properties, degradability, and long history of use in implants. PCL initially provides structural integrity, which subsequently degrades to offer the porous architecture necessary for tissue ingrowth.

In this study, 3DP HA/PCL composite granules were prepared using modified and proprietary techniques to form spherical granules approximately 2 mm in diameter, suitable for use as a bone graft for maxillary augmentation. Compared to the previously investigated large rectangular bars (19), the 3DP HA/PCL composite granules similarly exhibited a highly porous structure with interconnected spaces crucial for cellular infiltration and vascularization in bone regeneration processes, as evidenced by SEM images and micro-CT analysis. The microstructure comprises a three-dimensional architecture of HA crystals with varying sizes and morphologies, infiltrated or coated by PCL, yet retaining numerous micropores. The compressive load resistance of the 3DP HA/PCL composite granules was 7.55 ± 1.71 N. Due to the spherical shape of the granule, it was not straightforward to calculate the compressive strength. However, if the cross-sectional area at the largest diametral area was considered, the calculated compressive strength of the granule was approximately 2.4 MPa, which is about seven times greater than that of 3D HA granules alone. The compressive strength of cortical bone ranges from 100–230 MPa, while that of trabecular bone ranges from 2-12 MPa (29). The mechanical properties of 3DP HA/PCL composite granules are thus comparable to those of trabecular bone. Several studies have shown that the primary function of scaffolds is to act as structural templates that provide suitable substrates for cell proliferation, differentiation, and attachment, leading to new tissue formation. These processes depend on factors such as porosity, pore size, geometry, and interconnectivity. High porosity and large pores enhance bone ingrowth and osseointegration of the implant after surgery (30). Although the pore size of 3DP HA/PCL composite granules was not large, previous clinical studies have demonstrated that 3DP HA granules with small pore sizes can support bone regeneration through its highly porous structure and resorbability, which generate the necessary spaces for cells and bone ingrowth

during the bone healing process (26,27). It was anticipated that 3DP HA/PCL composite granules could also support bone regeneration by a similar mechanism, but can resist greater load during handling.

Bone density in dentistry is measured using CBCT images and expressed in Hounsfield Units (HU). This assessment of bone quality, crucial for predicting implant stability and prognosis, is typically categorized using Misch's classification (1988) into four groups: D1, D2, D3, and D4. This system aids clinicians in treatment planning and evaluating potential implant sites (3).

In this study, the initial mean Hounsfield Units (HU) at T1 for Xenograft and 3DP HA/PCL were 1059 ± 104.9 and 978.1 ± 118.8, respectively, showing no significant difference. These values are comparable to D2 bone density in Misch classification (5), indicating that both materials provide a suitable initial scaffold for bone regeneration in sinus augmentation procedures. However, significant differences emerged at 6 months (T2) and 12 months (T3) post-augmentation. The Xenograft group showed higher mean HU values (T2: 1303 ± 213.8, T3: 1523 ± 265.7) compared to the 3DP HA/PCL group (T2: 1048 ± 130.5, T3: 1184 ± 121.6). This divergence in bone density over time suggests different patterns of bone maturation and remodeling between the two materials. The Xenograft group demonstrated a progression from D2 to D1 bone density, indicating a rapid and robust bone formation process. This improvement in bone quality could be attributed to the osteoconductive properties of xenografts and their ability to integrate well with host bone. In contrast, The 3DP HA/PCL composite showed a slower, statistically non-significant increase in bone density from the T1 to T2 timepoints. However, it then exhibited a statistically significant accelerated rate of bone density gain between the T2 and T3 timepoints. This acceleration in bone density growth during the T2 to T3 period, while not matching the exact rate observed in the xenograft group, followed a similar overall trend of increased bone density during that time interval.

The findings were converted to percentages to facilitate a more comprehensive understanding of the results, By T3 revealed that the control group demonstrated a 44% increase in grafted bone density, compared to only 21% in the test group, suggesting superior bone formation with xenograft materials. These findings suggest that the bone healing and remodeling processes differ between Xenograft and 3DP HA/PCL materials. The Xenograft group's steady progression might be attributed to the osteoconductive properties of xenografts and their ability to integrate well with host bone (31). On the other hand, the 3DP HA/PCL group's accelerated late-phase density gain could be related to the unique properties of the 3DP HA/PCL composite fabricated from low-temperature phase transformation (24), including its biodegradation and interaction with surrounding tissues. Similar to previous study (27) that showed a healing index increased over time at 2 weeks, 1, 2, 3, and 6 months after block-graft with customized 3DP nanohydroxyapatite on implant site.

