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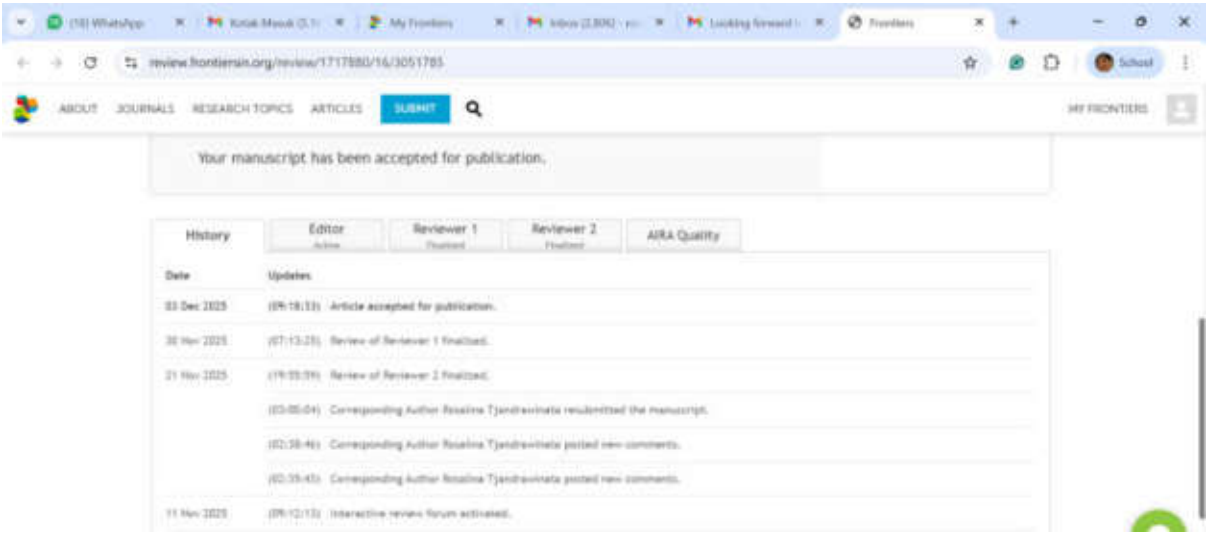

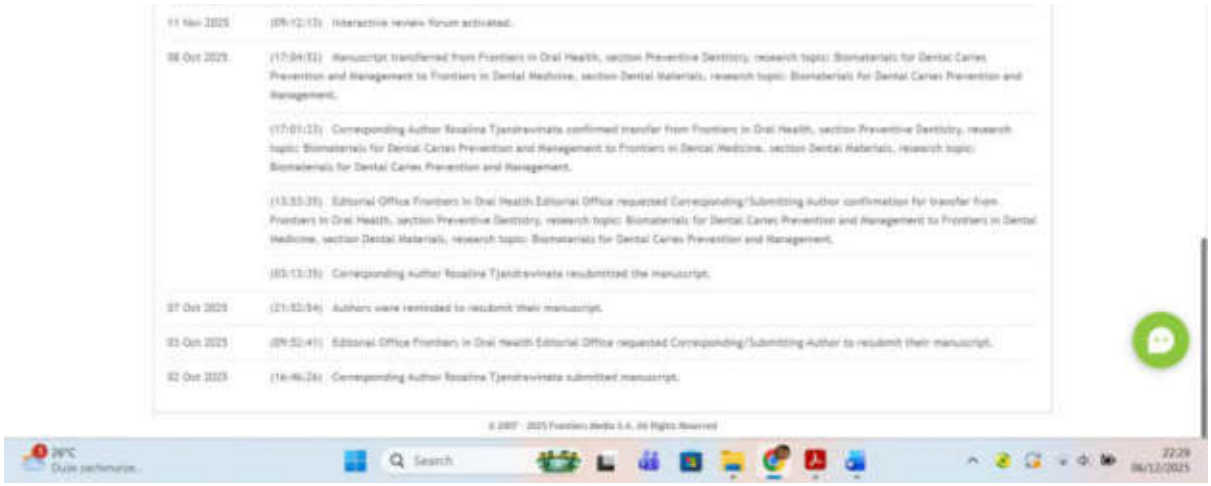

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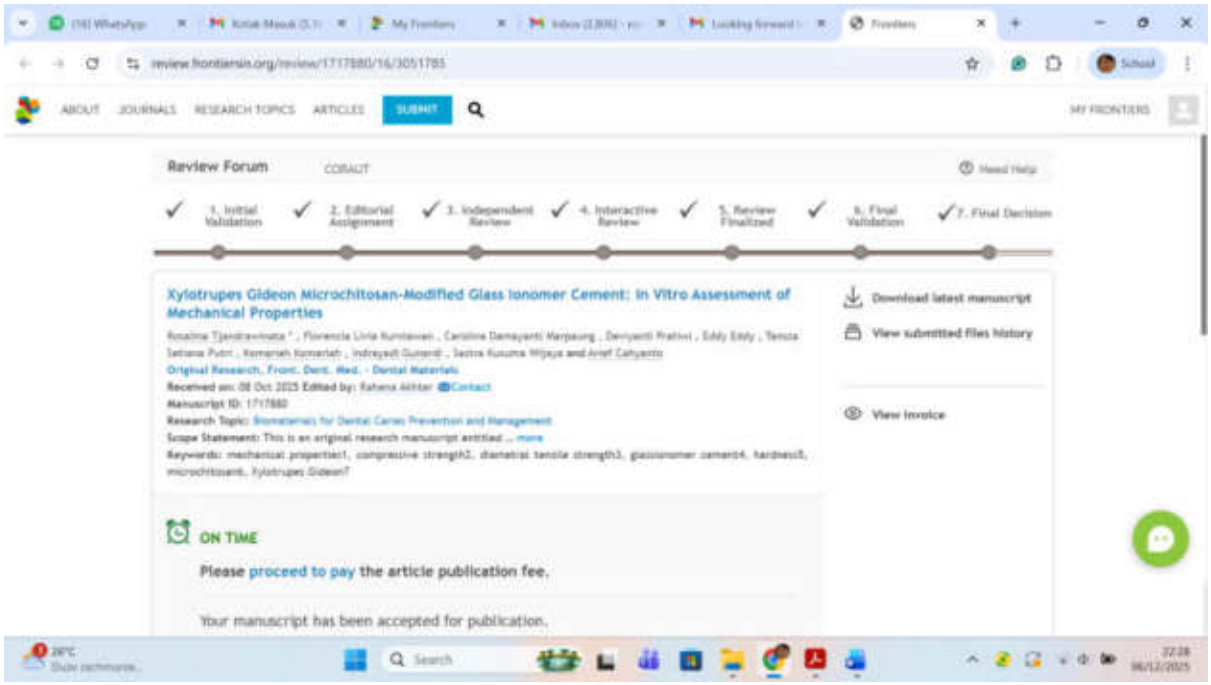

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Xylotrupes Gideon Microchitosan- Modified Glass Ionomer Cement: *In Vitro* Assessment of Biomechanical Properties

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Abstract

Background: Glass ionomer cements (GIC) are valued as inherent fluoride-releasing dental restorative materials, while chitosan binds to negatively charged enamel surfaces, promoting mineral deposition and strengthening teeth. This study aimed to evaluate the biomechanical properties of glass ionomer cement (GIC) modified with microchitosan derived from *Xylotrupes gideon*, using an *in vitro* experimental design.

Materials and Methods: Microchitosan was extracted from *Xylotrupes gideon* and incorporated into conventional GIC at 0.5%, 1% and 2% (w/w). Compressive strength, diametral tensile strength, and surface microhardness were measured using standard testing equipment after immersion in artificial saliva for 24 hours and 7 days. Statistical analysis was performed using one-way ANOVA followed by the Games-Howell post hoc test, with significance set at $p < 0.05$.

Results: The 1% microchitosan-modified GIC exhibited the most significant improvements compared to the unmodified control. After 7 days, compressive strength increased by 35.4%, diametral tensile strength by 51.3%, and surface hardness by 46.6% ($p<0.05$). These enhancements are attributed to microscale reinforcement and chemical bonding between microchitosan and the GIC matrix.

Conclusion: The addition of 1% microchitosan derived from *Xylotrupes gideon* significantly improved the biomechanical performance of GIC. This bioactive reinforcement shows promising

potential for clinical restorative applications, though further investigation into its long-term biocompatibility and fluoride release is warranted.

1 Introduction

Dental composite materials, including glass ionomer cements (GICs), are widely recognized in restorative dentistry for their versatility, biocompatibility, and ability to bond chemically to dental hard tissues. GICs, in particular, are valued for their inherent fluoride release, which aids in the prevention of tooth decay and supports long-term tooth preservation.(1, 2) These advantages make GICs ideal for pediatric dentistry and minimally invasive restorations where fluoride release and adhesion are critical; however, their adhesive performance varies significantly across formulations.

A major drawback of traditional GICs is their weak mechanical properties, including low compressive and tensile strength, poor wear resistance, and susceptibility to fracture under occlusal stress. These limitations preclude their use in stress-bearing restorations, particularly in posterior regions that are subjected to high occlusal loads.(3) The other limitations are primarily attributed to the intrinsic chemistry of the GIC matrix, particularly the brittle nature of the glass phase and the sensitivity of the acid-base setting reaction to moisture and dehydration.(4) Recent technology developments, particularly involving micro-sized chitosan and titanium dioxide, have demonstrated improved filler dispersion and particle–matrix bonding in glass ionomer cements, resulting in enhanced mechanical properties, matrix cohesion, fracture resistance, and bioactivity (5, 6) with chitosan enhancing particle-matrix bonding and decreasing microvoid formation.(7)

Such reinforcement strategies yield structurally robust cement matrices characterized by minimized porosity and enhanced resistance to fracture propagation. Studies have shown that the incorporation of chitosan significantly enhances compressive and flexural strength, consistent with previous findings on quaternized chitosan-coated nanoparticles and carboxymethyl chitosan derivatives (8, 9) thereby improving matrix homogeneity and resistance to fracture propagation.

A previous study showed that the addition of chitosan to GIC has a positive effect on microhardness and improves surface roughness, making it a promising additive for dental restorative materials.(10) This material reinforces the structural framework and enhances mechanical reliability, which in turn leads to improved fracture resistance, increased microhardness, and enhanced overall durability.(11) The reinforcement effect demonstrated temporal dependence, with mechanical improvements more evident following extended maturation periods. Its ability to improve both the mechanical and biological properties of restorative materials makes it a compelling additive in dental biomaterials research. The molecular features of chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose], a versatile biopolymer, are chemically inert, non-toxic, and possess robust and also broad antimicrobial activities due to its polycationic nature.(12) Characterized by amino and hydroxyl functional groups, chitosan enables ionic and hydrogen bonding that strengthens adhesion to dental substrates, improves mechanical strength through particle–matrix cohesion, and supports tissue compatibility via biocompatibility and regenerative potential (13) across a wide range of dental applications.

Chitosan exhibits remarkable physicochemical properties and good biocompatibility, forming bonds with human proteins, cells, and organs.(14) Chitosan exhibits multifunctionality in dental applications by contributing to mechanical reinforcement, promoting tissue regeneration, and acting as a bioactive agent in restorative formulations such as glass ionomer cements and adhesives. Recently, alternative sources of chitosan have emerged, including the exoskeletons of the *Xylotrupes gideon* beetle, which offer a sustainable and locally available biomaterial. Previous research indicates

that this chitosan, derived from insects, enhances GIC performance in acidic oral environments (with critical pH levels), showing promise for clinical applications.(15) These advantages support further investigation of its mechanical reinforcement potential in dental composites. Research indicates that this novel form of chitosan demonstrates superior mechanical enhancement capabilities when integrated into GICs, including increased compressive strength and Vickers hardness.(16) Additionally, the microstructure improves particle dispersion and matrix interaction, which are critical for achieving homogeneity and minimizing microvoid formation during the setting process.(7, 17)

In addition to mechanical benefits, chitosan may confer secondary biofunctional advantages, which warrant future exploration but were not addressed in the present study.(18) These properties may enhance the clinical performance of restorations by reducing bacterial colonization, improving marginal integrity, and supporting the healing of the pulp-dentin interface, in line with recent advancements in oral health technology.(17) Notably, such modifications do not appear to compromise GIC's core biofunctional properties, such as fluoride ion release and adhesion to dentin.(1, 11)

Therefore, this study aims to investigate the effect of *Xylotrupes gideon*-derived microchitosan incorporation on the mechanical properties of conventional GIC, including compressive strength, diametral tensile strength, and surface hardness, while also addressing its potential clinical feasibility, long-lasting nature, and biological functionality through the integration of eco-friendly materials in restorative dentistry.(7)

2 Materials and Methods

2.1 Synthesis and Characterization of Microchitosan from Xylotrupes gideon

Microchitosan (MCH) was synthesized from the exoskeletons of *Xylotrupes gideon* beetles collected in Bogor, West Java, Indonesia. All body parts were detached and dried for five days, followed by extraction using established chitin processing protocols—specifically, demineralization with 3N HCl, deproteinization with 3N NaOH, and deacetylation with 50% NaOH at 100 °C to obtain chitosan.

The resulting chitosan was converted into micro form via ionic gelation using sodium tripolyphosphate (TPP) in deionized water, stirred at 2500 rpm for four hours, intentionally omitting acetic acid to improve biocompatibility. A particle size analyzer (Horiba, Japan) revealed a spherical morphology with a size distribution ranging from 200 to 4000 nm, while SEM analysis also showed the form of chitosan particles as in Figure 1. The synthesized MCH was stored at 4 °C in airtight containers until use. (19)

The overall experimental workflow is illustrated in Figure 2, highlighting the synthesis of microchitosan from *Xylotrupes gideon* and its integration into glass ionomer cement for mechanical testing.

2.2 Preparation of Modified Glass Ionomer Cement

A commercially available Glass ionomer cement (Fuji IX Gold Label HS Posterior, GC, Tokyo, Japan) was used as the base material. The control group used unmodified GIC liquid, while the experimental groups consisted of 0.5%, 1%, and 2% (w/w) MCH added to the liquid component. The powder and respective liquid in each mixture were manually combined according to the

manufacturer's guidelines, with prior vortex mixing of the modified liquid to improve homogeneity, particularly at 2% concentration to achieve uniformity.(1, 20)

Compressive strength specimens were molded according to ISO 9917-1:2007: 3 mm diameter × 6 mm height, while the samples for diametrical tensile strength: 6 mm diameter × 4 mm thickness, and Vickers hardness: 6 mm diameter × 4 mm thickness. Specimen dimensions were confirmed using a digital caliper (Mitutoyo 500-196-30, Japan). After initial setting, all specimens were stored in 100% humidity at room temperature for 1 hour.

2.3 Conditioning and Storage

A total of 80 samples were prepared for each mechanical test and divided into four groups (n = 20 each), with each group further subdivided into two subgroups (n = 10) based on immersion time (24 hours and 7 days). A Fusayama-Meyer artificial saliva solution was prepared containing 0.4 g/L of NaCl, 0.4 g/L of KCl, 0.906 g/L of $CaCl_2 \cdot 2H_2O$, 0.690 g/L of $Na_2HPO_4 \cdot 2H_2O$, 0.005 g/L of $Na_2S \cdot 9H_2O$, and a pH of 6.75. Samples were placed in 10 mL of artificial saliva per specimen in sealed containers, then incubated at 37°C.

2.4 Mechanical Testing

Mechanical properties were evaluated using a universal testing machine (Shimadzu, Japan) equipped with a 5 kN load cell, following validated protocols.(21) Compressive and diametral tensile strength tests were conducted at a crosshead speed of 1 mm/min. Surface hardness was measured using a Shimadzu HMV-G microhardness tester (Kyoto, Japan), employing a micro Vickers diamond indenter with a 100 g load applied for 10 seconds (22) All mechanical testing adhered to ISO 9917-1:2007 guidelines and methodological frameworks.

2.5 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean and standard deviation, were calculated. Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was evaluated using Levene's test. When the assumption of homogeneity was violated, one-way ANOVA was followed by the Games–Howell post hoc test for pairwise comparisons.

Pearson correlation coefficients were calculated to examine relationships among mechanical parameters. A significant level of $p<0.05$ was applied throughout.

3 Results

The compressive, diametral tensile strength, and hardness test results for all test groups are presented in Figure 3 – 5, while Table 1 represents the significance value of the comparison between groups. The data from 240 specimens (with 20 specimens per group and 10 at different time points) were found to have a normal distribution, as confirmed by the Kolmogorov–Smirnov test ($p = 0.200$). The addition of microchitosan (MCH) at all concentrations resulted in statistically significant increases in both compressive and diametral tensile strength compared to the control group ($p < 0.05$).

3.1 Compressive Strength

The compressive strength results are presented in Figure 3. At 24 hours, the control GIC group recorded a mean strength of 127.14 ± 15.82 MPa. In comparison with the control group, the 0.5%

MCH group showed a slight reduction, while the 2% MCH group produced the highest value (130.01 ± 14.40 MPa, $p = 0.973$). The 1% MCH group was comparable to the control.

After 7 days of immersion, the control group decreased to 114.63 ± 21.39 MPa. In comparison with the control, all MCH groups improved. The highest value was observed in the 1% MCH, which peaked at 155.16 ± 19.08 MPa ($p = 0.002$). The 1% MCH group demonstrated the highest strength gain over time (35.4% increase).

3.2 Diametral Tensile Strength

The diametral tensile strength results are presented in Figure 4. The control at 24 hours showed a strength of 11.41 ± 3.16 MPa. Compared with the control, the 0.5% MCH group showed significantly lower strength, whereas 1% MCH and 2% MCH did not differ significantly from the control.

After 7 days, the control group decreased to 7.09 ± 2.70 MPa, while improvements were observed in all MCH groups, particularly in comparison with the control. The 1% MCH group showed the highest value at 10.73 ± 1.89 MPa ($p = 0.014$), which was 51% higher than the 7-day control.

3.3 Vickers Hardness

The Vickers microhardness test results at 24 hours are given in Figure 5. The Control GIC had a microhardness mean value of 63.92 ± 15.17 VHN. The MCH-modified groups demonstrated significantly increased hardness, with a peak at 1% MCH, which had a value of 135.30 ± 17.90 VHN, with a p-value of less than 0.001 for all.

All groups experienced a decrease in hardness after 7 days, while the 1% MCH maintained the highest residual value (46.95 ± 7.32 VHN, $p < 0.001$).

3.4 Trends and Correlation Analysis

The inclusion of microchitosan showed a consistent increase in mechanical properties, most notably at a concentration of 1%. While the 2% group showed strong initial values, its performance declined slightly over time, suggesting an upper threshold of reinforcement.

Pearson correlation analysis revealed strong positive correlations between microchitosan concentration and compressive strength ($r = 0.59$, $p = 0.002$), diametral tensile strength ($r = 0.57$, $p = 0.004$), and hardness ($r = 0.53$, $p = 0.011$). Seven-day specimens of compressive strength and diametral tensile strength showed a positive correlation ($r = 0.71$, $p = 0.001$). Additionally, compressive strength and hardness were positively correlated ($r = 0.64$, $p = 0.008$), indicating an interconnected strengthening effect.

4 Discussion

4.1 Summary of Findings

This study demonstrated that incorporating *Xylotrupes gideon*-derived microchitosan (MCH) into conventional GIC significantly improved its mechanical properties. The 1% MCH concentration produced the best results across all measurements, including compressive strength, diametral tensile strength, and surface hardness, particularly after 7 days of exposure to artificial saliva. The reinforcement effect appeared to be time-dependent, with greater effects noted after a more extended period of maturation.(23) The 0.5% MCH group showed modest effects, while the 2% MCH group,

despite initial enhancements, was assumed to be more prone to agglomeration, which undermined its long-term stability.(6) The results align with previous research findings that validate chitosan's ability to enhance GIC strength and durability, supporting the broader trend of dental composites reinforced with nanomaterials.(16)

The superior performance of 1% microchitosan (MCH) aligns with previous findings that nanochitosan outperforms other nanostructures (e.g., nano-silica, nano-zirconia) in glass ionomer cements (GICs), primarily due to its ability to form hydrogen bonds with the polyacrylic acid matrix, enhancing matrix cohesion and ion exchange. (1) Our results confirm the significant improvements in surface hardness and compressive strength compared to both unmodified controls and GICs modified with alternative nano-additives.(24) This dual enhancement—mechanical performance and biofunctionality—positions small particle chitosan as a promising candidate for stress-bearing restorations, particularly in Class I and V cavities where fluoride release and biocompatibility are critical.(25)

All groups experienced a decrease in hardness after 7 days, consistent with a previous study (26, 27), which reported a 40-45% decline in microhardness for traditional GICs after thermocycling. Notably, our 1% MCH group retained higher residual hardness (46.95 VHN), mirroring their findings that enhanced GICs resist degradation better than conventional formulations.

4.2 Mechanical Reinforcement

Mechanical improvements in mechanical properties can be attributed to the microscale dispersion and interfacial interactions between MCH and the GIC matrix. MCH particles (200–4000 nm) filled microvoids, similar to the homogenizing effect observed in nano-hydroxyapatite/chitosan composites (28) or microhybrid composite resin, resulting in a denser and more uniform matrix that reduced crack initiation and propagation. Furthermore, the amino and hydroxyl functional groups in chitosan form hydrogen and ionic bonds that interact with polyacrylic acid in GIC, thereby enhancing cross-link density and internal cohesion.(17) Unlike traditional reinforcement strategies, microchitosan targets weaknesses inherent in the acid–base setting mechanism, thereby supporting extended restoration durability.(4) The introduction of microchitosan may help mitigate these limitations by reinforcing the matrix at the molecular level.

The 1% concentration aligns with findings from previous studies, which have shown that 1–1.5% chitosan maximizes mechanical gains in GICs without agglomeration, underscoring the critical role of nanoparticle concentration in dental composites.(25) The good dispersion observed with 1% MCH may be attributed to its colloidal stability, indicated by a high zeta potential (+35 to +45 mV), which helps prevent particle aggregation and ensures uniform dispersion within the GIC matrix.

While zeta potential was not measured in this study, previous research has demonstrated that high surface charge in chitosan-modified glass ionomer cements contributes to improved nanoparticle dispersion, mechanical strength, and fluoride release. (23). Similarly, quaternized chitosan-coated nanoparticles have demonstrated enhanced interfacial bonding and uniform filler distribution, which may contribute to improved flexural strength and resistance to fracture in reinforced GIC systems.(8) These findings support the premise that colloidal stability is a key determinant of functional integration and mechanical performance in nano-enhanced restorative formulations.(3)

Our findings on the increase in Vickers surface hardness with the addition of microchitosan are consistent with previous research, which also reported a positive effect of chitosan on the microhardness of GIC.(10) The significant improvements in surface hardness observed in our study

are consistent with previous findings, which also confirmed that chitosan does not have an adverse effect on the microhardness of GIC. Their research, which compared chitosan to hydroxyapatite, found that the addition of chitosan was particularly effective at reducing surface roughness. (29) This supports our findings that the inclusion of micro-sized chitin derivatives, such as the one from *Xylotrupes gideon*, contributes to a more durable and homogeneous GIC matrix.

