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Hypochlorous acid as a potential cavity conditioner for caries-affected dentin

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To evaluate the effects of hypochlorous acid (HOCl) conditioning on bond strength to caries-affected dentin (CAD), antibacterial efficacy against *Streptococcus mutans* (*S. mutans*), and dental pulp cell compatibility. Extracted human molars with occlusal caries were pretreated with 50 ppm HOCl before adhesive application (Clearfil SE Bond 2), with untreated specimens serving as controls. Microtensile bond strength (μTBS) was evaluated after 24-hour storage and 10,000 thermocycles. Antibacterial efficacy was assessed by colony-forming unit (CFU) enumeration following *S. mutans* incubation with HOCl solution. Rat dental pulp cells were cultured with HOCl at concentrations of 0, 0.5, 1, 5, 10, and 50 ppm. Cell viability was assessed using Cell Counting Kit-8. Data underwent statistical analysis using one-way ANOVA with Tukey's post hoc test ($\alpha = 0.05$). HOCl pretreatment significantly enhanced μTBS to CAD (from 25.6 ± 4.3 to 35.8 ± 7.2, $p < 0.001$) and maintained bond stability after thermocycling (32.1 ± 4.5, $p > 0.05$). HOCl demonstrated significant antibacterial efficacy against *S. mutans* with substantial reduction of viable bacteria ($p < 0.001$). Additionally, HOCl maintained compatibility with dental pulp cells. HOCl shows a promise as a dentin conditioner, offering enhance bond performance to CAD with improved bond durability, while providing antibacterial benefits and maintaining biocompatibility.

Dental caries is a common chronic disease caused by dietary sugars, bacteria, and host factors. It progresses from enamel demineralization to dentin involvement, leading to structural damage and potential tooth loss if untreated. Understanding the characteristics of carious dentin layers is essential for effective management of dental caries[1]. The outer layer, referred to as caries-infected dentin (CID, Fig. 1a), is characterized by complete demineralization and a soft, cottage cheese-like consistency[1–3]. This layer is heavily contaminated with bacteria and denatured collagen[4], making it irreversibly damaged; therefore, it must be removed prior to restorative procedures[1]. Beneath the CID lies the inner layer known as caries-affected dentin (CAD, Fig. 1a), which is only partially demineralized and maintains some integrity of collagen, resulting in a firmer structure[1–3]. While CID is incapable of remineralization due to bacterial invasion and collagen breakdown, CAD can potentially remineralize under appropriate conditions, making it a preservable structure[1,5].

After the mechanical removal of CID, a smear layer forms over the CAD[5]. The smear layer is generated on the dentin surfaces when dental tissue is subjected to instrumentation, resulting in the formation of a thin layer of denatured debris. This smear layer, which shares similarities with the composition of the underlying dentin, contains a higher concentration of organic material (Fig. 1b) compared to normal dentin[5,6]. These organic components, particularly denatured collagen[4], can create a barrier that hinders resin infiltration and impairs the chemical interactions necessary for effective adhesive bonding. As a result, achieving durable adhesion between resin-based materials and CAD poses a significant challenge for adhesive dentistry.

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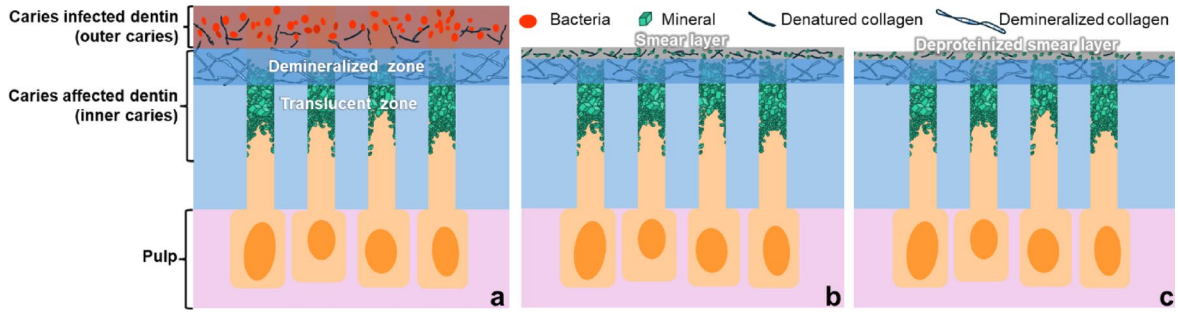

Fig. 1. Schematic of dentin caries and its smear layer.

Materials	Compositions	Application
Clearfil SE Bond 2 (Kuraray Noritake Dental, Tokyo, Japan) Batch: Primer 7K0218 Bond 7Q0333	Primer: 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-CQ, water Bond: 10-MDP, 2-HEMA, Bis-GMA, Bis-GMA, hydrophilic aliphatic dimethacrylate, dl-CQ, initiators, accelerators, silanated colloidal silica	1. Apply Primer and leave for 20 s 2. Dry with gentle air blow for 5 s 3. Apply Bond and make a uniform film using mild air blow 4. Light cure for 10 s
Clearfil AP-X (Kuraray Noritake Dental, Tokyo, Japan) Batch: CM0115	Bis-GMA, TEGDMA, CQ, photoinitiators, pigments, silanated barium glass, silanated silica	1. Apply resin composite in thickness less than 2 mm 2. Light cure for 20 s 3. Repeat 3 times

Table 1. Materials used in this study. *10-MDP* 10-methacryloyloxydecyl dihydrogen phosphate, *HEMA* 2-hydroxyethyl methacrylate, *CQ* camphorquinone, *Bis-GMA* bisphenol A-glycidyl methacrylate, *TEGDMA* triethyleneglycol dimethacrylate.