This is a preliminary report of the clinical evaluation of 3DP HA/PCL composite for maxillary sinus augmentation. Future analyses and reports should focus on correlating these bone density changes with clinical outcomes such as implant

stability, osseointegration, and long-term success rates. While bone density measurements indicated differences between groups, these results should be interpreted with caution as they cannot differentiate between new bone formation and residual graft materials. In contrast, histomorphometric analysis provides direct microscopic evidence of bone formation and is therefore more reliable for evaluating true osteogenic outcomes.

Conclusion

3DP HA/PCL composite demonstrates potential as an alternative bone graft material to deproteinized bovine bone mineral. While both deproteinized bovine bone mineral and 3DP HA/PCL composite materials demonstrate effectiveness in sinus augmentation but xenograft shows higher bone density results. However, their distinct patterns of bone density progression offer unique advantages. The choice between these materials should be based on a comprehensive consideration of the clinical scenario, patient factors, and desired timeline for implant placement.

Acknowledgements

This research was supported by Thammasat University research fund, Contract No. TUFT 93/64 and Thammasat University-National Science and Technology Development Agency Excellent Research Graduate Scholarship (T2.2-63-01). The authors would like to acknowledge the participants of the study and the staff of the Department of Implantology, Faculty of Dentistry, Thammasat University, for their assistance in conducting this clinical trial.

References

1. Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. J Clin Periodontol. 2002;29(Suppl 3):197-212; discussion 32-3.

2. Schropp L, Wenzel A, Kostopoulos L, Karring T. Bone healing and soft tissue contour changes following single-tooth extraction: a clinical and radiographic 12-month prospective study. Int J Periodontics Restorative Dent. 2003 ;23(4):313-23.

3. Goncalves SB, Correia J, Costa AC. Evaluation of dental implants using Computed Tomography. 2013 IEEE 3rd Portuguese Meeting in Bioengineering (ENBENG). 2013:1-4. doi:10.1109/ENBENG.2013.6518387.

4. Boyne PJ, James RA. Grafting of the maxillary sinus floor with autogenous marrow and bone. J Oral Surg. 1980;38(8):613-6.

5. Misch CE. Maxillary sinus augmentation for endosteal implants: organized alternative treatment plans. Int J Oral Implantol. 1987;4(2):49-58.

6. Danesh-Sani SA, Loomer PM, Wallace SS. A comprehensive clinical review of maxillary sinus floor elevation: anatomy, techniques, biomaterials and complications. Br J Oral Maxillofac Surg. 2016;54(7):724-30.

7. Lie SAN, Claessen RMMA, Leung CAW, Merten HA, Kessler PAWH. Non-grafted versus grafted sinus lift procedures for implantation in the atrophic maxilla: a systematic review and meta-analysis of randomized controlled trials. Int J Oral Maxillofac Surg. 2022;51(1):122-32.

8. Rickert D, Slater JJRH, Meijer HJA, Vissink A, Raghoebar GM. Maxillary sinus lift with solely autogenous bone compared to a combination of autogenous bone and growth factors or (solely) bone substitutes. a systematic review. Int J Oral Maxillofac Surg. 2012;41(2):160-7.

9. Cardoso CL, Curra C, Santos PL, Rodrigues MFM, Ferreira-Júnior O, de Carvalho PSP. Current considerations on bone substitutes in maxillary sinus lifting. Revista Clínica de Periodoncia, Implantología y Rehabilitación Oral. 2016;9(2):102-7.

10. Ginebra MP, Espanol M, Maazouz Y, Bergez V, Pastorino D. Bioceramics and bone healing. EFORT Open Rev. 2018;3(5):173-83.

11. Kattimani VS, Kondaka S, Lingamaneni KP. Hydroxyapatite–-Past, Present, and Future in Bone Regeneration. Bone and Tissue Regeneration Insights. 2016;7. doi:10.4137/BTRI.S36138

12. Zhao R, Xie P, Zhang K, Tang Z, Chen X, Zhu X, et al. Selective effect of hydroxyapatite nanoparticles on osteoporotic and healthy bone formation correlates with intracellular calcium homeostasis regulation. Acta Biomater. 2017;59: 338-50. doi: 10.1016/j.actbio.2017.07.009.