Incorporating carboxymethyl chitosan into the liquid phase of glass ionomer cement has been shown to significantly enhance flexural strength, fracture toughness, and compressive strength, particularly at low concentrations.(9) Their findings support the concept that chitosan derivatives can reinforce the GIC matrix through hydrogen bonding and improved interfacial cohesion.

Particle agglomeration is indicated by the decrease in mechanical properties at 2% MCH, as higher nanoparticle concentrations often disrupt dispersion uniformity (17) and concentrations below 0.5% are insufficient to enhance effective reinforcement. Enhanced powder-liquid interaction during setting, driven by MCH's high surface energy, likely contributed to the optimal gelation and final structure.(30)

MCH improves hardness and mechanical durability while preserving essential GIC properties, including adhesion.(31) Resin coatings significantly enhanced the viscoelastic behavior and environmental resistance of highly viscous glass ionomer cements.(32) While their approach focused on external surface protection, our findings suggest that internal reinforcement with chitosan may offer a comparable benefit by enhancing matrix cohesion and long-term mechanical stability without the need for additional coating layers. As stated earlier, shrimp shell-derived chitosan, when combined with hydrophilic fibers, produced a composite exhibiting a compressive strength of 32.67 MPa and a tensile strength of 17.18 MPa.(33) While their tensile values were higher, our 1% MCH-GIC formulation achieved a significantly greater compressive strength (155.16 MPa), highlighting the superior load-bearing potential of microchitosan in GIC matrices. These differences may reflect the influence of matrix type—composite resin vs. GIC—and underscore the versatility of chitosan-based reinforcements across dental materials.

While our study focused on the biomechanical properties of the microchitosan-modified GIC, it is important to note that chitosan offers additional benefits. Previous research has found that the inclusion of chitosan can enhance the shear bond strength of GIC to dentin, a critical factor for long-term clinical success.(34) This suggests that the microchitosan used in our study could provide similar bonding improvements, a topic that warrants further investigation.

4.3 Biofunctional Attributes and Fluoride Release

Biofunctional attributes refer to material properties that support biological compatibility, such as antimicrobial action, remineralization, and tissue integration. Although the present study did not assess antimicrobial effects or surface charge potential, these properties are relevant to the clinical application of nanochitosan-modified materials. Prior studies have reported that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation.(18, 35, 36) Additionally, *Xylotrupes gideon*-derived nanochitosan has demonstrated antibacterial activity and supported sustained fluoride release in restorative formulations.(19, 37) These findings from the literature suggest that biofunctional advantages may support the future clinical translation of nanochitosan-modified GICs.

Notably, previous studies have reported that the addition of nanochitosan (NCH) does not compromise fluoride release, a hallmark of glass ionomer cement (GIC) bioactivity.(16) NCH-

modified GICs have demonstrated enhanced cumulative fluoride release compared to conventional formulations, particularly under acidic conditions.(23) On the contrary, chitosan's hydrogel-like matrix may modulate fluoride diffusion, potentially supporting sustained release through ionic interactions and matrix permeability.(38)

Biocompatibility is another potential advantage of microchitosan (MCH), as supported by previous studies. Chitosan has been shown to promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammatory responses when used in proximity to pulp tissue.(39, 40) While these effects were not evaluated in the present study, they suggest that chitosan may support favorable biological responses. Future studies should investigate whether MCH's mucoadhesive properties(41) enhance marginal sealing *in vivo*.

Together, prior studies suggest that chitosan-modified GICs may offer multifunctional benefits, potentially combining improved mechanical durability with antibacterial effects (8, 18) although such bioactivity was not assessed in the present study.

4.4 Clinical Implications and Future Directions

The dual benefits of mechanical reinforcement and bioactivity make microchitosan-modified GICs a promising solution for various clinical applications. These include stress-bearing posterior restorations, atraumatic restorative treatment (ART), liners and bases in deep cavities, and potentially even as pulp-capping materials. This material addresses several limitations of conventional GICs, including weak bonding, limited regeneration, and poor bacterial resistance, while reinforcing the matrix and supporting fluoride release.(1, 16, 25)

Despite encouraging results, further research is warranted to validate these findings under clinical conditions. Recommended future studies include:

- *In vivo* assessment of pulp and tissue responses.
- Long-term durability testing under occlusal load and thermocycling.
- Evaluation of cytotoxicity and allergic responses in pediatric and medically compromised populations.
- Advanced modeling of fluoride release kinetics under cariogenic challenge.
- Optimization of setting and handling properties for clinical workflow.
- Dynamic immersion or pH cycling to better simulate oral conditions.

Additionally, the use of *Xylotrupes gideon*-derived microchitosan underscores a commitment to environmental sustainability in dental biomaterials. Clinically beneficial and environmentally sustainable, *Xylotrupes gideon*-derived microchitosan reduces reliance on marine sources and supports the development of localized, eco-conscious biomaterials.(1, 16) The eco-functional benefits of insect-derived chitosan include lower allergenic potential, effective integration into glass ionomer matrices, and enhanced fluoride release.(18) These attributes highlight *Xylotrupes gideon*-derived chitosan as a viable alternative for clinicians seeking bioactive and environmentally responsible reinforcement strategies in restorative dentistry. Microchitosan presents a sustainable alternative to conventional GIC modifiers and warrants further *in vivo* validation, biocompatibility testing, and clinical performance studies to support widespread clinical adoption.

5 Conclusion

Incorporating 1% microchitosan (MCH) from *Xylotrupes gideon* into glass ionomer cement (GIC) significantly enhanced its mechanical properties after 7 days of maturation in artificial saliva. This modification resulted in a 35% increase in compressive strength, a 51% improvement in tensile strength, and 46% increase in surface hardness. Although the 2% MCH group initially showed strong values, its performance declined over time, suggesting an upper threshold of reinforcement. This reduced performance was likely due to particle agglomeration, which undermined long-term stability. A 0.5% formulation produced inconclusive results, the optimized 1% formulation supports minimally invasive dentistry by prolonging restoration longevity and preserving tooth structure.

6 Ethics Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of the Faculty of Dentistry, Universitas Trisakti (Approval No: 006/Dosen/KEPK/FKG/06/2021).

7 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8 Author Contributions

AC: Writing – review & editing. CDM: Validation, Writing – original draft. DP: Investigation, Writing – original draft. EE: Supervision, Writing – original draft. FLK: Investigation, Methodology, Writing – original draft. Indrayadi Gunardi: Formal Analysis, Writing – original draft. Komariah Komariah: Resources, Writing – original draft. Rosalina Tjandrawinata: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. Sastra Kusuma Wijaya: Writing – review & editing. Tansza Setiana Putri: Visualization, Writing – original draft.

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Table

Table 1. Games-Howell significant value (*p*) for 24 hours (white) and 7 days (grey) compressive strength, diametral tensile strength and Vickers microhardness.

Compressive strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.223	0.002*	0.032*	7 days
MCH 0.5%	0.707		0.020*	0.473	
MCH 1%	0.671	0.234		0.155	
MCH 2%	0.973	0.476	0.848		
		24 hours			
Diametral Tensile Strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.133	0.014*	0.025*	7 days
MCH 0.5%	0.012*		0.674	0.825	
MCH 1%	0.445	0.118		0.992	
MCH 2%	0.900	0.000*	0.040*		
		24 hours			
Vickers Microhardness	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		1.000	0.000*	0.341	7 days
MCH 0.5%	0.000*		0.038*	0.913	
MCH 1%	0.000*	0.719		0.003*	
MCH 2%	0.000*	0.882	0.999		
		24 hours			

* Significant difference between groups with $p < 0,05$

Figure captions

Figure 1. Scanning electron microscope images of microchitosan from *Xylotrupes gideon*. A. 10,000× magnification. B. 2,000× magnification

Figure 2. Experimental workflow illustrating the synthesis of microchitosan from *Xylotrupes gideon* and its incorporation into glass ionomer cement for mechanical testing.

Figure 3. Compressive strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (**) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$).

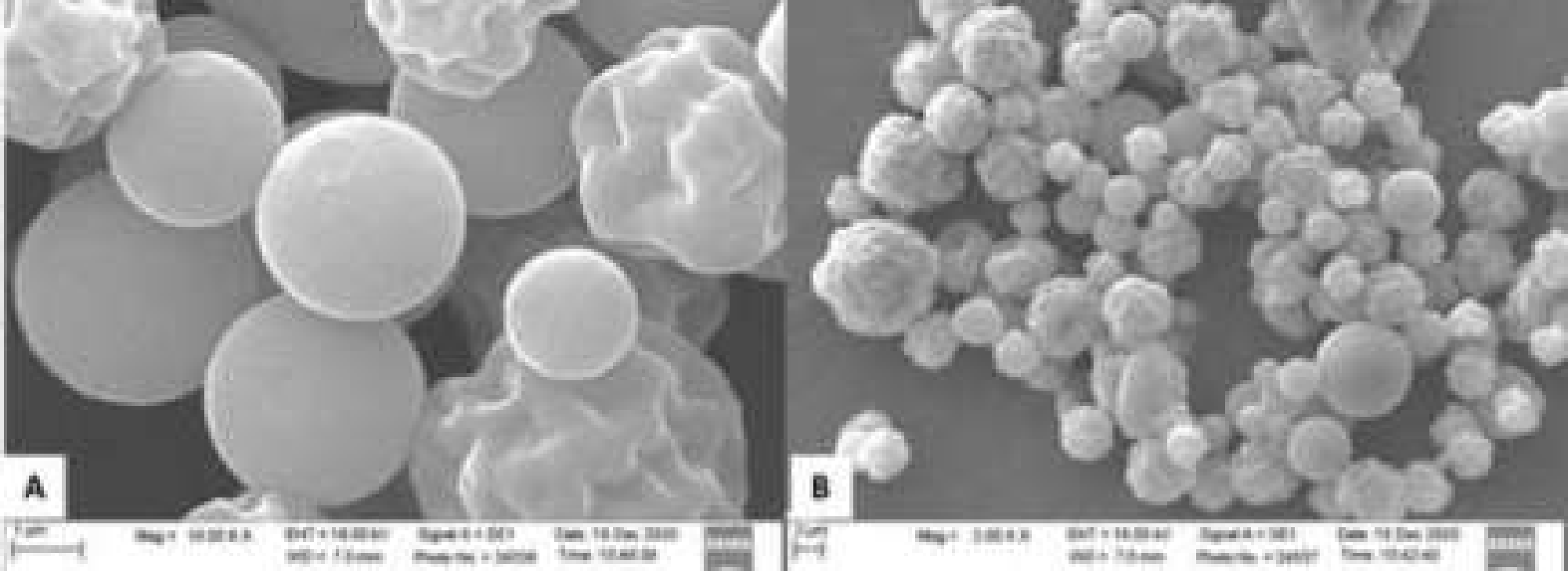
Figure 4. Diametral tensile strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (*,)**

show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$).

Figure 5. Vickers surface hardness (VHN) of microchitosan-modified glass ionomer cement (GIC) formulations following 24-hour and 7-day immersion in artificial saliva. Asterisks (*,) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$). The data clearly shows that the 1% microchitosan group exhibited the highest hardness values at both time points.**

Data Availability Statement

Data sharing may be shared on reasonable request to the corresponding author.


A
B

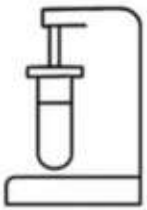
Exoskeleton drying

Demineralization
Deproteinization
Deacetylation

Chitosan

Ionic Gelation

Microchitosan
(MCH)



Samples of Modified
Glass Ionomer Cement

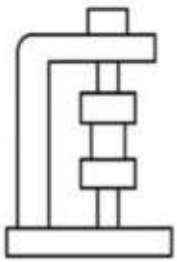
Control

0.5% (w/w) MCH

1 % (w/w) MCH

2 % (w/w) MCH

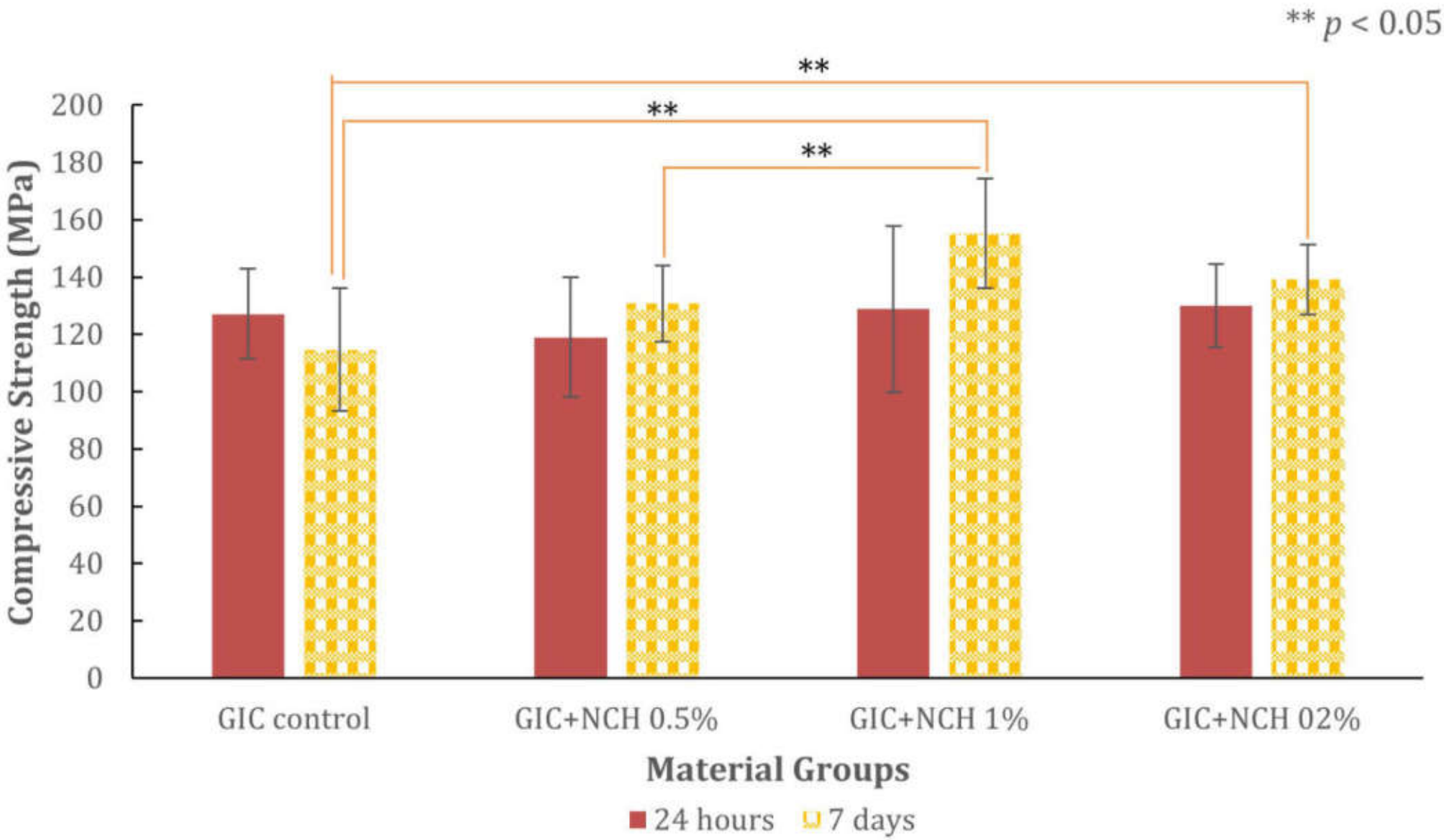
Incubation in Fusayama
Meyer Artificial Saliva
24 h and 7 d

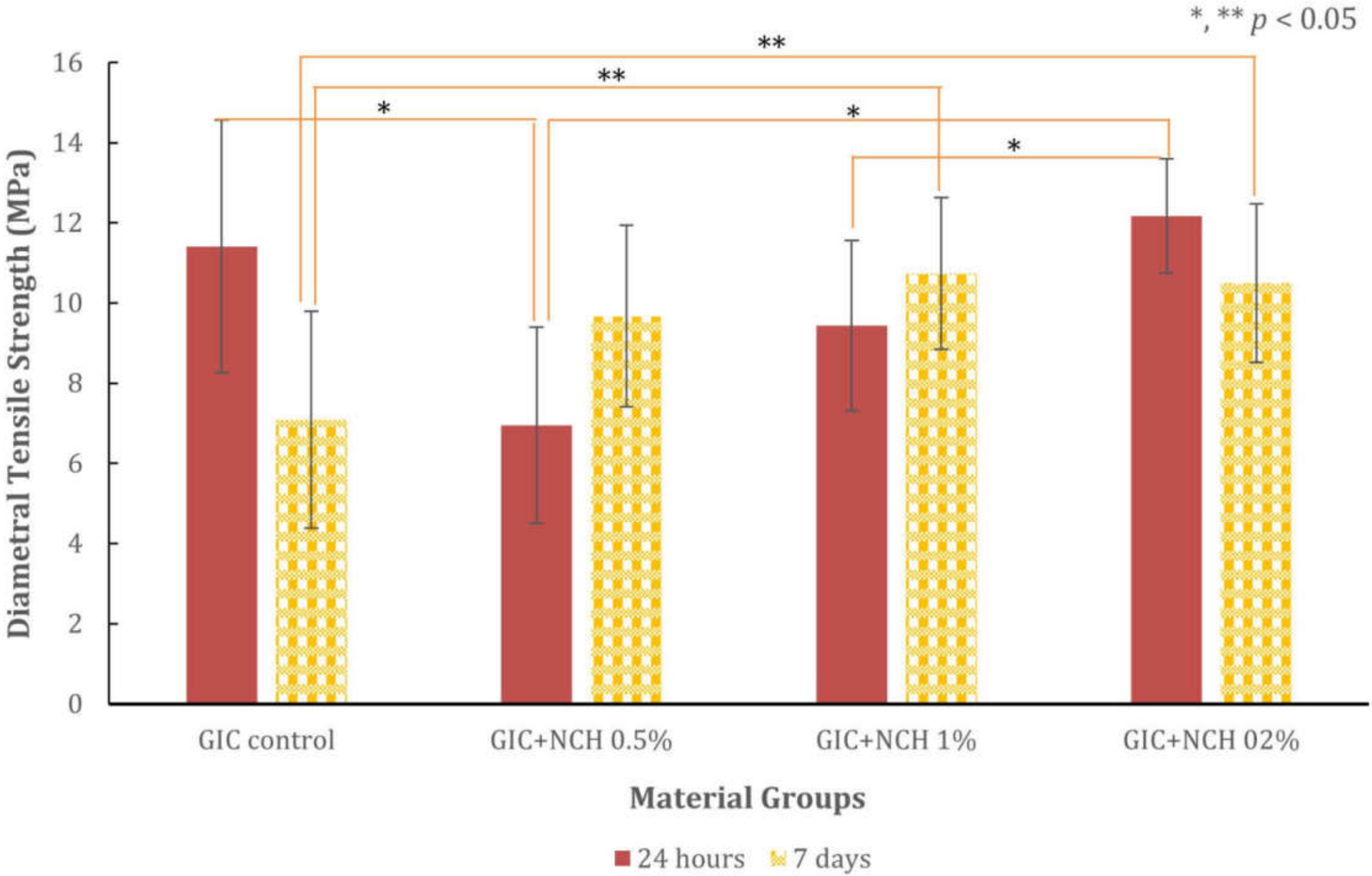


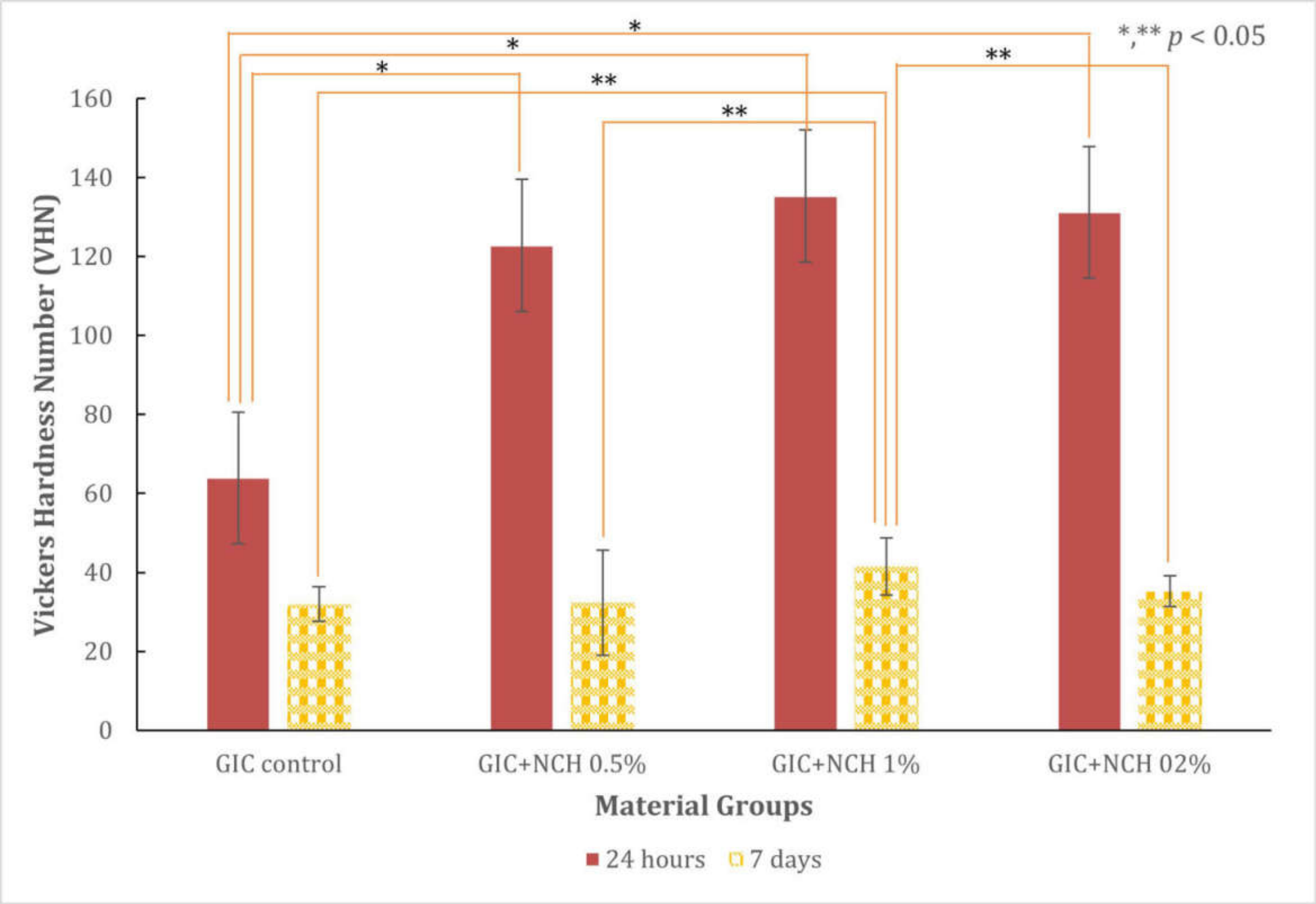
Compressive strength
Ø 3mm, t 6mm

Diametral tensile
strength
Ø 6 mm, t 4 mm

Vickers Micro Hardness
Ø 6 mm, t 4 mm


** $p < 0.05$
**
**
**
Compressive Strength (MPa)
200
180
160
140
120
100
80
60
40
20
0
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days


*, ** p < 0.05
**
**
*
*
*
16
14
12
10
8
6
4
2
0
Diametral Tensile Strength (MPa)
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days


*,** p < 0.05
*
*
*
**
**
**
160
140
120
100
80
60
40
20
0
Vickers Hardness Number (VHN)
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days

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Xylotrupes Gideon Microchitosan- Modified Glass Ionomer Cement: *In Vitro* Assessment of Biomechanical Properties

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Keywords: biomechanical[1], compressive strength[2], diametral tensile strength[3], glass ionomer cement[4], hardness[5], microchitosan[6], *Xylotrupes Gideon*[7]

Abstract

Background: Glass ionomer cements (GIC) are valued as inherent fluoride-releasing dental restorative materials, while chitosan binds to negatively charged enamel surfaces, promoting mineral deposition and strengthening teeth. This study aimed to evaluate the biomechanical properties of glass ionomer cement (GIC) modified with microchitosan derived from *Xylotrupes gideon*, using an *in vitro* experimental design.