In recent years, smear layer deproteinization has emerged as a promising strategy for improving dentin bond durability[7,8]. Among various agents explored for this purpose, hypochlorous acid (HOCl) has gained attention as a potential dentin conditioner[9–11]. HOCl, naturally produced by neutrophils during immune response, has demonstrated biocompatibility and antimicrobial properties in medical applications including eyelid cleaner, wound care and sanitization[12–14]. In adhesive dentistry, it has been proposed as an alternative method to remove organic components of the smear layer, potentially enhancing the penetration of adhesive systems and improving the quality of the hybrid layer[7–11]. Additionally, since bacterial contamination in CAD remains a clinical concern even after caries removal, HOCl antibacterial properties could provide simultaneous substrate disinfection during the bonding procedure.

Previous studies demonstrate HOCl effectively removes organic content[6,7,9,10], with Kunawarote et al. showing 50 ppm HOCl reduces CAD smear layer thickness (Fig. 1c)[6]. Furthermore, HOCl application has resulted in improved bond strength and significantly reduced nanoleakage[7–11]. However, while these findings are promising and pave the way for incorporating HOCl as a pre-treatment for bonding to CAD, knowledge gaps remain. Specifically, the effects of HOCl on bonding to CAD, its ability to disinfect carious bacteria, and its safety for dental pulp tissue remain insufficiently explored. As a strong oxidizing agent[13,15], HOCl raises valid concerns about its cytotoxic on dental pulp cells, especially if it penetrates dentinal tubules. Thus, a comprehensive understanding of HOCl's benefits related to bonding, disinfection, and cellular response is necessary for determining its clinical applicability and establishing safe concentration and application protocols.

This study aims to investigate the impact of HOCl on bonding to CAD, antibacterial efficacy, and the response of dental pulp cells. The evaluation examined microtensile bond strength after 24 h and thermocycles, S. mutans growth inhibition, and dental pulpal cell viability. The null hypotheses were that HOCl would not influence on (1) the μTBS to CAD, (2) the inhibition of S. mutans, and (3) cytocompatibility of pulpal cells. These parameters were selected as critical assessment criteria for HOCl's clinical application. Enhanced adhesion to CAD is fundamental for restoration durability, while antimicrobial properties are necessary to address residual bacteria that may persist following conventional caries excavation. Cytotoxicity evaluation establishes safety thresholds, particularly relevant in deep cavities where the pulp-dentin complex is vulnerable. This multifaceted investigation examines both therapeutic potential and biological compatibility, prerequisites for translating this approach into dental practice.

Materials and methods

Materials

The materials used in this study included an adhesive (Clearfil SEB 2; SEB, Kuraray Noritake Dental, Tokyo, Japan), and a resin composite (Clearfil AP-X shade A2; Kuraray Noritake Dental, Tokyo, Japan). An overview of the materials is presented in Table 1. A 50 ppm solution of HOCl (pH 6.8) was prepared by diluting a 500 ppm HOCl solution (Dent Zia, Tokuyama Dental, Tsukuba, Japan) with deionized water.

Tooth specimen preparation

Twenty extracted human molars with carious lesions confined to the occlusal surface were used in this study. The teeth were stored in a 0.1% thymol solution at 4 °C and were utilized within 3 months of extraction. The study was conducted in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments. The use of extracted human teeth in this study was approved by the Human Research Ethics Committee of the faculty of Dentistry Chulalongkorn University (HREC-DCU 2023-106). Informed consent was obtained from all subjects with the reason for tooth extraction.

The occlusal surface of each tooth was sectioned transversely at the mid-coronal third, perpendicular to its long axis, using a low-speed diamond saw (Isomet; Buehler Ltd., Lake Bluff, IL, USA) with water lubrication. After sectioning, the exposed surface was polished with 600-grit silicon carbide paper under water coolant to establish a uniform smear layer and remove any infected carious surface. Inclusion criteria required that the carious lesions extended at least halfway from the enamel-dentin junction toward the pulp chamber, with sufficient healthy dentin surrounding the lesion to serve as a control site for bonding procedures. The entire dentin surface was evaluated using sharp excavator tactile assessment, visual inspection, and caries detector solution (Nippon Shika Yakuhin Co., Ltd., Japan). Based on these criteria, the dentin was classified as either caries-affected dentin (firmer consistency with light-pink staining) or normal dentin (yellowish, hard texture without staining). This surrounding normal dentin provided the necessary control substrate for comparative bonding evaluations.

A standardized smear layer was created by polishing with 600-grit paper for 30 s under water coolant. In cases where the depth of the carious lesion was assessed to be either too shallow or too deep following bonding and sectioning, the tooth was replaced with another specimen that satisfied the inclusion criteria. An illustration of the specimen preparation is provided in Fig. 2.

Microtensile bond strength (μTBS) test

The dentin specimens were randomly allocated into two groups: no pretreatment (control) and HOCl-pretreatment ($n = 10$ teeth). The 50 ppm HOCl solution was applied gently with a microbrush for 30 s, followed by a wash with water for 30 s. After air-dried for 10 s, the adhesive was then applied to the treated dentin surfaces according to the manufacturer's instructions and light-cured for 10 s (1000 mW/cm², Valo Grand, Ultradent, South Jordan, UT, USA). Each increment of 2-mm-thick resin composite (Clearfil AP-X Flow, Kuraray Noritake Dental, Tokyo, Japan) was placed on the bonded surface and light-cured for 20 s. The teeth were subsequently stored in deionized water at 37 °C for 24 h.

