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1. Review Articles: a comprehensive article with technical knowledge collected from journals and/or textbooks which is profoundly criticized or analyzed, or tutorial with the scientific writing.

2. Case Reports: a clinically report of an update or rare case or case series related to dental field which has been carefully analyzed and criticized with scientific observation.

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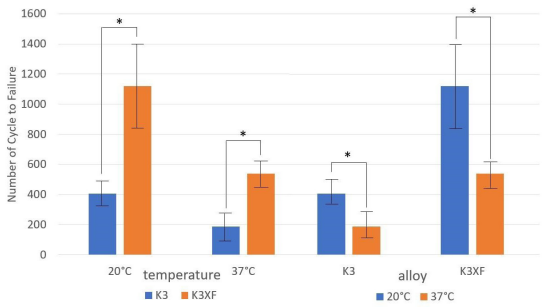
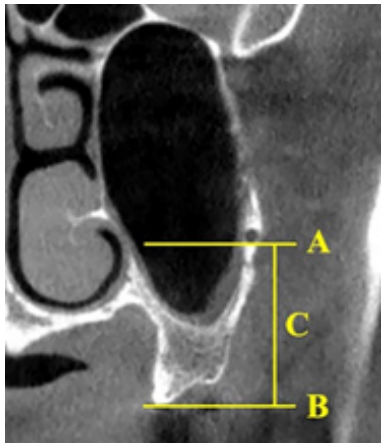
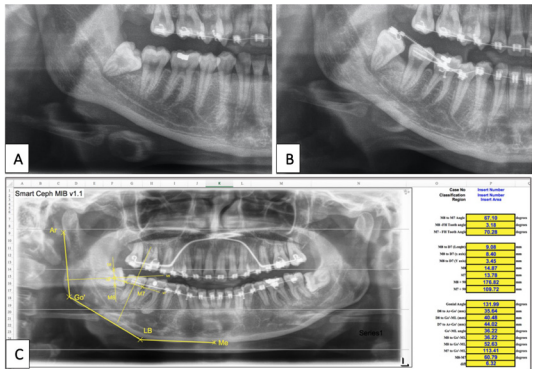
Line art	An image which is composed of line and text and is not contained of tonal or shading areas.		tif. of eps.	Monochrome 1-bit of RGB	900-1200 dpi
Half tone	A continuous tone photograph which does not compose of text.		tif.	RGB of Graycale	300 dpi
Combo	Combination of line art and half tone.		tif. of eps.	RGB of Graycale	500-900 dpi

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Sample of references from articles in Journals

- Authors

Zhao Y, Zhu J: *In vivo* color measurement of 410 maxillary anterior teeth. ***Chin J Dent Res*** 1998;1(3):49-51.

- Institutional authors

Council in Dental Materials and Devices. New American Dental Association Specification No.27 for direct filling resins. ***J Am Dent Assoc*** 1977;94(6):1191-4

- No author

Cancer in South Africa [editorial]. ***S Afr Med J*** 1994:84:15

Sample of references from books and other monographs

- Authors being writers

Neville BW, Damn DD, Allen CM, Bouquot JE. Oral and maxillofacial pathology. Philadelphia: WB Saunder; 1995. P. 17-20

- Authors being both writer and editor

Norman IJ, Redfern SJ, editors. Mental health care for the elderly people. New York: Churchill Livestone; 1996.

- Books with authors for each separate chapter

- Books with authors for each separate chapter and also have editor

Sanders BJ, Handerson HZ, Avery DR. Pit and fissure sealants; In: McDonald RE, Avery DR, editors. Dentistry for the child and adolescent. 7th ed. St Louis: Mosby; 2000. P. 373-83.

- Institutional authors

International Organization for Standardization. ISO/TR 11405 Dental materials-Guidance on testing of adhesion to tooth structure. Geneva: ISO; 1994.

Samples of references from academic conferences

- Conference proceedings

Kimura J, Shibasaki H, editors. R The Journal of the Dental Association of Thailand (JDAT): (ISSN 2408-1434) online open access and double-blind peer review journal and also supported by the Dental Association of Thailand advances in clinical neurophysiology. Proceeding of the 10th International Congress of EMG and Clinical Neuro physiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam; Elsevier; 1996.

- Conference paper

Hotz PR. Dental plague control and caries. In: Lang PN, Attstrom R, Loe H, editors. Proceedings of the European Work shop on Mechanical Plague Control; 1998 May 9-12; Berne, Switzerland. Chicago: Quintessence Publishing; 1998. p. 25-49.

- Documents from scientific or technical reports

Fluoride and human health. WHO Monograph; 1970. Series no.59.

Samples of reference from thesis

Muandmingsuk A. The adhesion of a composite resin to etched enamel of young and old teeth [dissertation]. Texas: The University of Texas, Dental Branch at Houston; 1974.

Samples of reference from these articles are only accepted in electronic format

- Online-only Article (With doi (digital identification object number))

Rasperini G, Acunzo R, Limiroli E. Decision making in gingival rec experience. ***Clin Adv Periodontics*** 2011;1: 41-52. doi:10.1902 cap.2011.1000002.

- Online only article (without doi)

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. ***Am J Nurs*** 2002; 102(6) [cited 2002 Aug 12] Available from: http://nursingworld.org/AJN/2002/june/WaWatch.htmArticle

Samples of references from patents/petty patents

- Patent

Pagedas AC, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1.

- Petty patent

Priprem A, inventor, Khon Kaen University. Sunscreen gel and its manufacturing process. Thailand petty patent TH1003001008. 2010 Sep 20.

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Front cover image: adapted from Figure 5 Computed tomography A) axial view **B) 3-dimensions showing areas of bone erosion with multiple air foci and hypodense collection in marrow cavity** C) axial view D) coronal view, presented complete obliteration of the right maxillary sinus (depicted by the white asterisk), partial obliteration of the left maxillary sinus exhibits (indicated by the white arrow). (see *Kaboosaya et al., page 112 for detail*)

Case Report

Invasive Maxillary Mucormycosis in Post-COVID-19: A Case Report and Review of Literatures

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Abstract

Patients with COVID-19 are predisposed to opportunistic fungal infections, particularly mucormycosis. This risk is heightened in individuals with specific systemic conditions, such as uncontrolled diabetes mellitus, immunocompromised status, or as a consequence of prescribed medications. Although mucormycosis typically presents acutely in the rhinocerebral region, originating from the upper turbinate and paranasal sinus, reported cases of mucormycosis in the jaws are scarce. This study presents a unique case involving a 60-year-old immunocompetent female with a history of COVID-19. Following discharge, she experienced mobility in the upper anterior teeth accompanied by pus discharge, leading to a diagnosis of maxillary mucormycosis upon histopathological investigation. Notably, the patient exhibited no prior immunocompromised status, emphasizing the need for heightened awareness even in immunocompetent individuals with a history of COVID-19. Globally, there has been an increase in fungal infections following the COVID-19 pandemic, with limited presentations. Prompt identification of maxillary mucormycosis post-SAR-CoV-2 infection is crucial for timely intervention. This study elucidates a case of COVID-19-associated mucormycosis (CAM) in an immunocompetent patient and reviews the existing medical literature on CAM affecting the jaw, thereby contributing to the understanding and management of this emerging issue.

Keywords: COVID-19, Fungal infection, Jaws, Mucormycosis

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Introduction

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread worldwide, prompting its classification as a global pandemic by the World Health Organization on March 11, 2020.[1] The clinical presentation of COVID-19 is highly variable, mirroring other pathogens, thus necessitating careful consideration in diagnosis. Prominent symptoms with sensitivities exceeding 50% include cough, sore throat, fever, myalgia or arthralgia, and headache.[2] Notably, approximately one-third of SARS-CoV-2 infections are asymptomatic, while others may progress to severe manifestations such as pneumonia, acute respiratory dis-

tress, and multi-organ failure.[3] Moreover, adding to this complexity, SARS-CoV-2 has been implicated in a spectrum of opportunistic infections, with mucormycosis caused by fungal pathogens emerging as a particularly invasive concern.

Mucormycosis is a rare, life-threatening opportunistic infection caused by fungi commonly known as black fungus. These fungi are widely distributed as decomposers of decaying organic matter in the environment. Transmission primarily occurs through the inhalation of spores,[4] with the rhino-orbital/rhino-cerebral region being the most frequently affected site of infection.[5] Mucormycosis predominantly affects males, with a mean age of approximately 51 years.[6] COVID-19 associated mucormycosis (CAM) has exhibited pronounced incidence in India, with reported cases predominantly observed in individuals undergoing treatment or recovering from SAR-CoV-2 infection, reaching rates approximately 70 times higher than the global data. The overall fatality rate varies between 16.3% and 61.9%, contingent on regional disparities.[4,7] Also, 35% of cases developed mucormycosis during active COVID-19 infections, while 65% manifested post-recovery.[8]

The development of mucormycosis in post-COVID-19 patients can be attributed to various factors. Underlying conditions notably latent diabetes mellitus serve as significant predisposing factors.[6,8-12] The administration of corticosteroids during COVID-19 treatment, aimed at modulating inflammation-mediated lung injury and mitigating the progression of respiratory failure, is identified as a prominent risk factor.[4,8,10-12] Additionally, other contributory risk factors include an immunocompromised host, hematological conditions such as leukemia and lymphoma, and individuals who have undergone solid-organ transplantation.[6] Chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), human immunodeficiency virus (HIV) infection.[5] and iron overload are also associated with an increased predisposition to mucormycosis.

Timely diagnosis and effective management of aggressive fungal co-infections associated with COVID-19 are imperative to mitigate fatal consequences and reduce mortality rates. However, there is a paucity of literature specifically addressing fungal co-infections in the oral region. This article aims to contribute to the understanding of such infections by presenting our experience with a case of maxillary mucormycosis in a female with a recent history of hospitalization for SARS-CoV-2 infection., The study details both the clinical presentation and histopathological characteristics of the case. Furthermore, the authors conducted a comprehensive review to identify reported cases of mucormycosis affecting the jaws as a consequence of COVID-19, providing valuable insights into the evolving landscape of this rare but serious complication.

Clinical case

A 60-year-old female with a history of hypertension, managed with atenolol (50 mg), was admitted to a cohort ward following the contraction of COVID-19. Her lungs scan showed bilateral reticulonodular infiltrates. Upon admission, she received favipiravir (200 mg), nine tablets every 12 hours on day 1, followed by favipiravir (200 mg), four tablets every 12 hours from day 2 to day 11. Dexamethasone (4 g) was given as two tablets every 12 hours from day 1 to day 10, followed by a shift to prednisolone (5 mg), four tablets every 12 hours from day 11 to day 13. Subsequently, the prednisolone dosage was reduced to two tablets every 12 hours from day 15 to day 18. On day 11, azithromycin (500 mg) was initiated at one tablet per day for a five-day course. Notably, the patient did not require intubation or mechanical ventilation and was discharged after a 19-day hospital stay.

Two months post-hospital discharge, the patient manifested mobility in the upper anterior teeth accompanied by pus discharge. The general dental practitioner conducted scaling and prescribed antibiotics—amoxicillin (500 mg) and metronidazole (400 mg)—to be taken three times a day. Unfortunately, after ten days, there was no improvement, prompting the patient to be referred to our department.

During the clinical examination, the patient complained of significant pain and discomfort, accompanied by mild diffuse swelling on the face. The overlying skin showed slight erythema without warmth (Fig.1A-1D).

Intraoral examination unveiled second- to third-degree mobility of teeth 15 to 25, with probing depths reaching approximately 5-6 mm. Gingival abscesses and fistulas were observed at 12, 14, and 22, although no shallow vestibule or facial space abscess was present (Fig. 2A). Radiographic assessments, including periapical (Fig. 2B-2D) and panoramic images (Fig. 2E), revealed generalized horizontal bone resorption. The initial diagnosis suggested generalized periodontitis, prompting the patient's referral for specialized periodontal treatment.

During a follow-up visit two weeks after the initial encounter, the patient presented with a palatal abscess and significant swelling in the buccal area (Fig. 3A-3B).

An incision and drainage procedure were performed on the hard palate and buccal mucosa (Fig 4A-4B), revealing few positive cocci in the Gram stain. Following this, the patient was prescribed amoxicillin/ clavulanate (1g), to be taken two tablets every 12 hours for a five-day course, and advised to use 0.12 % Chlorhexidine mouthwash.

However, five days later, the periodontist observed a lack of response to the electrical pulp test (EPT) in nearly all the upper teeth, along with an increase in mobility (upgrading from second to third mobility) in teeth 15-25. leading to the suspicion of a non-periodontitis-related cause. Consequently, further investigation using computed tomography (CT) was pursued.

Contrast-enhanced CT unveiled a distinctive radiological profile, showcasing heterogeneous density within the maxillary bone involving the alveolar process. The findings further revealed cortical disruption, extensive bone destruction (Fig. 5A-5B), and there was complete obliteration of the right maxillary sinus, while the left maxillary sinus exhibits partial obliteration (Fig. 5A-5B). Subsequently, the patient underwent a bone and soft tissue biopsy of the anterior maxilla under local anesthesia, revealing necrotic bone across the entire maxilla with minimal discharge.

Figure 1 *Clinical presentation on first visit; A) frontal view. B) worm's-eye view. C) left side, D) right side*

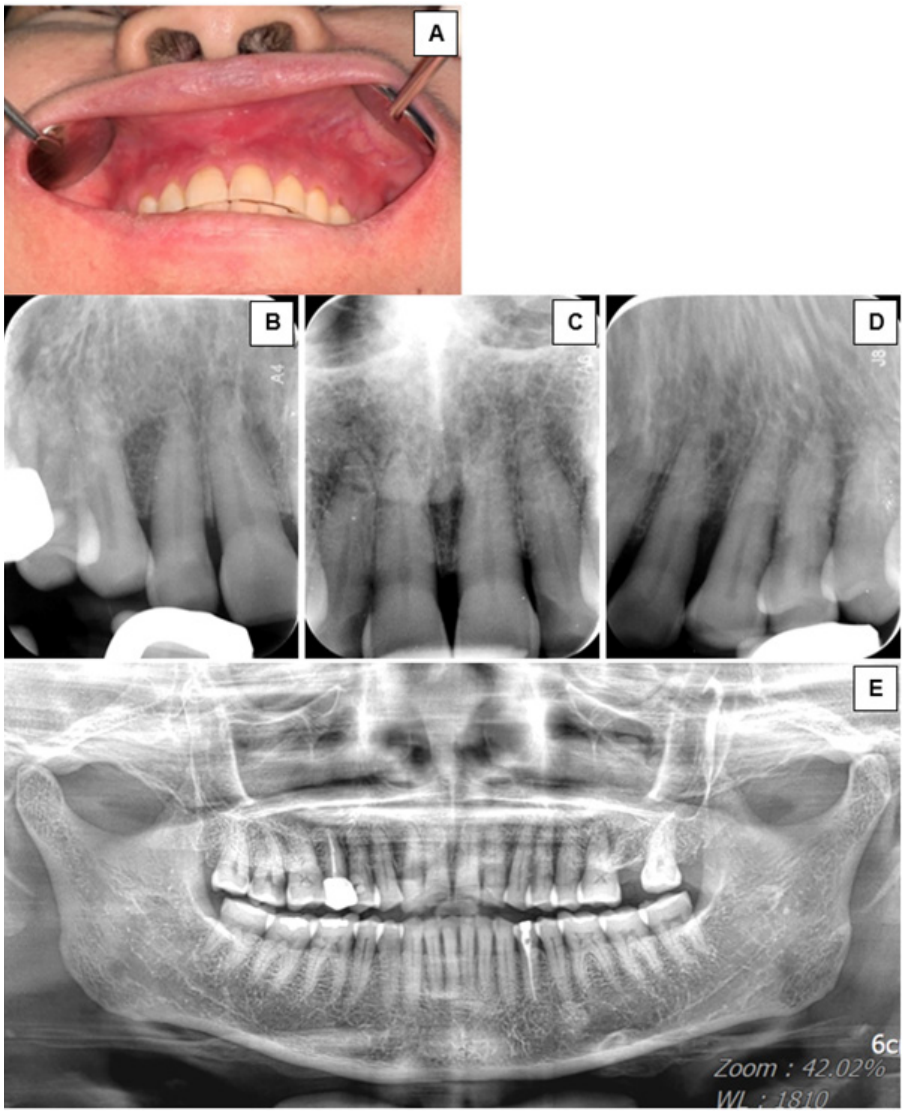

Figure 2 *Clinical presentation on first visit; A) upper vestibular area B) upper right C) middle D) upper left E) panoramic radiograph on first visit*

Figure 3 *Clinical presentation on second visit showed multiple draining sinuses A) buccal area, B) palatal area*