Materials and Methods: Microchitosan was extracted from *Xylotrupes gideon* and incorporated into conventional GIC at 0.5%, 1% and 2% (w/w). Compressive strength, diametral tensile strength, and surface microhardness were measured using standard testing equipment after immersion in artificial saliva for 24 hours and 7 days. Statistical analysis was performed using one-way ANOVA followed by the Games-Howell post hoc test, with significance set at $p < 0.05$.

Results: The 1% microchitosan-modified GIC exhibited the most significant improvements compared to the unmodified control. After 7 days, compressive strength increased by 35.4%, diametral tensile strength by 51.3%, and surface hardness by 46.6% ($p<0.05$). These enhancements are attributed to microscale reinforcement and chemical bonding between microchitosan and the GIC matrix.

Conclusion: The addition of 1% microchitosan derived from *Xylotrupes gideon* significantly improved the biomechanical performance of GIC. This bioactive reinforcement shows promising

potential for clinical restorative applications, though further investigation into its long-term biocompatibility and fluoride release is warranted.

1 Introduction

Dental composite materials, including glass ionomer cements (GICs), are widely recognized in restorative dentistry for their versatility, biocompatibility, and ability to bond chemically to dental hard tissues. GICs, in particular, are valued for their inherent fluoride release, which aids in the prevention of tooth decay and supports long-term tooth preservation.(1, 2) These advantages make GICs ideal for pediatric dentistry and minimally invasive restorations where fluoride release and adhesion are critical; however, their adhesive performance varies significantly across formulations.

A major drawback of traditional GICs is their weak mechanical properties, including low compressive and tensile strength, poor wear resistance, and susceptibility to fracture under occlusal stress. These limitations preclude their use in stress-bearing restorations, particularly in posterior regions that are subjected to high occlusal loads.(3) The other limitations are primarily attributed to the intrinsic chemistry of the GIC matrix, particularly the brittle nature of the glass phase and the sensitivity of the acid-base setting reaction to moisture and dehydration.(4) Recent technology developments, particularly involving micro-sized chitosan and titanium dioxide, have demonstrated improved filler dispersion and particle–matrix bonding in glass ionomer cements, resulting in enhanced mechanical properties, matrix cohesion, fracture resistance, and bioactivity (5, 6) with chitosan enhancing particle-matrix bonding and decreasing microvoid formation.(7)

Such reinforcement strategies yield structurally robust cement matrices characterized by minimized porosity and enhanced resistance to fracture propagation. Studies have shown that the incorporation of chitosan significantly enhances compressive and flexural strength, consistent with previous findings on quaternized chitosan-coated nanoparticles and carboxymethyl chitosan derivatives (8, 9) thereby improving matrix homogeneity and resistance to fracture propagation.

A previous study showed that the addition of chitosan to GIC has a positive effect on microhardness and improves surface roughness, making it a promising additive for dental restorative materials.(10) This material reinforces the structural framework and enhances mechanical reliability, which in turn leads to improved fracture resistance, increased microhardness, and enhanced overall durability.(11) The reinforcement effect demonstrated temporal dependence, with mechanical improvements more evident following extended maturation periods. Its ability to improve both the mechanical and biological properties of restorative materials makes it a compelling additive in dental biomaterials research. The molecular features of chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose], a versatile biopolymer, are chemically inert, non-toxic, and possess robust and also broad antimicrobial activities due to its polycationic nature.(12) Characterized by amino and hydroxyl functional groups, chitosan enables ionic and hydrogen bonding that strengthens adhesion to dental substrates, improves mechanical strength through particle–matrix cohesion, and supports tissue compatibility via biocompatibility and regenerative potential (13) across a wide range of dental applications.

Chitosan exhibits remarkable physicochemical properties and good biocompatibility, forming bonds with human proteins, cells, and organs.(14) Chitosan exhibits multifunctionality in dental applications by contributing to mechanical reinforcement, promoting tissue regeneration, and acting as a bioactive agent in restorative formulations such as glass ionomer cements and adhesives. Recently, alternative sources of chitosan have emerged, including the exoskeletons of the *Xylotrupes gideon* beetle, which offer a sustainable and locally available biomaterial. Previous research indicates

that this chitosan, derived from insects, enhances GIC performance in acidic oral environments (with critical pH levels), showing promise for clinical applications.(15) These advantages support further investigation of its mechanical reinforcement potential in dental composites. Research indicates that this novel form of chitosan demonstrates superior mechanical enhancement capabilities when integrated into GICs, including increased compressive strength and Vickers hardness.(16) Additionally, the microstructure improves particle dispersion and matrix interaction, which are critical for achieving homogeneity and minimizing microvoid formation during the setting process.(7, 17)

In addition to mechanical benefits, chitosan may confer secondary biofunctional advantages, which warrant future exploration but were not addressed in the present study.(18) These properties may enhance the clinical performance of restorations by reducing bacterial colonization, improving marginal integrity, and supporting the healing of the pulp-dentin interface, in line with recent advancements in oral health technology.(17) Notably, such modifications do not appear to compromise GIC's core biofunctional properties, such as fluoride ion release and adhesion to dentin.(1, 11)

Therefore, this study aims to investigate the effect of *Xylotrupes gideon*-derived microchitosan incorporation on the mechanical properties of conventional GIC, including compressive strength, diametral tensile strength, and surface hardness, while also addressing its potential clinical feasibility, long-lasting nature, and biological functionality through the integration of eco-friendly materials in restorative dentistry.(7)

2 Materials and Methods

2.1 Synthesis and Characterization of Microchitosan from Xylotrupes gideon

Microchitosan (MCH) was synthesized from the exoskeletons of *Xylotrupes gideon* beetles collected in Bogor, West Java, Indonesia. All body parts were detached and dried for five days, followed by extraction using established chitin processing protocols—specifically, demineralization with 3N HCl, deproteinization with 3N NaOH, and deacetylation with 50% NaOH at 100 °C to obtain chitosan.

The resulting chitosan was converted into micro form via ionic gelation using sodium tripolyphosphate (TPP) in deionized water, stirred at 2500 rpm for four hours, intentionally omitting acetic acid to improve biocompatibility. A particle size analyzer (Horiba, Japan) revealed a spherical morphology with a size distribution ranging from 200 to 4000 nm, while SEM analysis also showed the form of chitosan particles as in Figure 1. The synthesized MCH was stored at 4 °C in airtight containers until use. (19)

The overall experimental workflow is illustrated in Figure 2, highlighting the synthesis of microchitosan from *Xylotrupes gideon* and its integration into glass ionomer cement for mechanical testing.

2.2 Preparation of Modified Glass Ionomer Cement

A commercially available Glass ionomer cement (Fuji IX Gold Label HS Posterior, GC, Tokyo, Japan) was used as the base material. The control group used unmodified GIC liquid, while the experimental groups consisted of 0.5%, 1%, and 2% (w/w) MCH added to the liquid component. The powder and respective liquid in each mixture were manually combined according to the

manufacturer's guidelines, with prior vortex mixing of the modified liquid to improve homogeneity, particularly at 2% concentration to achieve uniformity.(1, 20)

Compressive strength specimens were molded according to ISO 9917-1:2007: 3 mm diameter × 6 mm height, while the samples for diametrical tensile strength: 6 mm diameter × 4 mm thickness, and Vickers hardness: 6 mm diameter × 4 mm thickness. Specimen dimensions were confirmed using a digital caliper (Mitutoyo 500-196-30, Japan). After initial setting, all specimens were stored in 100% humidity at room temperature for 1 hour.

2.3 Conditioning and Storage

A total of 80 samples were prepared for each mechanical test and divided into four groups (n = 20 each), with each group further subdivided into two subgroups (n = 10) based on immersion time (24 hours and 7 days). A Fusayama-Meyer artificial saliva solution was prepared containing 0.4 g/L of NaCl, 0.4 g/L of KCl, 0.906 g/L of $CaCl_2 \cdot 2H_2O$, 0.690 g/L of $Na_2HPO_4 \cdot 2H_2O$, 0.005 g/L of $Na_2S \cdot 9H_2O$, and a pH of 6.75. Samples were placed in 10 mL of artificial saliva per specimen in sealed containers, then incubated at 37°C.

2.4 Mechanical Testing

Mechanical properties were evaluated using a universal testing machine (Shimadzu, Japan) equipped with a 5 kN load cell, following validated protocols.(21) Compressive and diametral tensile strength tests were conducted at a crosshead speed of 1 mm/min. Surface hardness was measured using a Shimadzu HMV-G microhardness tester (Kyoto, Japan), employing a micro Vickers diamond indenter with a 100 g load applied for 10 seconds (22) All mechanical testing adhered to ISO 9917-1:2007 guidelines and methodological frameworks.

2.5 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean and standard deviation, were calculated. Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was evaluated using Levene's test. When the assumption of homogeneity was violated, one-way ANOVA was followed by the Games–Howell post hoc test for pairwise comparisons.

Pearson correlation coefficients were calculated to examine relationships among mechanical parameters. A significant level of $p<0.05$ was applied throughout.

3 Results

The compressive, diametral tensile strength, and hardness test results for all test groups are presented in Figure 3 – 5, while Table 1 represents the significance value of the comparison between groups. The data from 240 specimens (with 20 specimens per group and 10 at different time points) were found to have a normal distribution, as confirmed by the Kolmogorov–Smirnov test ($p = 0.200$). The addition of microchitosan (MCH) at all concentrations resulted in statistically significant increases in both compressive and diametral tensile strength compared to the control group ($p < 0.05$).

3.1 Compressive Strength

The compressive strength results are presented in Figure 3. At 24 hours, the control GIC group recorded a mean strength of 127.14 ± 15.82 MPa. In comparison with the control group, the 0.5%

MCH group showed a slight reduction, while the 2% MCH group produced the highest value (130.01 ± 14.40 MPa, $p = 0.973$). The 1% MCH group was comparable to the control.

After 7 days of immersion, the control group decreased to 114.63 ± 21.39 MPa. In comparison with the control, all MCH groups improved. The highest value was observed in the 1% MCH, which peaked at 155.16 ± 19.08 MPa ($p = 0.002$). The 1% MCH group demonstrated the highest strength gain over time (35.4% increase).

3.2 Diametral Tensile Strength

The diametral tensile strength results are presented in Figure 4. The control at 24 hours showed a strength of 11.41 ± 3.16 MPa. Compared with the control, the 0.5% MCH group showed significantly lower strength, whereas 1% MCH and 2% MCH did not differ significantly from the control.

After 7 days, the control group decreased to 7.09 ± 2.70 MPa, while improvements were observed in all MCH groups, particularly in comparison with the control. The 1% MCH group showed the highest value at 10.73 ± 1.89 MPa ($p = 0.014$), which was 51% higher than the 7-day control.

3.3 Vickers Hardness

The Vickers microhardness test results at 24 hours are given in Figure 5. The Control GIC had a microhardness mean value of 63.92 ± 15.17 VHN. The MCH-modified groups demonstrated significantly increased hardness, with a peak at 1% MCH, which had a value of 135.30 ± 17.90 VHN, with a p-value of less than 0.001 for all.

All groups experienced a decrease in hardness after 7 days, while the 1% MCH maintained the highest residual value (46.95 ± 7.32 VHN, $p < 0.001$).

3.4 Trends and Correlation Analysis

The inclusion of microchitosan showed a consistent increase in mechanical properties, most notably at a concentration of 1%. While the 2% group showed strong initial values, its performance declined slightly over time, suggesting an upper threshold of reinforcement.

Pearson correlation analysis revealed strong positive correlations between microchitosan concentration and compressive strength ($r = 0.59$, $p = 0.002$), diametral tensile strength ($r = 0.57$, $p = 0.004$), and hardness ($r = 0.53$, $p = 0.011$). Seven-day specimens of compressive strength and diametral tensile strength showed a positive correlation ($r = 0.71$, $p = 0.001$). Additionally, compressive strength and hardness were positively correlated ($r = 0.64$, $p = 0.008$), indicating an interconnected strengthening effect.

4 Discussion

4.1 Summary of Findings

This study demonstrated that incorporating *Xylotrupes gideon*-derived microchitosan (MCH) into conventional GIC significantly improved its mechanical properties. The 1% MCH concentration produced the best results across all measurements, including compressive strength, diametral tensile strength, and surface hardness, particularly after 7 days of exposure to artificial saliva. The reinforcement effect appeared to be time-dependent, with greater effects noted after a more extended period of maturation.(23) The 0.5% MCH group showed modest effects, while the 2% MCH group,

despite initial enhancements, was assumed to be more prone to agglomeration, which undermined its long-term stability.(6) The results align with previous research findings that validate chitosan's ability to enhance GIC strength and durability, supporting the broader trend of dental composites reinforced with nanomaterials.(16)

The superior performance of 1% microchitosan (MCH) aligns with previous findings that nanochitosan outperforms other nanostructures (e.g., nano-silica, nano-zirconia) in glass ionomer cements (GICs), primarily due to its ability to form hydrogen bonds with the polyacrylic acid matrix, enhancing matrix cohesion and ion exchange. (1) Our results confirm the significant improvements in surface hardness and compressive strength compared to both unmodified controls and GICs modified with alternative nano-additives.(24) This dual enhancement—mechanical performance and biofunctionality—positions small particle chitosan as a promising candidate for stress-bearing restorations, particularly in Class I and V cavities where fluoride release and biocompatibility are critical.(25)

All groups experienced a decrease in hardness after 7 days, consistent with a previous study (26, 27), which reported a 40-45% decline in microhardness for traditional GICs after thermocycling. Notably, our 1% MCH group retained higher residual hardness (46.95 VHN), mirroring their findings that enhanced GICs resist degradation better than conventional formulations.

4.2 Mechanical Reinforcement

Mechanical improvements in mechanical properties can be attributed to the microscale dispersion and interfacial interactions between MCH and the GIC matrix. MCH particles (200–4000 nm) filled microvoids, similar to the homogenizing effect observed in nano-hydroxyapatite/chitosan composites (28) or microhybrid composite resin, resulting in a denser and more uniform matrix that reduced crack initiation and propagation. Furthermore, the amino and hydroxyl functional groups in chitosan form hydrogen and ionic bonds that interact with polyacrylic acid in GIC, thereby enhancing cross-link density and internal cohesion.(17) Unlike traditional reinforcement strategies, microchitosan targets weaknesses inherent in the acid–base setting mechanism, thereby supporting extended restoration durability.(4) The introduction of microchitosan may help mitigate these limitations by reinforcing the matrix at the molecular level.

The 1% concentration aligns with findings from previous studies, which have shown that 1–1.5% chitosan maximizes mechanical gains in GICs without agglomeration, underscoring the critical role of nanoparticle concentration in dental composites.(25) The good dispersion observed with 1% MCH may be attributed to its colloidal stability, indicated by a high zeta potential (+35 to +45 mV), which helps prevent particle aggregation and ensures uniform dispersion within the GIC matrix.

While zeta potential was not measured in this study, previous research has demonstrated that high surface charge in chitosan-modified glass ionomer cements contributes to improved nanoparticle dispersion, mechanical strength, and fluoride release. (23). Similarly, quaternized chitosan-coated nanoparticles have demonstrated enhanced interfacial bonding and uniform filler distribution, which may contribute to improved flexural strength and resistance to fracture in reinforced GIC systems.(8) These findings support the premise that colloidal stability is a key determinant of functional integration and mechanical performance in nano-enhanced restorative formulations.(3)

Our findings on the increase in Vickers surface hardness with the addition of microchitosan are consistent with previous research, which also reported a positive effect of chitosan on the microhardness of GIC.(10) The significant improvements in surface hardness observed in our study

are consistent with previous findings, which also confirmed that chitosan does not have an adverse effect on the microhardness of GIC. Their research, which compared chitosan to hydroxyapatite, found that the addition of chitosan was particularly effective at reducing surface roughness. (29) This supports our findings that the inclusion of micro-sized chitin derivatives, such as the one from *Xylotrupes gideon*, contributes to a more durable and homogeneous GIC matrix.

Incorporating carboxymethyl chitosan into the liquid phase of glass ionomer cement has been shown to significantly enhance flexural strength, fracture toughness, and compressive strength, particularly at low concentrations.(9) Their findings support the concept that chitosan derivatives can reinforce the GIC matrix through hydrogen bonding and improved interfacial cohesion.

Particle agglomeration is indicated by the decrease in mechanical properties at 2% MCH, as higher nanoparticle concentrations often disrupt dispersion uniformity (17) and concentrations below 0.5% are insufficient to enhance effective reinforcement. Enhanced powder-liquid interaction during setting, driven by MCH's high surface energy, likely contributed to the optimal gelation and final structure.(30)

MCH improves hardness and mechanical durability while preserving essential GIC properties, including adhesion.(31) Resin coatings significantly enhanced the viscoelastic behavior and environmental resistance of highly viscous glass ionomer cements.(32) While their approach focused on external surface protection, our findings suggest that internal reinforcement with chitosan may offer a comparable benefit by enhancing matrix cohesion and long-term mechanical stability without the need for additional coating layers. As stated earlier, shrimp shell-derived chitosan, when combined with hydrophilic fibers, produced a composite exhibiting a compressive strength of 32.67 MPa and a tensile strength of 17.18 MPa.(33) While their tensile values were higher, our 1% MCH-GIC formulation achieved a significantly greater compressive strength (155.16 MPa), highlighting the superior load-bearing potential of microchitosan in GIC matrices. These differences may reflect the influence of matrix type—composite resin vs. GIC—and underscore the versatility of chitosan-based reinforcements across dental materials.

While our study focused on the biomechanical properties of the microchitosan-modified GIC, it is important to note that chitosan offers additional benefits. Previous research has found that the inclusion of chitosan can enhance the shear bond strength of GIC to dentin, a critical factor for long-term clinical success.(34) This suggests that the microchitosan used in our study could provide similar bonding improvements, a topic that warrants further investigation.

4.3 Biofunctional Attributes and Fluoride Release

Biofunctional attributes refer to material properties that support biological compatibility, such as antimicrobial action, remineralization, and tissue integration. Although the present study did not assess antimicrobial effects or surface charge potential, these properties are relevant to the clinical application of nanochitosan-modified materials. Prior studies have reported that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation.(18, 35, 36) Additionally, *Xylotrupes gideon*-derived nanochitosan has demonstrated antibacterial activity and supported sustained fluoride release in restorative formulations.(19, 37) These findings from the literature suggest that biofunctional advantages may support the future clinical translation of nanochitosan-modified GICs.

Notably, previous studies have reported that the addition of nanochitosan (NCH) does not compromise fluoride release, a hallmark of glass ionomer cement (GIC) bioactivity.(16) NCH-

modified GICs have demonstrated enhanced cumulative fluoride release compared to conventional formulations, particularly under acidic conditions.(23) On the contrary, chitosan's hydrogel-like matrix may modulate fluoride diffusion, potentially supporting sustained release through ionic interactions and matrix permeability.(38)

Biocompatibility is another potential advantage of microchitosan (MCH), as supported by previous studies. Chitosan has been shown to promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammatory responses when used in proximity to pulp tissue.(39, 40) While these effects were not evaluated in the present study, they suggest that chitosan may support favorable biological responses. Future studies should investigate whether MCH's mucoadhesive properties(41) enhance marginal sealing *in vivo*.

Together, prior studies suggest that chitosan-modified GICs may offer multifunctional benefits, potentially combining improved mechanical durability with antibacterial effects (8, 18) although such bioactivity was not assessed in the present study.

4.4 Clinical Implications and Future Directions

The dual benefits of mechanical reinforcement and bioactivity make microchitosan-modified GICs a promising solution for various clinical applications. These include stress-bearing posterior restorations, atraumatic restorative treatment (ART), liners and bases in deep cavities, and potentially even as pulp-capping materials. This material addresses several limitations of conventional GICs, including weak bonding, limited regeneration, and poor bacterial resistance, while reinforcing the matrix and supporting fluoride release.(1, 16, 25)

Despite encouraging results, further research is warranted to validate these findings under clinical conditions. Recommended future studies include:

- *In vivo* assessment of pulp and tissue responses.
- Long-term durability testing under occlusal load and thermocycling.
- Evaluation of cytotoxicity and allergic responses in pediatric and medically compromised populations.
- Advanced modeling of fluoride release kinetics under cariogenic challenge.
- Optimization of setting and handling properties for clinical workflow.
- Dynamic immersion or pH cycling to better simulate oral conditions.

Additionally, the use of *Xylotrupes gideon*-derived microchitosan underscores a commitment to environmental sustainability in dental biomaterials. Clinically beneficial and environmentally sustainable, *Xylotrupes gideon*-derived microchitosan reduces reliance on marine sources and supports the development of localized, eco-conscious biomaterials.(1, 16) The eco-functional benefits of insect-derived chitosan include lower allergenic potential, effective integration into glass ionomer matrices, and enhanced fluoride release.(18) These attributes highlight *Xylotrupes gideon*-derived chitosan as a viable alternative for clinicians seeking bioactive and environmentally responsible reinforcement strategies in restorative dentistry. Microchitosan presents a sustainable alternative to conventional GIC modifiers and warrants further *in vivo* validation, biocompatibility testing, and clinical performance studies to support widespread clinical adoption.

5 Conclusion

Incorporating 1% microchitosan (MCH) from *Xylotrupes gideon* into glass ionomer cement (GIC) significantly enhanced its mechanical properties after 7 days of maturation in artificial saliva. This modification resulted in a 35% increase in compressive strength, a 51% improvement in tensile strength, and 46% increase in surface hardness. Although the 2% MCH group initially showed strong values, its performance declined over time, suggesting an upper threshold of reinforcement. This reduced performance was likely due to particle agglomeration, which undermined long-term stability. A 0.5% formulation produced inconclusive results, the optimized 1% formulation supports minimally invasive dentistry by prolonging restoration longevity and preserving tooth structure.