The bonded specimens were sectioned perpendicularly to the bond interface into five to eight 0.7-mm-thick slab-shaped specimens using a low-speed diamond saw with water cooling (Isomet, Buehler; Lake Bluff, IL, USA). On lateral view, the slightly discolored dentin was classified as caries-affected dentin, while the surrounding yellow dentin was classified as normal dentin. Three to four slabs from each type of dentin were collected to obtain a total of 30 slabs for each pretreated dentin condition.

The resin-dentin slab specimens were then divided into two subgroups: those stored for 24 h and those subjected to artificial aging through 10,000 thermocycles ($n = 15$ slabs). The design of sample size and thermocycling was performed according to the guidelines established by the Academy of Dental Materials[12], with temperature cycling between 5 and 55 °C, including a dwell time of 30 s in each bath and a transfer time of five seconds. After the designated aging procedure, the slabs were hand-trimmed to an hourglass shape with

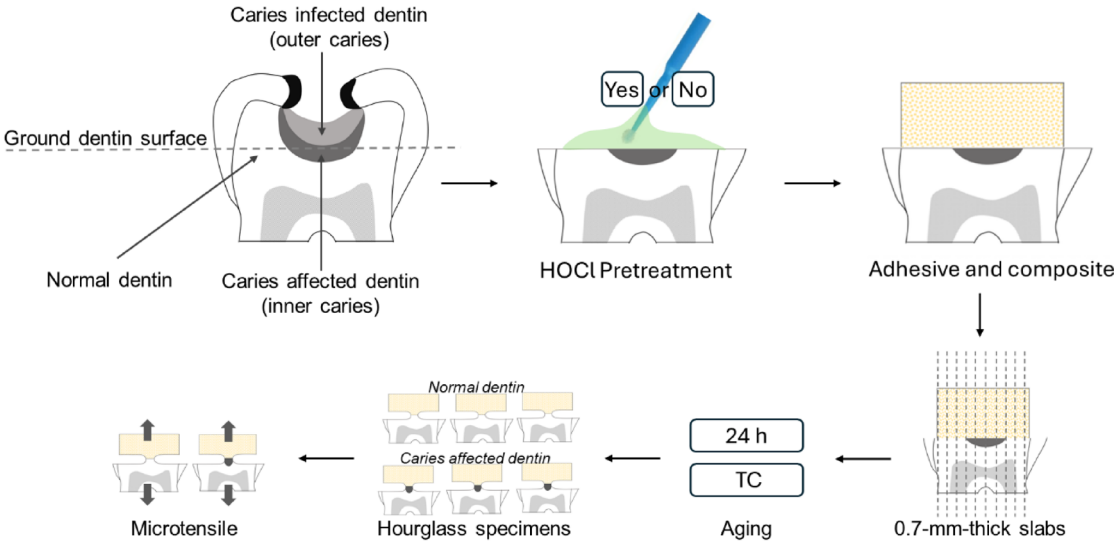

Fig. 2. Illustration of sample preparation and study design of microtensile bond strength test.

approximately 1 mm^2 cross-sectional areas of isolated normal or caries-affected dentin, using a fine diamond bur. The hourglass specimens were then attached to a universal testing machine (EZ-SX Test, Shimadzu, Kyoto, Japan) and subjected to μTBS test at a crosshead speed of 1 mm/min. Illustration of sample preparation and study design is shown in Fig. 2.

IBM SPSS version 29.0 was used for all data analyses. The hourglass specimens were considered statistical units ($n = 15$). The Shapiro–Wilk and Levene's tests indicated that the μTBS data were normally distributed and had homogeneous variance, respectively. The data were analyzed using three-way ANOVA (independent variables: dentin substrate, HOCl pretreatment, and thermocycling) followed by Tukey's post hoc tests. Bonding durability was assessed by comparing μTBS after 24 h and after thermocycling in each group using t-tests. The threshold for statistical significance was set at $p < 0.05$.

Failure mode

Following the microtensile bond strength testing, all fractured specimens were desiccated in a desiccator at room temperature for 24 h, sputter-coated with gold, and observed using SEM (JSM-IT100, JEOL, Tokyo, Japan). Fracture patterns were categorized into four distinct modes: (1) adhesive failure, where more than 80% of the fracture occurred at the adhesive-dentin interface; (2) dentin cohesive failure, where more than 80% of the fracture was within the dentin substrate; (3) resin cohesive failure, where more than 80% of the fracture occurred within the adhesive layer or composite material; and (4) mixed failure, characterized by a combination of failure patterns, with none exceeding 80%. Statistical evaluation of failure mode distribution was performed using Pearson's chi-square test at a significance level of 0.05.