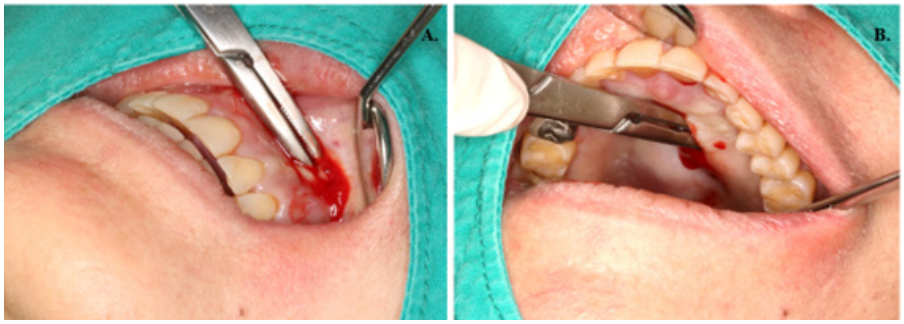

Figure 4 *Incision and drain procedure under local anesthesia A) buccal area, B) palatal area*

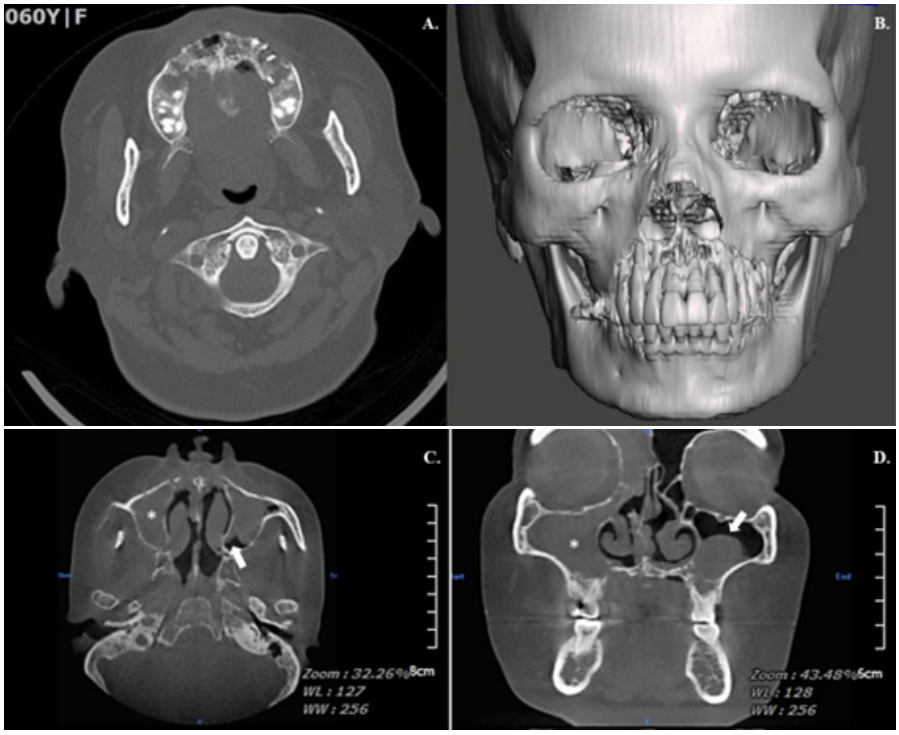

Figure 5 *Computed tomography A) axial view B) 3-dimensions showing areas of bone erosion with multiple air foci and hypodense collection in marrow cavity. C) axial view D) coronal view, presented complete obliteration of the right maxillary sinus (depicted by the white asterisk), partial obliteration of the left maxillary sinus exhibits (indicated by the white arrow).*

A negative acid-fast stain for bacterial infection was observed. However, the culture results demonstrated the presence of numerous Serratia marcescens and Klebsiella pneumonia. Histopathological analysis confirmed the presence of non-septate hyphae in the hard tissue (Fig. 6A-6C), further validated by periodic acid-Schiff (PAS) staining, which highlighted positive fungal non-septate hyphae of relatively large size (Fig. 4D). This distinct histopathological finding strongly supported the diagnosis of a deep fungal infection, specifically indicative of mucormycosis.

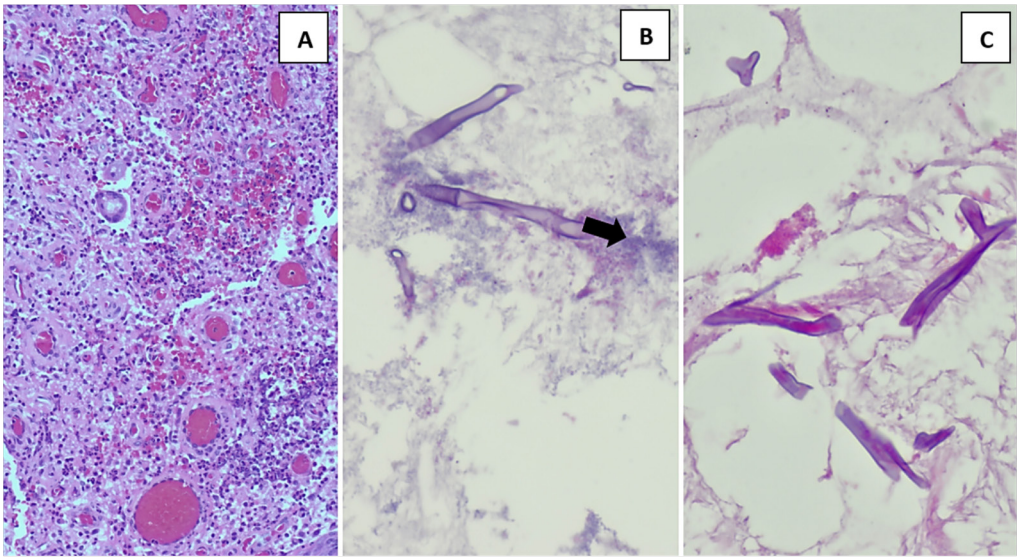

Figure 6 *Histopathology of biopsy: A) Haematoxylin and Eosin (H&E) at 20x magnification. B) H&E image at 100x magnification showing the large, non-septate, right-angle fungal hyphae (black arrow). C) Periodic Acid Schiff (PAS) images at 100x magnification showing broad, non-septate fungal hyphae*

Figure 7 *A.-B.) clinical presentation, C.) panoramic radiograph at 8th months post-maxillectomy*

Following the laboratory report, the serum chemistry of the patient exhibited glucose levels at 87 mg/dL, sodium at 137 mEq/L, potassium at 4.5 mEq/L, chloride at 99 mEq/L, total CO_2 at 25 mEq/L, and a morning cortisol level of 14.7 ug/dl. Due to limitations at the initial medical facility, the patient was referred to a tertiary care teaching hospital. Diagnostic sinonasal endoscopy revealed destruction of the nasal septum at the posterior bony part, along with darkened discoloration of the mucosa from the right nasal floor to the septum, not extending to the inferior meatus. Subsequently, an urgent maxillectomy and surgical debridement were performed, with concurrent administration of ceftriaxone (2 g) intravenously once daily and systemic antifungal treatment. The antifungal regimen consisted of amphotericin B administered intravenously as a 60-mg dose in 5% dextrose in water (500 ml) infused over a six-hour period. Premedication immediately before amphotericin B administration included chlorpheniramine 10 mg intravenously and paracetamol 500 mg orally, along with a 500-ml intravenous drip of normal saline over six hours.

The wound healed by secondary intention, resulting in oro-antral communication. During a follow-up visit four months after discharge, the patient remained asymptomatic with no signs of infection (Fig. 7A-7C).

Materials and Methods

A comprehensive literature search was conducted using the PubMed interface of MEDLINE, Web of Science, and Scopus to identify English language case reports, case series, and observational studies related to oral mucormycosis. The search strategy involved the use of the terms "[COVID-19] OR [SARS-CoV-2] OR [SARS-CoV2] OR [2019-nCoV] AND [Mucormycosis] OR [Mucorales] OR [Black fungus]" within the publication timeline of December 1, 2019, to April 30, 2022. Additionally, the authors manually reviewed references in pertinent articles. This review specifically focused on CAM cases involving jaws with active or recent SAR-CoV-2 infection. Cases with concurrent sino-maxillary involvement were excluded, as the pathogenesis may differ from isolated maxillary and mandibular region involvement.

Results

Following a meticulous screening process, fourteen articles were identified encompassing 90 cases that manifested mucormycosis of the jaws as a result of COVID-19. A comprehensive overview of patient characteristics and oral manifestations, highlighting predisposing conditions, is presented in Table 1.[13-28]

The age range of the affected individuals spanned from 29 to 77 years, with males constituting 74.44% of the entire patient cohort. The onset of symptoms after COVID-19 detection varied, ranging from one day to 159 days. Notably, diabetes mellitus emerged as a prevalent underlying condition in almost all the cases reviewed, with 24 cases reported in the maxillary area, one case in the mandibular region, and one article (Suresh *et al.*[25]) failing to specify the exact affected area. Additionally, a notable occurrence of mandibular mucormycosis was identified in a young, previously healthy adult post-COVID-19 infection. Twenty-one articles noted a potential association between steroid administration during COVID-19 and the occurrence of mucormycosis, albeit employing varying descriptions. Specifically, 13 articles reported steroid use during coronavirus treatment, while eight articles mentioned steroid use during COVID-19 treatment. Clinical symptoms predominantly manifested in the maxilla. While some articles lacked specific details regarding the fungal agent causing osteonecrosis, non-septate right-angle branched fungal hyphae emerged as the most encountered pathogen.

Table 1 *Characteristics of studies included in the review*

Author, year/ location	Study design	Number of case (oral/ total)	Onset of symptom after COVID-19 detection	Gender	Age (years)	Clinical characteristic at the time of presentation	Risk factors	Histopathologic features	Remark
Ahmed *et al.* 2021[13]/ Egypt	Case series	21/21	14 days	M=11 F=10	58±12 (mean)	-Palatal lesion	N/A	N/A	-
Mendhe *et al.* 2021[14]/ India	Case report	1/1		M	31	-Pain and swelling at right maxilla with multiple draining sinuses	N/A	N/A	-No information of COVID-19 infection
Ambereen *et al.* 2021[15]/ India	Case report	1/1	20 days	M	39	-Numbness at left lower lip -Pain and pus discharge at left posterior mandibular teeth	-Steroid use during COVID-19 treatment	-Broad non-septate right-angle branched fungal hyphae	-
Jawanda *et al.* 2021[17]/ India	Case report	1/1	120 days	M	70	-Pain at right maxilla	- Diabetes mellitus - Steroid use during COVID-19 treatment	-Broad non-septate right-angle branched fungal hyphae (mixed infections (Mucormycosis; black fungus, Actinomycosis; yellow fungus, and Candidiasis; white fungus)	-
Pathak *et al.* 2021[18]/ India	Case report	1/1	60 days	M	65	-Necrotic ulceration at hard palate	- Diabetes mellitus - Hypertension	-Numerous large non-septate righ angle branched fungal hyphae	-
Pauli *et al.* 2021[19]/ Brazil	Case report	1/1	8 days	F	50	-Painful bone-exposed ulcer at hard plate	- Diabetes mellitus	- Large non-septate right-angle branched fungal hyphae	-
Venugopal&Marya 2021[21]/ Cambodia	Case report	1/1	8 days	F	53	-Painless lesion at palate	-Diabetes mellitus	-Large non-septate with thin walls and branched hyphae	-
Krishna *et al.* 2021[22]/ India	Case reports	2/2	N/A	M	34	-Pain and swelling at right maxilla	- Diabetes mellitus - Hypertension	-Fungal osteomyelitis (not specific)	-
			N/A	M	50	-Swelling at right malar region	- Uncontrolled diabetes mellitus	-Non-septate right-angle branched fungal hyphae	-
Guptal&Dosi. 2021[23]/ India	Case reports	2/2	30 days	M	58	-Teeth pain and mobility at right maxilla	-Steroid use during COVID-19 treatment	N/A	-
			N/A	M	60	-Teeth pain and mobility at right maxilla	- Diabetes mellitus -Steroid use during COVID-19 treatment	N/A	-

Table 1 *Characteristics of studies included in the review (cont.)*

Author, year/ location	Study design	Number of case (oral/ total)	Onset of symptom after COVID-19 detection	Gender	Age (years)	Clinical characteristic at the time of presentation	Risk factors	Histopathologic features	Remark
Ahmed *et al.* 2021[24]/ Egypt	Case series	14/14	14-30 days	M	45	-Anterior maxillary bone exposure	- Post COVID-19 diabetes mellitus	N/A	-
				M	35	-Right posterior palatal bone exposure	- Post COVID-19 diabetes mellitus	N/A	-
				M	65	-Right and left maxillary bone	- Diabetes mellitus	N/A	-
				M	48	-Right and left maxillary bone exposure	- Diabetes mellitus	N/A	-
				M	76	-Right maxilla bone exposure	- Diabetes mellitus	N/A	-
				F	55	-Left palatal bone exposure	- Diabetes mellitus	N/A	-
				F	61	-Left palatal bone + alveolar bone exposure	- Diabetes mellitus	N/A	-
				M	45	Alveolar + anterior palatal bone exposure	- Post COVID-19 diabetes mellitus	N/A	-
				M	52	-Anterior palatal bone exposure	- Diabetes mellitus	N/A	-
				F	53	-Necrosis of premaxilla	- Diabetes mellitus	N/A	-
				M	29	-Premaxilla + left palatal and alveolar bone exposure	- Post COVID-19 diabetes mellitus	N/A	-
				M	77	-Right and left palatal bone exposure	- Diabetes mellitus	N/A	-
				F	49	-Anterior and right palatal + alveolar bone exposure	- Post COVID-19 diabetes mellitus	N/A	-
				M	69	-Right palatal bone exposure	- Diabetes mellitus	N/A	-
Suresh *et al.* 2022[25]/ India	Observa-tional study	39	1-159 days (mean=53.38)	M=32 F=7	50.69	-Teeth pain (n=32) -Teeth mobility (n=38) -Gingival swelling (n=27) -Sinus opening with pus discharge (n=27) -Ulceration or blackish discoloration of hard plate (n=12)	- Diabetes mellitus (n=32) - Steroid use during COVID-19 treatment (n=21)	-Mucorale spp. (n=33) -Aspergillus fumigatus (n=2) -Curvulria lunata (n=2) - Mucorale spp.+Candida albicans (n=1) - Mucorale spp.+ Aspergillus fumigatus (n=1)	

Table 1 *Characteristics of studies included in the review (cont.)*

Author, year/ location	Study design	Number of case (oral/ total)	Onset of symptom after COVID-19 detection	Gender	Age (years)	Clinical characteristic at the time of presentation	Risk factors	Histopathologic features	Remark
Ingle *et al.* 2022[26]/ India	Case report	1/1	25 days	M	74	-Teeth mobility at left maxilla	-Uncontrolled diabetes mellitus -Steroid use during COVID-19 treatment	-Numerous non-septate hyphae with and without branching in the granulation and necrotic tissues	-Superimposed with ameloblastoma
Chugh *et al.* 2022[27]/ India	Case series	3/4	60 days	M	64	-Pain at posterior right mandible and numbness at right lower lip	-Diabetes mellitus -Steroid use during COVID-19 treatment	-Non-septate hyaline ribbon like right angled branching fungal hyphae	-
			60 days	M	35	-Pain, paresthesia and teeth mobility at left mandible	-Steroid use during COVID-19 treatment	-Fungal hyphae (not specific)	-
			60 days	M	35	-Multiple sinuses with pus discharge at labial gingiva of left maxilla with mobility of dentoalveolar complex	-Steroid use during COVID-19 treatment	-Broad non-septate right-angle branched fungal hyphae and thin septate acute-angle branched fungal hyphae	- Possibility of mixed mucor and aspergillus infection
Fakhar *et al.* 2022[28]/ Iran	Case reports	2/2	30 days	M	35	-Teeth mobility at right maxilla	- Uncontrolled diabetes mellitus	-Broad septate fungal hyphae	-
			30 days	M	40	-Teeth mobility at right maxilla	- Diabetes mellitus	-Broad ribbon-like branched fungal hyphae	-

Discussion

COVID-19 is a new disease that was first documented in December 2019 in China. Previous articles have reported a potential increased risk of developing invasive fungal infection (mucormycosis) in COVID-19 patients.[4,7,10,11,29] A prospective cohort evaluation of 135 COVID-19 patients stated that the fungal infection incidence was 26.7%. The mortality rates in COVID-19-associated patients with and without fungal disease were 53% and 31%, respectively.[30] According to this review, fungal infection of the jaws related to SARS-CoV-2 infection has a male predisposition. The previous article mentioned that the incidence of mucormycosis was not gender-dependent.[31] Nonetheless, the notably higher number of males affected by mucormycosis-related COVID-19 may be attributed to the higher incidence of COVID-19 cases identified in males.[6,9]

Mucormycosis, an angio-invasive fungal infection, causes thrombi formation, reduces blood supply, and leads to blood vessel necrosis.[33] COVID-19 appears to increase susceptibility to mucormycosis, as seen in autopsies by Auckermann *et al.*[34], where COVID-19 induced endothelialitis, microvascular thrombosis, and disrupted cell membranes. Thrombi may provide iron, thereby promoting fungal growth. Elevated cytokine levels, particularly interleukin-6, in COVID-19 patients increase ferritin levels, leading to excess free iron.[4] This iron overload contributes to tissue damage and necrosis.