6 Ethics Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of the Faculty of Dentistry, Universitas Trisakti (Approval No: 006/Dosen/KEPK/FKG/06/2021).

7 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

8 Author Contributions

AC: Writing – review & editing. CDM: Validation, Writing – original draft. DP: Investigation, Writing – original draft. EE: Supervision, Writing – original draft. FLK: Investigation, Methodology, Writing – original draft. Indrayadi Gunardi: Formal Analysis, Writing – original draft. Komariah Komariah: Resources, Writing – original draft. Rosalina Tjandrawinata: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. Sastra Kusuma Wijaya: Writing – review & editing. Tansza Setiana Putri: Visualization, Writing – original draft.

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Table

Table 1. Games-Howell significant value (*p*) for 24 hours (white) and 7 days (grey) compressive strength, diametral tensile strength and Vickers microhardness.

Compressive strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.223	0.002*	0.032*	7 days
MCH 0.5%	0.707		0.020*	0.473	
MCH 1%	0.671	0.234		0.155	
MCH 2%	0.973	0.476	0.848		
		24 hours			
Diametral Tensile Strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.133	0.014*	0.025*	7 days
MCH 0.5%	0.012*		0.674	0.825	
MCH 1%	0.445	0.118		0.992	
MCH 2%	0.900	0.000*	0.040*		
		24 hours			
Vickers Microhardness	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		1.000	0.000*	0.341	7 days
MCH 0.5%	0.000*		0.038*	0.913	
MCH 1%	0.000*	0.719		0.003*	
MCH 2%	0.000*	0.882	0.999		
		24 hours			

* Significant difference between groups with $p < 0,05$

Figure captions

Figure 1. Scanning electron microscope images of microchitosan from *Xylotrupes gideon*. A. 10,000× magnification. B. 2,000× magnification

Figure 2. Experimental workflow illustrating the synthesis of microchitosan from *Xylotrupes gideon* and its incorporation into glass ionomer cement for mechanical testing.

Figure 3. Compressive strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (**) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$).

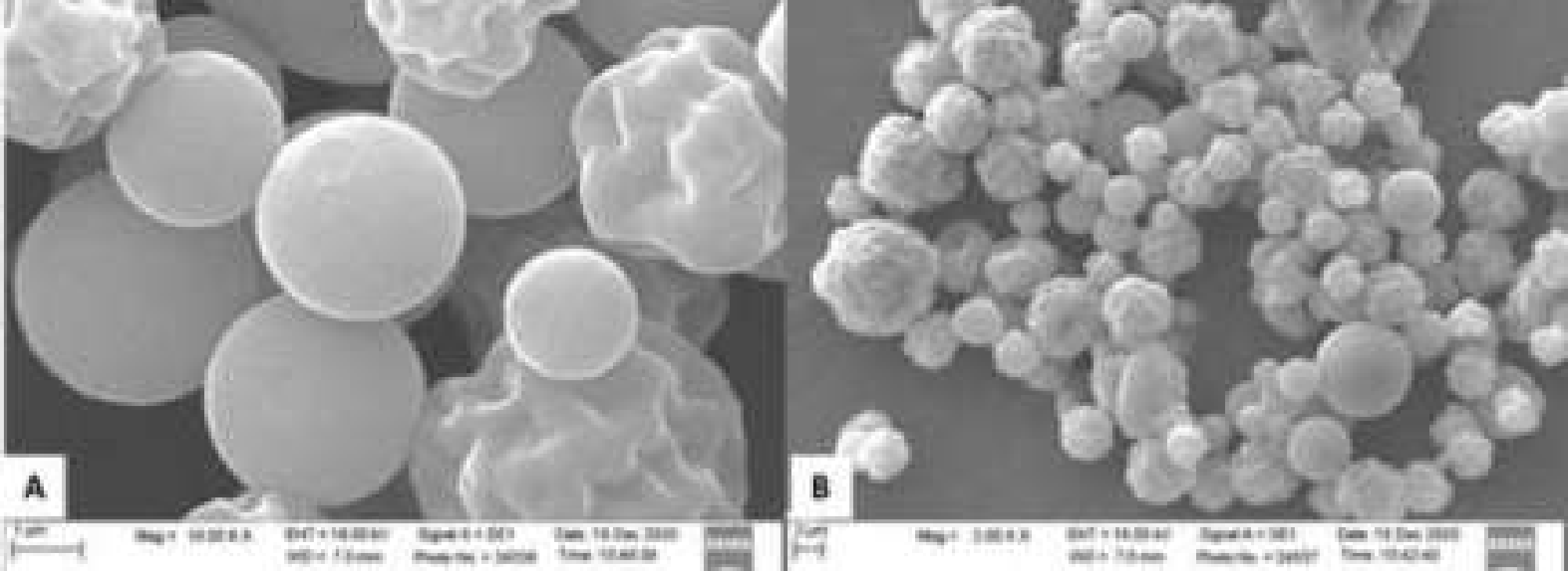
Figure 4. Diametral tensile strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (*,)**

show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$).

Figure 5. Vickers surface hardness (VHN) of microchitosan-modified glass ionomer cement (GIC) formulations following 24-hour and 7-day immersion in artificial saliva. Asterisks (*,) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$). The data clearly shows that the 1% microchitosan group exhibited the highest hardness values at both time points.**

Data Availability Statement

Data sharing may be shared on reasonable request to the corresponding author.


A
B

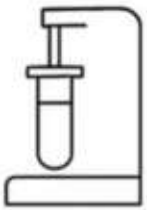
Exoskeleton drying

Demineralization
Deproteinization
Deacetylation

Chitosan

Ionic Gelation

Microchitosan
(MCH)



Samples of Modified
Glass Ionomer Cement

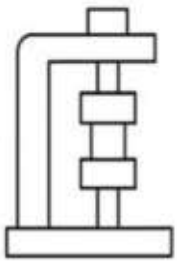
Control

0.5% (w/w) MCH

1 % (w/w) MCH

2 % (w/w) MCH

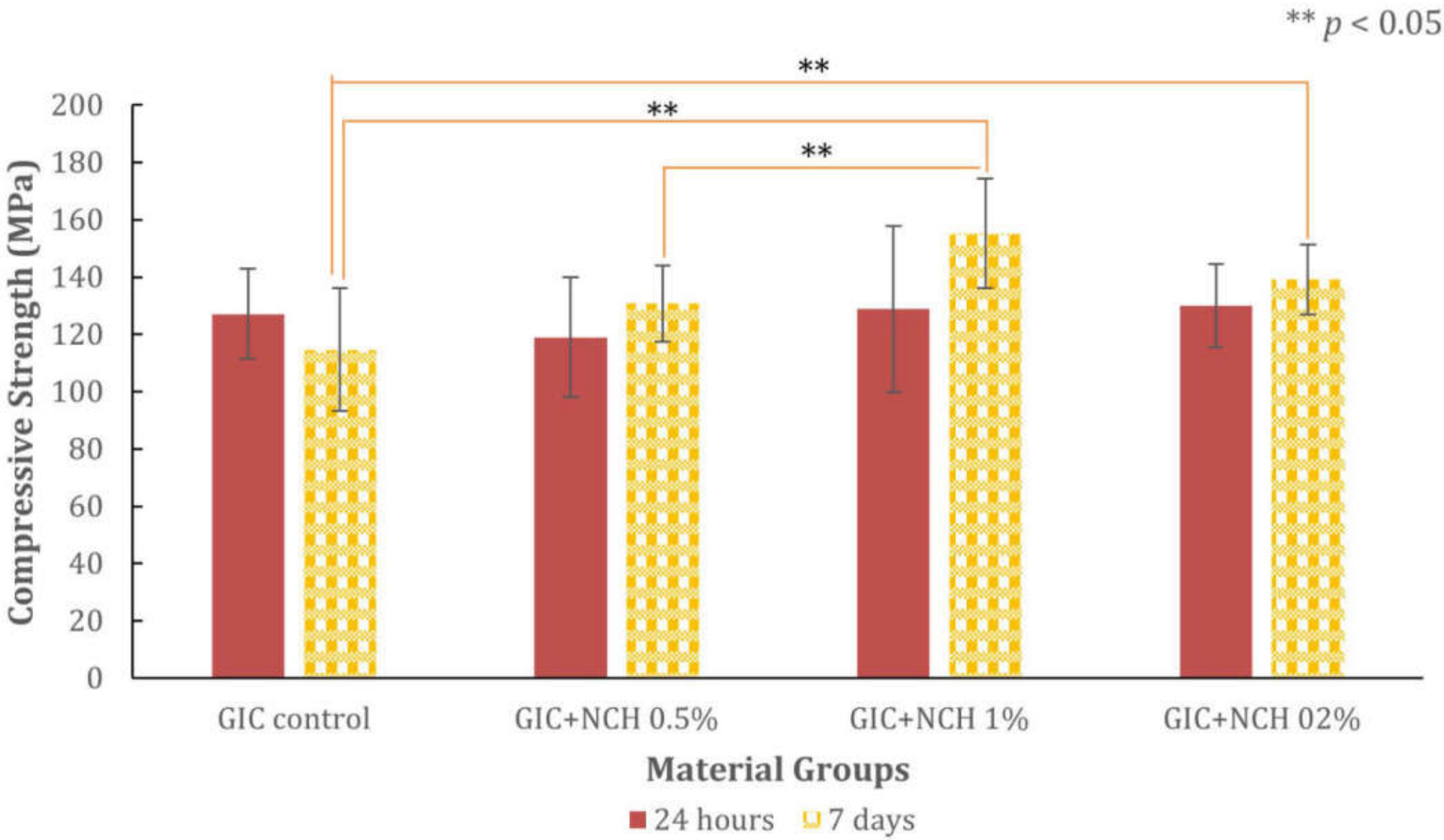
Incubation in Fusayama
Meyer Artificial Saliva
24 h and 7 d

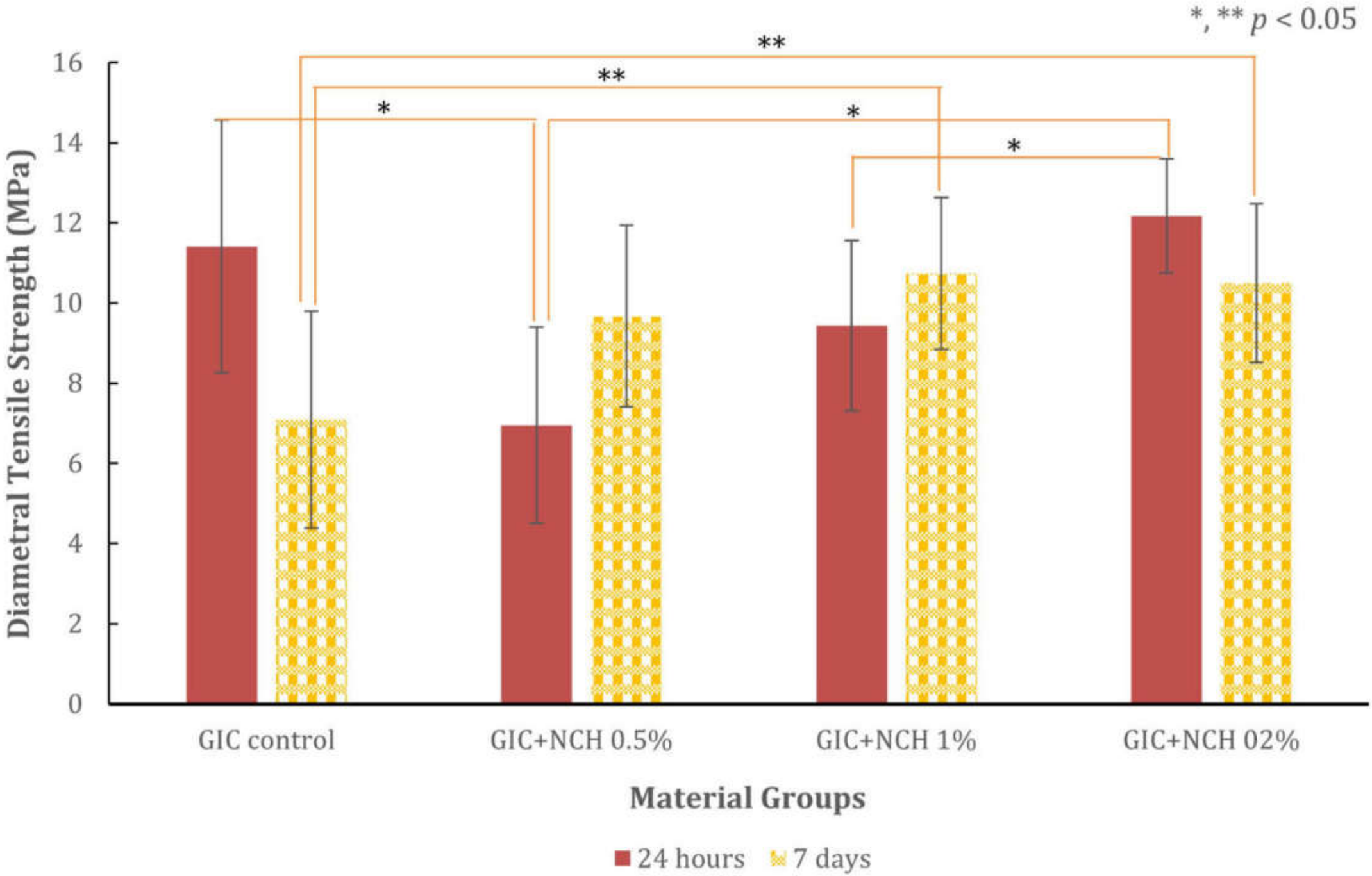


Compressive strength
Ø 3mm, t 6mm

Diametral tensile
strength
Ø 6 mm, t 4 mm

Vickers Micro Hardness
Ø 6 mm, t 4 mm


** $p < 0.05$
**
**
**
Compressive Strength (MPa)
200
180
160
140
120
100
80
60
40
20
0
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days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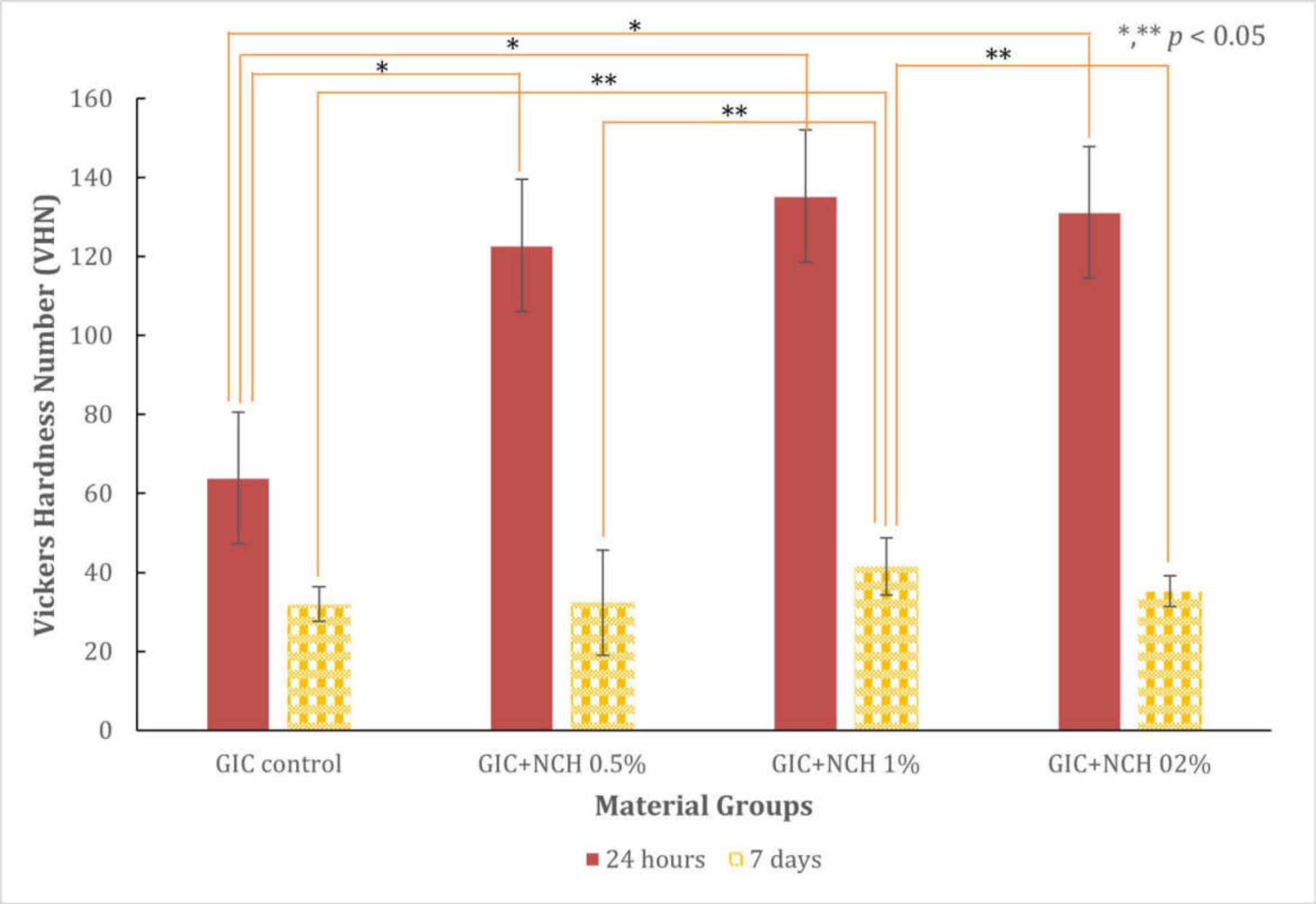
Diametral Tensile Strength (MPa)
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days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160
140
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Vickers Hardness Number (VHN)
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days

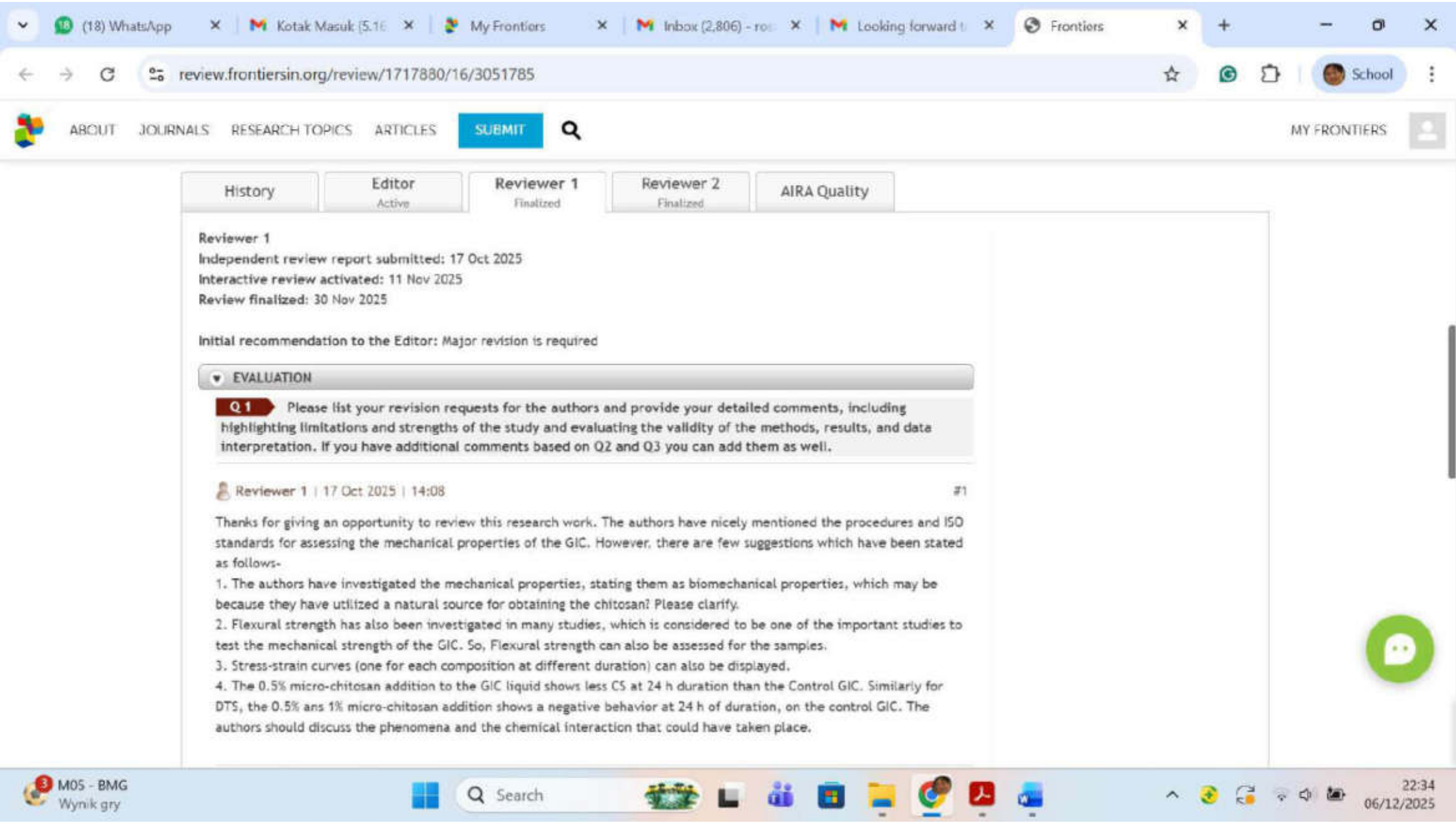
3. Bukti Review

Reviewer 1 (17 Oktober 2025)

Reviewer 2 (8 November 2025)

Jawaban atas review (21 November 2025)