Antibacterial effect

Streptococcus mutans NTCT10449 was cultured in brain heart infusion (BHI; Becton Dickinson, Sparks, MD) broth at 37 ℃ overnight. The bacterial suspension was then adjusted to approximately 2×10^7 colony-forming units (CFU)/mL using phosphate-buffered saline (Wako, Fujifilm, Osaka, Japan). HOCl solution at 500 ppm was diluted in PBS to prepare a working solution of HOCl at 100 ppm. Fifty microliters (μL) of the prepared bacterial suspension and 50 μL of HOCl solution (100 ppm) were added to each well of a sterile, flat-bottom 96-well microtiter plate, resulting in a final HOCl concentration of 50 ppm in a total volume of 100 μL per well. BHI broth without HOCl served as the control group. The plate was incubated at 37 °C for 30 s. Following incubation, the suspensions were serially diluted, and 10 μL of each dilution was plated onto BHI agar. The agar plates were incubated anaerobically at 37 °C for 24 h, and the number of bacterial colonies was counted. The experiment was performed in triplicate.

Cell viability assay

The rat dental pulp cells (RPC-C2A), a clonal cell line derived from Wistar rat incisor pulp, were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum[16,17]. The cells (5×10^4 cell/well were seeded and incubated for 24 h in a 5% CO2 incubator at 37 °C. In a clinical situation, the HOCl conditioner may not directly contact dental pulp cells, as it can penetrate dentinal tubules and may be diluted by dentinal fluid. Therefore, in this study, HOCl solutions were prepared in serial concentrations of 0.5, 1, 5, 10, and 50 ppm by diluting the eluate in deionized water and subsequently further diluting it in culture medium (1:9 ratio). Control groups were treated with fresh medium without HOCl.

Six wells were allocated for each test solution to cell proliferation. After incubating the cells with the test solutions for 24 h, cytotoxicity was assessed using the 2-(2-Methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2 H-tetrazolium, mono-sodium salt (WST-8; Cell Counting Kit-8; Dojindo, Tokyo, Japan), following the manufacturer's instructions ($n = 6$ wells). Ten microliters of the kit's solution were added to each well and incubated for 30 min. Absorbance was measured at 450 nm using an iMark Microplate Reader (BIO-RAD). According to the ISO 10993-5:2009 standard, cell viability values were calculated; cell viability (%) = 100 × (OD_{test}/OD_{blank}), where OD_{test} is the absorbance of the test solution and OD_{blank} is the absorbance of the blank. The data were analyzed statistically using one-way ANOVA followed by Tukey's post hoc test, with a significance level set at 0.05.

Results

μTBS

Means and standard deviations of μTBS are presented in Table 2. The three-way ANOVA revealed that μTBS was significantly influenced by dentin substrate ($p < 0.001$), HOCl pretreatment ($p < 0.001$), and thermocycling ($p < 0.001$). No significant interactions between the factors were observed ($p > 0.05$). Without HOCl pretreatment,

Substrate	Pretreatment	24-hour storage	Thermocycling
Normal dentin	No	41.6 ± 6.7^{Aa}	39.1 ± 3.8^{Aa}
	HOCl	51.0 ± 7.8^{Ba}	47.6 ± 5.7^{Ba}
Caries affected dentin	No	25.6 ± 4.3^{Ca}	19.7 ± 4.5^{Cb}
	HOCl	35.8 ± 7.2^{Aa}	32.1 ± 4.5^{Da}

Table 2. Means and standard deviations of microtensile bond strength (MPa). Different capital letters indicate statistically significant differences in each column and different lowercase letters indicate statistically significant differences in each row ($p < 0.05$).

normal dentin exhibited significantly higher 24 h μTBS compared to caries-affected dentin (CAD) ($p < 0.001$). However, HOCl pretreatment significantly increased the 24 h μTBS of both normal dentin ($p = 0.002$) and CAD ($p < 0.001$). Thermocycling did not significantly reduce μTBS in normal dentin, regardless of whether HOCl pretreatment was applied ($p > 0.05$). In contrast, thermocycling significantly decreased the μTBS of CAD ($p = 0.03$), although HOCl pretreatment was shown to stabilize μTBS in CAD, approaching significance ($p = 0.06$).

Failure mode

The distributions of failure modes are presented in Fig. 3. SEM analysis of the fractured surfaces (Figs. 4, 5, 6 and 7) revealed that mixed failures prevailed in almost all groups. There were no significant differences in failure mode distribution among the groups ($p = 0.67$).

Antimicrobial effect

The concentration of viable S. mutans after a 30-second incubation with 50 ppm HOCl and the control is presented in Fig. 8. In the absence of HOCl treatment, the bacterial concentration was 8.9×10^7 CFU/mL. Following treatment with HOCl, the bacterial concentration was significantly reduced to 5.8×10^6 ($p < 0.05$), demonstrating the antimicrobial efficacy of HOCl.

Cell viability

The results of cell viability are presented in Fig. 9. HOCl concentrations of 0.5, 1, 5, and 10 ppm maintained cell viability levels statistically similar to to the control group ($p > 0.05$). While, the 50 ppm HOCl group showed a significant reduction in cell viabilty compared to control ($p < 0.001$), the mean viability remained above 70%, which is considered ono-toxic according to ISO10993-5(2009) standard.

Discussion

The results of this study provide important insights into the effects of HOCl as a potential dentin conditioner in adhesive dentistry. Our findings revealed that HOCl pretreatment significantly increased bond strength to CAD and maintained it after thermocycling. HOCl demonstrated a antibacterial effect on *S. mutans*. Additionally, demonstrated favorable compatibility with dental pulp cells. Based on these outcomes, the null hypotheses were partially rejected.