Several studies have indicated that diabetes and diabetic ketoacidosis were the most significant risk factors observed in the majority of mucormycosis in COVID-19 cases.[4,5] In this review, 13 of 16 articles mentioned diabetes as a risk factor. The current case, while lacking a diabetes history, presented with hypertension and corticosteroid immunosuppression during COVID-19 treatment. Cardiovascular disease was also mentioned as a risk factor for mucormycosis.[5] A systematic review of 144 COVID-19 and mucormycosis co-infection cases reported hypertension in 34.3%. However, hypertension and diabetes often co-exist in many patients. However, because hypertension and diabetes often coexist, attributing mucormycosis solely to hypertension might be overestimated due to the high prevalence of diabetes.[10]

Steroid use during COVID-19 treatment was stated as a risk factor related to fungal infection in eight of the 16 articles. Corticosteroids, such as dexamethasone, have proven effective in treating COVID-19 and reducing mortality in severe cases.[35] However, corticosteroids could cause drug-induced hyperglycemia, impairing granulocyte phagocytic capacity and potentially contributing to opportunistic fungal infections.[36] Some authors have suggested stopping steroid prescription in non-hypoxemic COVID-19 patients and limiting the steroid dose and duration in hypoxic patients.[37]

SARS-CoV-2 utilizes angiotensin-converting enzyme II (ACE2) for cell entry,[38] and its distribution, including in the lung, heart, kidney, oral mucosa, and tongue epithelial cells, suggests potential infection risks.[39] This indicates a direct impact on the oral cavity, making it a potentially high-risk area for COVID-19 susceptibility and a possible transmission route.[40] In this review, most cases reported mucormycosis in the maxilla, in contrast with only two cases in the mandible. The maxilla was rare osteomyelitis, attributed to its vascularity, thin cortical bone, and porous architecture. Notably, maxillary cases are often presented with hallmark signs such as pain and tooth mobility. These reviewed cases suggest an elevated incidence of mucormycosis in the jaws due to the combination of COVID-19 infection, diabetes mellitus, and steroid use during coronavirus treatment.

CAM onset varied from three10 to 90 days after confirmation,11 averaging around 25.6 ± 21 days.10 In a prospective study of 95 post-COVID patients, 98% developed invasive mucormycosis within 20 days of recovery. At the time of presentation, 91.5% of patients complained of local facial pain, 9.5% reported pain in the upper teeth, and 2.1% experienced pain in the lower teeth along with loosening.[12] From this review, the average onset of symptoms was approximately 30-60 days after COVID-19 detection. In the current case, maxillary infection symptoms emerged

around day 30 post-COVID confirmation, with a mucormycosis diagnosis on day 87.

Microscopy and culture methods continue to serve as the gold standard for identifying pathogenic fungi. These techniques offer several advantages, including the ability to identify specific species, thereby facilitating differentiation between septate molds (such as Aspergillus spp.), non-septate molds (such as Mucorales), and yeasts (such as Candida spp.). Moreover, these methods enable the assessment of antifungal resistance.[41] However, they are associated with certain drawbacks, including low sensitivity. Cultures from biopsy specimens or blood often yield negative results, and positive cultures may sometimes result from contamination rather than true infection. Additionally, these methods are characterized by long turnaround times.[42]

Quantitative polymerase chain reaction (qPCR) or real-time PCR in blood samples is now acknowledged as a non-invasive tool for early mucormycosis diagnosis. The assay demonstrated a sensitivity of 99.29% and a specificity of 83.84% for the diagnosis of invasive mucormycosis.[43] Quantitative polymerase chain reaction (qPCR) was conducted using both tissue samples, which were also utilized for cultures, and blood samples. The detection of circulating Mucorales DNA in serum or plasma holds superiority as it can be initiated promptly upon clinical suspicion of the diagnosis, and it may be carried out in all patients, including those unable to undergo a biopsy. qPCR is now highly valued for diagnosing fungal infections due to its easy handling, rapid turnaround time (approximately 3 hours), specificity, and cost-effectiveness.[44]

Successful management of mucormycosis encompasses both surgical and medical interventions. A standard daily dose of Liposomal amphotericin B suggested by current guidelines of less than 5 mg/kg/day, is considered the drug of choice for invasive mucormycosis,[32] with several studies reporting overall survival rates of up to 72%.[45] However, a significant limitation of amphotericin B is that it can only be administered parenterally, and its safety profile is associated with increased nephrotoxicity and electrolyte imbalances.[46] In such cases, newer triazoles, namely posaconazole and isavuconazole, are recommended.[32]

In vitro studies have shown that posaconazole exhibits varying activity against Mucorales, with its effectiveness being species-dependent.[47] Posaconazole is currently available only as an oral suspension and is preferably taken with a high-fat meal to enhance bioavailability.[48] These dietary requirements pose challenges for critically ill patients who may not be able to eat. On the other hand, isavuconazole is a newer broad-spectrum antifungal agent with minimum inhibitory concentration (MIC) values for Mucorales that are 2- to 4-fold higher than those of posaconazole.[49] Isavuconazole is available in both intravenous and oral formulations, is associated with less hepatotoxicity and no nephrotoxicity, and exhibits excellent oral bio-availability with no specific food requirements.[46]

To the best of our knowledge, this is the first literature review on fungal infections of the jaws related to COVID-19. For the case report, this initial presentation of the patient mimicked chronic periodontitis. Computed tomography was chosen over cultures and plain imaging for detecting mucormycosis. A definitive diagnosis relied on histological investigation from a biopsy, which detailed fungus morphology, tissue reaction, and blood vessel invasion. Treatment involved addressing underlying conditions, extensive surgical debridement, and antifungal therapy (amphotericin B). Aggressive debridement, facial reconstruction, and supportive therapy were crucial for restoring the patient's quality of life.

The precise pathogenesis remains unknown. Mucormycosis is an exceedingly rare infection in healthy individuals. It is plausible that the patient may have inadvertently consumed contaminated food. Concurrently, the patient had been administered corticosteroids during COVID-19 treatment, comprising dexamethasone (16 mg/day for ten days), followed by prednisolone (40 mg/day for three days) and then prednisolone (20 mg/day for three days). A systematic review and meta-analysis of 958 cases revealed that the majority of patients with CAM were treated with corticosteroids (78.5%), particularly dexamethasone, which was the most commonly prescribed drug (46.6%). These findings are consistent with the treatment regimen of our patient.[50]

Due to the common presenting complaints of CAM of the jaws, such as gingival swelling (18.5%) and palatal necrosis or ulcer (12.7%),[51] prompt detection is facilitated, enabling immediate treatment initiation, and potentially reducing mortality rates. The overall mortality rate of CAM related to the head and neck area was 38.32%,[52] which was lower than that of pulmonary mucormycosis (80%),[53] and disseminated mucormycosis (96%).[54] However, limitations of this study include the inability to differentiate outcomes based on glycemic control status due to the lack of information on HbA1C and limited data on the fungal culture.

Conclusion

Typical pain and swelling in the oral region should raise suspicion for a cautious diagnosis in individuals with a history of previous COVID-19 infection. Biopsy plays a crucial role as a diagnostic tool in such cases. It is crucial to differentiate mucormycosis from other infections to initiate early and appropriate treatment, thereby improving outcomes. Patients with suspected mucormycosis should be referred immediately to a facility with the highest care level. This article aims to raise awareness among clinicians about this rare yet potentially fatal fungal infection.

Ethical approval and consent to participate

The research project was approved by the Human Research Ethics Committee of the Faculty of Dentistry, XXX (HREC-XXX 2022-065). A written consent for publication was obtained from the patient.

Consent for publication

The authors affirm and agree to the submission of this paper. The human research participants provided informed consent for publication of the images in Fig. 1A-1D, Fig. 2A-2E, Fig. 3A-3B, Fig. 4A-4B, Fig. 5A-5D, Fig. 6A-6Cc, and Fig. 7A-7C.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Boosana Kaboosaya, Napat Damrongsirirat, Saraporn Koosrivinij and Atiphan Pimkhaokham. The first draft of the manuscript was written by Boosana Kaboosaya, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

The authors declare that data supporting the findings of this study are available.

Declaration of Competing interests

All authors confirm they have no competing interests in the publication of this paper.

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Original Articles

Effect of Variations in Translucency of CAD/CAM Lithium-disilicate Ceramic and Abutment Color on Optical Color of Veneer Restoration

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Abstract

This study aimed to evaluate color differences of different translucencies of CAD/CAM lithium-disilicate ceramics on backgrounds with different values. Low translucency (LT) and medium translucency (MT) rectangular-shaped specimens (0.6 mm thickness, shade A1) were fabricated from IPS e.max CAD (EMC; Ivoclar Vivadent) and Amber Mill (AM; HASS). Six specimens of EMC-MT, EMC-LT, AM-MT, and AM-LT were fabricated, resulting in 24 specimens in total. The specimens were placed on six background colors (1M1(reference), 1M2, 2M2, 3M2, 4M2, and 5M2) made of light-cured resin composite (Vita VM LC Base Dentine, Vita Zahnfabrik) using glycerine as a medium. The CIE L*a*b* coordinates were evaluated using a spectrophotometer (Ultrascan PRO, Hunter Lab) and calculated using the CIEDE2000 formula to determine the color difference (ΔE_{00}) and translucency parameter (TP_{00}) of the materials. Mean ΔE_{00} values were statistically analyzed using a two-way analysis of variance (ANOVA) and Bonferroni post-hoc tests (α=0.05), then qualitatively analyzed to consider the perceptibility threshold (PT) and acceptability threshold (AT). Mean TP_{00} values were analyzed by an independent *t*-test (α=0.05). The results revealed that the ΔE_{00} values significantly increased as darker backgrounds were used. The significantly higher ΔE_{00} values of MT compared to LT were presented when EMC specimens were placed on 2M2, 3M2, 4M2, and 5M2 backgrounds. The ΔE_{00} values below AT were found in the specimens placed on the 1M2 background. The TP_{00} values showed that MT had a statistically significant higher value compared to LT in both EMC and AM, and values from AM were higher than EMC. In conclusion, the masking ability of specimens was lower when placed on darker backgrounds. For the 2M2 darker background, IPS e.max CAD in low translucency showed better masking ability than medium translucency. Amber Mill showed more translucency and poorer masking properties than IPS e.max CAD.

Keywords: CAD/CAM block, Ceramic translucency, Ceramic veneers, Color difference, Lithium disilicate ceramic

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Introduction

Ceramic laminate veneer restorations exhibited good clinical long-term success.[1-3] One of the materials used for fabricating ceramic veneers is lithium disilicate. This material has good physical properties and optical characteristics that mimic a natural tooth. Its excellent biomechanical properties allow the thickness of restorations to be minimal in the anterior areas, resulting in the conservation of dental tissues.[4, 5] Studies have shown that restorations fabricated with a lithium disilicate material exhibited very high survival and success rates.[6, 7] Nowadays, computer-assisted design/computer-assisted manufacturing (CAD/CAM) technology has shown to improve clinical predictability with quick and reliable results.[8]

CAD/CAM lithium disilicate ceramic blocks, such as IPS e.max CAD (Ivoclar Vivadent, Liechtenstein) and Amber Mill (HASS, Korea), are the materials that combine both benefits from lithium disilicate and CAD/CAM technology and have been well used in several regions of the world. These materials are available in various translucencies. The translucency levels recommended to fabricate monolithic restoration for veneer were high, medium, and low.[9, 10] Different translucency levels of the lithium disilicate materials might result differently in the final color of the restoration when being restored on abutments with the same color.[11-13] Some challenges for fabricating ceramic veneers are matching the optical color of adjacent natural teeth and creating a life-like restoration. Many factors may influence the optical color of the restorations, such as ceramic translucency, ceramic thickness, abutment color, cement color, and cement thickness.[11-16]

Many studies compared low and high translucency lithium disilicate used for veneers, and it was shown that high translucency lithium disilicate was unsuitable for masking dark color backgrounds. This translucency was more suitable in situations where a small amount of color change from the background was preferred.[11, 13, 17, 18] However, there are limited studies on medium translucency lithium disilicate in shade reproduction or masking ability compared to low translucency lithium disilicate.

Many previous studies have shown that the optical properties of these materials are complex.[11-13] Precisely specified color-matching standards for the ceramic laminate veneers still have not been established. Former studies showed that the traditional color reproduction protocol was unsuitable for all ceramic veneers.[17, 18] There is limited information regarding various translucencies of CAD/CAM lithium disilicate materials on different values of background shades.[19] A previous study has shown that the value of the underlying background probably had a stronger effect than chroma on the final color of restorations.[11] However, in most of the previous studies, background abutments were fabricated from resin materials with shade corresponding to the Vita classic shade guide, therefore, lacking proper order in value.[11, 12]

Therefore, the purpose of this study was to evaluate color differences of veneer restorations when using different translucencies of CAD/CAM lithium disilicate ceramics on backgrounds with different values. The null hypothesis was that the translucency of CAD/CAM ceramic materials and the value of the background would not affect the optical color of veneer restorations, while translucency values would not be different among the medium and low translucent materials.

Materials and Methods

Medium translucency (MT) and low translucency (LT) rectangular-shaped specimens, 0.6 mm thickness, were fabricated from IPS e.max CAD (EMC; Ivoclar Vivadent, Liechtenstein) and Amber Mill (AM; HASS, Korea) size C14, shade A1. Four groups of ceramic specimens were EMC-MT, EMC-LT, AM-MT, and AM-LT, consisting of six specimens in each group, therefore there were 24 specimens in total. The specimens were fabricated by sectioning the ceramic blocks with a water-cooled precision diamond saw (Isomet low-speed saw, Buehler, USA), and polished using a polishing machine (Minitech 233, Presi, France) at 100 rpm for 30 sec under water cooling with 600- and 800-grit SiC paper on both sides. The outer surface of the ceramic specimens

was finely polished with a 1200-grit SiC paper. A thickness of 0.6 (±0.05) mm was verified using a digital micrometer (IP-65 Digimatic Micrometer; Mitutoyo, Japan). Crystallization and glaze firing of the specimens were performed simultaneously in a furnace (Programat P700, Ivoclar Vivadent, Liechtenstein), according to the manufacturer's instructions. To simulate the natural variations in the appearance of dentin and to comply with the Vita 3D-Master color organization system (Vita Zahnfabrik, Germany), a light-curing composite material for extraoral application (Vita VM LC Base Dentine, Vita Zahnfabrik, Germany) in shades 1M1, 1M2, 2M2, 3M2, 4M2, and 5M2 was used to fabricate background specimens. The 4 mm thick, rectangular-shaped backgrounds were fabricated using a mold, and polymerized by a light curing unit (Solidilite V, Shofu Dental, USA) according to the manufacturer's instructions. The 1M1 shade was used as a reference background (Fig. 1).