13. LeGeros RZ. Calcium phosphate-based osteoinductive materials. Chem Rev. 2008;108(11): 4742-53.

14. Zhiwei J, Bin L, Shengyi X, Haopeng M, Yuan Y, Weimin Y. 3D printing of HA / PCL composite tissue engineering scaffolds. Advanced Industrial and Engineering Polymer Research. 2019;2(4):196-202.

15. Stevanovic S, Chavanne P, Braissant O, Pieles U, Gruner P, Schumacher R. Improvement of mechanical properties of 3d printed hydroxyapatite scaffolds by polymeric infiltration. Bioceram. Dev. Appl. 2013;3:10-2. doi: 10.4172/2090-5025.S1-012.

16. Kim BS, Yang SS, Park H, Lee SH, Cho YS, Lee J. Improvement of mechanical strength and osteogenic potential of calcium sulfate-based hydroxyapatite 3-dimensional printed scaffolds by ε-polycarbonate coating. J Biomater Sci Polym Ed. 2017;28(13):1256-70.

17. BaoLin G, Ma PX. Synthetic biodegradable functional polymers for tissue engineering: a brief review. Sci China Chem. 2014;57(4):490-500.

18. Taecha-Apaikun K, Pisek P, Suwanprateeb J, Thammarakcharoen F, Arayatrakoollikit U, Angwarawong T, et al. In vitro resorbability and granular characteristics of 3D-printed hydroxyapatite granules versus allograft, xenograft, and alloplast for alveolar cleft surgery applications. Proc Inst Mech Eng H. 2021;235(11):1288-96.

19. Suwanprateeb J, Thammarakcharoen F, Hobang N. Enhancement of Mechanical Properties of 3D printed Hydroxyapatite by Combined Low and High Molecular Weight Polycaprolactone Sequential Infiltration. J. Mater. Sci. 570 Mater. Med. 2016;27(11):171. doi: 10.1007/s10856-016-5784-4.

20.Hemstapat R, Suwanprateeb J, Sriwatananukulkit O, Luangwattanawilai T, Desclaux SS. Study of bone regeneration of a porous hydroxyapatite scaffolds infiltrated with polycaprolactone and polycaprolactone membrane in a rat model. [Study report: Sponsored by the National Metal and Materials Technology Center] Bangkok: Department of Pharmacology, Mahidol University; 2019.

21. Suwanprateeb J, Thammarakcharoen F, Wasoontararat K, Suvannapruk W. Influence of printing parameters on the transformation efficiency of 3D-printed plaster of paris to hydroxyapatite and its properties. Rapid Prototyping J. 2012;18(6):490-9.

22. Kijartorn P, Thammarakcharoen F, Suwanprateeb J, Buranawat B. The use of three dimensional printed hydroxyapatite granules in alveolar ridge preservation. Key Eng Mater. 2017;751:663–7. doi:10.4028/www.scientific.net/KEM.751.663.

23. Thammarakcharoen F, Suwanprateeb J. Effect of process parameters on biomimetic deposition of calcium phosphate on 3D printed hydroxyapatite. Key Eng Mater. 2017;751:599–604.

24. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharmaceutical Statistics. 2005;4(4):287-91.

25. Suwanprateeb J, Suvannapruk W, Wasoontararat K. Low temperature preparation of calcium phosphate structure via phosphorization of 3D-printed calcium sulfate hemihydrate based material. J Mater Sci Mater Med. 2010;21(2):419-29.

26. Kijartorn P, Wongpairojpanich J, Thammarakcharoen F, Suwanprateeb J, Buranawat B. Clinical evaluation of 3D printed nano-porous hydroxyapatite bone graft for alveolar ridge preservation: A randomized controlled trial. J Dent Sci. 2022;17(1):194-203.

27. Mekcha P, Wongpairojpanich J, Thammarakcharoen F, Suwanprateeb J, Buranawat B. Customized 3D printed nanohydroxyapatite bone block grafts for implant sites: A case series. J Prosthodont Res. 2023;67(2):311-320.

28. Suwanprateeb J, Thammarakcharoen F, Hobang N. Enhancement of mechanical properties of 3D printed hydroxyapatite by combined low and high molecular weight polycaprolactone sequential infiltration. J Mater Sci Mater Med. 2016;27(11):171. doi:10.1007/s10856-016-5784-4.

29. Loh QL, Choong C. Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size. Tissue Eng Part B Rev. 2013;19(6):485-502.

30. Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials. 2005;26(27):5474-91.