The enhanced bond strength and durability to CAD following HOCl pretreatment can be primarily attributed to its deproteinizing effect. HOCl effectively reduces the organic components within the smear layer covering dentin surfaces[6], thereby facilitating adhesive monomer infiltration and promoting chemical interaction between the monomers and underlying dentin substrate[5,7]. While previous studies have raised concerns about residual oxidizing molecules potentially interfering with adhesive polymerization[7,8,11,18,19], HOCl presents minimal challenges in this regard[9]. Research suggests that these residual molecules can be effectively eliminated through adequate wash-out time[7,9]. Additionally, the impact HOCl significantly influenced by the concentration, application time, and adhesive systems[6,9–11,18].

The antimicrobial activity of HOCl was evaluated at 50 ppm, a concentration previously established for optimal dentin bonding[6]. *S. mutans* was tested as the primary cariogenic bacteria that initiates dental caries. This study demonstrates HOCl effectiveness at this lower concentration and short application time at 30 s. Previous studies with higher concentrations of 100–500 ppm showed bacteriostatic effects against various

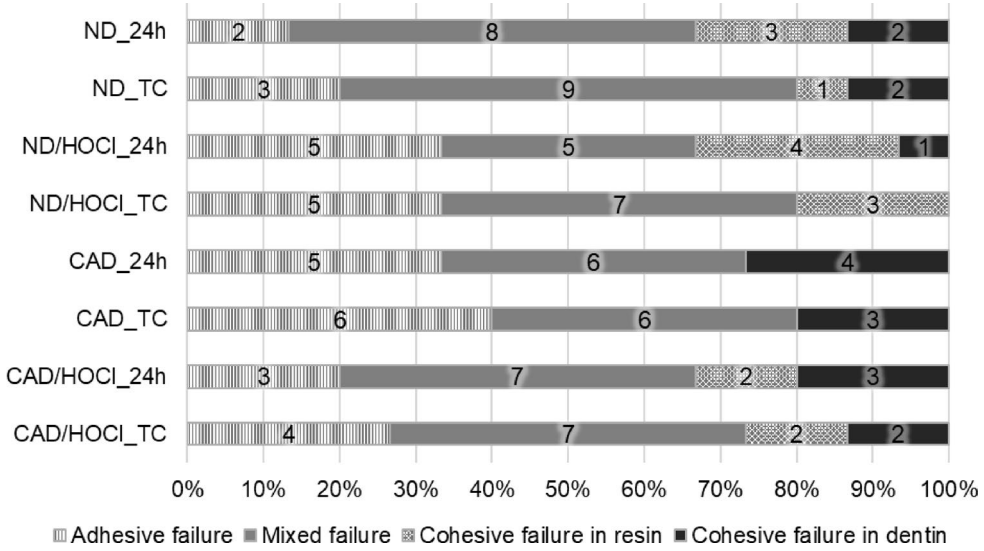

Fig. 3. Distributions of failure modes in each group. Mixed failures prevailed in all groups, and the distributions of failure modes were statistically similar. Numbers within the bars indicate the number of specimens with the respective failure mode. *ND* normal dentin, *CAD* caries-affected dentin, *HOCl* hypochlorous acid, *24 h* 24-hour storage, *TC* thermocycling.

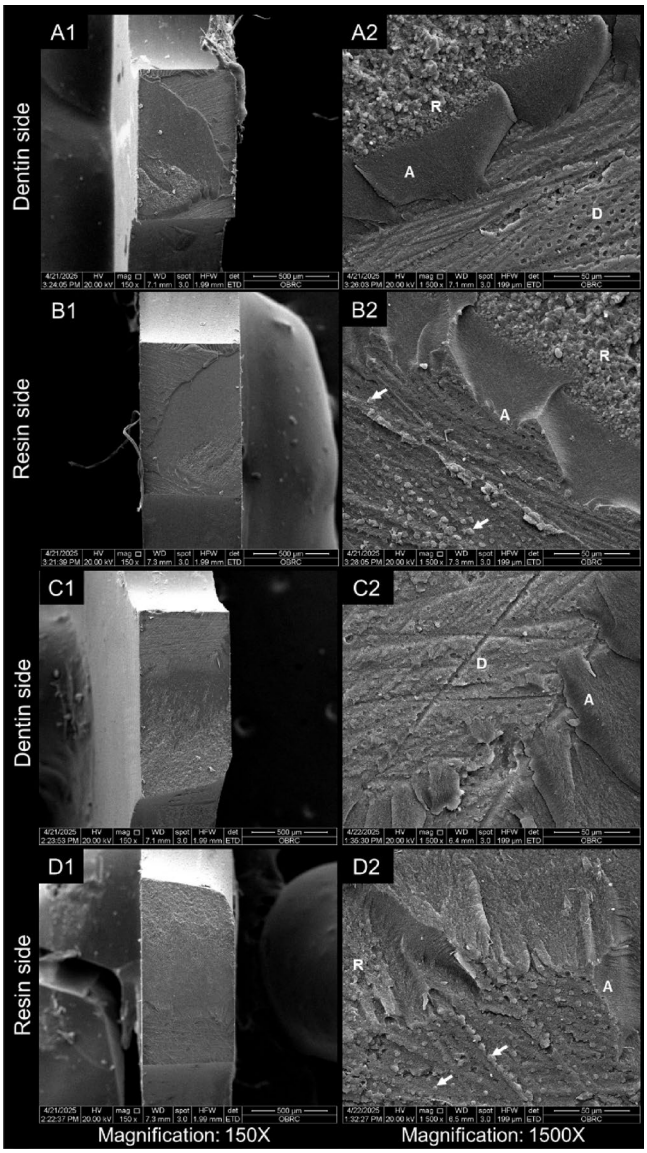

Fig. 4. Representative SEM images of fractography of the bonded normal dentin without pretreatment after 24-hour water storage (**A1-2**,**B1-2**) and thermocycling (**C1-2**,**D1-2**). Arrows indicate resin tags. *A* adhesive, *D* dentin, *R* resin composite.