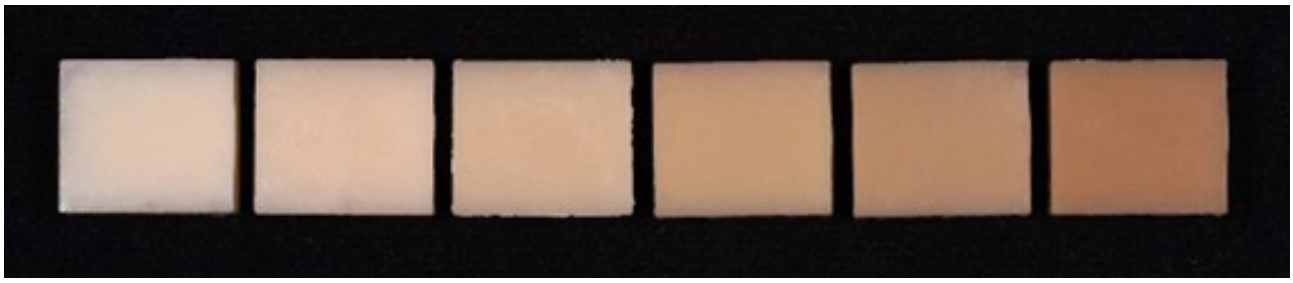

Figure 1 *Different background colors from left to right: 1M1 (reference), 1M2, 2M2, 3M2, 4M2, and 5M2*

All-ceramic specimens were placed on all six different color backgrounds using glycerine as a medium to exclude the effect of cement color and to minimize light scattering between the specimens and the backgrounds (Fig. 2).[20, 21] The color expressed in CIE L*a*b* coordinates was recorded at the center of the specimen using a spectrophotometer (Ultrascan PRO, Hunter Lab, USA) with a 7 mm diameter port on a standard white background under CIE standard illuminant D65, which represented typical daylight. Color coordinates of materials placed on a 1M1 background, a reference, and color coordinates of the materials on other backgrounds were calculated in the CIEDE2000 formula to determine the color difference (ΔE_{00}) values. CIELAB is classically the standard parameter for total color difference between two objects. However, CIEDE2000 (ΔE_{00}) formula was used in this study since it was formulated to improve the correction between perceived and computed color differences. CIEDE2000 is also the most recent international standard recommended by the CIE.[22] In addition, translucency parameter (TP_{00}) values of the lithium disilicate specimens were also calculated from the CIEDE2000 color difference metric by measuring the L*a*b* coordinate differences between the materials positioned over black and white backgrounds. Greater TP_{00} values meant higher translucency of the materials.

Figure 2 *Ceramic specimen over shaded resin composite background*

The ΔE_{00} data was collected and analyzed using the statistical software SPSS version 29 (SPSS, Chicago, IL, USA). Normal distribution was determined by the Shapiro-Wilk test. The homogeneity of variance was tested using Levene's test. Two-way repeated ANOVA and Bonferroni post-hoc multiple comparison tests were used to identify the effect of translucency, abutment color, and their interactions on the mean ΔE_{00} data and to detect significant differences in the mean ΔE_{00} values among the groups.

The *P* value < 0.05 was considered a statistically significant difference. Additionally, ΔE_{00} data were qualitatively analyzed considering the perceptibility threshold (PT) and acceptability threshold (AT). The PT and AT were set at 0.8 and 1.8, respectively. If the ΔE_{00} value was at or below PT, it represented an excellent match; if the difference was between PT and AT, it represented an acceptable match; and if the difference was above AT, it represented an unacceptable match.[23] The difference in translucency parameter (TP_{00}) values between MT and LT specimens was evaluated and analyzed by an independent *t*-test (α=0.05).

Results

The Shapiro-Wilk test indicated that the data were normally distributed. The mean and standard deviation of the color difference (ΔE_{00}) values of each tested group are shown in Tables 2 and 3. The lowest mean ΔE_{00} value was obtained from EMC-LT placed on a 1M2 background, and the highest mean ΔE_{00} value was demonstrated from AM-MT placed on a 5M2 background. Two-way ANOVA revealed that the color of backgrounds, levels of translucency, and their interactions had statistically significant influences on ΔE_{00} values (P<0.001) of EMC groups (Table 1). On the other hand, for AM groups, only the color of backgrounds had a statistically significant influence on ΔE_{00} values (P<0.001). The translucency and their interaction did not significantly influence ΔE_{00} values (P=0.875 and 0.805, respectively) (Table 1).

According to Bonferroni post-hoc multiple comparison tests, when the background color was 1M2, the ΔE_{00} values between the reference background color 1M1 and the tested background color 1M2 of EMC-LT did not have a statistically significant difference from EMC-MT (P=0.062) (Table 2). For other tested background colors, EMC-LT had a statistically significant lower mean ΔE_{00} value than EMC-MT (Table 2). On the other hand, AM-MT and AM-LT did not have statistically significantly different ΔE_{00} values on any tested background colors (Table 3). Statistically, ΔE_{00} values significantly increased as darker backgrounds were used for both translucencies of EMC and AM (Tables 2 and 3). None of the test groups could provide ΔE_{00} values below PT. The ΔE_{00} values below AT were found in all groups of specimens placed on the 1M2 background color, while specimens placed on other background colors had ΔE_{00} values higher than AT (Fig. 3). The comparison of mean TP_{00} values showed that MT had statistically significantly higher mean TP_{00} values than LT in both EMC and AM groups. The TP_{00} values from AM groups seemed much more elevated than EMC groups (Table 4).

Table 1 *Two-way repeated ANOVA, showing results of the effect of backgrounds (A), translucency (B), and their interactions (A x B) on the mean ΔE_{00} values of IPS e.max CAD (EMC) and Amber Mill (AM)*

Source	Sum of Squares	df	Mean Square	F	*P* Value
EMC					
Backgrounds (A)	49.152	4	12.288	1319.433	< 0.001
Translucency (B)	1.389	1	1.389	110.703	< 0.001
A x B	0.488	4	0.122	13.106	< 0.001
AM					
Backgrounds (A)	94.208	4	23.552	3902.473	< 0.001
Translucency (B)	0.041	1	0.041	0.025	0.875
A x B	0.010	4	0.002	0.403	0.805

Table 2 *Mean and standard deviation of ΔE_{00} value between EMC specimens placed on 1M1 background (reference) and those placed on 1M2, 2M2, 3M2, 4M2, and 5M2 backgrounds*

Background color	Translucency		*P* Value
	EMC-MT	EMC-LT	
1M2	1.61 (0.13) [a]	1.43 (0.16) [a]	0.062
2M2	2.19 (0.06) [b]	1.99 (0.10) [b]	0.002
3M2	2.64 (0.07) [c]	2.39 (0.11) [c]	< 0.001
4M2	3.11 (0.04) [d]	2.88 (0.09) [d]	< 0.001
5M2	4.52 (0.10) [e]	3.86 (0.07) [e]	< 0.001

Different superscript lowercase letters within columns represent significant differences (P<0.05) between background colors.

EMC, IPS e.max CAD; MT, medium translucency; LT, low translucency.

Table 3 *Mean and standard deviation of ΔE_{00} value between AM specimens placed on 1M1 background (reference) and those placed on 1M2, 2M2, 3M2, 4M2, and 5M2 backgrounds*

Background color	Translucency		*P* Value
	AM-MT	AM-LT	
1M2	1.80 (0.08) [a]	1.73 (0.10) [a]	0.251
2M2	2.65 (0.05) [b]	2.64 (0.03) [b]	0.844
3M2	3.04 (0.09) [c]	2.96 (0.05) [c]	0.073
4M2	3.73 (0.05) [d]	3.68 (0.04) [d]	0.073
5M2	5.52 (0.15) [e]	5.47 (0.05) [e]	0.410

Different superscript lowercase letters within columns represent significant differences (P<0.05) between background colors.

AM, Amber Mill; MT, medium translucency; LT, low translucency.

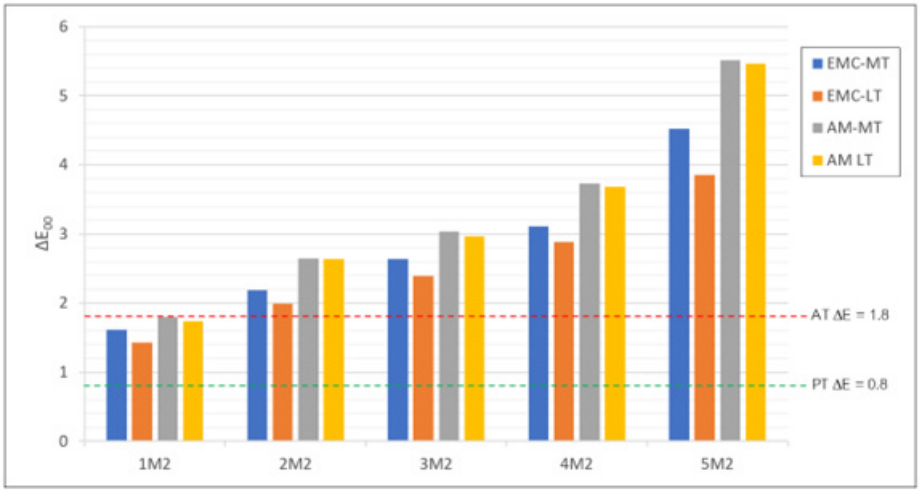

Figure 3 *Graph showing the mean color differences (ΔE_{00}) between the reference background color 1M1 and background colors 1M2, 2M2, 3M2, 4M2, and 5M2 of IPS e.max CAD (EMC) and Amber Mill (AM) with medium translucency (MT) and low translucency (LT). The green and red lines indicate the levels of the perceptibility threshold (PT) and acceptability threshold (AT), respectively*

Table 4 *Mean and standard deviation of TP00 value from medium translucency (MT) and low translucency (LT) specimens of both IPS e.max CAD (EMC) and Amber Mill (AM)*

Brand	Translucency		*P* Value
	MT	LT	
EMC	9.00 (0.15)	8.31 (0.08)	< 0.001
AM	19.56 (0.40)	17.98 (0.36)	< 0.001

Discussion

The present study showed that both translucency and background color affected the optical color of veneer restorations for EMC, but AM was only influenced by the background and not by translucency. The MT specimens showed higher TP_{00} values than LT specimens in both EMC and AM. Thus, the null hypothesis was partially rejected. For the color of the background, the results showed an increase in color differences as the background color became darker for both translucencies of EMC and AM. The results agreed with the findings of other studies that changing the abutment tooth color from lighter to darker caused an increase in color difference.[14, 24-26] In this study, when the color of the background was 1M2, all the groups of lithium disilicate specimens in shade A1 with 0.6 mm thickness could mask the 1M2 background color (ΔE_{00} value below AT) (Fig. 3), and both translucencies of the materials showed no significant different ΔE_{00} value from each other (Tables 2 and 3). Therefore, in clinical situations, when a patient has a light color abutment, both MT and LT materials may be chosen to fabricate veneer. However, when the background colors were changed to 2M2, 3M2, 4M2, and 5M2, ΔE_{00} values of all specimen groups were higher than AT, showing the lack of masking ability. Even though the specimens could not mask these backgrounds, for EMC, the LT group showed a significantly lower ΔE_{00} value than the MT group (Table 2). This might imply that when the background color was in shade 2M2 or darker, the LT materials could be used to achieve better masking properties, and possibly giving a more acceptable color match. The result conformed to the TP_{00} values that EMC-LT had significantly lower values than EMC-MT (Table 4), which could mean that EMC-LT was more opaque than EMC-MT and might be the reason why EMC-LT provided a better masking ability compared to EMC-MT. This was similar to previous studies which reported that LT lithium disilicate materials had a lower TP value when compared with other translucencies that were intended for monolithic veneer restorations.[27-29] Other studies also reported that LT materials appeared more opaque than other tested materials and had better masking properties.[11, 26]

On the other hand, for AM groups, AM-MT and AM-LT were not significantly different in the ΔE_{00} values from each other on any tested background color (Table 3). This could mean that AM-LT did not have a superior masking ability than AM-MT, even though the TP_{00} value of AM-LT was also significantly lower than AM-MT. This might be explained by the material's translucency, in which the TP_{00} value of AM showed a much higher value than that of EMC (Table 4). Thus, AM specimens were more translucent, which caused the compromised masking effect of its LT specimens. Conforming to the previous studies, thin veneer was not suitable for dark color backgrounds.[11,25] Other studies that included thicker ceramic also found that thicker ceramic was more opaque and provided better background coverage than thinner ceramic.[20,24,30,31] However, increasing ceramic thickness may not be suitable in some clinical situations, especially for veneers. Other previous studies found that luting cement may help improve the masking ability and make an acceptable shade reproduction.[14,24,32] The results from Igiel *et al.* demonstrated that using a high-value resin cement could help veneer specimens to mask background colors up to shade 3M2.[24]

There were some limitations of the present *in vitro* study. This study only used one shade of lithium

disilicate ceramic (shade A1) and did not involve the effect of cement color; therefore, the results may not be relevant to other ceramic colors and colored luting cement. Additionally, one ceramic thickness was studied, and thicker or thinner ceramic may show a different effect on the final optical properties of the restorations. Moreover, this study used resin composite to fabricate the background specimens that might have different optical properties compared to natural teeth. Therefore, further studies are suggested using other shades and thicknesses of ceramic specimens along with the effect of luting cement on natural tooth abutments.

Conclusion

Within the limitation of this study, background colors had influences on optical color of veneer restorations. When the background color was 1M2, a considerably light background color, both medium translucency and low translucency of CAD/CAM lithium disilicate glass-ceramics (IPS e.max CAD and Amber Mill) performed acceptable masking properties. For the background color 2M2 and darker, low translucency IPS e.max CAD showed better masking ability than medium translucency IPS e.max CAD. Amber Mill showed much more translucency and poorer masking properties than IPS e.max CAD.

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Original Articles

Root Canal Angulation Change When Using Reciprocating Files in Different Temperatures

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Abstract

Heat-treated NiTi files express different properties at body temperature depending on the type of heat treatment. The aim of this study was to evaluate the reduction of root canal angulation using Blue, Gold and M wire NiTi file systems at different temperatures in simulated severely curved root canals. Eighty-eight resin models with 45° curved root canals were divided into eight groups based on temperature during instrumentation and file type: WaveOne Gold primary file (WOG), WaveOne primary file (WO), Reciproc Blue file (RPB), and Reciproc file (RP) at room temperature (25°C), with four additional groups at intracanal temperature (35°C). Resin models and handpieces were mounted on a customized fixed stand with restricted vertical handpiece movement in a temperature-controlled incubator. The sample preparations were conducted consistently in terms of cycle and amplitude by one operator. Curvature angulation of the canal was analyzed by using ImageJ according to Schneider's method. Mean differences in curvature degrees before and after instrumentation were compared within each group. The results showed that both temperature and file type affected the reduction in canal angulation ($p < 0.001$). WOG and RPB showed the lowest mean angulation reduction, followed by RP and WO ($p < 0.001$). There was a significant difference between 25°C and 35°C instrumentation in the WO and RP groups ($p < 0.001$), but not in the WOG or RPB groups ($P = 0.65, 0.17$). In severely curved canals, reduction of canal angulation after preparation was influenced by file type especially with M-wire (WO, RP) and temperature (35°C).

Keywords: Blue wire, Gold wire, M wire, Root canal angulation reduction, Temperature

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Introduction

A prognosis for endodontic treatment depends on successfully eliminating microorganisms from the affected root canals. Therefore, thoroughly preparing the root canal system would enhance the efficiency of irrigants and medicaments and optimal filling. Certain errors, such as ledges, transportation, zipping, and perforation, may occur during mechanical instrumentation, especially with stainless-steel files in a severely curved canal. Rotary

nickel-titanium (NiTi) files, developed in 1988,[1] were designed to prevent these errors. Because of its flexibility, it could follow and conform to the original canal's shape and maintain the original foramen in its natural position.

Conventional NiTi alloys have been the raw material for the NiTi rotary files for several years. However, file separation due to cyclic fatigue failure sometimes occurs when the files are subjected to repeated bending and rotation within the curved canal. Improvement in metallurgy, heating, and cooling of the alloys significantly reduced cyclic fatigue failure and improved safety in curve canals. The first generation of heat-treated NiTi files is known as M-wire (Dentsply: Maillefer, Switzerland) and R-phase wire (SybronEndo: California, USA). In cooperation with the heat-treated files, the concepts of single-use and reciprocation motion were introduced as WaveOne (WO, Dentsply: Maillefer, Switzerland) and Reciproc (RP, VDW: Munich, Germany). They are more resistant to cyclic fatigue but still exhibit the same property as conventional NiTi alloy, which is the stress-induced martensitic property. This means that the deformed shape recovers to its original shape when the external force is removed.[2-5]

With a different heat-treating process, the degree of martensitic condition varies among each file system. The NiTi alloy of gold and blue wire as WaveOne Gold; WOG, (Dentsply: Maillefer, Switzerland) and Reciproc Blue; RPB, (SybronEndo: California, USA) are at a more martensitic phase at room temperature.[6] They can be deformed because of the reorientation of the martensitic crystalline structure, but they will recover their shape on heating above the transformation temperature, becoming more austenitic at higher temperatures.[5] Metallurgically, gold heat-treated files are more ductile, providing higher flexibility than conventional NiTi files and first-generation M wire files.[7]

Most of the previous cyclic fatigue and shaping ability studies of the NiTi files were performed at room temperature (20-25°C).[6,8,9] The results showed that the heat-treated file could maintain the working length, conform to the original canal anatomy, and have less canal transportation when compared to the conventional NiTi rotary files.[9-12] To simulate conditions closer to the natural environment within the root canal system, the temperature of approximately 35°C or 95°F was used in the *in vitro* cyclic fatigue experiments.[13,14] Results showed a reduction of cyclic fatigue of the heat-treated file, except for the Hyflex EDM file, at body temperature as compared to room temperature.[13]

The effect of temperature change might alter the file property. When flies have an austenitic state at body temperature, they become stiffer and have less fatigue resistance. This might be the cause of canal transportation or file separation in patients. However, how body temperature affects the ability of the heat-treated NiTi files to maintain the original root canal shape, especially in a severe curve canal, is still unknown. This study aimed to compare the change of root canal angulation before and after instrumentation with RP, WO, RPB, and WOG at the environment temperature of 25°C and 35°C in the resin model with a simulated 45° root canal.