Reviewer 1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AIRA Quality
Reviewer 1
Independent review report submitted: 17 Oct 2025
Interactive review activated: 11 Nov 2025
Review finalized: 30 Nov 2025
Initial recommendation to the Editor: Major revision is required
EVALUATION
Q 1
Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.
Reviewer 1 | 17 Oct 2025 | 14:08
#1
Thanks for giving an opportunity to review this research work. The authors have nicely mentioned the procedures and ISO standards for assessing the mechanical properties of the GIC. However, there are few suggestions which have been stated as follows-
1. The authors have investigated the mechanical properties, stating them as biomechanical properties, which may be because they have utilized a natural source for obtaining the chitosan? Please clarify.
2. Flexural strength has also been investigated in many studies, which is considered to be one of the important studies to test the mechanical strength of the GIC. So, Flexural strength can also be assessed for the samples.
3. Stress-strain curves (one for each composition at different duration) can also be displayed.
4. The 0.5% micro-chitosan addition to the GIC liquid shows less CS at 24 h duration than the Control GIC. Similarly for DTS, the 0.5% ans 1% micro-chitosan addition shows a negative behavior at 24 h of duration, on the control GIC. The authors should discuss the phenomena and the chemical interaction that could have taken place.
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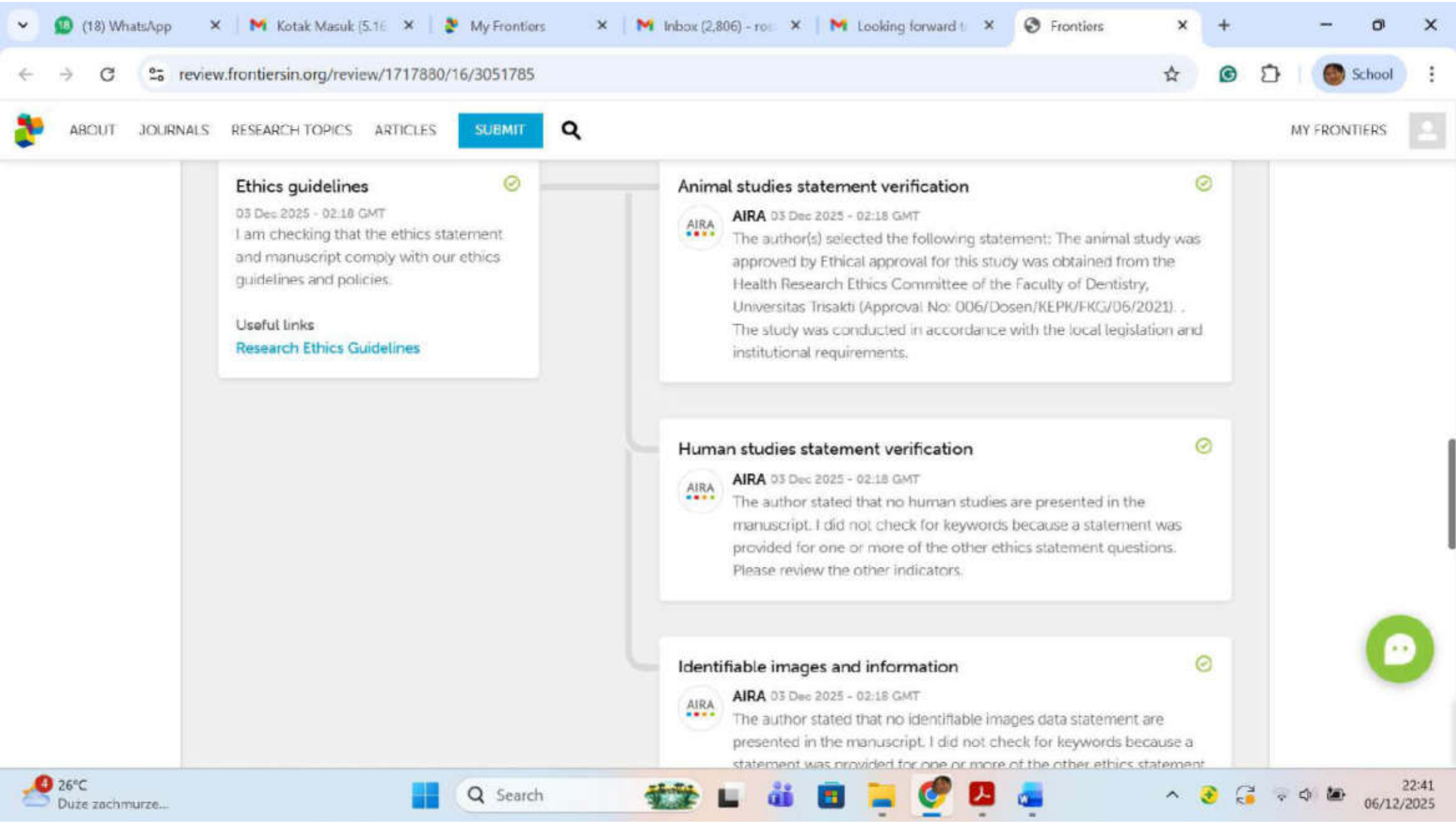
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- Yes

QUALITY ASSESSMENT

Q 3	Rigor	
Q 4	Quality of the writing	
Q 5	Overall quality of the content	
Q 6	Interest to a general audience	

Declaration of AI use

Q 7 Did you use any AI tools to assist you with reviewing this manuscript or writing this report?

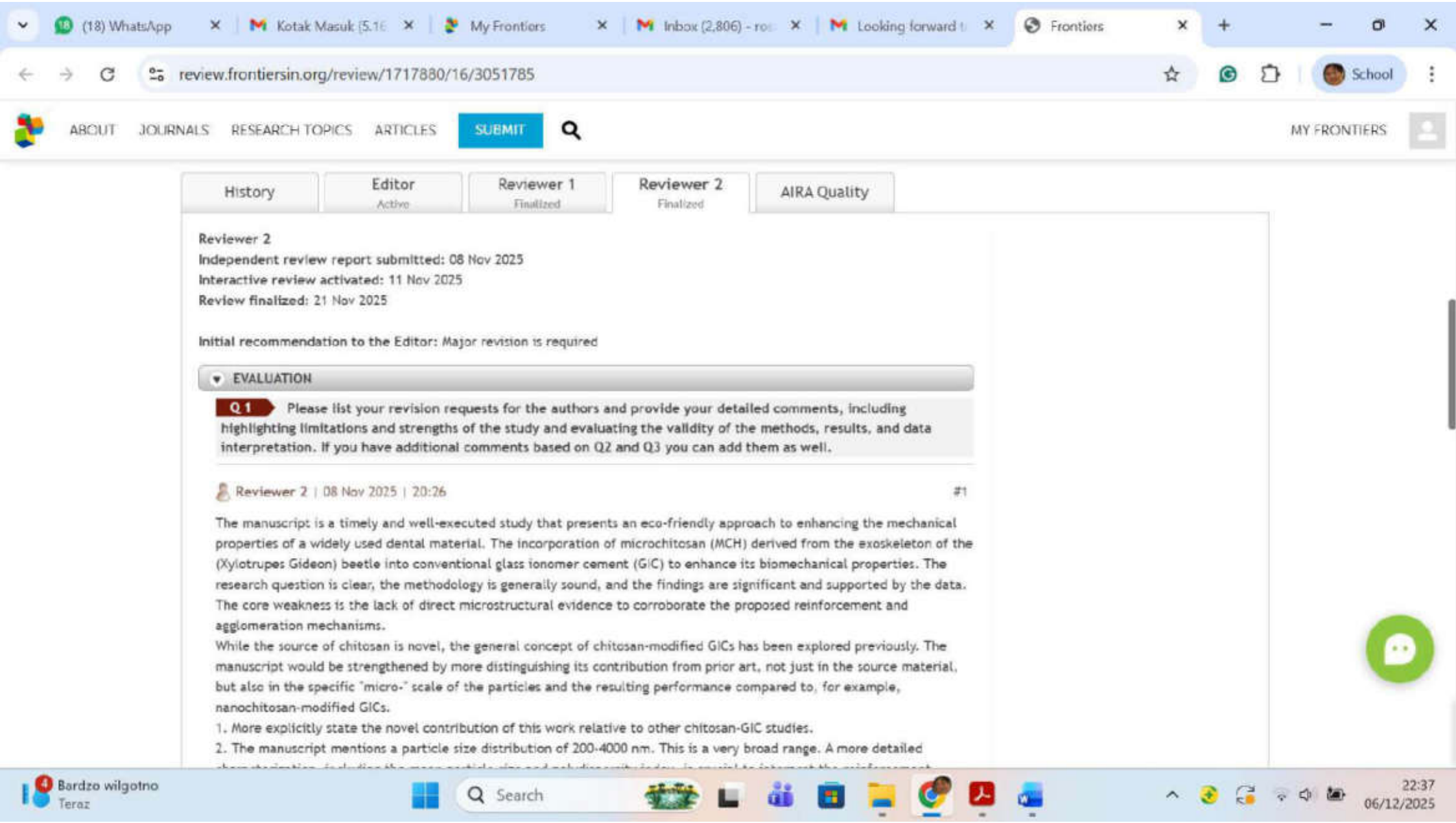
Reviewer 1 | 17 Oct 2025 | 14:08 #1

No

Corresponding Author: Rosalina Tjandrawinata | 21 Nov 2025 | 02:35 #2

Thank you for your justification

Reviewer 2



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Reviewer 2
Independent review report submitted: 08 Nov 2025
Interactive review activated: 11 Nov 2025
Review finalized: 21 Nov 2025

Initial recommendation to the Editor: Major revision is required

EVALUATION

Q 1 **Please list your revision requests for the authors and provide your detailed comments, including highlighting limitations and strengths of the study and evaluating the validity of the methods, results, and data interpretation. If you have additional comments based on Q2 and Q3 you can add them as well.**

Reviewer 2 | 08 Nov 2025 | 20:26 #1

The manuscript is a timely and well-executed study that presents an eco-friendly approach to enhancing the mechanical properties of a widely used dental material. The incorporation of microchitosan (MCH) derived from the exoskeleton of the (Xylotrupes Gideon) beetle into conventional glass ionomer cement (GIC) to enhance its biomechanical properties. The research question is clear, the methodology is generally sound, and the findings are significant and supported by the data. The core weakness is the lack of direct microstructural evidence to corroborate the proposed reinforcement and agglomeration mechanisms.

While the source of chitosan is novel, the general concept of chitosan-modified GICs has been explored previously. The manuscript would be strengthened by more distinguishing its contribution from prior art, not just in the source material, but also in the specific "micro-" scale of the particles and the resulting performance compared to, for example, nanochitosan-modified GICs.

1. More explicitly state the novel contribution of this work relative to other chitosan-GIC studies.
2. The manuscript mentions a particle size distribution of 200-4000 nm. This is a very broad range. A more detailed

Bardzo wilgotno Teraz | Search | 22:37 06/12/2025

1. More explicitly state the novel contribution of this work relative to other chitosan-GIC studies.
2. The manuscript mentions a particle size distribution of 200-4000 nm. This is a very broad range. A more detailed characterization, including the mean particle size and polydispersity index, is crucial to interpret the reinforcement mechanism accurately. The term "microchitosan" is used, but particles at the lower end of this range (200 nm) are arguably nanoscale. This terminology should be clarified or justified.
3. The method states that the liquid was vortex-mixed "particularly at 2% concentration to achieve uniformity." This implies that dispersion was a challenge at higher concentrations, which aligns with the observed agglomeration and performance decline. A more standardized and detailed description of the mixing protocol for all groups is necessary for reproducibility.
4. While the sample size (n=10 per subgroup) is standard, a brief justification or power analysis would strengthen the methodological reporting.
5. On page 7, line 158, it states the 2% group had the highest value at 24 hours, but the text also says this was not significant (p=0.973). This could be phrased more clearly to avoid misleading the reader
6. The discussion is thorough but somewhat lengthy. Some paragraphs could be consolidated for conciseness.
7. The discussion on the decline in performance of the 2% group due to agglomeration is reasonable. However, providing SEM images of the fractured composite surfaces for different groups, would greatly enhance the manuscript.
8. The authors correctly note that biofunctional properties (antibacterial effect, fluoride release) were not assessed. The speculation in the discussion (Section 4.3) is informed by literature but should be tempered, as the study's own data cannot support these claims. A statement recommending this for future work would be appropriate here.
9. Contextualize the absolute values of the improved mechanical properties. For instance, how does a compressive strength of ~155 MPa compare to the requirements for permanent, stress-bearing restorations?

Corresponding Author: Rosalina Tjandrawinata | 21 Nov 2025 | 02:38 #2

We appreciate your positive and detailed review of our work, as well as your recognition of its eco-friendly approach and clinical significance. We address each of your comments in the attachment file and have revised the manuscript accordingly.

Corresponding Author: Rosalina Tjandrawinata | 21 Nov 2025 | 02:38 #2

We appreciate your positive and detailed review of our work, as well as your recognition of its eco-friendly approach and clinical significance. We address each of your comments in the attachment file and have revised the manuscript accordingly.

Review supporting file - 1015221

Q 2 Check List

Reviewer 2 | 08 Nov 2025 | 20:26 #1

a. Is the quality of the figures and tables satisfactory?
- Yes

b. Does the reference list cover the relevant literature adequately and in an unbiased manner?
- Yes

c. Are the statistical methods valid and correctly applied? (e.g. sample size, choice of test)
- Yes

d. Is a statistician required to evaluate this study?
No answer given.

e. Are the methods sufficiently documented to allow replication studies?
- No

No answer given.

e. Are the methods sufficiently documented to allow replication studies?
- No

QUALITY ASSESSMENT

Q 3	Rigor	
Q 4	Quality of the writing	
Q 5	Overall quality of the content	
Q 6	Interest to a general audience	

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Reviewer 2 | 08 Nov 2025 | 20:26 #1

No

Corresponding Author: Rosalina Tjandrawinata | 21 Nov 2025 | 02:38 #2

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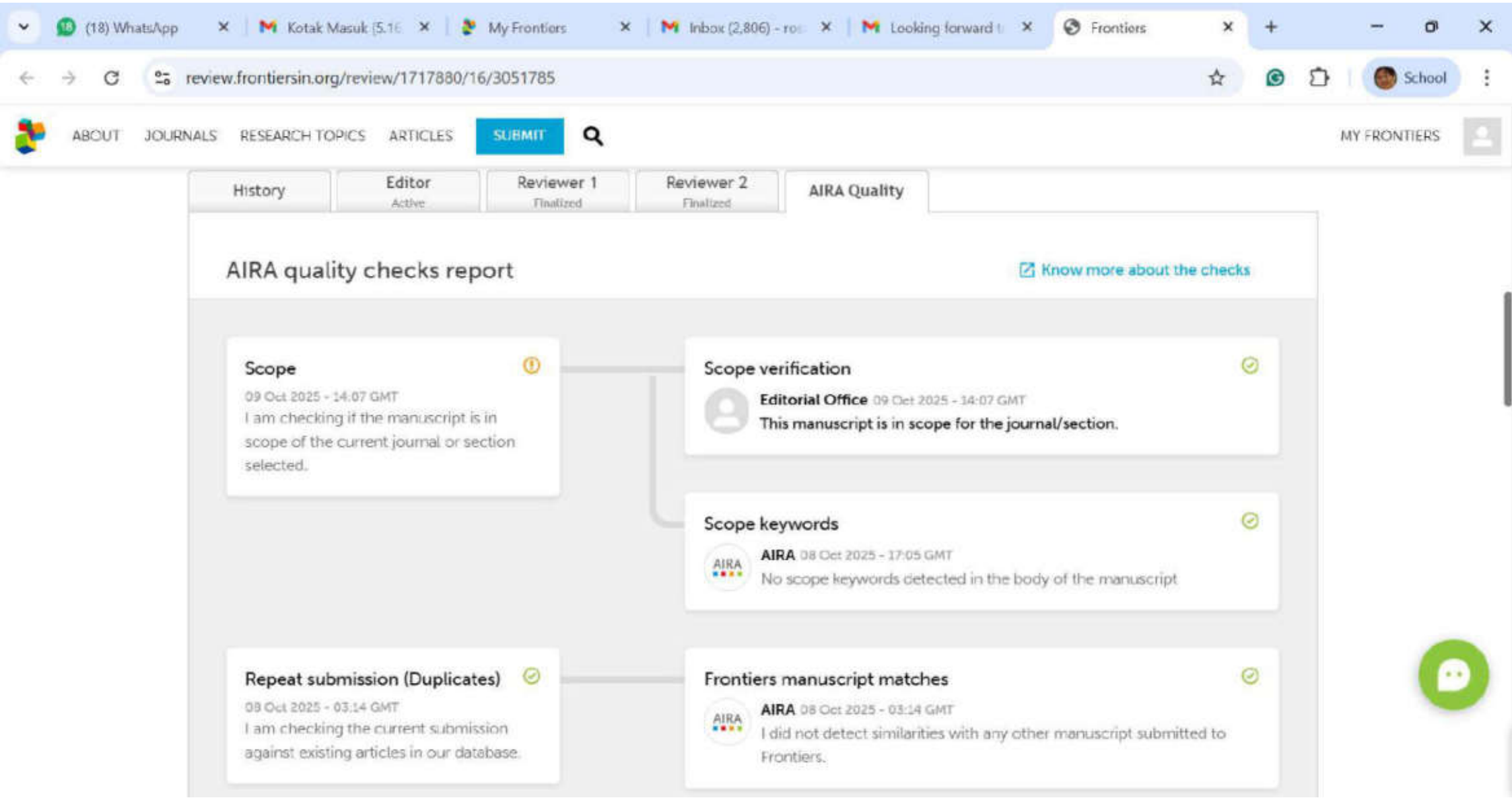
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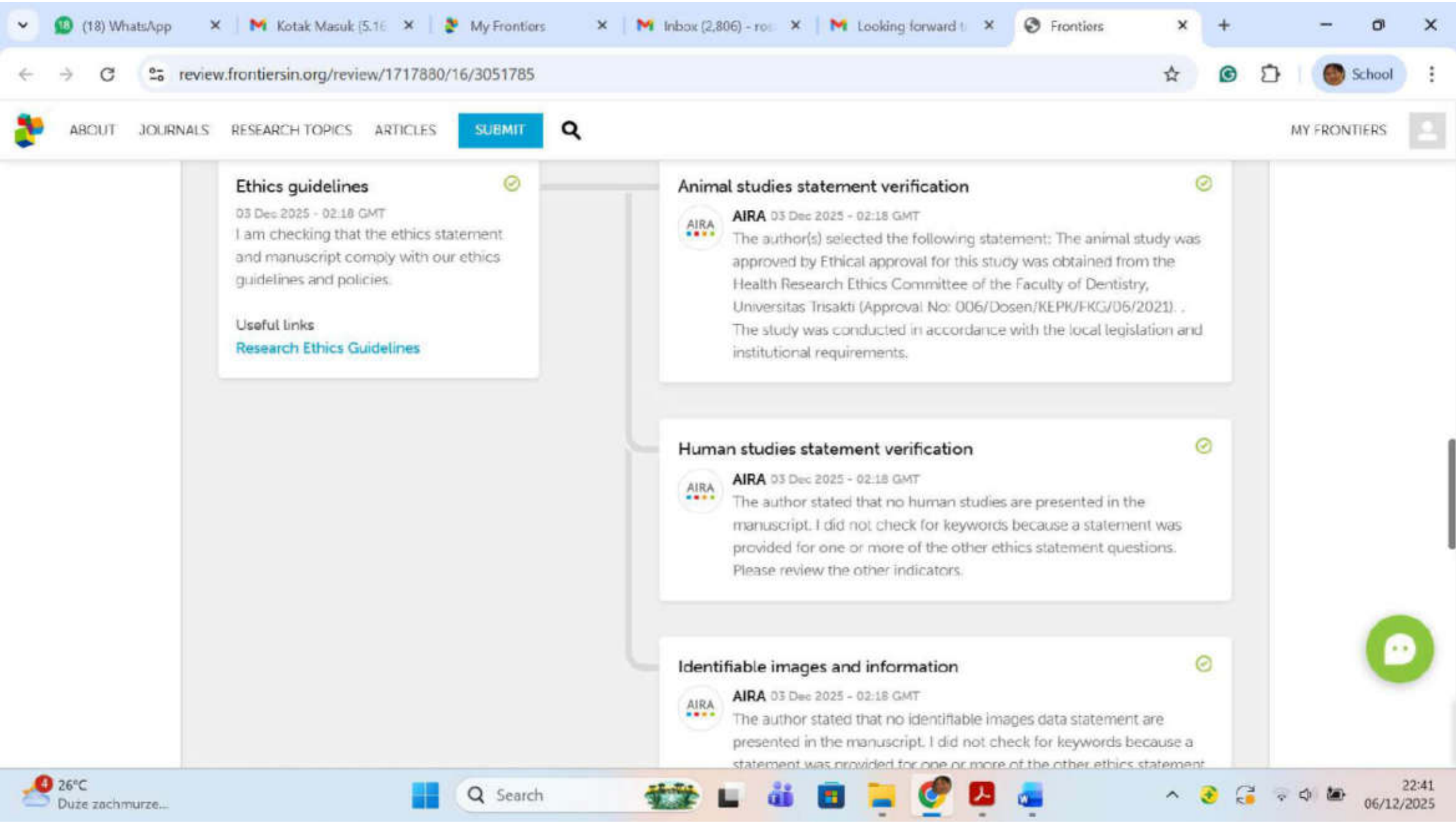
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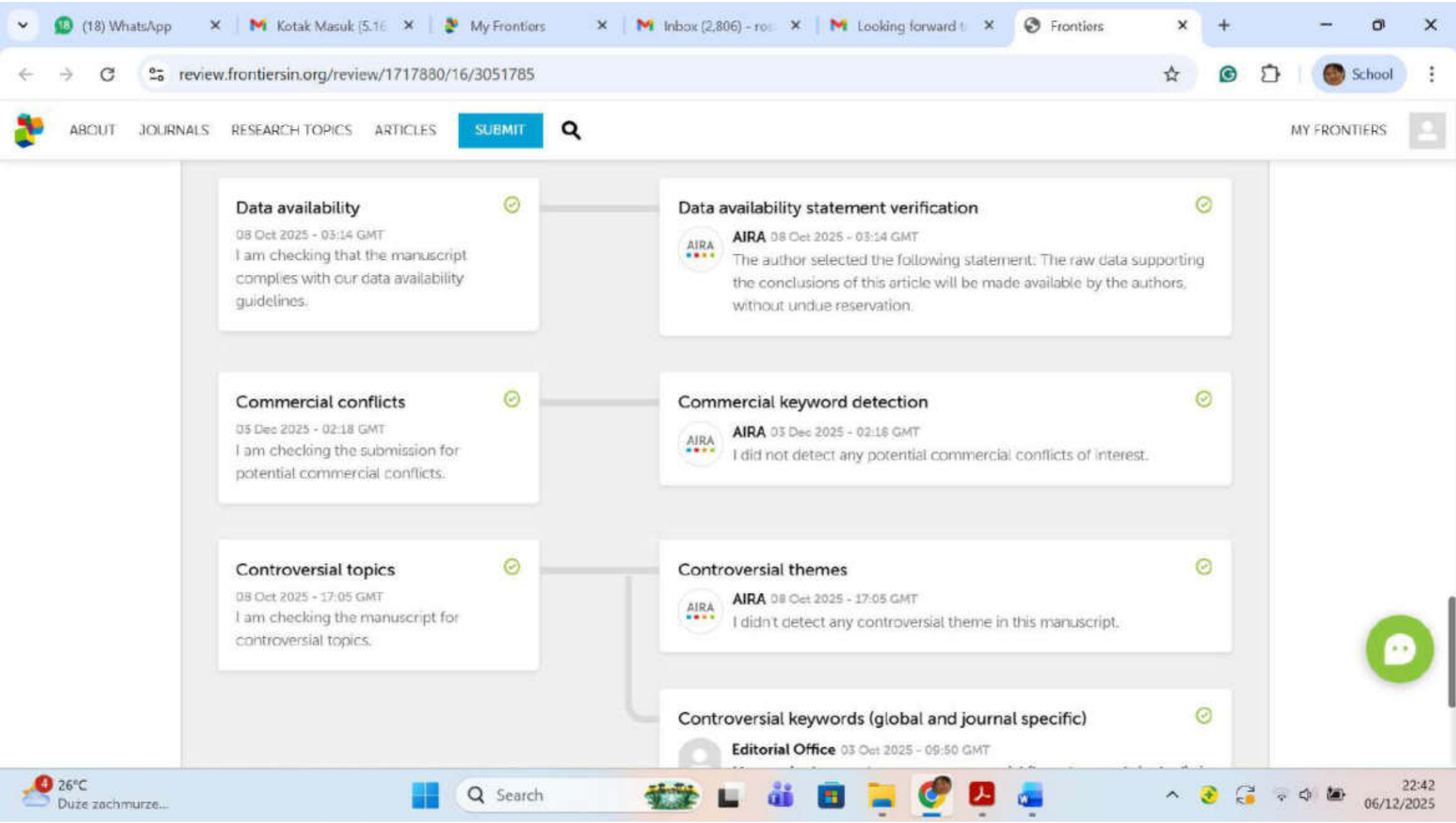
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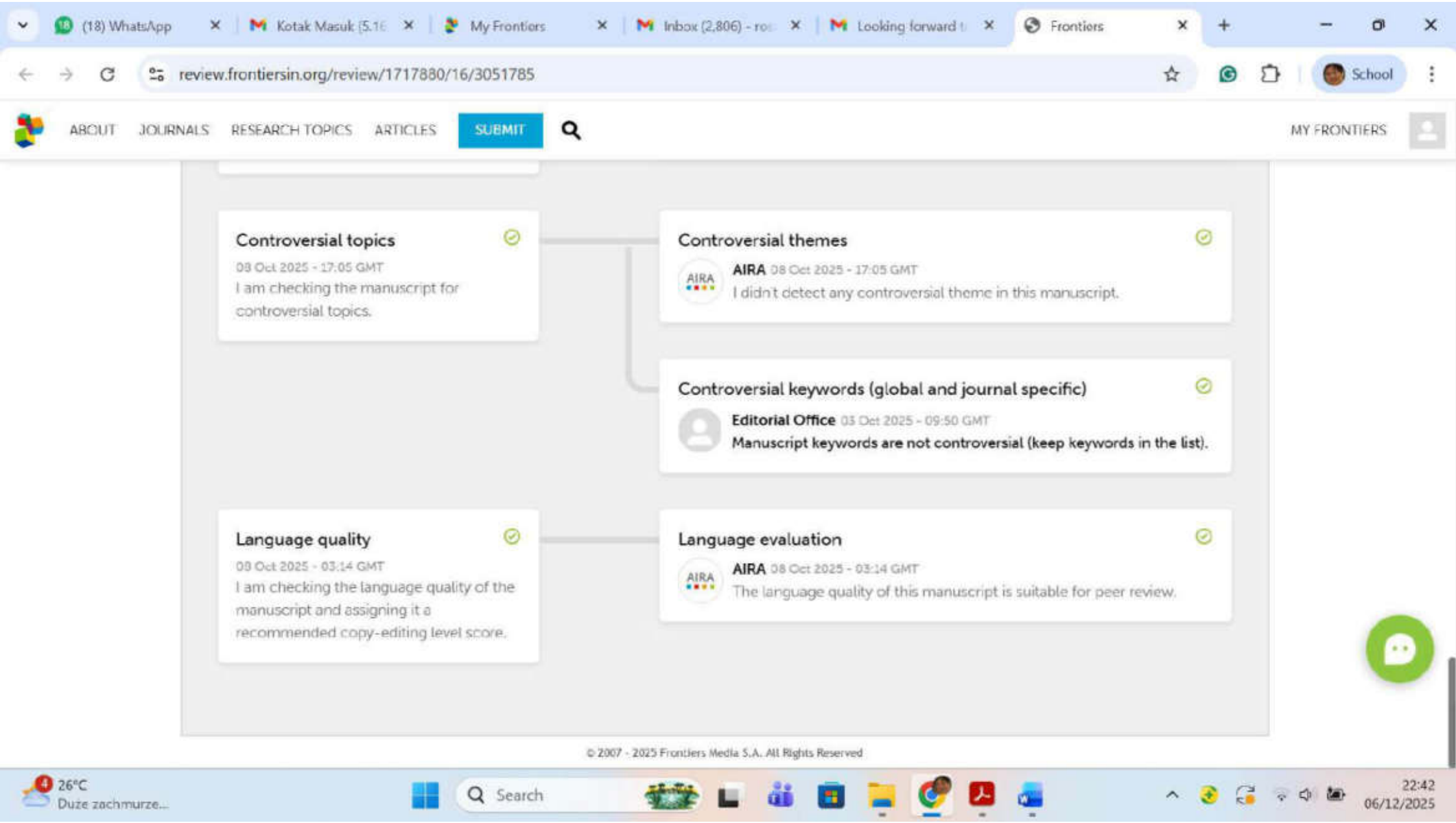
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2	21 Nov 2025	Rosalina Tjandrawinata	Xylotrupes Gideon Microchitosan-Modified Glass Ionomer Cement: In Vitro Assessment of Mechanical Properties Abstract | 1717880_Manuscript.PDF | 1717880_Manuscript.BIB | 1717880_Manuscript.DOCX | 1717880_Manuscript | Figure 1.JPEG | Figure 2.JPEG | Figure 3.JPEG | Figure 4.JPEG | Figure 5.JPEG | Figure 6.JPEG
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History | Editor Active | Reviewer 1 Finalized | Reviewer 2 Finalized | AIRA Quality