oral bacteria including *A. actinomycetcmcomitans*, *P. gingivalis*, *S. mutans* and *S. sanguinis*[20]. The variation in antimicrobial efficacy across different concentrations may be attributed to experimental conditions, as previous studies evaluated adherent bacteria on plates while this study examined planktonic bacteria[20–22]. While this study focused on planktonic S. mutans to demonstrate supplementary antibacterial potential of HOCl as part of cavity conditioner, we acknowledge that biofilm models, which exhibit higher resistance to antimicrobial agents, would provide more clinically relevant data. Future studies should incorporate biofilm models to better replicate the complex microbial environment in caries-affected dentin.

Despite reported reductions in bacterial counts within affected dentin, bacterial activity remains a clinical concern that requires attention during restorative procedures[23,24]. While various chemical treatments have been explored, finding an agent that enhances both bonding effectiveness and provides antimicrobial properties during cavity preparation remains crucial[25]. The lower HOCl concentration reduces potential cytotoxicity concerns, particularly important given dentin proximity to pulpal tissue. The 50 ppm concentration minimizes cytotoxicity concerns while maintaining antimicrobial effectiveness with 30-second application time, suggesting HOCl potential as a dentin pretreatment agent with disinfectant properties. Furthermore, in clinical settings, cavity conditioners are typically applied after caries excavation, where biofilms covering the surface are already removed. Previous clinical studies also reported significantly lower bacterial counts in dentin than in plaque or biofilm[26], supporting our model. Thus, the 30-second application time of HOCl was chosen to reflect a realistic chairside protocol. Although minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) values were not measured in this study, prior literature reports MIC of HOCl against pathogens in the range

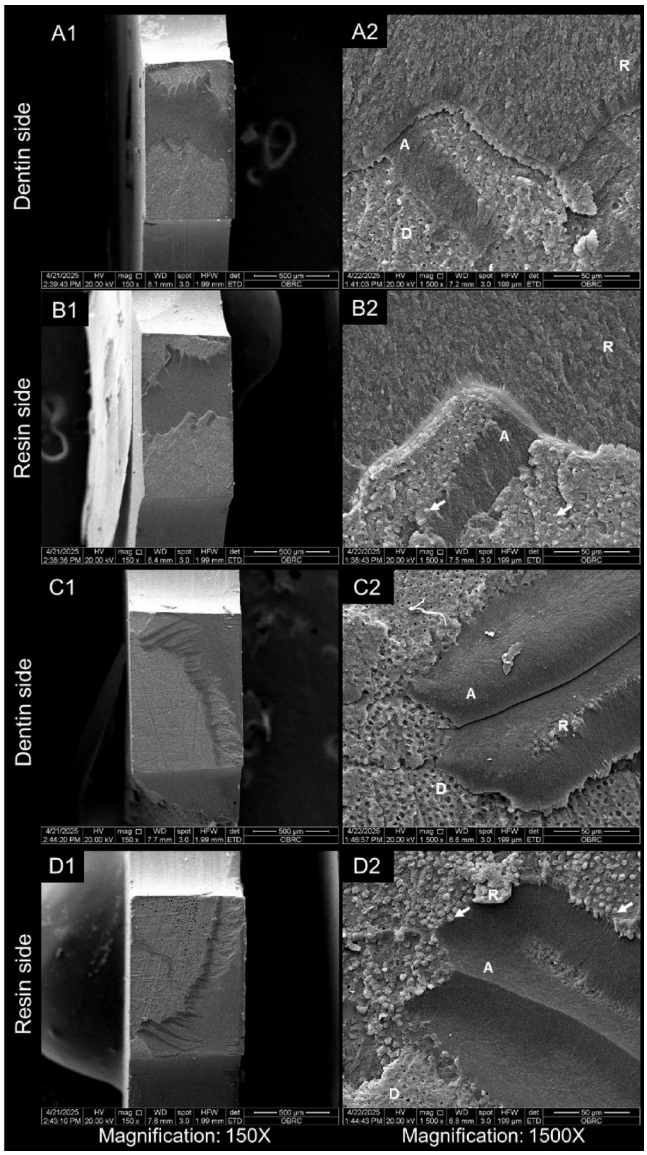

Fig. 5. Representative SEM images of fractography of the bonded normal dentin with HOCl-pretreatment after 24-hour water storage (**A1-2**,**B1-2**) and thermocycling (**C1-2**,**D1-2**). Arrows indicate resin tags. *A* adhesive, *D* dentin, *R* resin composite.

of 0.99–1.63 mM (approximately 50–85 ppm)[27]. Since this study focused on the bonding context, the primary aim was to test whether the selected HOCl concentration for dentin conditioning could simultaneously provide antibacterial effects. The observed bacterial reduction confirms its supplementary antimicrobial benefit.