Material and method

Resin models simulating 45°curved root canals (Nissin E-ENDR001 Series; Nissin Dental Products, Kyoto, Japan) were employed in this study. Prior to instrumentation, a glide path was confirmed using a K-file #15/.02 (Dentsply Sirona, Ballaigues, Switzerland). The resin models, with the glide path file inserted at the working length, were placed on a custom fixed stand and photographed at 8x magnification using a stereo-microscope (stereomicroscope system SZ 61, OLYMPUS corporation, Japan) to obtain pre-instrumentation images.

A total of 88 resin models were divided into eight experimental groups based on temperature and file type: WaveOne Gold primary file (WOG), WaveOne primary file (WO), Reciproc Blue file (RPB), and Reciproc file (RP) at room temperature (25°C), with four additional groups at intracanal temperature (35°C). All four file types were used as single-use instruments. The resin model, along with a 6:1 contra angle handpiece powered by a torque-control motor (X-smart plus; Dentsply Sirona, Ballaigues, Switzerland), was mounted on a custom fixed

setting within a temperature-controlled incubator (Fig. 1). The incubator maintained a temperature of either 25°C or 35°C with 95% humidity, ensuring strict temperature control within a +/-1°C. Prior to preparation, verification was conducted using a thermocouple to recheck the temperature. The handpiece speed and torque were set according to the manufacturer's instructions. The WaveOne All program was configured for the WOG and WO groups, while the Reciproc All program was set for the RPB and RP groups. The mounted handpiece was moved up and down by operator, limited to the vertical direction only by custom fixed setting. Each rotary file was inserted into the root canal, and three pecking motions with a 3 mm amplitude were performed for three cycles, with the operator reaching the working length. The duration of one cycle was controlled not to exceed 5 seconds. After each cycle of instrumentation, the root canal was irrigated with 1 mL of distilled water that had been incubated in the same incubator at a consistent temperature. The temperature accuracy was confirmed using a thermocouple before applying it with a needle gauge 30 into the canal. Patency was ensured by employing a K-file #10/.02. The debris was cleaned from the cutting blade of the file using a moist, clean gauze.

After root canal preparation, the clean and dry resin models, with the rotary file inserted at the working length, were photographed under the stereomicroscope at 8x magnification using the same custom fixed stand as pre-instrumentation images. These images served as post-instrumentation images. The angulation of the canal curvature in the superimposed pre- and post-instrumentation images was analyzed using the ImageJ program (National Institutes of Health, Bethesda, MD) based on Schneider's technique. The difference in canal curvature angulation before and after instrumentation was compared among each group.

Data analysis was performed using IBM SPSS Statistics for Windows version 28.0 (IBM, Armonk, NY, USA). The Shapiro-Wilk test was used to assess the normal distribution of the data. The mean difference in root canal angulation between pre- and post-instrumentation images was compared among the groups using the Kruskal-Wallis test. The effect of file type and temperature on the difference in angulation was analyzed using a univariate test with Bonferroni correction. Additionally, comparisons of the difference in angulation for each temperature within the same file type were conducted using the Mann-Whitney U test. The significance level was set at 5% (.05).

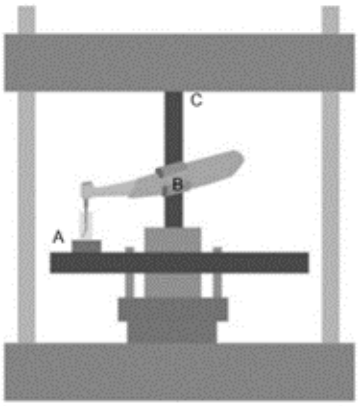

Figure 1 *Illustration of the customized device used for standardization of operator-related-variables. A; custom stand for fixed resin block, B; handpiece holder, C; vertical axis with a handle for operator to move handpiece upward and downward only*

Results

After root canal preparation at 35°C, the results showed that 5 out of 11 resin models in the RP group and 9 out of 11 resin models in the WO group exhibited transportation from the original canal (Fig. 2E and 2F). Transportation began at an average distance of 3.82±0.47 mm and 4.02±0.49 mm, shortened from the working length in the RP and WO groups, respectively. Conversely, in the RP and WO groups at 25°C, as well as in the WOG and RPB groups at both temperatures, canal preparation conformed to the root canal curvature.

Root canal preparation with each file system at both temperatures resulted in varying degrees of canal angulation reduction. The difference in curvature degrees between pre- and post-instrumentation was calculated and compared using the Kruskal-Wallis test (P = .05). The mean angulation reduction for each group is presented in Table 1. At both temperatures, the order of angulation

reduction from lowest to highest was RPB, WOG, RP, and WO, respectively. The mean angulation reduction in the WO and RP groups was statistically higher than that in the WOG and RPB groups ($P < .05$), but there was no statistically significant difference ($P > .05$) between the WO and RP groups and the WOG and RPB groups.

Within groups of the same file type, only the WO and RP groups exhibited a statistically significant difference in root canal angulation reduction when comparing instrumentation at 35°C and 25°C (Mann-Whitney U test, $P < .001$) (Fig. 3). The results of the univariate analysis showed that the factors of file type and temperature during canal preparation significantly influenced the angulation change ($P < .001$), with the file type factor having a stronger effect on the degree of angulation reduction compared to the temperature factor.

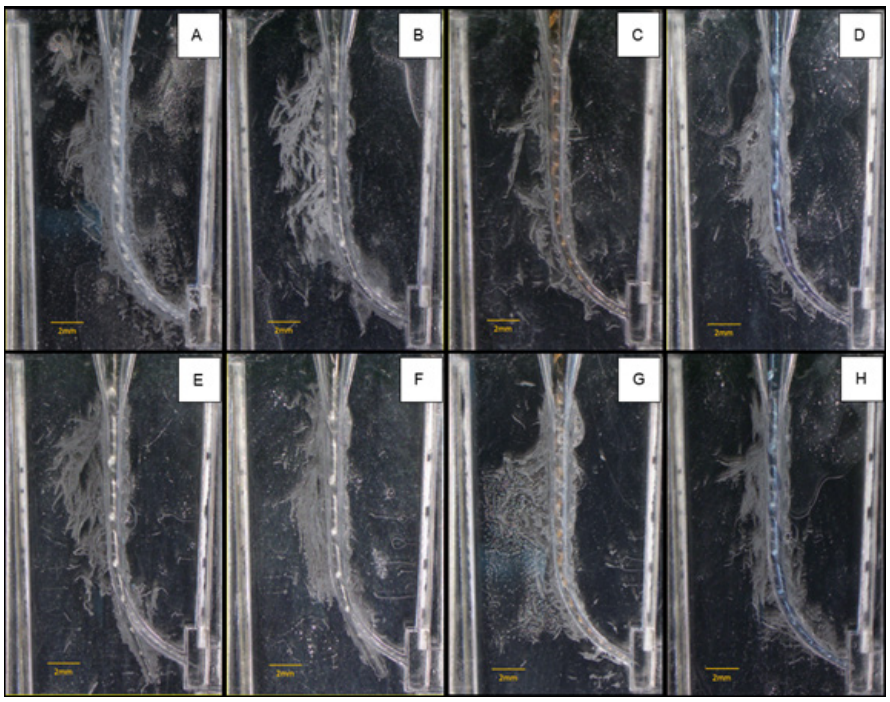

Figure 2 *The superimposed images pre-and post-instrumentation were obtained from imageJ program at 25°C A; Reciproc, B; WaveOne, C; WaveOne Gold and D; Reciproc Blue and 35°C E; Reciproc, F; WaveOne, G; WaveOne Gold and H; Reciproc Blue*

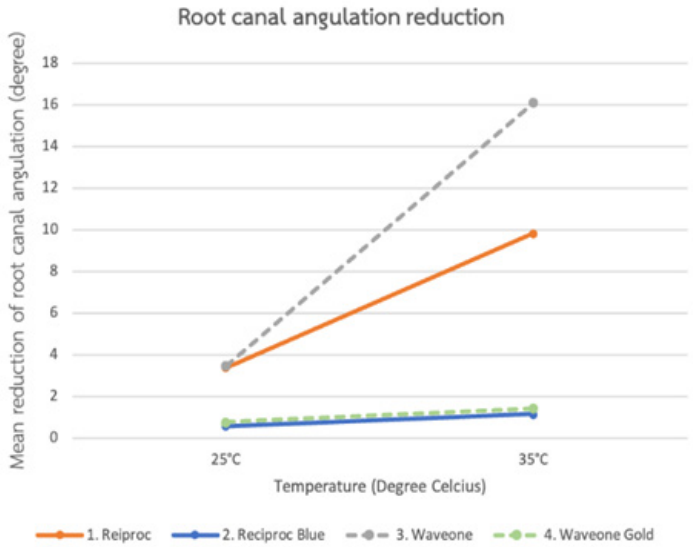

Figure 3 *The mean of root canal angulation (degree) produced by instrumentation with 4 different endodontic file types in different temperatures (25°C and 35°C) ((*); The mean difference was significantly different at different temperature)*

Table 1 *Root canal preparation with each file system at both temperatures resulted in varying degrees of reduction in root canal curvature*

Group	Reduction in root canal curvature angulation		*P*-value (Angulation change between 25°C and 35°C)
	Mean ± SD at 25°C	Mean ± SD at 35°C	
1. RP	3.383 ± 0.203 [a]	9.844 ± 2.223 [a]	<0.001*
2. RPB	0.571 ± 0.116 [b]	1.069 ± 1.029 [b]	0.652
3. WO	3.477 ± 0.258 [a]	16.108 ± 2.896 [a]	0.008*
4. WOG	0.766 ± 0.255 [b]	1.434 ± 0.346 [b]	0.171

The Mann-Whitney U test for the significance of the preparation temperature (25° or 35°C) on the mean reduction in root canal curvature angulation after using 4 different endodontic file systems (RP; Reciproc, RPB; Reciproc Blue, WO; WaveOne and WOG; WaveOne Gold). (*) = data was statistically significant different at different temperature (P-value < .001) The same letters show differences were not statistically significant (P > .05) in comparison with different groups in the same column

Discussion

This present study investigated the ability of four NiTi endodontic file systems in shaping severely curved canal resin models under different temperatures based on root canal angulation changes. Based on the results of this study, WOG and RPB showed significantly less canal angulation change compared to WO and RP at room temperature (25°C). The degree of angulation change ranged from 0.57-0.76 and 3.383-3.47 degrees fromRPB-WOG and RP-WO respectively. This finding contrasts with previous studies that showed no significant difference in the ability of WO, RP, WOG, and RPB to maintain the curved canal of extracted human molar teeth.[9,10] Previous studies reported only a 1.3 to 1.9 degree of angulation change after canal preparation, with WOG and RPB exhibiting the lowest values. The difference in the study's outcome was attributed to the use of severely curved resin models, which simulated an extreme situation to highlight the limitations of instrument properties in this study.

In accordance with prior research findings, it has been consistently observed that the intracanal temperature remains equivalent to the body temperature of 35°C.[15-17] Based on the aforementioned study, the simulated intracanal temperature in this study was set to 35°C to simulate the working temperature of the rotary file. At 35°C, the angulation reduction in the WO and RP groups (16.11 and 9.84 degrees, respectively) exhibited a statistically significant difference in root canal angulation reduction compared to the WO and RP groups at 25°C, as well as the WOG and RPB groups at both temperatures. The prevalence of canal transportation observed in the RP and WO groups at body temperature was 45.45% and 81.81%, respectively. Conversely, at 25°C, no observable canal transportation was noted in either the RP or WO groups. The severely curved root canal with an angulation of 45 degrees and the higher intracanal temperature during preparation in this study are considered to play an important role in canal transportation.

The point at which rotary file transportation commenced was observed to be 3.82±0.47 mm and 4.02±0.49 mm short of the working length in the RP and WO groups, respectively. The presence of canal curvature in this resin block, beginning 4 mm short of the working length, confirms that both WO and RP exhibited transportation from the curvature's initiation point when utilized in severely curved root canals at 35°C. This scenario may significantly impede the thorough debridement of the root canal system, should it arise in actual clinical practice.[18]

This study demonstrated the effect of temperature on the ability of NiTi files to maintain severely curved root canals during instrumentation. Previous studies have also shown that increased temperatures can affect the properties of the rotary file. The increased temperature to 35°C can affect the cyclic fatigue resistance in all file systems, such as RP, RPB, WO, and WOG, but at different levels. Therefore, the properties of M-wire are more affected by temperature than gold and blue wire.[6,13,14,22,23] The

NiTi alloy of WOG and RPB has undergone multiple heat-treating processes and is characterized by a more martensitic phase at room temperature. The gold and blue heat-treated files can be deformed due to the reorientation of the martensitic crystalline structure but can recover their shape upon heating above the transformation temperature.[5] However, at body temperature, the phase composition of WOG is a mixed state of martensite and R-phase.[13] Metallurgically, gold heat-treated files are more ductile and provide higher flexibility than conventional NiTi files and the first generation of M-wire files.[7] On the other hand, WO and RP are manufactured from M-wire NiTi, which contains an austenite microstructural phase with fewer amounts of martensite and without R-phase at body temperature.[2,19,20] The austenite start (As) to finish (Af) temperature of M-Wire has been found to be around 5 to 50 °C, respectively.[3,21] Therefore, M-wire still exhibits a shape memory effect and can restore its shape at either room or body temperature.

The different cross-sections and tapers of the rotary files may also play a role in maintaining canal curvature during root canal preparation. The WO file has a convex triangular cross-sectional design, while WOG has a cross-section with a parallelogram structure and two cutting edges.[24] WOG has the smallest core area compared to WO and RPB.[6] Instruments with larger tapers, especially in the apical portion like WO, tend to cause more transportation due to decreased flexibility, while those with lesser taper like WOG show less transportation.[9,25] However, with reciprocal motion, reciprocating rotary NiTi files are associated with a decreased incidence of file fracture,[26] increased fatigue lifespan, and preservation of the original canal anatomy.[27] The results of the RP and RPB groups at 35°C may reflect the effect of different materials, even if they have the same design, convex S-shape cross-section, diameter, taper, and kinematics. The thermal treatment of RPB was associated with a finer structure, smaller grains, increased fracture resistance, and reduced hardness and lower elastic modulus compared to RP.[4]

A simulated severely curved resin model may not fully reflect the clinical conditions under which endodontic instruments operate due to differences in hardness between resin and human teeth.[28] However, resin models provide standardized samples because they are produced with the same diameter, taper, curvature, and material. The credibility of resin blocks as an ideal experimental model for analyzing endodontic preparation techniques has been validated.[29,30] In this study, the focus was on the different mechanical properties of each instrument system at different temperatures, so other confounding factors needed to be eliminated. Previous studies have shown that the more severe the curvature, the more root canal deviation or shortening of working length occurs.[29,31,32] The elastic memory of the file provides a restoring force that straightens it when it has been deformed by the curvature. This restoring force leads to canal transportation and prevents the instrument from remaining perfectly centered within the canal.[33] The thermal treatment of files may improve their flexibility, increasing resistance during the preparation of curved canals.[34]

Hence, in severely curved root canals, consideration should be given to the utilization of conventional or austenitic files, as they pose a risk of inducing straightening or transportation of the root canal in clinical contexts. Therefore, further studies should investigate alternative materials for resin models that closely mimic the mechanical properties of human teeth or explore patient-specific 3D-printed root canal models, aiming to provide a more accurate representation of clinical conditions while maintaining standardization. Additionally, the comparison of endodontic instrument performance in resin models to their performance in actual clinical settings requires further study to ensure validity.

Conclusion

The reduction in root canal angulation after instrumentation was influenced by file type and temperature. WaveOne and Reciproc created a significant reduction in root canal angulation, especially at 35°C, while WaveOne

Gold and Reciproc Blue can maintain the original canal for both temperatures.

Conflict of interest : None disclosed.