Reviewer 2
Independent review report submitted: 08 Nov 2025
Interactive review activated: 11 Nov 2025
Review finalized: 21 Nov 2025

Gmail

Rosalina Tjandrawinata <rosatjandrawinata@gmail.com>

Independent review report submitted (ID 1717880)

2 pesan

Frontiers in Dental Medicine Editorial Office <dentalmedicine.editorial.office@frontiersin.org> 17 Oktober 2025 pukul 21.08
Balas Ke: Frontiers in Dental Medicine Editorial Office <dentalmedicine.editorial.office@frontiersin.org>
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A new report has been submitted by Reviewer 1.

As soon as all reviewers have submitted their comments, you will be able to access the reports in the review forum, enabling you to start your revisions.

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Manuscript title: Xylotrupes Gideon Microchitosan-Modified Glass Ionomer Cement: In Vitro Assessment of Biomechanical Properties
Manuscript ID: 1717880
Authors: Rosalina Tjandrawinata, Florencia Livia Kurniawan, Carolina Damayanti Marpaung, Deviyanti Pratiwi, Eddy Eddy, Tansza Setiana Putri, Komariah Komariah, Indrayadi Gunardi, Sastra Kusuma Wijaya and Arief Cahyanto
Journal: Frontiers in Dental Medicine, section Dental Materials
Article type: Original Research
Submitted on: 02 Oct 2025

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Rosalina Tjandrawinata <rosatjandrawinata@gmail.com> 18 Oktober 2025 pukul 08.33
Kepada: Sastra Kusuma Wijaya <skwijaya@gmail.com>

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Rosalina Tjandrawinata <rosatjandrawinata@gmail.com>

Independent review report submitted (ID 1717880)

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Article type: Original Research
Submitted on: 02 Oct 2025

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Rosalina Tjandrawinata <rosatjandrawinata@gmail.com>

Looking forward to your revisions on Xylotrupes Gideon Microchitosan-Modified Glass Ionomer Cement: In Vitro Assessment of Biomechanical Properties (ID: 1717880)

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Dear Rosalina,

I see that you will shortly be submitting the revisions of your paper currently under review: Xylotrupes Gideon Microchitosan-Modified Glass Ionomer Cement: In Vitro Assessment of Biomechanical Properties [ID: 1717880].

Need some help? The Peer Review team at Frontiers will be happy to assist you! Please reach out to us via email if you are having any difficulties.

Or, if you need more time to respond to the feedback provided and resubmit your revised manuscript, please do use the "Request Extension" button on the Review Forum to extend your deadline: Review Forum

I am looking forward to your response in the coming days.

With very best wishes,

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RESPONSE TO THE FIRST REVIEWER COMMENTS

We appreciate the reviewer for constructive comments and detailed review of our work. We address each of your comments below and have revised the manuscript accordingly.

Q1.

Comment: The authors have investigated the mechanical properties, stating them as biomechanical properties, which may be because they have utilized a natural source for obtaining the chitosan? Please clarify.

Response: Thank you for highlighting this potential source of confusion. In the original version, we used the term *"biomechanical properties"* in a broad sense to describe the mechanical behaviour of a bio-derived restorative material tested under conditions simulating the oral environment (artificial saliva, 37 °C). However, the actual outcome measures in this study are strictly *mechanical* parameters (compressive strength, diametral tensile strength, and Vickers hardness) assessed according to ISO 9917-1:2007.

To avoid misinterpretation:

1. We have replaced "biomechanical properties" with "mechanical properties" in the Abstract, Materials and Methods, Results, Discussion, and Keywords wherever it referred specifically to the measured outcomes.
2. We have retained the term "biomechanical performance" only in the broader, conceptual context (e.g., in the Clinical Implications) where we discuss how these mechanical properties may support the overall biomechanical behaviour of restorations in the oral cavity.
3. We also added a brief clarifying sentence in the Introduction stating that, in this work, "mechanical properties" specifically denote compressive strength, diametral tensile strength, and surface hardness measured in vitro according to ISO standards.

Revision:

4.3 Clinical Implications and Future Directions

……..

Finally, the use of *Xylotrupes gideon*–derived microchitosan highlights an environmentally conscious approach to dental biomaterials. Insect-based chitosan offers a locally available, potentially less allergenic alternative to marine sources and integrates effectively into GIC matrices.(1, 16, 18) Microchitosan thus represents a sustainable modifier that can enhance the mechanical performance of GIC while reducing reliance on traditional marine-derived additives. Such improvements in mechanical properties are critical for the biomechanical performance of GIC restorations in the oral cavity. Comprehensive biocompatibility testing, biofunctional evaluation, and clinical trials are necessary to confirm the safety and effectiveness of this approach and to support its broader clinical implementation.

……..

Q2.

Comment: Flexural strength has also been investigated in many studies, which is considered to be one of the important studies to test the mechanical strength of the GIC. So, Flexural strength can also be assessed for the samples.

Response: We agree that flexural strength is an important mechanical parameter for GICs, especially for stress-bearing clinical applications. In the present work, we limited our testing to compressive strength, diametral tensile strength, and surface hardness, all performed according to ISO 9917-1:2007 and commonly used protocols for conventional GICs.

1. We have acknowledged in the Discussion (Clinical Implications and Future Directions subsection) that the absence of flexural strength data is a limitation of this study.
2. We added a statement specifying that three-point or biaxial flexural strength testing will be included in a follow-up investigation focusing on a broader mechanical characterization of Xylotrupes-derived microchitosan–modified GICs.

Revision:

4.3 Clinical Implications and Future Directions

Within the limitations of this in vitro study, *Xylotrupes gideon*–derived microchitosan appears to be a promising internal reinforcement for conventional GIC, particularly at a 1% loading. The observed improvements in strength and hardness, together with the material's fluoride-releasing nature, support potential use in stress-bearing posterior restorations, atraumatic restorative treatment (ART), and liners or bases in deep cavities, provided that adequate adhesion and long-term behaviour are confirmed.(1, 16, 27) However, the absence of flexural strength data represents an important limitation, as flexural performance is highly relevant to the clinical loading conditions of GIC restorations. To address this, future work will incorporate three-point or biaxial flexural strength testing as part of a broader mechanical characterization of *Xylotrupes*-derived microchitosan–modified GICs.

…….

Q3.

Comment: Stress-strain curves (one for each composition at different duration) can also be displayed.

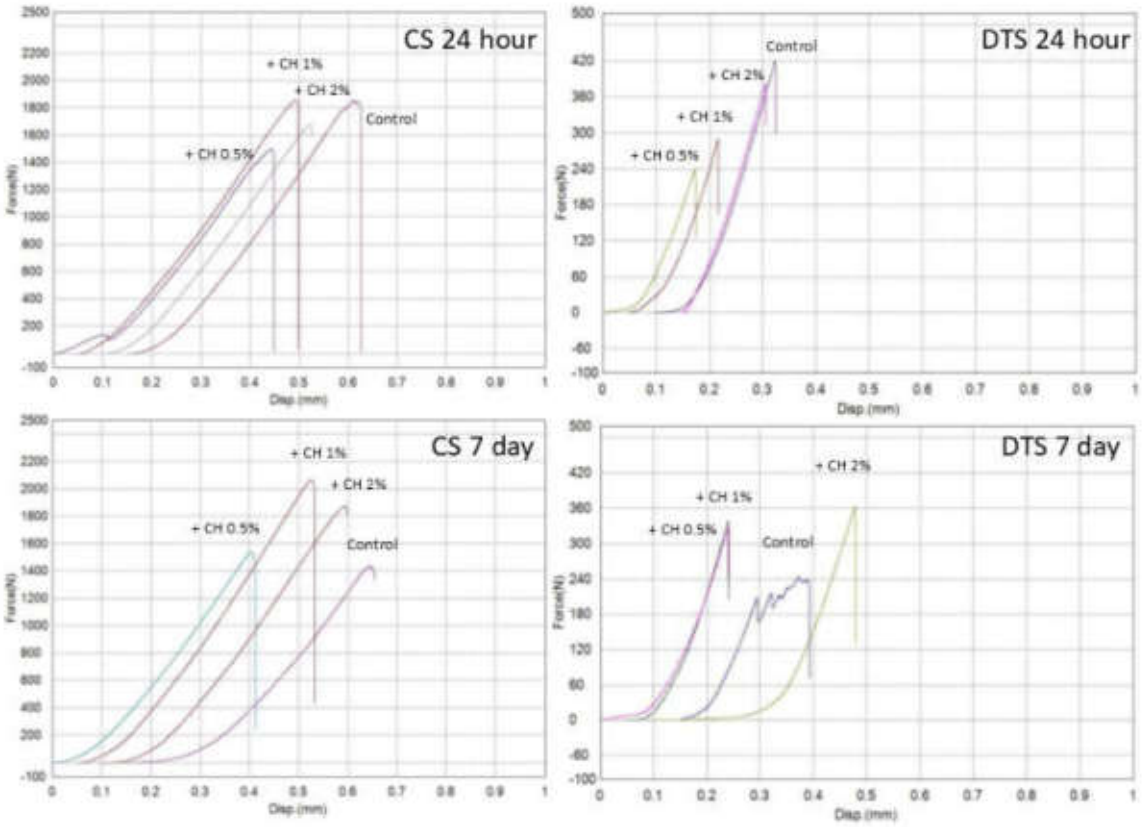
Response: We appreciate this helpful suggestion. In the original submission, we summarized the mechanical behavior primarily as mean strength values and standard deviations to align with ISO-based reporting. In the revised manuscript, we have now made fuller use of the load-displacement data generated by the universal testing machine:

1. We converted the load-displacement data to engineering stress–strain curves and added representative curves for each composition at both 24 hours and 7 days, as shown in Figure 5.

2. In the Results section, we briefly describe these curves, noting that all groups exhibit predominantly linear elastic behaviour followed by abrupt failure consistent with brittle, cement-like fracture, which is typical for conventional GICs.
3. In the Mechanical Reinforcement subsection of the Discussion**,** we now refer to these stress–strain curves to support the interpretation that 1% MCH provides the most favourable combination of strength and stiffness, whereas 2% MCH shows evidence of overloading and reduced reinforcement efficiency.

We hope that the inclusion of these stress–strain curves addresses your suggestion and provides a clearer visualization of the mechanical behaviour of each group over time.

Revision:



3 Results

The compressive strength, diametral tensile strength, and hardness results for all test groups are presented in Figures 3–6, while Table 1 summarizes the significance values for comparisons between groups. The data from 240 specimens (20 per group, 10 per time point) were normally distributed, as determined by the Kolmogorov–Smirnov test ($p = 0.200$). Representative load–displacement (stress–strain–type) curves for each group and time point are shown in Figure 5. In agreement with the numerical data, the addition of microchitosan (MCH) at all concentrations resulted in statistically significant increases in both compressive and diametral tensile strength compared with the control group at 7 days ($p < 0.05$).

3.1 Compressive Strength

The compressive strength results are presented in Figure 3. At 24 hours, the control GIC group recorded a mean strength of 127.14 ± 15.82 MPa. Compared with the control, the 0.5% MCH group showed a slight reduction, whereas the 2% MCH group showed a numerically higher mean compressive strength (130.01 ± 14.40 MPa); however, this difference was not statistically significant ($p = 0.973$). The 1% MCH group was comparable to the control at this early time point.

After 7 days of immersion, the control group decreased to 114.63 ± 21.39 MPa. In contrast, all MCH groups showed higher values than the control. The highest compressive strength was observed in the 1% MCH group, which reached 155.16 ± 19.08 MPa ($p = 0.002$), representing a 35.4% increase over the 7-day control.

The corresponding compressive load-displacement curves (Figure 5, CS 24 h and CS 7 d) reveal predominantly linear elastic behaviour, followed by abrupt failure in all groups, consistent with brittle, cement-like fracture. At 24 hours, the slopes of the curves are broadly similar across groups, with only modest differences in peak load. After 7 days, the curves for all MCH groups shift towards higher peak loads compared with the control, with the 1% MCH group showing the highest peak load and largest displacement at failure, reflecting its superior strength and energy-absorbing capacity. The 0.5% MCH group exhibits the lowest peak load, while the 2% MCH and control curves lie between 0.5% and 1% MCH, mirroring the numerical ranking of compressive strength.

3.2 Diametral Tensile Strength

The diametral tensile strength results are presented in Figure 4. At 24 hours, the control group showed a mean strength of 11.41 ± 3.16 MPa. Compared with the control, the 0.5% MCH group showed significantly lower strength, whereas the 1% and 2% MCH groups did not differ significantly from the control.

After 7 days, the control group decreased to 7.09 ± 2.70 MPa, while all MCH groups showed higher values. The 1% MCH group had the highest diametral tensile strength (10.73 ± 1.89 MPa, $p = 0.014$), corresponding to a 51% increase relative to the 7-day control.

The diametral tensile load-displacement curves (Figure 5, DTS 24 h and DTS 7 d) corroborate these trends. At 24 hours, the control curve demonstrates the highest peak load, with the curves for the MCH groups clustered slightly below, reflecting the small or non-significant differences in DTS. By 7 days, the curves for all MCH-modified groups shift upward relative to the control, indicating higher peak loads at fracture. The 1% and 2% MCH groups in particular show steeper initial slopes and higher maxima than the control, whereas the 0.5% MCH curve occupies an intermediate position. This pattern is consistent with the 7-day DTS values, where 1% MCH provides the most pronounced reinforcement.

Q4.

Comment: The 0.5% micro-chitosan addition to the GIC liquid shows less CS at 24 h duration than the Control GIC. Similarly, for DTS, the 0.5% and 1% micro-chitosan addition

shows a negative behavior at 24 h of duration, on the control GIC. The authors should discuss the phenomena and the chemical interaction that could have taken place.

Response: Thank you for drawing attention to this important early-time behavior. We agree that the transient reduction in some 24-hour values requires clearer discussion.

In the Results, we already mention that some 24-hour values did not exceed the control, while the 7-day values showed pronounced improvement. In the revised Discussion (Mechanical Reinforcement subsection), we have now added a dedicated paragraph explaining the likely mechanisms, summarised as follows:

1. At early maturation (24 h), the microchitosan particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disrupting optimal ionic cross-linking within the GIC matrix.
2. This competition, along with the introduction of additional interfaces, may result in slightly reduced initial network rigidity and localized microvoids, leading to the observed modest decreases in compressive and diametral tensile strength at 24 hours for some concentrations.
3. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and the microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1 % group.

Revision:

4.2 Mechanical Reinforcement

……..……..

At early maturation (24 h), however, MCH particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disturbing optimal ionic cross-linking within the GIC matrix. This competition, together with the introduction of additional interfaces, may reduce initial network rigidity and create localized microvoids, which is consistent with the modest reductions or limited improvements in compressive and diametral tensile strength observed at 24 hours for some concentrations. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1% group.

……

RESPONSE TO THE SECOND REVIEWER COMMENTS

We appreciate your positive and detailed review of our work, as well as your recognition of its eco-friendly approach and clinical significance. We address each of your comments below and have revised the manuscript accordingly.

Reviewer 2:
The manuscript is a timely and well-executed study that presents an eco-friendly approach to enhancing the mechanical properties of a widely used dental material. The incorporation of microchitosan (MCH) derived from the exoskeleton of the (Xylotrupes Gideon) beetle into conventional glass ionomer cement (GIC) to enhance its biomechanical properties. The research question is clear, the methodology is generally sound, and the findings are significant and supported by the data. The core weakness is the lack of direct microstructural evidence to corroborate the proposed reinforcement and agglomeration mechanisms.
While the source of chitosan is novel, the general concept of chitosan-modified GICs has been explored previously. The manuscript would be strengthened by more distinguishing its contribution from prior art, not just in the source material, but also in the specific "micro-" scale of the particles and the resulting performance compared to, for example, nanochitosan-modified GICs.

Q1.
Comment: More explicitly state the novel contribution of this work relative to other chitosan-GIC studies.

Response: Thank you for this important point. We have strengthened the description of novelty in both the abstract and introduction.

Revision: We yellow-highlight the changes.

Abstract

Background: Glass ionomer cements (GIC) are valued as inherent fluoride-releasing dental restorative materials, while chitosan binds to negatively charged enamel surfaces, promoting mineral deposition and strengthening teeth. This study aimed to evaluate the biomechanical properties of GIC modified with microchitosan derived from *Xylotrupes gideon*, using an *in vitro* experimental design. This study uniquely employs micro-scaled chitosan derived from the exoskeleton of *Xylotrupes gideon*, an insect-based, locally sourced, and environmentally sustainable alternative to conventional marine chitosan, to reinforce a conventional GIC.

Materials and Methods: Microchitosan was extracted from *Xylotrupes gideon* and incorporated into conventional GIC at 0.5%, 1% and 2% (w/w). Compressive strength, diametral tensile strength, and surface microhardness were measured using standard testing equipment after immersion in artificial saliva for 24 hours and 7 days. Statistical analysis was performed using one-way ANOVA followed by the Games-Howell post hoc test, with significance set at $p < 0.05$.

Results: The 1% microchitosan-modified GIC exhibited the most significant improvements compared to the unmodified control. After 7 days, compressive strength increased by 35.4%, diametral tensile strength by 51.3%, and surface hardness by 46.6% ($p<0.05$). These

enhancements are attributed to microscale reinforcement and chemical bonding between microchitosan and the GIC matrix.