The present study demonstrated that 50 ppm HOCl maintained cell viability above the critical 70% threshold, indicating favorable biocompatibility for dental applications described in the standard ISO 10993-5. This finding aligns with previous studies demonstrating the cytocompatibility nature of HOCl-based treatments[12–14]. The clinical safety profile of HOCl as a dentin conditioning agent is supported by multiple factors. Of particular importance is the inherent structure of caries-affected dentin (CAD), where partially occluded dentinal tubules create a natural barrier that reduces dentin permeability, thereby limiting direct HOCl exposure to pulpal cells[1–3]. Moreover, the proposed clinical protocol specifies a short 30-second application period, during which HOCl primarily interacts with the superficial smear layer rather than penetrating deeply into the dentin substrate[6,9–11]. The combination of maintained cellular viability, restricted penetration through partially occluded tubules, and short application duration collectively supports the biological safety of HOCl conditioning for dental pulp cells.

Future investigations should explore HOCl application in more complex microbial communities found in caries-affected dentin to better represent clinical conditions. Research must establish optimal HOCl concentrations that balance deproteinizing efficacy, antimicrobial effectiveness, and cellular compatibility. Clinical validation through in vivo studies remains essential to confirm safety and efficacy. Understanding cellular response mechanisms could provide insights for maximizing therapeutic benefits while minimizing

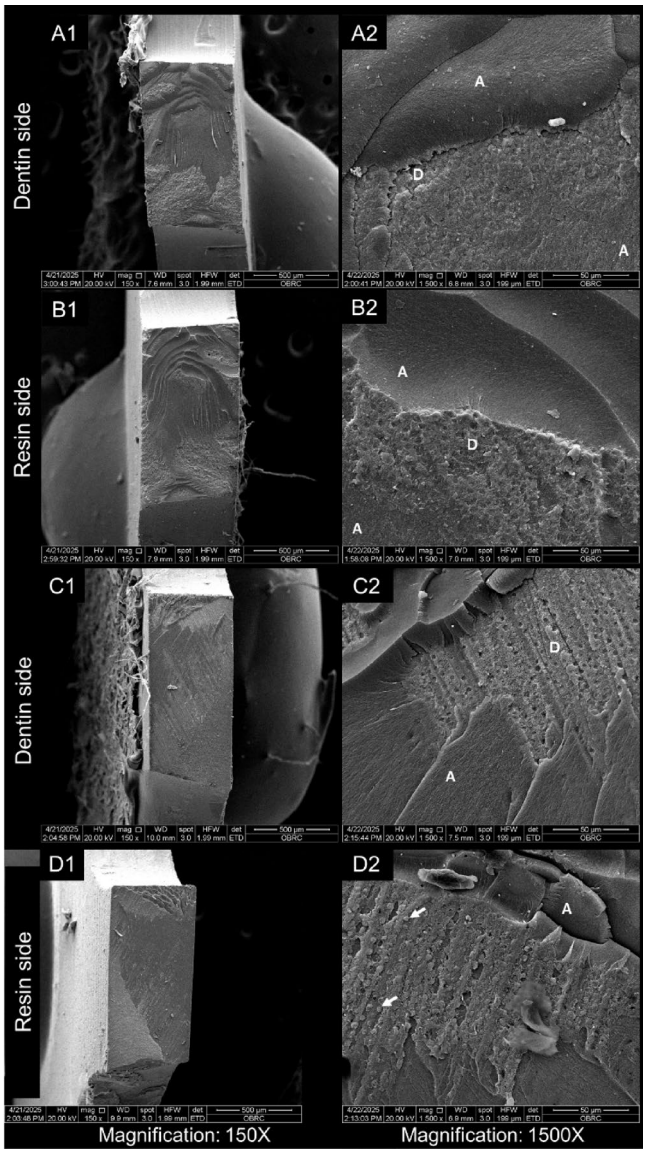

Fig. 6. Representative SEM images of fractography of the bonded caries-affected dentin without pretreatment after 24-hour water storage (**A1-2**,**B1-2**) and thermocycling (**C1-2**,**D1-2**). Arrows indicate resin tags. *A* adhesive, *D* dentin.

cytotoxicity. Development of standardized application protocols will facilitate clinical implementation and reproducible outcomes.

The clinical implications of these findings are significant. HOCl shows promise as a dentin conditioning agent due to its deproteinizing and antimicrobial properties, as well as its ability to enhance bond strength. However, precise control of its concentration is essential to avoid adverse cellular effects on cells.

Conclusion

HOCl emerges as a promising dentin conditioning agent that offers multiple clinical advantages. The significant improvements in bond strength, coupled with its potent antibacterial properties. Importantly, these benefits are achieved while preserving pulpal cell viability, suggesting HOCl could represent a valuable addition to contemporary dental adhesive protocols.