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Original Article

The Effectiveness of Probiotic Milk Tablets in Preventing Dental Caries Among Young Children: A Cluster Randomized Control Trial- 12-Months Results

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Abstract

The research aimed to assess the effectiveness of probiotic milk tablets in preventing dental caries in children attending 12 selected kindergartens in Phayao Province, Thailand, from June 2022 to May 2023. The goal was to determine if probiotic-fortified milk tablets could serve as a viable method for preventing dental caries in children aged 2-5 years. Study participants needed to meet specific inclusion criteria, including being aged 2-5 years, attending one of the designated daycare centers, and having parental consent. Exclusion criteria encompassed conditions like cow's milk allergy, serious medical issues such as heart disease or asthma, oral and jaw abnormalities, the inability to undergo oral health assessments, or developmental delays. The participants were divided into control and treatment groups, with the treatment group receiving three milk tablets daily. Both groups received oral hygiene guidance. The milk tablets contained the probiotic *Lactobacillus rhamnosus* SD11. Each participant underwent oral examinations conducted by the dentists at the beginning of the study and subsequently every four months for 12 months to detect the presence of caries. The minimum required number of study participants was calculated to be 208 based on the estimated caries prevalence in the study population. Initially, 260 participants (136 males) were enrolled in the study, with 130 in the control group and 130 in the intervention group. The mean numbers of caries per participant in the control group at 4, 8, and 12 months were 5.74, 5.78, and 7.34, respectively. In contrast, the mean numbers of caries in the intervention group at 4, 8, and 12 months were 3.56, 3.33, and 3.43, respectively. Statistical analysis indicated that the differences in the mean caries numbers between the control and intervention groups at 4, 8, and 12 months were statistically significant (p=0.019, p=0.018, and p=0.001, respectively). In summary, the intervention group showed significantly fewer caries cases compared to the control group. Consequently, this intervention be considered for the study group to reduce caries. However, further research is needed to determine if this intervention can be extended to other populations of children of similar age, different age groups and multi-center study.

Keywords: Dental caries prevention, Probiotic milk tablets, Young children

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Introduction

Dental caries in primary teeth is a significant global public health concern (WHO, 2019). In Thailand, the prevalence of dental caries among children aged 3-5 years is alarmingly high, with 52.9% among 3-year-olds and a staggering 75.6% among 5-year-olds.[1] A study conducted in Phayao Province, Thailand, reported a dental caries prevalence of 51.2% among 3-year-old children.[2] Typically, dental caries treatment for children under five5 years of age in this region involves tooth extraction, while restorative procedures and caries control are less common. Providing dental care for young children poses several challenges, including behavioral management and the logistical difficulties parents face in taking time off work, resulting in irregular dental visits and suboptimal care.[3]

Given the considerable challenges in treating dental caries, effective strategies for preventing caries in primary teeth are crucial. Primary preventive measures primarily revolve around maintaining good oral hygiene.[4] These strategies also include patient education, dietary management[5], the use of fluoride-based toothpaste or other fluoride applications[6,7] antibacterial agents like chlorhexidine varnish, and the application of dental sealants.[8] Another approach to preventing dental caries in primary teeth involves the use of probiotics. Probiotics can be incorporated into various dairy products, such as including powdered milk, yogurt, lozenges, and milk tablets.[9] Several studies[10-12] have reported successful caries prevention using probiotic products.

Despite the promising outcomes of probiotic-based interventions, there is limited published data on the effectiveness of using probiotics milk tablets, especially in preventing dental caries among children. This study aims to assess the effectiveness of employing probiotics in the form of milk tablets to prevent dental caries in primary teeth among children aged 2-5 years. The objective is to determine whether probiotic-infused milk tablets offer a viable approach to preventing dental caries in this specific population.

Materials and Methods

Study design

The research employed a single-blinded, cluster-randomized controlled design.

Study subject inclusion and exclusion criteria

The study included children aged 2-5 years who attended one of the 12 kindergartens in Phayao Province, northern Thailand, during June 2022-May 2023, and whose parents or guardians provided consent for their participation. Exclusion criteria consisted of a history of cow's milk allergy, cardiac conditions, asthma, craniofacial abnormalities, inability to undergo oral health examination, or developmental disabilities

Participants

The minimum number of participants required for the study was determined using the following formula:

$$n/group = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \, 2\sigma^2 \, xIF}{(\mu_1 - \mu_2)^2}$$

where
- Z_{α} = Z-score at the desired level of statistical significance (α) = 0.05; Z_{α} = 1.96.
- Z_{β} = Z-score for the desired statistical power (1 - β) = 0.2; Z_{β} = 0.84.
- σ^2 = Variance of the group of 3-year-old children in the province of Phayao = 1.00 (assumed to be the same for both intervention group and control groups).
- μ_1 = Expected mean number of decayed, missing or filled tooth surfaces (dmfs) among those receiving probiotics. This was estimated by expert opinion to be = 0.84.
- μ_2 = Expected mean number of dmfs among those in the control group. This was based on data collected from children aged 3 years in Phayao Province = 1.34.
- IF = Inflation factor (IF) = 1.65.
- n = Minimum sample size for each study group (control and intervention groups) = 104 (total of 208 for the 2 groups).

This research assumed a dropout rate of 20%, resulting in a total sample size of 260 participants, with 130 children in each group. The intervention group participants were provided with 3 milk tablets daily by their caregivers, which contained the probiotic Lactobacillus rhamnosus SD11, and they also received instructions on maintaining oral hygiene. In contrast, the control group received only oral hygiene instructions. (Fig. 1).

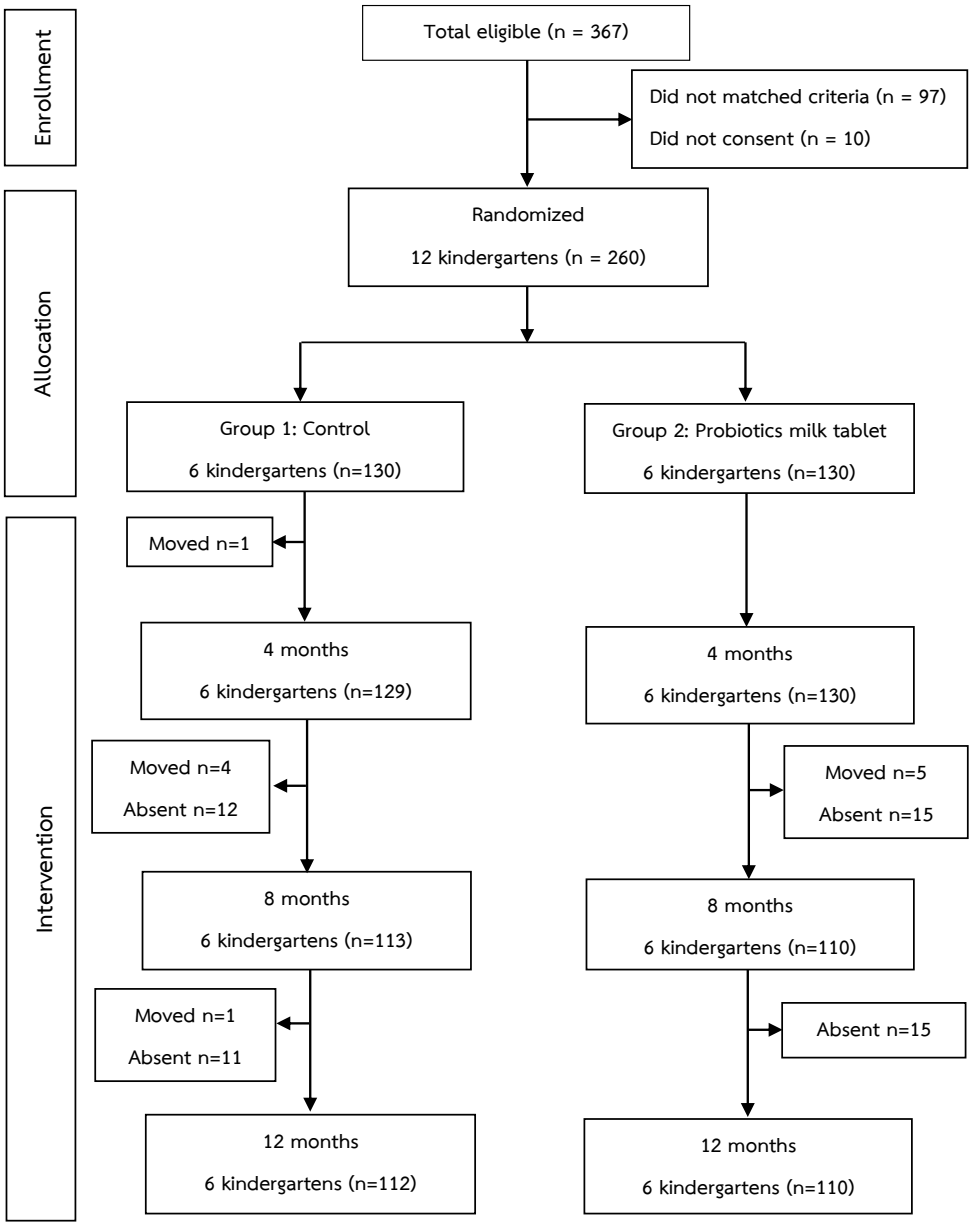

Figure 1 *Flow of study, the random allocation and attrition of participants over the course of the 12-months intervention duration*

Clinical examination

Each study participant underwent dental examinations conducted by a dentist at the beginning of the study and subsequently at 4, 8, and 12 months after the study initiation. These examinations followed the standard criteria set by the World Health Organization (WHO) in 2013 and involved the use of a dental mirror and probe. Prior to the examination, each participant brushed their teeth.

To assess the reliability of the dental examinations, inter-examiner reliability between the two study dentists was evaluated before the study, yielding a weight Kappa value of 0.89. Additionally, the intra-examiner reliability of the two study dentists was assessed, resulting in Kappa

values of 0.91 and 0.92. Importantly, the dentists conducted their examinations without knowing which of the groups each participant belonged, ensuring blinding.

Ethical clearance

The study obtained ethical clearance from the Research Ethics Review Committee for Studies Involving Human Research Participants at Chulalongkorn University Faculty of Medicine. The Institutional Review Board reference number is IRB No.721/64 COA No.1613/2022. Additionally, the study was registered with the Thai Clinical Trial Registry under reference number TCTR20220329003. All children included in the study had written informed consent obtained from their parents or guardians prior to their participation in the study.

Statistical analysis

The age and gender of the participants were recorded as well as the number of dmfs and carious surfaces at baseline, 4 months, 8 months and 12 months. These data were presented using means and standard deviations. Comparisons of the mean numbers of dmfs at baseline, 4 months, 8 months and 12 months were made using difference analyses and relationship comparisons were made using the Fisher's exact test and generalized linear mixed models. This study was conducted as a randomized controlled trial, and the statistical analysis used adheres to the intention-to-treat (ITT) principle, which measures all participants who were part of the study, regardless of whether they dropped out.[13]

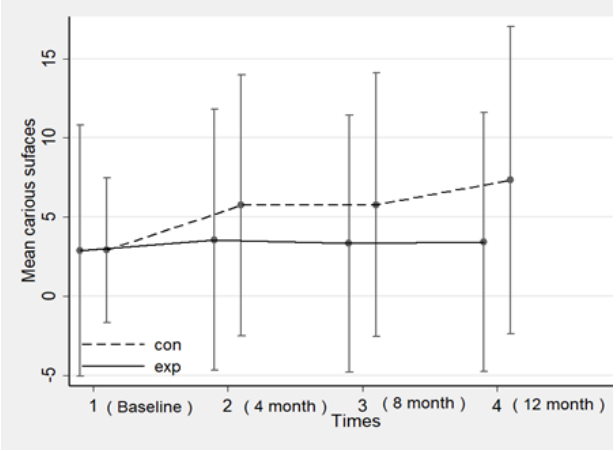

Figure 2 *Mean carious surfaces values at baseline, 4 months, 8 months and 12 months*

Data analysis was conducted using the STATA program, version 18.0 (StataCorp 2023. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC)

Definitions

In this study, specific terms are defined as follows:

1. Normal Teeth: These refer to teeth that do not have any cavities or carious lesions.
2. Carious surfaces: These are teeth surfaces that exhibit soft or softened enamel or dentin, and where the tooth structure can be displaced or effected using a dental probe and not including inactive or non-cavitated caries. Carious surfaces essentially have cavities or lesions.
3. Effectiveness: This term measures the ability of a particular intervention to prevent the formation of cavities, and it is evaluated by comparing the outcomes of the intervention group with those of a control group that receives no intervention. In essence, effectiveness assesses how well the intervention can prevent the development of dental cavities.

Results

Clinical examination

The study included a total of 260 participants, with 130 in the control group and 130 in the intervention group, comprising 136 males and 124 females in both groups. There were no significant differences between the control and intervention groups in terms of mean age (3.01±0.57 and 3.04±0.61 years, respectively), gender distribution (68 males and 62 females in both groups), mean dmfs scores (3.52±4.09 and 3.24±3.83, respectively), and the mean numbers of caries surfaces at baseline (2.92±4.57 and 2.88±7.93, respectively) (Table 1).

The mean numbers of carious surfaces at 0, 4, 8, and 12 months in the control group were 2.92±4.57, 5.74±8.24, 5.78±8.31, and 7.34±9.70, while in the intervention group, they were 2.88±7.93, 3.56±8.25, 3.33±8.13, and 3.43±8.17. The p-values for differences were 0.96, 0.019, 0.018, and 0.001, respectively (Table 2). These results indicate that the number of carious surfaces increased over time in both study groups, but the increase was more rapid in the control group than in the intervention group.

The mean dmfs scores at 0, 4, 8, and 12 months in the control group were 6.88±8.92, 10.16±10.79, 9.68±11.09, and 11.36±12.10, while in the intervention group, they were 5.05±9.82, 6.30±9.98, 5.78±10.18, and 5.93±10.29. The p-values were 0.14, 0.002, 0.002, and <0.001, respectively. The reduction in dmfs observed at the 8-month examination might be due to the possibility that dental caries lesions may have transitioned from an active to an inactive state. (Table 3).

These findings indicate that there was a significant difference in dmfs scores between the control and intervention groups, with the intervention group showing lower scores, particularly at 4, 8, and 12 months.

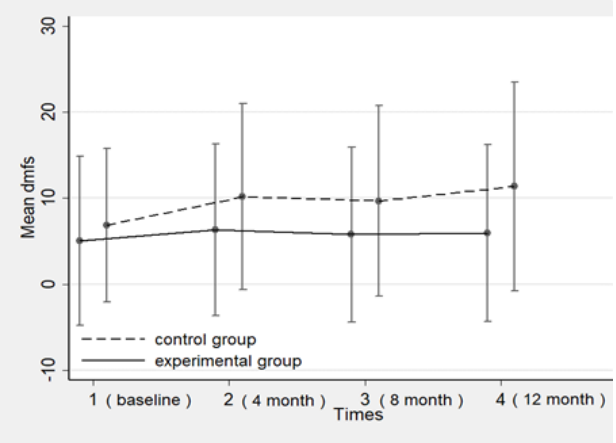

Figure 3 *Mean of decayed missing filled surfaces of decidous teeth (dmfs) values over the course of 12 months*

Table 1 *Baseline characteristics of study participants by study group (n=260)*

Characteristics	Groups	
	Control	Intervention
Number of children	130	130
Age in years (Mean ± SD)	3.01 ± 0.57	3.04 ± 0.61
Gender		
Male, *n*	68	68
Female, *n*	62	62
dmfs score (Mean ± SD)	3.52 ± 4.09	3.24 ± 3.83
Numbers carious surfaces (Mean ± SD)	2.92 ± 4.57	2.88 ± 7.93

Note: no difference in Baseline characteristics data.