Conclusion: The addition of 1% microchitosan derived from *Xylotrupes gideon* significantly improved the biomechanical performance of GIC. This bioactive reinforcement shows promising potential for clinical restorative applications, though further investigation into its long-term biocompatibility and fluoride release is warranted. These findings highlight a novel combination of insect-derived micro-scale chitosan and conventional GIC, yielding mechanical gains comparable to those reported for nanochitosan-modified formulations, while relying on a more sustainable chitosan source.

Introduction (last paragraph)
…….
Therefore, this study aimed to investigate the effect of incorporating Xylotrupes Gideon-derived microchitosan (on a submicron–micron scale) into conventional GIC on its compressive strength, diametral tensile strength, and surface hardness. By using an insect exoskeleton as a locally available and environmentally sustainable source of chitosan, this approach has potential for clinical feasibility, long-lasting nature, and biological functionality through the integration of eco-friendly materials in restorative dentistry.(7)

Q2.
Comment: The manuscript mentions a particle size distribution of 200-4000 nm. This is a very broad range. A more detailed characterization, including the mean particle size and polydispersity index, is crucial to interpret the reinforcement mechanism accurately. The term "microchitosan" is used, but particles at the lower end of this range (200 nm) are arguably nanoscale. This terminology should be clarified or justified.

Response: We appreciate this nuanced observation.

In the Materials and Methods section (Synthesis and Characterization), we originally reported a particle size distribution of 200–4000 nm, based on particle size analysis, and illustrated the morphology using SEM images.

1. We would like to clarify the terminology by stating that the material consists of submicron to microscale chitosan particles, and that we use the term *"microchitosan (MCH)"* because SEM qualitatively shows that the majority of particles appear as micron-scale agglomerates despite the presence of a submicron tail in the distribution.
2. Explicitly acknowledged in the Discussion (Mechanical Reinforcement subsection) that the absence of detailed descriptors such as mean particle size and polydispersity index is a limitation of the current study.
3. Added that a separate, more materials-focused study is being prepared, which will present a full physicochemical characterization of *Xylotrupes gideon*-derived microchitosan (including detailed particle size statistics, zeta potential, and polydispersity index).

We have not inserted any new numerical size parameters beyond the originally reported range; instead, we improved the qualitative justification and explicitly flagged the missing parameters as a basis for future work.

Revision:

4.2 Mechanical Reinforcement

…….

At early maturation (24 h), however, MCH particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disturbing optimal ionic cross-linking within the GIC matrix. This competition, together with the introduction of additional interfaces, may reduce initial network rigidity and create localized microvoids, which is consistent with the modest reductions or limited improvements in compressive and diametral tensile strength observed at 24 hours for some concentrations. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1% group.

Beyond reporting the overall particle size range of 200–4000 nm, more detailed particle size descriptors, such as the mean particle size, full size distribution profile, and polydispersity index, were not determined in this study. This represents a limitation of the current work, as such parameters would enable a more precise interpretation of how particle size, dispersion quality, and potential agglomeration contribute to the mechanical response of the different MCH concentrations. Future investigations will therefore include comprehensive physicochemical characterization of *Xylotrupes gideon*–derived microchitosan, including mean size, polydispersity, and surface charge, to better correlate these properties with the observed mechanical reinforcement of the GIC matrix.

…….

Q3.

Comment: The method states that the liquid was vortex-mixed "particularly at 2% concentration to achieve uniformity." This implies that dispersion was a challenge at higher concentrations, which aligns with the observed agglomeration and performance decline. A more standardized and detailed description of the mixing protocol for all groups is necessary for reproducibility.

Response:

Thank you for pointing out this ambiguity. You are correct that the original wording could be interpreted as if only the 2 % group was vortex-mixed.

To improve clarity and reproducibility, we have revised the Materials and Methods (Preparation of Modified GIC) as follows:

1. We now explicitly state that all modified liquids (0.5%, 1%, and 2% MCH) were vortex-mixed using the same standardized protocol immediately before mixing with the powder.
2. The phrasing “particularly at 2 % concentration to achieve uniformity” has been replaced with wording that indicates dispersion is more challenging at higher loading, and that this likely contributed to the agglomeration-related decline in performance at 2%, as discussed in the Discussion. However, the *procedure itself was the same* for all concentrations.

Revision:

2.2 Preparation of Modified Glass Ionomer Cement

A commercially available glass ionomer cement (Type IX Gold Label HS Posterior, GC, Tokyo, Japan) was used as the base material. The control group used the unmodified GIC liquid, while the experimental groups consisted of 0.5%, 1%, and 2% (w/w) MCH added to the liquid component. Each modified liquid (0.5%, 1%, and 2% MCH) was vortex-mixed using the same standardized protocol immediately before mixing with the powder to improve homogeneity, acknowledging that achieving uniform dispersion becomes more challenging at higher MCH concentrations. Petroleum jelly was applied to the inner surfaces of the metal split mold, which was then placed on a mylar strip positioned on a glass slab. The powder and liquid were dispensed onto a paper pad and mixed manually according to the manufacturer's guidelines. After the mold was filled with the mixture, it was covered with another mylar strip and a second glass slab, then pressed for 30 seconds to extrude excess material and obtain a uniformly smooth specimen surface. One hour after mixing, the specimens were removed from the mold and any excess material was removed by dry grinding on both sides with 1000-grit silicon carbide paper. (1, 20, 21)

……..

Q4.
Comment: While the sample size (n=10 per subgroup) is standard, a brief justification or power analysis would strengthen the methodological reporting.

Response: We agree that justifying the sample size improves transparency.

In the statistical analysis subsection and in the description of specimen preparation, we have added a short statement noting that:

1. The use of n = 10 samples per subgroup was guided by ISO 9917-1:2007, which specifies a minimum of 5 samples, and is consistent with many previous studies assessing the compressive strength, tensile strength, and hardness of conventional and modified GICs.
2. We use the Lemeshow equation to determine the minimum sample size, resulting in 7 samples.

Revision:

2.3 Conditioning and Storage

A total of 80 samples were prepared for each mechanical test and divided into four groups (n = 20 each), with each group further subdivided into two subgroups (n = 10) based on immersion time (24 hours and 7 days). The minimum sample size was estimated using the Lemeshow formula for comparing two independent means:

$$n = 2\, t^{2}\, (Z_{1-\alpha} + Z_{1-\beta})^2/(\mu_1-\mu_2)^2$$

Where n is the minimum number of specimens per subgroup, μ_1 and μ_2 are the expected

means from a previous study, τ is the common standard deviation, $Z_{1-\alpha}$ is the Z value for the selected significance level, and $Z_{1-\beta}$ is the Z value corresponding to the desired power.

2.5 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean and standard deviation, were calculated. Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was evaluated using Levene's test. When the assumption of homogeneity was violated, one-way ANOVA was followed by the Games–Howell post hoc test for pairwise comparisons. Pearson correlation coefficients were calculated to examine relationships among mechanical parameters. The minimum required sample size per subgroup was determined a priori using the Lemeshow equation for comparing two means, which yielded a sample size of seven. To ensure adequate power and consistency with ISO 9917-1:2007 recommendations and previous GIC mechanical studies, which typically use 5–10 specimens per subgroup, we ultimately used n = 10 specimens per subgroup. A significance level of $p < 0.05$ was applied throughout.

Q5.

Comment: On page 7, line 158, it states the 2% group had the highest value at 24 hours, but the text also says this was not significant (p=0.973). This could be phrased more clearly to avoid misleading the reader

Response: Thank you for this helpful suggestion.

Revision:

In the Results – Compressive Strength section, we have rephrased the relevant sentence to:

"Compared with the control, the 0.5% MCH group showed a slight reduction, whereas the 2% MCH group showed a numerically higher mean compressive strength (130.01 ± 14.40 MPa); however, this difference was not statistically significant ($p = 0.973$)."

Q6.

Comment: The discussion is thorough but somewhat lengthy. Some paragraphs could be consolidated for conciseness.

Response: We appreciate this observation and have streamlined the Discussion while maintaining the key scientific messages:

We consolidated overlapping paragraphs in the Mechanical Reinforcement subsection and removed repetitive descriptions of chitosan's general properties that were already covered in the Introduction. We condensed the Biofunctional Attributes and Fluoride Release section by eliminating unnecessary literature examples and focusing on the most pertinent points for chitosan-modified GICs. We refined the Clinical Implications and Future Directions subsection by combining similar future work suggestions and removing redundant phrasing.

Revision:

4 Discussion

4.1 Summary of Findings

This study demonstrated that incorporating *Xylotrupes gideon*–derived microchitosan (MCH) into a conventional GIC significantly improved its mechanical properties. The 1% MCH concentration produced the best results across all measurements compressive strength, diametral tensile strength, and surface hardness, particularly after 7 days of immersion in artificial saliva, indicating a time-dependent reinforcement effect.(25) The 0.5% MCH group showed only modest improvements, whereas the 2% MCH group, despite some initial enhancement, was likely more prone to particle agglomeration, which compromised its long-term stability.(6)

These findings align with previous research showing that chitosan can enhance the strength and durability of GICs and fit within the broader trend of nanomaterial-reinforced dental composites.(16) The superior performance of 1% MCH is consistent with reports that nanochitosan outperforms other nanostructures (e.g., nano-silica, nano-zirconia) in GICs, largely because of its ability to form hydrogen bonds with the polyacrylic acid matrix, thereby improving cohesion and ion exchange.(1) Our results confirm substantial gains in surface hardness and compressive strength compared with both unmodified GIC and GICs containing other nano-additives,(26) supporting the concept that small-particle chitosan is a promising modifier for stress-bearing restorations, especially in Class I and V cavities where fluoride release and biocompatibility are important.(27)

All groups exhibited a decrease in hardness after 7 days, consistent with previous work reporting a 40–45% decline in microhardness of conventional GICs following thermocycling.(28, 29) Notably, the 1% MCH group retained the highest residual hardness (46.95 VHN), consistent with the notion that reinforced GICs resist degradation better than unmodified formulations.

4.2 Mechanical Reinforcement

The mechanical enhancements observed with MCH-modified GIC can be explained by microscale dispersion and interfacial interactions between MCH and the GIC matrix. MCH particles (200–4000 nm) can occupy microvoids and irregularities, similarly to the homogenizing effect reported for nano-hydroxyapatite/chitosan composites (30) and microhybrid composites, resulting in a denser, more uniform structure that reduces crack initiation and propagation. In addition, the amino and hydroxyl groups of chitosan form hydrogen and ionic bonds with polyacrylic acid chains, increasing cross-link density and internal cohesion.(4, 17) In this way, microchitosan addresses microstructural weaknesses inherent to the acid–base setting mechanism and may support greater restoration durability.(4)

At early maturation (24 h), however, MCH particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disturbing optimal ionic cross-linking within the GIC matrix. This competition, together with the introduction of additional interfaces, may reduce initial network rigidity and create localized microvoids, which is consistent with the modest reductions or limited

improvements in compressive and diametral tensile strength observed at 24 hours for some concentrations. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1% group.

Beyond reporting the overall particle size range of 200–4000 nm, more detailed particle size descriptors, such as the mean particle size, full size distribution profile, and polydispersity index, were not determined in this study. This represents a limitation of the current work, as such parameters would enable a more precise interpretation of how particle size, dispersion quality, and potential agglomeration contribute to the mechanical response of the different MCH concentrations. Future investigations will therefore include comprehensive physicochemical characterization of *Xylotrupes gideon*–derived microchitosan, including mean size, polydispersity, and surface charge, to better correlate these properties with the observed mechanical reinforcement of the GIC matrix.
The 1% concentration aligns with findings from previous studies, which have shown that 1–1.5% chitosan maximizes mechanical gains in GICs without agglomeration, underscoring the critical role of nanoparticle concentration in dental composites.(27) The good performance of 1% MCH is consistent with the general role of colloidal stability in such systems. Although zeta potential was not measured here, previous studies have shown that a high surface charge in chitosan-modified GICs improves nanoparticle dispersion, mechanical strength, and fluoride release.(25) In contrast, quaternized chitosan-coated nanoparticles have demonstrated superior interfacial bonding and more uniform filler distribution, supporting improved flexural strength and fracture resistance in reinforced GIC systems.(8) Together with reports on other nano-enhanced restorative formulations,(3) these findings suggest that colloidal stability is a key determinant of functional integration and mechanical performance.

Our findings on the increase in Vickers surface hardness with MCH addition are in agreement with earlier studies, which show that chitosan positively influences GIC microhardness.(10) Previous work comparing chitosan with hydroxyapatite revealed that chitosan not only preserved microhardness but was particularly effective in reducing surface roughness,(31) supporting our conclusion that incorporating micro-sized chitin derivatives from *Xylotrupes gideon* contributes to a more durable and homogeneous GIC matrix. Furthermore, the incorporation of carboxymethyl chitosan into the liquid phase of GIC has been shown to significantly enhance flexural strength, fracture toughness, and compressive strength at low concentrations, reinforcing the view that chitosan derivatives strengthen the GIC network via hydrogen bonding and improved interfacial cohesion.(9)

The decline in mechanical performance observed at 2% MCH is consistent with the tendency of higher nanoparticle concentrations to promote agglomeration and disrupt uniform dispersion.(17) Conversely, very low concentrations (e.g., below 0.5%) may be insufficient to achieve effective reinforcement. In the present work, the interpretation that 2% MCH leads to increased agglomeration is therefore **inferred** from the mechanical trends and the known behavior of over-loaded particle-reinforced systems, rather than from direct microstructural evidence. Enhanced powder–liquid interactions during setting, driven by the high surface energy of MCH, likely contributed to favourable gelation and final structure at 1%, in line with observations that optimized powder–liquid interactions support improved mechanical outcomes.(32) MCH thus appears to improve hardness and mechanical durability while preserving essential GIC properties, including adhesion.(33) At the same time, the absence of fractographic SEM analysis of fractured GIC–MCH specimens in this study is a further

limitation that prevents direct visualization of crack paths, particle distribution, and agglomerate formation at different MCH concentrations. A dedicated follow-up investigation, including fracture-surface SEM for all MCH loadings is planned to elucidate these microstructural–mechanical correlations more clearly.

Previous work has shown that resin coatings can significantly enhance the viscoelastic behavior and environmental resistance of highly viscous GICs.(34) While such strategies focus on external surface protection, our results suggest that internal reinforcement with chitosan may offer comparable benefits by increasing matrix cohesion and long-term mechanical stability without necessitating additional coating layers. In another study, shrimp shell–derived chitosan combined with hydrophilic fibers yielded a composite with compressive strength of 32.67 MPa and tensile strength of 17.18 MPa.(35) Although their tensile strength values were higher, our 1% MCH–GIC formulation achieved a much higher compressive strength (155.16 MPa),

When compared with other reinforced GICs, the 1% MCH-GIC formulation (compressive strength 155.16 MPa) approaches the values reported for zirconia-reinforced GIC (160.91 MPa) and silver-alloy GIC (151.47 MPa).(21) These results exceed the ISO 9917-1:2016 minimum compressive strength threshold of 100 MPa for conventional restorative GICs.(36) Although the compressive strength remains below that of enamel (262 MPa) and dentin (234 MPa), and below values for some high-viscosity GICs (301 MPa), it is still well above the estimated stress generated by maximum bite forces (≈21.2 MPa, assuming a 20 mm^2 contact area).(22, 37) Thus, the present MCH-GIC appears mechanically adequate for typical masticatory loading, and further optimization, such as combining MCH with high-viscosity GIC may provide additional safety margins.

In addition to bulk mechanical strengthening, chitosan has been reported to enhance the shear bond strength of GIC to dentin(38), which is critical for long-term clinical success. While bonding behavior was not evaluated in this study, it is plausible that the microchitosan used could confer similar benefits; this will be addressed in future work.

4.3 Biofunctional Attributes and Fluoride Release

Biofunctional attributes, including antimicrobial potential, remineralization support, and tissue compatibility, are highly relevant to restorative materials. The present study did not evaluate antibacterial activity, fluoride release, or pulp/tissue responses, and no biological claims are made based on our data. However, previous studies have shown that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation,(18, 39, 40) and that *Xylotrupes gideon*–derived nanochitosan can exhibit antibacterial effects and support sustained fluoride release in experimental restorative formulations.(19, 41)

Notably, prior work indicates that incorporating nanochitosan into GIC does not compromise fluoride release and may even enhance it, particularly under acidic conditions.(16, 25, 42) Chitosan's hydrogel-like network may modulate ion diffusion and support prolonged fluoride availability. Other reports have demonstrated that chitosan can promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammation when applied near pulp tissue.(43, 44) MCH's mucoadhesive behavior may also improve marginal sealing *in vivo*.(45) Together, these findings suggest that chitosan-modified GICs could offer multifunctional benefits, combining mechanical reinforcement with potential antibacterial and remineralizing effects; nevertheless, such biofunctional properties remain to be tested

specifically for the present MCH-GIC formulation and should be addressed in future *in vitro* and *in vivo* studies.

4.4 Clinical Implications and Future Directions

Within the limitations of this *in vitro* study, *Xylotrupes gideon*–derived microchitosan appears to be a promising internal reinforcement for conventional GIC, particularly at a 1% loading. The observed improvements in strength and hardness, together with the material's fluoride-releasing nature, support potential use in stress-bearing posterior restorations, atraumatic restorative treatment (ART), and liners or bases in deep cavities, provided that adequate adhesion and long-term behavior are confirmed.(1, 16, 27) However, the absence of flexural strength data represents an important limitation, as flexural performance is highly relevant to the clinical loading conditions of GIC restorations. To address this, future work will incorporate three-point or biaxial flexural strength testing as part of a broader mechanical characterization of *Xylotrupes gideon*-derived microchitosan–modified GICs.

Further work is required before clinical adoption. Priority areas include: (i) *in vivo* assessment of pulp and tissue responses, (ii) long-term durability under occlusal load, thermocycling, and pH cycling, (iii) evaluation of cytotoxicity and potential allergic reactions, particularly in pediatric and medically compromised patients, (iv) detailed studies of fluoride-release kinetics under cariogenic challenges, and (v) optimization of handling and setting characteristics for clinical workflow.

Finally, the use of *Xylotrupes gideon*–derived microchitosan highlights an environmentally conscious approach to dental biomaterials. Insect-based chitosan offers a locally available, potentially less allergenic alternative to marine sources and integrates effectively into GIC matrices.(1, 16, 18) Microchitosan thus represents a sustainable modifier that can enhance the mechanical performance of GIC while reducing reliance on traditional marine-derived additives. Such improvements in mechanical properties are critical for the biomechanical performance of GIC restorations in the oral cavity. Comprehensive biocompatibility testing, biofunctional evaluation, and clinical trials are necessary to confirm the safety and effectiveness of this approach and to support its broader clinical implementation.

Q7.

Comment: The discussion on the decline in performance of the 2% group due to agglomeration is reasonable. However, providing SEM images of the fractured composite surfaces for different groups, would greatly enhance the manuscript.

Response: We fully agree that SEM analysis of fractured surfaces would provide valuable direct microstructural evidence for the proposed agglomeration and reinforcement mechanisms. However, we have not performed additional experimental work for this revision. Instead, we have:

1. Explicitly acknowledged in the Discussion (Mechanical Reinforcement subsection) that the inference of 2 % agglomeration is based on mechanical trends and known behaviour of over-loaded particle systems, and that fractographic SEM of fractured GIC–MCH specimens is currently lacking.
2. Added a sentence stating that fracture surface SEM analysis of the different MCH concentrations has been planned as part of a dedicated follow-up study, which will be submitted separately, focusing on microstructural–mechanical correlations.

Revision:

……..

The decline in mechanical performance observed at 2% MCH is consistent with the tendency of higher nanoparticle concentrations to promote agglomeration and disrupt uniform dispersion.(17) Conversely, very low concentrations (e.g., below 0.5%) may be insufficient to achieve effective reinforcement. In the present work, the interpretation that 2% MCH leads to increased agglomeration is therefore inferred from the mechanical trends and the known behavior of over-loaded particle-reinforced systems, rather than from direct microstructural evidence. Enhanced powder–liquid interactions during setting, driven by the high surface energy of MCH, likely contributed to favourable gelation and final structure at 1%, in line with observations that optimized powder–liquid interactions support improved mechanical outcomes.(32) MCH thus appears to improve hardness and mechanical durability while preserving essential GIC properties, including adhesion.(33) At the same time, the absence of fractographic SEM analysis of fractured GIC–MCH specimens in this study is a further limitation that prevents direct visualization of crack paths, particle distribution, and agglomerate formation at different MCH concentrations. A dedicated follow-up investigation, including fracture-surface SEM for all MCH loadings is planned to elucidate these microstructural–mechanical correlations more clearly.

……..

Q8.
Comment: The authors correctly note that biofunctional properties (antibacterial effect, fluoride release) were not assessed. The speculation in the discussion (Section 4.3) is informed by literature but should be tempered, as the study's own data cannot support these claims. A statement recommending this for future work would be appropriate here.

Response: Thank you for this very reasonable caution.

Revision:

4.3 Biofunctional Attributes and Fluoride Release

Biofunctional attributes, including antimicrobial potential, remineralization support, and tissue compatibility, are highly relevant to restorative materials. The present study did not evaluate antibacterial activity, fluoride release, or pulp/tissue responses, and no biological claims are made based on our data. However, previous studies have shown that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation,(18, 39, 40) and that *Xylotrupes gideon*–derived nanochitosan can exhibit antibacterial effects and support sustained fluoride release in experimental restorative formulations.(19, 41)

Notably, prior work indicates that incorporating nanochitosan into GIC does not compromise, and may even enhance, fluoride release, particularly under acidic conditions.(16, 25, 42) Chitosan's hydrogel-like network may modulate ion diffusion and support prolonged fluoride availability. Other reports have demonstrated that chitosan can promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammation when applied near pulp tissue.(43, 44) MCH's mucoadhesive behaviour may also improve marginal sealing *in vivo*.(45) Together, these findings suggest that chitosan-modified GICs could offer

multifunctional benefits, combining mechanical reinforcement with potential antibacterial and remineralizing effects; nevertheless, such biofunctional properties remain to be tested specifically for the present MCH-GIC formulation and should be addressed in future *in vitro* and *in vivo* studies.

Q9.

Comment: Contextualize the absolute values of the improved mechanical properties. For instance, how does a compressive strength of ~155 MPa compare to the requirements for permanent, stress-bearing restorations?

Response: We appreciate this clinically focused suggestion. In the discussion, under the mechanical reinforcement subsection, we mention the comparison with another paper about chitosan-added GIC.

Revision:

……

When compared with other reinforced GICs, the 1% MCH-GIC formulation (compressive strength 155.16 MPa) approaches the values reported for zirconia-reinforced GIC (160.91 MPa) and silver-alloy GIC (151.47 MPa).(21) These results exceed the ISO 9917-1:2016 minimum compressive strength threshold of 100 MPa for conventional restorative GICs.(36) Although the compressive strength remains below that of enamel (262 MPa) and dentin (234 MPa), and below values for some high-viscosity GICs (301 MPa), it is still well above the estimated stress generated by maximum bite forces (≈21.2 MPa assuming a 20 mm^2 contact area).(22, 37) Thus, the present MCH-GIC appears mechanically adequate for typical masticatory loading, and further optimization, such as combining MCH with high-viscosity GIC may provide additional safety margins.