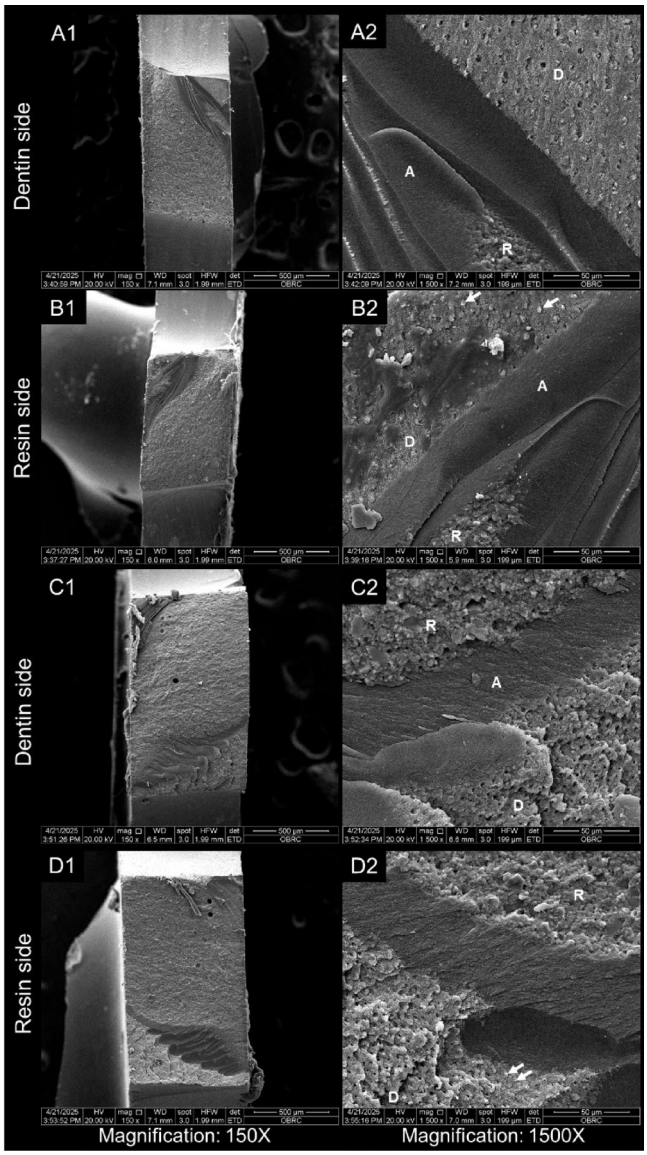

Fig. 7. Representative SEM images of fractography of the bonded caries-affected dentin with HOCl-pretreatment after 24-hour water storage (**A1-2**,**B1-2**) and thermocycling (**C1-2**,**D1-2**). Arrows indicate resin tags. *A* adhesive, *D* dentin, *R* resin composite.

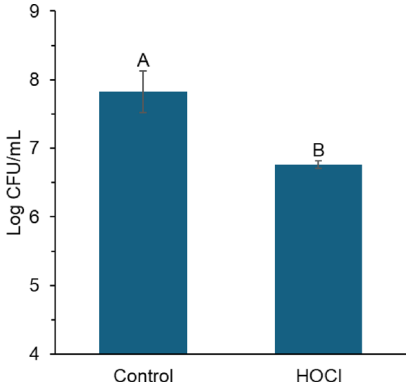

Fig. 8. Means and standard deviations of CFU. Different letters indicate statistically significant differences ($p < 0.001$).

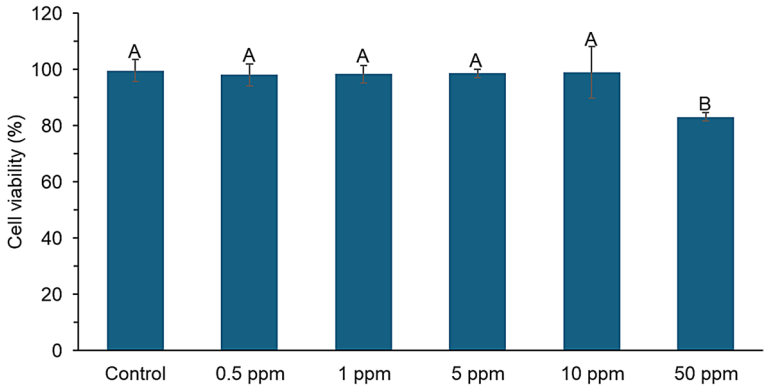

Fig. 9. Means and standard deviations of cell viability (%). Different letters indicate statistically differences ($p < 0.001$).

Data availability

Data available on reasonable request from the corresponding author.

Received: 20 February 2025; Accepted: 6 June 2025
Published online: 01 July 2025

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Acknowledgements
This research project was supported by Faculty Research Grant, Faculty of Dentistry, Chulalongkorn University (DRF 67_025, Kittisak Sanon). Junji Tagami is supported by The Second Century Fund, Chulalongkorn University (C2F).

Author contributions
K.S. Principal investigator, conceptualization, methodology, data collection, data analysis, visualization, resource, funding, wrote manuscriptN.E. Co-investigator, wrote manuscriptN.H. Methodology, resource, validation, wrote manuscriptY.T. Methodology, resource, wrote manuscriptN.S. Validation, data analysis, wrote manuscriptS.T Visualization, wrote manuscriptY.S Resource, wrote manuscript J.T. Supervision, conceptualization, data analysis, resource, wrote manuscriptP.T. Co-investigator, project coordinator, methodology, data analysis, visualization, resource, wrote manuscript.

Funding
This research project was supported by Faculty Research Grant, Faculty of Dentistry, Chulalongkorn University (DRF 67_025). Junji Tagami is supported by The Second Century Fund, Chulalongkorn University (C2F).

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
The use of extracted human teeth in this study was approved by the Human Research Ethics Committee of the faculty of Dentistry Chulalongkorn University (HREC-DCU 2023 – 106). Informed consent was obtained from all subjects with the reason for tooth extraction. The experiments involving the animal cell line (RPC-C2A) were conducted in accordance with standard laboratory protocols and did not require additional ethics approval as they utilized an established cell line.

Additional information
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