Table 2 *Data of Mean carious surfaces at baseline, 4, 8 and 12 months by study groups (n=260)*

Time of investigation	numbers carious surfaces (Mean ± SD)		Difference in mean numbers of carious surfaces (Intervention - Control)	95% confidence interval	*p* - value
	Control group	Intervention group			
Baseline	2.92 ± 4.57	2.88 ± 7.93	-0.04 ± 0.86	(-1.73, 1.65)	0.96
4 months	5.74 ± 8.24	3.56 ± 8.25	-2.18 ± 0.93	(-4.01, -0.36)	0.019*
8 months	5.78 ± 8.31	3.33 ± 8.13	-2.45 ± 1.03	(-4.47, -0.42)	0.018*
12 months	7.34 ± 9.70	3.43 ± 8.17	-3.91 ± 1.16	(-6.18, -1.63)	0.001*

** p < 0.05*
Note: Analysis with generalized linear mixed model.

Table 3 *Assessing the difference in mean dmfs (decayed, missing, and filled surfaces) at the initial point, 4 months, 8 months, and 12 months during the longitudinal analysis.*

Time of oral investigation	dmfs (Mean ± SD)		Mean difference of dmfs (Intervention - Control)	95% confidence interval	*p* - value
	Control group	Intervention group			
Baseline	6.88 ± 8.92	5.05 ± 9.82	-1.84 ± 1.22	(-4.22, 0.55)	0.14
4 months	10.16 ±10.79	6.30 ± 9.98	-3.86 ± 1.32	(-6.45, -1.28)	0.002*
8 months	9.68 ± 11.09	5.78 ± 10.18	-3.91 ± 1.47	(-6.79, -1.02)	0.002*
12 months	11.36 ± 12.10	5.93 ± 10.29	-5.43 ± 1.66	(-8.69, -2.17)	< 0.001**

** p <0.05*
*** p < 0.001*
Note: Analysis with generalized linear mixed model.

Discussion

In this study, there were significant differences in caries prevention outcomes observed at 4, 8, and 12 months. The group that consumed probiotic milk tablets showed statistically significant reductions in carious lesions compared to the control group. This difference was most pronounced at 8 and 12 months, with the control group demonstrating a clear increase in carious surfaces over time. This study did not utilize the ICDAS criteria.[14] for evaluating caries progression. However, it is important to recognize that employing a combined approach that includes both the probing method and the ICDAS criteria could potentially provide more comprehensive data.

These discoveries align with earlier research, particularly when compared to a 2014 study that investigated probiotic tablets versus placebo tablets in adolescents aged 12 to 17 over a three-month duration. Intriguingly, that study revealed no statistically significant variances in caries prevention at the three-month[15], which contrasts with our study. This discrepancy may be attributed to differences in study duration and methods of assessing carious lesions.

Furthermore, the study in 2016, which compared the effects of probiotic milk with standard milk in young children over a 10-month period, found that the probiotic milk group demonstrated significantly better caries prevention outcomes[16], consistent with our results.

Another study in 2017 investigated the use of Probiotic Yogurt and Xylitol-Containing Chewing Gum for caries prevention in 50 female school students over a period of 12 months. The levels of *S. mutans*, a cariogenic bacteria, was measured and it was found that both interventions led to statistically significant reductions in *S. mutans* counts.[17] The outcomes of that study align with our results, that the reduction in cariogenic bacterium may be related to the caries prevention, where children receiving probiotic interventions had fewer carious lesions compared to the control group.

The study conducted in 2020, which involved using probiotics mixed with milk powder for children aged 3-4 years, yielded results consistent with our study. In this research, participants were divided into three groups: one group received the probiotic-mixed milk powder every day, another group received it three times a week, and the control group consumed regular milk powder for six months. The outcomes were measured for 12 months, and it was found that the group receiving probiotics daily or three times a week demonstrated statistically significant improvements in caries prevention compared to the control group.[18] These findings align with the observations of the study that probiotic interventions can lead to better caries prevention outcomes.

Conclusion

The study provides further evidence that probiotic interventions, such as probiotic milk tablets, can be effective in preventing dental caries in young children. However, it is important to note that while this intervention shows promise, it should be considered as part of a comprehensive approach to oral health, and further research is needed to explore its applicability to different populations and age groups, as well as its long-term effects.

Acknowledgement

The researcher would like to express our gratitude to the children, parents, guardians, and caregivers who participated in this research and generously contributed their time and cooperation and also extend our sincere thanks to Dr. Thanapoom Rattananupong, Professor Dr. Narin Hiransuthikul, Faculty of Medicine, Chulalongkorn University and all other individuals involved in this study, whose names are not mentioned here. This study was a part of the cost-effectiveness of probiotics to prevent dental caries among young children in Phayao province, Thailand.

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Original Article

Microleakage of Class II Restoration Using Short Fiber-Reinforced Flowable Resin Composite with a Universal Adhesive

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Abstract

With short-fiber reinforced resin composite (SFRC), improved mechanical strength and good workability could be achieved within a single material. However, there remains concerns related to microleakage, which could restrict the application of this material in certain clinical situations. Thus, the aim of the study is to investigate microleakage of class II cavities restored with SFRC compared to other resin composites, in a simulated aging environment using thermocycling. Class II cavities were prepared in 80 premolars at the cementoenamel junction. Each group, consisting of 10 specimens, was restored with different materials: bulk-fill flowable SFRC (EverX Flow), bulk-fill SFRC (EverX Posterior), flowable bulk-fill resin composite (Tetric N-flow), and conventional resin composite (Filtek Z350XT). The specimens were divided into two subgroups: one underwent thermocycling of 20,000 cycles, while the other did not. All specimens were subjected to the dye penetration test and then assessed for microleakage scores. In the non-thermocycling group, no significant differences in microleakage scores were observed. In the thermocycling group, EverX Posterior showed a significant difference in microleakage scores compared to Tetric N-flow ($p = 0.018$) and all other tested materials (all $p < 0.001$). However, there was no significant difference in microleakage scores between EverX Posterior and Filtek Z350XT ($p = 0.714$), or between Filtek Z350XT with Tetric N-flow ($p = 0.951$). In conclusion, when restored with a universal adhesive, bulk-fill flowable SFRC achieved the highest microleakage score compared to other tested resin composite after thermocycling. In addition, all materials showed a significantly higher microleakage score after thermocycling. The materials could be ranked in ascending order of susceptibility to microleakage after aging by bulk-fill SFRC, conventional resin composite, flowable bulk-fill resin composite, and bulk-fill flowable SFRC, respectively.

Keyword: Flowable Resin Composite, Microleakage, Short Fiber-reinforced Resin Composite, Thermocycling

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Introduction

With the increasing demands of both esthetic and functional aspects of the dental restorative material, resin composite and adhesive technologies are rapidly evolving. The incorporation of fiber into resin composite material stems from industrial demands for high-strength material that could withstand stress in load-bearing areas. Consequently, short fiber-reinforced resin composite (SFRC) was introduced in 2013 as EverX Posterior (GC Corporation, Tokyo, Japan). Multiple studies reported that this material showed superior mechanical properties compared to conventional resin composite in many aspects.[1-4]

In recent years, further improvements have been made to improve the workability and handling properties of SFRC. In 2019, flowable SFRC (EverX flow; GC Corporation, Tokyo, Japan) has been introduced, combining the advantage of fiber reinforcement and good flowability in the same material, by changing the length and diameter of the fibers, the content percentage of fiber, particulate fillers, and the resin matrix. Moreover, flowable SFRC could also be placed in bulk up to 5.5 mm according to the manufacturer, reducing technical sensitivities and chair-time for restoration of extensive cavities.

However, it is known that one of the problems of flowable resin composite is the polymerization shrinkage due to high monomer content. Flowable SFRC is not an exception. An *in vitro* study found that, although flowable SFRC was superior to conventional bulk-fill resin composite in multiple aspects, such as flexural strength and fracture toughness, it exhibited more water sorption and polymerization shrinkage stress, which may lead to microleakage and post-operative sensitivity.[5] In addition, it was shown that polymerization shrinkage stress tremendously weakened the performance and longevity of the restorations.[6]

Even though there are numerous studies supporting SFRC superior properties, studies concerning flowable SFRC are still sparse. Criteria regarding selection and use of contemporary class of restorative material is always of benefit. Therefore, the objective of this study was to investigate the microleakage of class II cavities restored with SFRC compared to other resin composites, in a simulated aging environment using thermocycling.

Materials and Methods

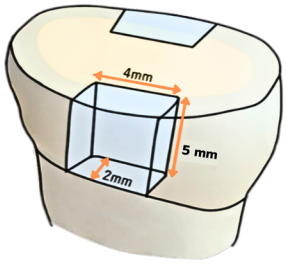

Figure 1 *Sample preparation and dimension of class II slot cavities*

Sample Size Calculation

From the experimental design of this research; sample size calculation from G*Power 3.1.9.7, f-test, ANOVA: fixed effects, omnibus, one-way, with the level of significance (α) as 0.05, the power of study (1-B) as 80%, number of groups as 8, effect size f as 0.51697347-9; total sample size calculated was 64, thus, the sample size per group was 8. To decrease the systematic error, the sample size was increased 20% of the calculated sample size, resulting in a total sample size of 80, which was 10 per group.

Specimen Preparations

With the approval of the ethics committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2022-048), eighty sound human maxillary premolars extracted with informed consent, for orthodontic reasons, were selected with similar occlusal table size (±1 mm) in both buccolingual and mesiodistal dimensions. All teeth were visualized under a microscope at 2x magnification to ensure that teeth with dental caries, fracture lines, or defects were excluded. Soft tissue remaining and dental calculus were cleaned with an ultrasonic scaler, and then stored in a 0.1 % thymol solution at room temperature. The root portion of the teeth were coated and sealed with molten sticky wax 3 mm from the apexes. Each tooth was assigned a single number from 1 to 80, and then randomly distributed into 8 groups (n=10 per group) using

the Microsoft Excel randomized function. Occlusal table of each tooth was flattened using high-speed, super-coarse diamond burs (837H 060; Meisinger, Germany) with water irrigation. All teeth were measured that a 5 mm cavity depth could be prepared with gingival margin at CEJ, to ensure similar quality of dentin substrate among specimens.[10]

Standardized Class II slot on mesial and distal surfaces (OM and OD) was prepared by one operator, using high-speed, medium-grit diamond cylinder burs (837 025 Meisinger, Germany) with copious water irrigation. The bur was replaced after every five preparations.[11] The occlusal dimension of the cavity was in total of 4 mm in buccolingual dimension, with 2 mm gingival floor width and 5 mm axial height with the floor of the cavity at the CEJ level (Figure 1). All internal line angles were rounded using round-end cylinder burs (842 016 Meisinger, Germany. Additional measurements using a digital caliper were made to ensure standardization of all cavity preparations with the sensitivity of 0.1 mm.

Restorative procedure

All prepared teeth were subjected to the same bonding procedures using a selective enamel etching protocol (Fig. 2). Enamel was etched with a 32 % phosphoric acid (Scotchbond Universal Etching Gel; 3M ESPE, USA) for 15 seconds, thoroughly rinsed with water for 15 seconds, and dried by a blotting technique with clean dental sponges to avoid over-drying of the dentin surface. Then, the universal adhesive resin (Scotchbond Universal; 3M ESPE, USA) was thoroughly applied in a single layer with a microbrush, gently rubbed for 20 seconds and dried with a gentle stream of air until the adhesive surface appeared immobile, followed by light curing for 10 seconds (1,200 mW/cm^3 by Bluephase N; Ivoclar Vivadent, Liechtenstein) as per the instructions of the manufacturer. The light curing unit was calibrated using a radiometer (Bluephase Meter II; Ivoclar Vivadent, Liechtenstein) after each day of use to standardize the light intensity. After the bonding procedure, a metal matrix band and a Tofflemire matrix retainer were used for cavity restoration.

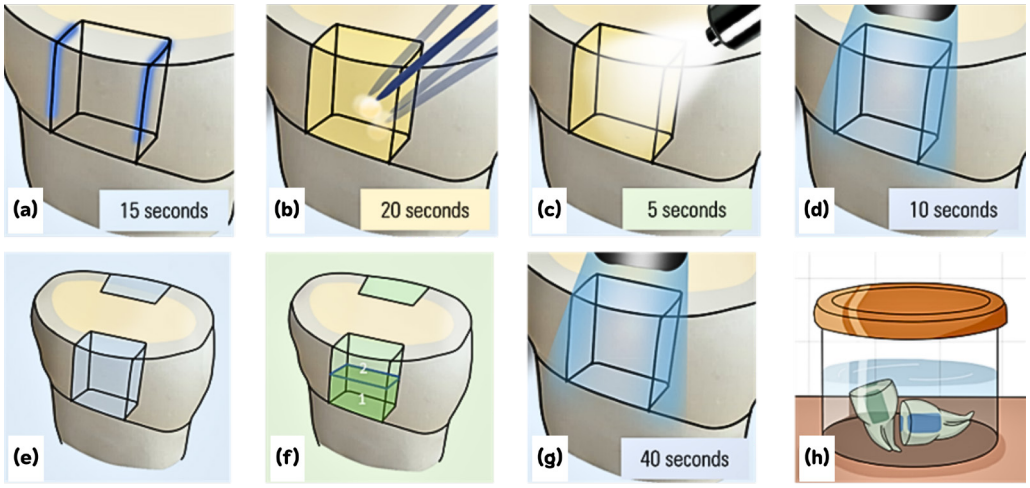

Figure 2 *Restorative Procedures: (a) Selective etching for 15 seconds, rinsed with water and blot dried, (b) Applied the adhesive for 20 seconds and gently rubbed to the prepared tooth (c) Gently air dried to evaporate the solvent, (d) Light cured the adhesive layer for 10 seconds, (e) EXF group was restored as a bulk, (f) EXP, TNF, Z350 groups were restored using a horizontal increment technique, (g) Light cured from occlusal side for 40 seconds for every increment placed, and then from buccal and lingual sides after matrix removal, (h) Specimens stored in distilled water at room temperature for 24 hours*

For group 1, a SFRC bulk fill flowable resin composite (EverX flow; GC Corporation, Japan) was injected as a bulk for the entire cavity, starting from the deepest part of the proximal slot, and light-cured for 40 seconds from occlusal side. Then, the matrix was removed, and additional light curing was made from buccal and lingual sides of the tooth for 40 seconds each.

For groups 2, 3, and 4, the restorations were performed using a horizontal incremental technique consisting of 2 increments. For group 2, a SFRC bulk fill resin composite (EverX Posterior; GC Corporation, Japan) and group 3, a bulk fill flowable resin composite (Tetric N-Flow Bulk-Fill; Ivoclar Vivadent, Liechtenstein) was injected into the prepared cavities. For group 4, a conventional resin composite (Filtek Z350 XT; 3M ESPE, USA) was used as the control group. The cavities were restored using composite filling and packing instruments to ensure that the restorative material was properly placed and packed evenly. Then, light curing was performed in the same manner as group 1. All specimens were polished with a series of aluminum oxide discs and wheels (Sof-Lex discs and Sof-Lex wheels; 3M ESPE, USA). The materials used in this study and their composition are listed in Table 1.

The specimens were stored in distilled water at room temperature for at least 24 hours. The teeth in each group were then divided into 2 subgroups for testing: (a) without thermocycling, and (b) with thermocycling. The groups without thermocycling were subjected to microleakage test after 24-hour storage in distilled water. The other groups underwent thermocycling before being subjected to a microleakage test.

Table 1 *Composition of restorative materials according to manufactures' information*

Test Materials (shade)	Composition	Manufacturer	Lot number
EverX Flow; EXF (Translucent)	● Resin: Bis-MEPP, TEGDMA, UDMA ● Filler: Micrometer scale glass fiber filler (average length 140 µm, diameter 6 µm), barium glass ● Filler load: 70 wt%, 46 vol%	GC Corporation, Tokyo, Japan	1911231
EverX Posterior; EXP (Translucent)	● Resin: bis-GMA, PMMA, TEGDMA ● Filler: Micrometer scale glass fiber filler (average length 800 µm, diameter 17 µm), barium glass ● Filler load: 74.2 wt%, 53.6 vol%	GC Corporation, Tokyo, Japan	2112031
Tetric N-Flow Bulk-Fill; TNF (IVW)	● Resin: Bis-GMA, UDMA ● Filler: Barium glass fillers, YbF3, mixed oxides, silicon dioxide, copolymers ● Filler load: 68 wt%, 46.4 vol%	Ivoclar Vivadent, Schaan, Liechtenstein	Z03CBL
Filtek Z350 XT; Z350 (A1)	● Resin: Bis-GMA, UDMA, TEGDMA and Bis-EMA ● Filler: ZrO2/SiO2 nanocluster, SiO2 nanofiller ● Filler load: 82 wt%, 60 vol%	3M ESPE, St. Paul, Minnesota, USA	NF24321
Scotchbond Universal; SBU	● Etchant: 32% phosphoric acid (pH = 0.1) ● Adhesive: 2-HEMA, 10-MDP, dimethacrylate resins, Vitrebond copolymer, silane, filler, ethanol, water, initiators (pH = 2.7)	3M ESPE, St. Paul, Minnesota, USA	8563601

Thermocycling Test

The designated specimens were subjected to thermocycling (Thermo Cycling Unit, KMITL, Thailand) in distilled water between 5°C and 55°C with a 30-second dwell time for 20,000 cycles. Subsequently, all specimens were observed under a stereomicroscope with a 5x magnification for signs of crack and fracture. The specimens would

be rated as failure if debonding or fracture occurred, then excluded from the microleakage test.