31. Ferraz MP. Bone Grafts in Dental Medicine: An Overview of Autografts, Allografts and Synthetic Materials. Materials (Basel). 2023;16(11):4117. doi: 10.3390/ma16114117.

Corresponding author:
Assoc.Prof.Dr. Borvornwut Buranawat
Research Unit in Innovations in Periodontics, Oral Surgery and advanced technology in Implant Dentistry, Faculty of Dentistry, Thammasat University, Pathum Thani, 12120, Thailand
Tel: (668) 5997 7669
E-mail: borvornw@tu.ac.th

ใบแจ้งความจำนงลงโฆษณาในวิทยาสารทันตแพทยศาสตร์
มหาวิทยาลัยศรีนครินทรวิโรฒ

เขียนที่.....

วันที่.....เดือน.....พ.ศ.....

เรียน บรรณาธิการวิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

ข้าพเจ้า..... ตำแหน่ง.....

บริษัท/ห้าง/ร้าน..... โทรศัพท์.....

ที่อยู่.....

.....

มีความประสงค์ลงโฆษณาในหนังสือ “วิทยาสารทันตแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ” ประจำปี พ.ศ. ..... (ปีละ 1 ฉบับ)

เนื้อที่ที่ต้องการโฆษณา จำนวน ..... หน้า (โปรดดูรายละเอียดและทำเครื่องหมายด้านล่าง)

พร้อมกันนี้ได้มอบ สไลด์ ..... อัน เพลท ..... ชิ้น

ภาพ ..... ชิ้น ใบแทรก ..... แผ่น

อื่น ๆ (โปรดระบุ) .....

มาเพื่อดำเนินการต่อไปด้วย

ลงชื่อ .....

(.....)

อัตราค่าโฆษณา (ต่อวิทยาสาร 1 ฉบับ)

- ☐ ปกหน้าด้านใน (4 สี) 12,000 บาท
- ☐ ปกหลังด้านใน (4 สี) 12,000 บาท
- ☐ หน้าในเต็มหน้า (ขาว-ดำ) 6,000 บาท
- ☐ หน้าในครึ่งหน้า (ขาว-ดำ) 4,000 บาท
- ☐ ใบแทรก 7,000 บาท

Survival after 3-year of Partial Pulpotomy Using Bioactive Cements as Pulp Capping Materials in Adult's Permanent Teeth with Carious Pulp Exposure

Thanwarat Thonthanan Warit Powcharoen Dujrudee Chinwong Patchanee Chuveera

---

The Study of Satisfaction on Chewing Jelly with Coconut Oil for Oral Moisturization in the Elderly: A Pilot study

Panurak Kaewnoi Serena S. Sakoolnamarka Sorasun Rungsiyanont

Human Osteoclasts Enhance Osteogenic Differentiation of Bone Stromal Cells from Mandibular Tori

Sumit Suamphan Anupong Makeudom Ekapong Dechtham Piyanat Sangangam Suttichai Krisanaprakornkit Chidchanok Leethanakul

---

Effect of saliva storage conditions on bacterial DNA quantification by real-time polymerase chain reaction

Jantipa Jobsri Rutchanoo Chansamart Krittiphat Rungnapapaisarn Wanida Yokliant Donlayawan Tidpraman Vijai Sensakul

---

Effect of Building Orientation on Marginal Gap and Internal Fit of The Implant-Supported 3D-Printed Provisional Crown

Atittaya Chaowthawee Patcharanun Chaiamornsup Pornpot Jiangkongkho Yosnarong Sirimethawong

---

Knowledge and Attitudes Towards Emergency Management of Traumatic Dental Injuries Among Elementary School Teachers in Sakaeo, Thailand

Kanchana Hemantakul Praewpat Pachimsawat Pornpoj Fuangtharnthip

---

The Remineralization Effect of Theobromine and CPP-ACP on Enamel of Primary Molars

Krairavee Theeranut Varisara Sirimaharaj Sithichai Wanachantarak

---

The accuracy of single tooth mini dental implant placement using computer assisted surgical guide: A randomized clinical trial comparative study

Voravan Vorasubin Weerapan Aunmeungthong Pathawee Khongkhunthian

---

Fabrication and Clinical Evaluation of a Novel 3D printed Hydroxyapatite/Polycaprolactone Composite (Novel 3DP HA/PCL) for Maxillary Sinus Augmentation: A Preliminary Study

Poommarin Thammanoonkul Suwit Limpattamapanee Faungchat Thammarakcharoen Jintamai Suwanprateeb Borvornwut Buranawat

---