Microleakage test

All designated untested specimens and all survived specimens from the thermocycling test were then subjected to a microleakage test. An adhesive tape, 5x10 mm in dimension, was used to cover the gingival margin of proximal surfaces on both the mesial and distal sides of the restoration. The rest of the surface was coated with 2 layers of nail varnish and left to dry for 24 hours. After the removal of adhesive tapes, the specimens were immersed in a 50 % silver nitrate solution at room temperature for 24 hours, followed by a photo-developing solution for 8 hours under fluorescent light in a dark container. The specimens were then removed from dye solution and gently rinsed under running water for 5 minutes without interfering with proximal parts.

Evaluation of microleakage score

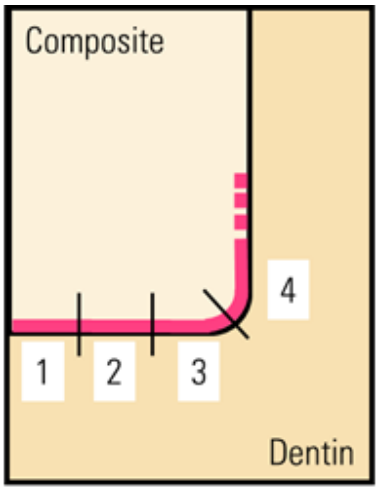

Figure 3 *Schematic representation of scoring scale. Pink line indicated the degree of dye penetration at the gingival margin and axial wall. Score 0; no dye penetration. Score 1; dye penetration up to one-third of the gingival wall. Score 2; dye penetration up to two-third of the gingival wall. Score 3; dye penetration up to full length of the gingival wall. Score 4; dye penetration up to the whole length of the gingival wall and along the axial wall*

The specimens were cut through a bucco-lingual plane. Then, each separated part was sectioned mesiodistally in a vertical plane using a low-speed diamond saw (ISOMET 1000, Buehler, Binghamton, NY, USA) with constant water cooling. With 2 cuts, a total of 4 proximal reading surfaces from both mesial and distal sides were obtained. The sections were evaluated for the degree of dye penetration under a stereomicroscope (SZ 61, Olympus, Japan) at a 40x magnification, based on degree of dye penetration at gingival margin and axial wall (Fig. 3).[12]

Statistics

All statistical analyses were performed using an IBM SPSS Statistics version 26.0 (SPSS, Chicago, IL, USA). The level of significance of 0.05 was used for all analyses. For the analysis of microleakage scores, Kruskal-Wallis with Dunn post-hoc tests were used to compare between studied material groups. The Mann-Whitney U-test was used to compare the effect of thermocycling between study groups.

Results

The frequency distribution and percentages of microleakage scores for each material in non-thermocycling and thermocycling groups are presented in Table 2. Kruskal-Wallis test revealed no statistically significant difference in microleakage scores among 4 materials in the non-thermocycling groups ($p = 0.151$). In contrast, a statistically significant difference was observed among the materials in the thermocycling groups ($p < 0.001$), in which pairwise comparison showed that there was a statistically significant difference in the microleakage score between EXP and TNF ($p = 0.018$), and EXF with all other tested materials (all $p < 0.001$). However, there was no statistically significant difference in the microleakage score between EverX Posterior with Z350 ($p = 0.714$), and Z350 with TNF ($p = 0.951$).

Table 2 *Frequency distribution of microleakage score (percentages) in non-thermocycling group and thermocycling group for all tested materials*

Material	Test	Microleakage score (% within group)					Total
		0	1	2	3	4	
Z350	NT	0	33 (82.5%)	6 (15%)	1 (2.5%)	0	40
	T	0	19 (47.5%)	14 (35%)	6 (15%)	1 (2.5%)	40
TNF	NT	0	34 (85%)	6 (15%)	0	0	40
	T	0	15 (37.5%)	11 (27.5%)	9 (22.5%)	5 (12.5%)	40
EXP	NT	0	39 (97.5%)	1 (2.5%)	0	0	40
	T	0	29 (72.5%)	7 (17.5%)	3 (7.5%)	1 (2.5%)	40
EXF	NT	0	36 (90%)	4 (10%)	0	0	40
	T	0	0	8(20%)	10(25%)	22(55%)	40

In addition, the Mann-Whitney U test found that the microleakage scores of the thermocycling groups were higher than the non-thermocycling groups with a statistically significant difference. In the non-thermocycling group, there was no statistically significant difference in the microleakage score for each material (p = 0.154), with the median microleakage score of one across all groups. In contrast to the thermocycling group, EXP exhibited the lowest median microleakage score of 1, followed by Z350 and TNF which exhibited a median microleakage score of 2. EXF exhibited the highest median microleakage score of 4, as shown in table 3.

Table 3 *Median value and interquartile range (IQR) of microleakage score for each material in both groups*

Material	Median (IQR)		*P*-value
	NT	T	
Z350	1.00 (0)^{a}	2.00 (1)^{AB}	0.001
TNF	1.00 (0)^{a}	2.00 (2)^{B}	<0.001
EXP	1.00 (0)^{a}	1.00 (1)^{A}	0.002
EXF	1.00 (0)^{a}	4.00 (1)^{C}	<0.001
P-value	0.154	<0.001	

Different superscript letters indicated a statistically significant different at a 0.05 level of significance (P ≤ 0.05)

Discussion

The results from the study indicated that class II slot cavities restored with short fiber-reinforced flowable resin composite showed a significantly higher microleakage score than other tested materials in the thermocycling group. In addition, class II slot cavities that underwent thermocycling showed a significantly higher microleakage score than the non-thermocycling group, regardless of materials tested.

Microleakage was defined as an invasion of bacteria and fluids between the cavity walls and restorative material, which many researchers indicated as the primary cause of recurrent caries, tooth hypersensitivity, and pulpal inflammation.[12,13] Microleakage occurred from the micrometer gap at the margin of the cavity, resulting from several factors including properties of restorative material, filling techniques, bonding procedures, cavity configuration and its substrate.[14] In addition, different CTE between the resin composite material and the dental structure would generate stresses at the bonding interface, potentially leading to degradation and failure.[14] This phenomenon was simulated by thermocycling in our study. However, it is important to consider the multifactorial nature of microleakage, which resulted from the previously mentioned factors.

Despite efforts to control the dental substrate through inclusion and exclusion criteria, and bonding procedures through a streamlined restorative process using a universal bonding agent and a single operator, these factors could still potentially influence the microleakage score. Therefore, the microleakage score of this study could not be entirely attributed to the performance of the tested restorative material, but rather to the overall tooth-restoration complex.

From the results, all materials showed a significantly higher microleakage score after the aging process compared to non-aging, regardless of the material tested. Thermocycling test was regarded as the most frequently used method to simulate thermal changes and hydrolytic activities in the intraoral environment, which tested bond durability of the tooth-restoration.[15] Thermocycling procedures in various studies were different in aspect of temperature, number of cycles, and dwell time, rendering it challenging to compare results from different studies.[15] In regard to the numbers of cycle, long-term function could not be simulated if the number of cycles was too low.[15-17] It was estimated by Gale *et al.* that 10,000 cycles represented 1 year of service.[16] In the present study, 20,000 cycles was used to represent 2 years of function, and to ensure that the number of cycles was sufficient to accelerate aging process of the tested restorative materials. Thus, the increased microleakage score after aging could be the result of repeated stress and degradation from accelerated aging by thermocycling, leading to more tooth-and-restoration degradation compared to the non-thermocycling group.

From this study, the lowest microleakage score from both groups was achieved by EverX Posterior, which was the representative of the short fiber-reinforced resin composite (SFRC). SFRC had been extensively studied for its superior mechanical properties, which include a higher fracture resistance,[1] fatigue limits[2] and flexural strength.[18] From a previous study by Tsujimoto *et al.,* EverX Posterior exhibited the lowest volumetric shrinkage compared to other bulk fill resin composites.[4] In addition, Patel *et al.* concluded that SFRC obtained the lowest microleakage score with good homogeneity of the restoration compared to bulk fill and conventional resin composite when subjected to artificial aging by thermocycling, which was in concordance with this study.[9]

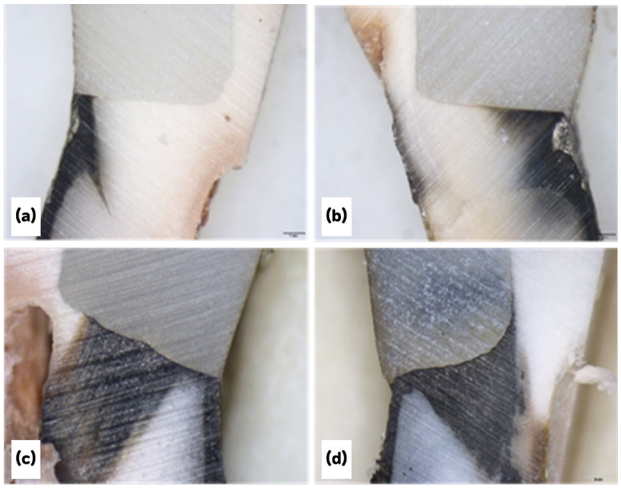

Figure 4 *Stereo micrographs of the specimens, represented microleakage score 1 (a), 2 (b), 3 (c) and 4 (d)*

The superior performance of SFRC was speculated to be the result of the incorporation of randomly oriented millimeter-scale E-glass fibers into the material. With its randomized orientation, the resin matrix could not shrink along the length of the fibers. Thus, its initial dimension could be mostly maintained,[3] which decreased overall volumetric contraction of the composite.[19] Furthermore, the inclusion of short-fiber could increase resistance to microcracking of the restoration, reducing polymerization shrinkage stress leading to a lower microleakage score.[19] However, from this present study, its flowable counterparts (EverX Flow) performed differently from EverX Posterior and other tested materials in terms of a microleakage score. Although EverX Flow exhibited comparable microleakage to other tested materials without thermocycling, it exhibited the highest microleakage with a statistically significant difference after undergoing thermocycling.

SFRC flowable bulk-fill resin composite, represented by EverX Flow, had been developed to ease the complexity of the restorative process, while maintaining improved mechanical properties from glass-fibers.[3] However, some differences between SFRC bulk-fill and its flowable version could be noted. For instance, the average diameter and length of glass fiber were 17 μm and 800 μm in SFRC, while

the average diameter and length of glass fibers were 6 μm and 140 μm in SFRC flowable respectively. It is generally known that critical fiber length, aspect ratio, fiber orientation and amount of fiber loading could influence the mechanical properties of SFRC.[2] Critical length is the minimal length of fiber that can effectively reinforce the polymer matrix by transferring stress to the fiber, which could be as much as 50 times the diameter of the fiber.[20] However, the diameter of EverX Flow was 6 μm, therefore, the critical fiber length should be approximately 300 μm. In addition, the aspect ratio, which is the ratio of fiber length to diameter, affects tensile strength, flexural modulus and reinforcing efficacy of SFRC.[21] For optimal stress transfer, the aspect ratio should be in the range of 30-94.[21] From the calculations, EverX Posterior would have an aspect ratio of 47 when determined from its fiber average length. Meanwhile, EverX Flow would have an aspect ratio of 23.3, which is less than the recommended aspect ratio for optimal stress transfer.[21] This could compromise shrinkage stress alleviation of the EverX Flow. Thus, for these reasons, EverX Posterior and EverX Flow might perform differently in terms of polymerization shrinkage and microleakage score as observed in this study.

Another difference between EverX Posterior and EverX Flow was the number of particulate fillers and fiber content. Generally, flowable materials required lower filler loading by volume to adjust the viscosity of the material, thus making them flow properly.[22] EverX Posterior had a total inorganic and filler content of 76 wt%/57 vol%, meanwhile EverX Flow had a total inorganic and filler content of 70 wt%/46 vol%. A higher amount of resin matrix could lead to higher polymerization contraction, which may compromise adhesion between bonding material, restoration, and cavity walls.[23] Lower filler loading of EverX Flow, compared to EverX Posterior, might contribute to a more polymerization contraction, leading to a higher microleakage score as seen in this study. Unlike EverX Posterior, there are no other studies comparing the microleakage score of EverX Flow to other materials. However, there are some studies observing various different parameters between EverX Posterior and EverX Flow. For instances, a study by Lassila *et al.* revealed that EverX Flow exhibited higher shrinkage stress value compared to EverX Posterior.[5] Also, Magne *et al.* stated that EverX Flow had more shrinkage-induced cuspal deformation despite more favorable failure modes.[24] Nevertheless, further investigation and more clinical trials should be done before confirming its performance.Otherwise, it seems reasonable to use other resin composite materials as an outer layer to protect EverX Flow from an intraoral environment, as seen in the recommendation of the manufacturer.

Universal adhesives have gained popularity in dentistry due to their simplified procedure which reduced technical sensitivity and application time. Nevertheless, adhesion to dentin remained a challenge.[25] In this present study, Scotchbond Universal adhesive was used. With a pH of 2.7, it is considered to be a mild acidic adhesive, which can be used in both total-etch and self-etch modes. Due to its less acidic composition, adhesion to enamel might be compromised when used in self-etch mode.[26] In this study, selective etching technique was performed, meaning the etchant was applied exclusively to enamel on the proximal cavosurface of the prepared cavity, excluding dentin on the internal cavity walls. Although many studies concluded that a mild self-etch adhesive was currently recommended for dentin adhesion,[27] the content of acidic monomers could affect bond stability over time.[25,28] A previous study by Perdigaõ *et al.* found a deterioration of marginal adaptation from baseline to 18-months using Scotchbond Universal in self-etch mode compared to total-etch mode, with no difference in the clinical retention rate.[29] Also, a 5-year clinical evaluation from Matos *et al.* discovered that the clinical performance of the universal adhesive in total-etch mode was superior to self-etch mode, and selective etching was recommended.[30] From this present study, the dentin surface of the specimens were not treated with phosphoric etchant. Thus, the bonding interface between the universal adhesive and dentin could deteriorate, potentially leading to an increased microleakage score from all materials after the aging process. However, it is important to note that the most favorable

etching mode remained a highly controversial topic, and diverse results have been reported in various studies.[25,28,30]

Apart from the materials, restorative techniques may have some degree of effect on the microleakage score in this present study. The manufacturer of EverX flow claimed that it could be filled as a single bulk of 5.5 mm, thus all samples in this material group were filled as a bulk for the entire cavity, contrary to other material groups which were filled incrementally because of their lesser depth of cure. Currently, there was no study comparing the effects of different filling techniques using SFRC bulk-fill materials. Nevertheless, several studies had examined different parameters associated with both the bulk-fill technique and incremental techniques using other bulk-fill resin composites. From a study by Mulder *et al.,* evaluating the shrinkage of bulk-fill flowable composites, restored with bulk-fill and incremental technique compared to conventional resin composite using electronic mercury dilatometer, reported that even though all tested bulk-fill flowable resin composites can be filled up to 4 mm as recommended by the manufacturer, their volumetric shrinkage was higher than that of a conventional resin composite restored incrementally in 2 mm layers, including the bulk-fill resin composites when restored incrementally in 2 mm layers themselves. Therefore, the standard increment technique was still advisable even when using bulk-fill materials.[31] On the other hand, a study by Han *et al.* demonstrated no difference in the microtensile bond strength for class II cavities restored with bulk-fill resin composites using different filling techniques.[32] Additionally, a systematic review and meta-analysis by Kunz *et al.* showed similar clinical performance in class II restorations in posterior teeth for both incremental and bulk-fill techniques.[33] It is important to note that, due to variations in materials, specimens, and methodologies used, the comparison of these results should be done with caution.

It should be acknowledged that this study had certain limitations. The current experimental design utilized only four restorative materials from each category and only one universal adhesive system. Consequently, the findings of this study may not be inferred to other product and adhesive systems. To address this limitation, a future experimental design should include a wider range of products from different brands, such as other adhesive systems, bulk fill resin composite, and flowable resin composite products. Ultimately, randomized controlled trials may be the most essential source of information to help clinicians make decisions regarding material and technique choices in different clinical situations.

Conclusion

Within the limitations of the present study, it can be concluded that short fiber-reinforced bulk-fill flowable resin composites achieved the highest microleakage score compared to other tested resin composite, with a universal adhesive after the aging process by thermocycling. In addition, all materials showed a significantly higher microleakage score after the aging process. The materials could be ranked in ascending order of susceptibility to microleakage after aging by short fiber-reinforced bulk-fill resin composites, conventional resin composite, flowable bulk-fill resin composite, and short fiber-reinforced bulk-fill flowable resin composites, respectively.

Clinical Implications

The recently developed short fiber-reinforced bulk-fill flowable resin composite aimed to simplify the restorative process while maintaining the improved mechanical properties of glass fibers. However, because of its susceptibility to microleakage, it may not be advisable to use this material alone as a definitive restoration.

Conflict of Interest

The authors declare that they have no conflict of interest.

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