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Xylotrupes Gideon Microchitosan-Modified Glass Ionomer Cement: *In Vitro* Assessment of Mechanical Properties

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Keywords: mechanical properties$_1$, compressive strength$_2$, diametral tensile strength$_3$, glass ionomer cement$_4$, hardness$_5$, microchitosan$_6$, *Xylotrupes gideon*$_7$

Abstract

Background: Glass ionomer cements (GIC) are valued as inherent fluoride-releasing dental restorative materials, while chitosan binds to negatively charged enamel surfaces, promoting mineral deposition and strengthening teeth. This study aimed to evaluate the mechanical properties of GIC modified with microchitosan derived from *Xylotrupes gideon*, using an *in vitro* experimental design. This study uniquely employs micro-scaled chitosan derived from the exoskeleton of Xylotrupes gideon, an insect-based, locally sourced, and environmentally sustainable alternative to conventional marine chitosan, to reinforce a conventional GIC.

Materials and Methods: Microchitosan was extracted from *Xylotrupes gideon* and incorporated into conventional GIC at 0.5%, 1% and 2% (w/w). Compressive strength, diametral tensile strength, and surface microhardness were measured using standard testing equipment after immersion in artificial saliva for 24 hours and 7 days. Statistical analysis was performed using one-way ANOVA followed by the Games-Howell post hoc test, with significance set at $p < 0.05$.

Results: The 1% microchitosan-modified GIC exhibited the most significant improvements compared to the unmodified control. After 7 days, compressive strength increased by 35.4%, diametral tensile strength by 51.3%, and surface hardness by 46.6% ($p<0.05$). These enhancements are attributed to microscale reinforcement and chemical bonding between microchitosan and the GIC matrix.

Conclusion: The addition of 1% microchitosan derived from *Xylotrupes gideon* significantly improved the mechanical performance of GIC. This bioactive reinforcement shows promising potential for clinical restorative applications, though further investigation into its long-term biocompatibility and fluoride release is warranted. These findings highlight a novel combination of insect-derived micro-scale chitosan and conventional GIC, yielding mechanical gains comparable to those reported for nanochitosan-modified formulations while relying on a more sustainable chitosan source.

1 Introduction

Dental composite materials, including glass ionomer cements (GICs), are widely recognized in restorative dentistry for their versatility, biocompatibility, and ability to bond chemically to dental hard tissues. GICs, in particular, are valued for their inherent fluoride release, which aids in the prevention of tooth decay and supports long-term tooth preservation.(1, 2) These advantages make GICs ideal for pediatric dentistry and minimally invasive restorations where fluoride release and adhesion are critical; however, their adhesive performance varies significantly across formulations.

A major drawback of traditional GICs is their weak mechanical properties, including low compressive and tensile strength, poor wear resistance, and susceptibility to fracture under occlusal stress. These limitations preclude their use in stress-bearing restorations, particularly in posterior regions that are subjected to high occlusal loads.(3) The other limitations are primarily attributed to the intrinsic chemistry of the GIC matrix, particularly the brittle nature of the glass phase and the sensitivity of the acid-base setting reaction to moisture and dehydration.(4) Recent technology developments, particularly involving micro-sized chitosan and titanium dioxide, have demonstrated improved filler dispersion and particle–matrix bonding in glass ionomer cements, resulting in enhanced mechanical properties, matrix cohesion, fracture resistance, and bioactivity (5, 6) with chitosan enhancing particle-matrix bonding and decreasing microvoid formation.(7)

Such reinforcement strategies yield structurally robust cement matrices characterized by minimized porosity and enhanced resistance to fracture propagation. Studies have shown that the incorporation of chitosan significantly enhances compressive and flexural strength, consistent with previous findings on quaternized chitosan-coated nanoparticles and carboxymethyl chitosan derivatives (8, 9) thereby improving matrix homogeneity and resistance to fracture propagation.

A previous study showed that the addition of chitosan to GIC has a positive effect on microhardness and improves surface roughness, making it a promising additive for dental restorative materials.(10) This material reinforces the structural framework and enhances mechanical reliability, which in turn leads to improved fracture resistance, increased microhardness, and enhanced overall durability.(11) The reinforcement effect demonstrated temporal dependence, with mechanical improvements more evident following extended maturation periods. Its ability to improve both the mechanical and biological properties of restorative materials makes it a compelling additive in dental biomaterials research. The molecular features of chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose], a versatile biopolymer, are chemically inert, non-toxic, and possess robust, broad antimicrobial activities due to its polycationic nature.(12) Characterized by amino and hydroxyl functional groups, chitosan enables ionic and hydrogen bonding that strengthens adhesion to dental substrates, improves mechanical strength through particle–matrix cohesion, and supports tissue compatibility via biocompatibility and regenerative potential (13) across a wide range of dental applications.

Chitosan exhibits remarkable physicochemical properties and good biocompatibility, forming bonds with human proteins, cells, and organs.(14) Chitosan exhibits multifunctionality in dental applications by contributing to mechanical reinforcement, promoting tissue regeneration, and acting as a bioactive agent in restorative formulations such as glass ionomer cements and adhesives. Recently, alternative sources of chitosan have emerged, including the exoskeletons of the *Xylotrupes gideon* beetle, which offer a sustainable and locally available biomaterial. Previous research indicates that this chitosan, derived from insects, enhances GIC performance in acidic oral environments (with critical pH levels), showing promise for clinical applications.(15) These advantages support further investigation of its mechanical reinforcement potential in dental composites. Research indicates that this novel form of chitosan demonstrates superior mechanical enhancement capabilities when integrated into GICs, including increased compressive strength and Vickers hardness.(16) Additionally, the microstructure improves particle dispersion and matrix interaction, which are critical for achieving homogeneity and minimizing microvoid formation during the setting process.(7, 17)

In addition to mechanical benefits, chitosan may confer secondary biofunctional advantages, which warrant future exploration but were not addressed in the present study.(18) These properties may enhance the clinical performance of restorations by reducing bacterial colonization, improving marginal integrity, and supporting the healing of the pulp-dentin interface, in line with recent advancements in oral health technology.(17) Notably, such modifications do not appear to compromise GIC's core biofunctional properties, such as fluoride ion release and adhesion to dentin.(1, 11)

Therefore, this study aimed to investigate the effect of incorporating *Xylotrupes gideon*-derived microchitosan (on a submicron–micron scale) into conventional GIC on its mechanical properties, including compressive strength, diametral tensile strength, and surface hardness. By using an insect exoskeleton as a locally available and environmentally sustainable source of chitosan, this approach has potential for clinical feasibility, long-lasting nature, and biological functionality through the integration of eco-friendly materials in restorative dentistry.(7)

2 Materials and Methods

2.1 Synthesis and Characterization of Microchitosan from *Xylotrupes gideon*

Microchitosan (MCH) was synthesized from the exoskeletons of *Xylotrupes gideon* beetles collected in Bogor, West Java, Indonesia. All body parts were detached and dried for 5 days, followed by extraction using established chitin-processing protocols: demineralization with 3N HCl, deproteinization with 3N NaOH, and deacetylation with 50% NaOH at 100 °C to obtain chitosan.

The resulting chitosan was converted into microform via ionic gelation with sodium tripolyphosphate (TPP) in deionized water, stirred at 2500 rpm for 4 hours, intentionally omitting acetic acid to improve biocompatibility. A particle size analyzer (Horiba, Japan) revealed a spherical morphology with a size distribution of 200-4000 nm, while SEM analysis also confirmed the morphology of the chitosan particles, as shown in Figure 1. The synthesized MCH was stored at 4 °C in airtight containers until use. (19)

The overall experimental workflow is illustrated in Figure 2, highlighting the synthesis of microchitosan from *Xylotrupes gideon* and its integration into glass ionomer cement for mechanical testing.

2.2 Preparation of Modified Glass Ionomer Cement

A commercially available Glass ionomer cement (Type IX Gold Label HS Posterior, GC, Tokyo, Japan) was used as the base material. The control group used unmodified GIC liquid, while the experimental groups consisted of 0.5%, 1%, and 2% (w/w) MCH added to the liquid component. Each modified liquid (0.5%, 1%, and 2% MCH) was vortex-mixed using the same standardized protocol immediately before mixing with the powder to improve homogeneity, acknowledging that achieving uniform dispersion becomes more challenging at higher MCH concentrations. Petroleum jelly was applied to the inner surfaces of the metal split mold. Then the mold was placed on a mylar strip, which was placed on a glass slab. The powder and liquid were dispensed on a paper pad and mixed manually according to the manufacturer's guidelines. After filling with the mixture, the mold was covered with another mylar strip, then covered with a second glass slab, and pressed for 30 seconds to extrude the excess material and obtain a uniformly smooth specimen surface. One hour after mixing, the specimens were removed from the mold, and any excess material was removed by dry grinding on both sides with 1000-grit silicon carbide paper. (1, 20, 21)

Compressive strength specimens were molded according to ISO 9917-1:2007: 3 mm diameter × 6 mm height, while the specimens for diametrical tensile strength: 6 mm diameter × 4 mm thickness, and Vickers hardness: 6 mm diameter × 4 mm thickness. Specimen dimensions were confirmed using a digital caliper (Mitutoyo 500-196-30, Japan). After initial setting, all specimens were stored in 100% humidity at room temperature for 1 hour. Compressive strength was calculated, in MPa, using the following equation: C = (4p)/(πd2) where p is the maximum force applied, in Newton; d is the average measured diameter of the specimen, in mm. The diametral tensile strength was also calculated in MPa, using the equation DTS = (2F)/(πdt), where F is the applied load, D is the diameter of the disk, and t is the thickness of the disk.(22)

2.3 Conditioning and Storage

A total of 80 specimens were prepared for each mechanical test and divided into four groups (n = 20 each), with each group further subdivided into two subgroups (n = 10) based on immersion time (24 hours and 7 days). The minimum specimen size was estimated using the Lemeshow formula for comparing two independent means:

$$n = 2\,\tau^2 (Z_{1-\alpha} + Z_{1-\beta})^2/(\mu_1 - \mu_2)^2$$

Where n is the minimum number of specimens per subgroup, μ_1 and μ_2 are the expected means from a previous study, τ is the common standard deviation, $Z_{1-\alpha}$ is the Z value for the selected significance level, and $Z_{1-\beta}$ is the Z value corresponding to the desired power.

A Fusayama-Meyer artificial saliva solution was prepared containing 0.4 g/L of NaCl, 0.4 g/L of KCl, 0.906 g/L of $CaCl_2 \cdot 2H_2O$, 0.690 g/L of $Na_2HPO_4 \cdot 2H_2O$, 0.005 g/L of $Na_2S \cdot 9H_2O$, and a pH of

6.75. Each specimen was immersed in 10 mL of artificial saliva in a sealed container and incubated at 37 °C.

2.4 Mechanical Testing

Mechanical properties were evaluated using a universal testing machine (Shimadzu, Japan) equipped with a 5 kN load cell, following validated protocols.(23) Compressive and diametral tensile strength tests were conducted at a crosshead speed of 1 mm/min. Surface hardness was measured using a Shimadzu HMV-G microhardness tester (Kyoto, Japan), employing a micro Vickers diamond indenter with a 100 g load applied for 10 seconds (24) All mechanical testing adhered to ISO 9917-1:2007 guidelines and methodological frameworks.

2.5 Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean and standard deviation, were calculated. Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was evaluated using Levene's test. When the assumption of homogeneity was violated, one-way ANOVA was followed by the Games–Howell post hoc test for pairwise comparisons. Pearson correlation coefficients were calculated to examine relationships among mechanical parameters. The minimum required specimen size per subgroup was determined a priori using the Lemeshow equation for comparing two means, yielding a specimen size of 7. To ensure adequate power and consistency with ISO 9917-1:2007 recommendations and previous GIC mechanical studies, which typically use 5–10 specimens per subgroup, we ultimately used n = 10 specimens per subgroup. A significance level of $p < 0.05$ was applied throughout.

3 Results

The compressive, diametral tensile strength, and hardness test results for all test groups are presented in Figures 3–6, while Table 1 presents the significance values for comparisons between groups. The data from 240 specimens (20 per group and 10 at different time points) were found to be normally distributed, as confirmed by the Kolmogorov–Smirnov test ($p = 0.200$). The addition of microchitosan (MCH) at all concentrations resulted in statistically significant increases in both compressive and diametral tensile strength compared to the control group ($p < 0.05$).

3.1 Compressive Strength

The compressive strength results are presented in Figure 3. At 24 hours, the control GIC group recorded a mean strength of 127.14 ± 15.82 MPa. Compared with the control, the 0.5% MCH group showed a slight reduction, whereas the 2% MCH group showed a numerically higher mean compressive strength (130.01 ± 14.40 MPa); however, this difference was not statistically significant ($p = 0.973$). The 1% MCH group was comparable to the control at this early time point.

After 7 days of immersion, the control group decreased to 114.63 ± 21.39 MPa. In contrast, all MCH groups showed higher values than the control. The highest compressive strength was observed in the 1% MCH group, which reached 155.16 ± 19.08 MPa ($p = 0.002$), representing a 35.4% increase over the 7-day control.

The corresponding compressive load-displacement curves (Figure 5, CS 24 h and CS 7 d) reveal predominantly linear elastic behavior, followed by abrupt failure in all groups, consistent with brittle,

cement-like fracture. At 24 hours, the slopes of the curves are broadly similar across groups, with only modest differences in peak load. After 7 days, the curves for all MCH groups shift towards higher peak loads compared with the control, with the 1% MCH group showing the highest peak load and largest displacement at failure, reflecting its superior strength and energy-absorbing capacity. The 0.5% MCH group exhibits the lowest peak load, while the 2% MCH and control curves lie between 0.5% and 1% MCH, mirroring the numerical ranking of compressive strength.

3.2 Diametral Tensile Strength

The diametral tensile strength results are presented in Figure 4. At 24 hours, the control group showed a mean strength of 11.41 ± 3.16 MPa. Compared with the control, the 0.5% MCH group showed significantly lower strength, whereas the 1% and 2% MCH groups did not differ significantly from the control.

After 7 days, the control group decreased to 7.09 ± 2.70 MPa, while all MCH groups showed higher values. The 1% MCH group had the highest diametral tensile strength (10.73 ± 1.89 MPa, $p = 0.014$), corresponding to a 51% increase relative to the 7-day control.

The diametral tensile load-displacement curves (Figure 5, DTS 24 h and DTS 7 d) corroborate these trends. At 24 hours, the control curve demonstrates the highest peak load, with the curves for the MCH groups clustered slightly below, reflecting the small or non-significant differences in DTS. By 7 days, the curves for all MCH-modified groups shift upward relative to the control, indicating higher peak loads at fracture. The 1% and 2% MCH groups in particular show steeper initial slopes and higher maxima than the control, whereas the 0.5% MCH curve occupies an intermediate position. This pattern is consistent with the 7-day DTS values, where 1% MCH provides the most pronounced reinforcement.

3.3 Vickers Hardness

The Vickers microhardness test results at 24 hours are given in Figure 6. The Control GIC had a microhardness mean value of 63.92 ± 15.17 VHN. The MCH-modified groups demonstrated significantly increased hardness, with a peak at 1% MCH (135.30 ± 17.90 VHN; $p < 0.001$).

All groups experienced a decrease in hardness after 7 days, while the 1% MCH maintained the highest residual value (46.95 ± 7.32 VHN, $p < 0.001$).

3.4 Trends and Correlation Analysis

The inclusion of microchitosan resulted in a consistent increase in mechanical properties, particularly at 1%. While the 2% group showed strong initial values, its performance declined slightly over time, suggesting an upper threshold of reinforcement.

Pearson correlation analysis revealed strong positive correlations between microchitosan concentration and compressive strength ($r = 0.59$, $p = 0.002$), diametral tensile strength ($r = 0.57$, $p = 0.004$), and hardness ($r = 0.53$, $p = 0.011$). Seven-day specimens of compressive strength and diametral tensile strength showed a positive correlation ($r = 0.71$, $p = 0.001$). Additionally, compressive strength and hardness were positively correlated ($r = 0.64$, $p = 0.008$), indicating an interconnected strengthening effect.

4 Discussion

4.1 Summary of Findings

This study demonstrated that incorporating *Xylotrupes gideon*–derived microchitosan (MCH) into a conventional GIC significantly improved its mechanical properties. The 1% MCH concentration produced the best results across all measurements compressive strength, diametral tensile strength, and surface hardness, particularly after 7 days of immersion in artificial saliva, indicating a time-dependent reinforcement effect.(25) The 0.5% MCH group showed only modest improvements, whereas the 2% MCH group, despite some initial enhancement, was likely more prone to particle agglomeration, which compromised its long-term stability.(6)

These findings align with previous research showing that chitosan can enhance the strength and durability of GICs and fit within the broader trend of nanomaterial-reinforced dental composites.(16) The superior performance of 1% MCH is consistent with reports that nanochitosan outperforms other nanostructures (e.g., nano-silica, nano-zirconia) in GICs, largely because of its ability to form hydrogen bonds with the polyacrylic acid matrix, thereby improving cohesion and ion exchange.(1) Our results confirm substantial gains in surface hardness and compressive strength compared with both unmodified GIC and GICs containing other nano-additives,(26) supporting the concept that small-particle chitosan is a promising modifier for stress-bearing restorations, especially in Class I and V cavities where fluoride release and biocompatibility are important. (27)

All groups exhibited a decrease in hardness after 7 days, consistent with previous work reporting a 40–45% decline in microhardness of conventional GICs following thermocycling. (28, 29) Notably, the 1% MCH group retained the highest residual hardness (46.95 VHN), consistent with the notion that reinforced GICs resist degradation better than unmodified formulations.

4.2 Mechanical Reinforcement

The mechanical enhancements observed with MCH-modified GIC can be explained by microscale dispersion and interfacial interactions between MCH and the GIC matrix. MCH particles (200–4000 nm) can occupy microvoids and irregularities, similarly to the homogenizing effect reported for nano-hydroxyapatite/chitosan composites (30) and microhybrid composites, resulting in a denser, more uniform structure that reduces crack initiation and propagation. In addition, the amino and hydroxyl groups of chitosan form hydrogen and ionic bonds with polyacrylic acid chains, increasing cross-link density and internal cohesion.(4, 17) In this way, microchitosan addresses microstructural weaknesses inherent to the acid–base setting mechanism and may support greater restoration durability.(4)

At early maturation (24 h), however, MCH particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disturbing optimal ionic cross-linking within the GIC matrix. This competition, together with the introduction of additional interfaces, may reduce initial network rigidity and create localized microvoids, which is consistent with the modest reductions or limited improvements in compressive and diametral tensile strength observed at 24 hours for some concentrations. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1% group.

Beyond reporting the overall particle size range of 200–4000 nm, more detailed particle size descriptors, such as the mean particle size, full size distribution profile, and polydispersity index, were not determined in this study. This represents a limitation of the current work, as such parameters would enable a more precise interpretation of how particle size, dispersion quality, and potential agglomeration contribute to the mechanical response of the different MCH concentrations. Future investigations will therefore include comprehensive physicochemical characterization of *Xylotrupes gideon*–derived microchitosan, including mean size, polydispersity, and surface charge, to better correlate these properties with the observed mechanical reinforcement of the GIC matrix.

The 1% concentration aligns with findings from previous studies, which have shown that 1–1.5% chitosan maximizes mechanical gains in GICs without agglomeration, underscoring the critical role of nanoparticle concentration in dental composites.(27) The good performance of 1% MCH is consistent with the general role of colloidal stability in such systems. Although zeta potential was not measured here, previous studies have shown that a high surface charge in chitosan-modified GICs improves nanoparticle dispersion, mechanical strength, and fluoride release. (25) In contrast, quaternized chitosan-coated nanoparticles have demonstrated superior interfacial bonding and more uniform filler distribution, supporting improved flexural strength and fracture resistance in reinforced GIC systems.(8) Together with reports on other nano-enhanced restorative formulations,(3) these findings suggest that colloidal stability is a key determinant of functional integration and mechanical performance.

Our findings on the increase in Vickers surface hardness with MCH addition are in agreement with earlier studies, which show that chitosan positively influences GIC microhardness.(10) Previous work comparing chitosan with hydroxyapatite revealed that chitosan not only preserved microhardness but was particularly effective in reducing surface roughness,(31) supporting our conclusion that incorporating micro-sized chitin derivatives from *Xylotrupes gideon* contributes to a more durable and homogeneous GIC matrix. Furthermore, the incorporation of carboxymethyl chitosan into the liquid phase of GIC has been shown to significantly enhance flexural strength, fracture toughness, and compressive strength at low concentrations, reinforcing the view that chitosan derivatives strengthen the GIC network via hydrogen bonding and improved interfacial cohesion.(9)

The decline in mechanical performance observed at 2% MCH is consistent with the tendency of higher nanoparticle concentrations to promote agglomeration and disrupt uniform dispersion. (17) Conversely, very low concentrations (e.g., below 0.5%) may be insufficient to achieve effective reinforcement. In the present work, the interpretation that 2% MCH leads to increased agglomeration is therefore inferred from the mechanical trends and the known behavior of over-loaded particle-reinforced systems, rather than from direct microstructural evidence. Enhanced powder–liquid interactions during setting, driven by the high surface energy of MCH, likely contributed to favorable gelation and final structure at 1%, in line with observations that optimized powder–liquid interactions support improved mechanical outcomes.(32) MCH thus appears to improve hardness and mechanical durability while preserving essential GIC properties, including adhesion.(33) At the same time, the absence of fractographic SEM analysis of fractured GIC–MCH specimens in this study is a further limitation that prevents direct visualization of crack paths, particle distribution, and agglomerate formation at different MCH concentrations. A dedicated follow-up investigation, including fracture-surface SEM for all MCH loadings is planned to elucidate these microstructural–mechanical correlations more clearly.

Previous work has shown that resin coatings can significantly enhance the viscoelastic behavior and environmental resistance of highly viscous GICs.(34) While such strategies focus on external surface protection, our results suggest that internal reinforcement with chitosan may offer comparable benefits by increasing matrix cohesion and long-term mechanical stability without necessitating additional coating layers. In another study, shrimp shell–derived chitosan combined with hydrophilic fibers yielded a composite with compressive strength of 32.67 MPa and tensile strength of 17.18 MPa.(35) Although their tensile strength values were higher, our 1% MCH–GIC formulation achieved a much higher compressive strength (155.16 MPa),

When compared with other reinforced GICs, the 1% MCH-GIC formulation (compressive strength 155.16 MPa) approaches the values reported for zirconia-reinforced GIC (160.91 MPa) and silver-alloy GIC (151.47 MPa).(21) These results exceed the ISO 9917-1:2016 minimum compressive strength threshold of 100 MPa for conventional restorative GICs.(36) Although the compressive strength remains below that of enamel (262 MPa) and dentin (234 MPa), and below values for some high-viscosity GICs (301 MPa), it is still well above the estimated stress generated by maximum bite forces (≈21.2 MPa, assuming a 20 mm^2 contact area).(22, 37) Thus, the present MCH-GIC appears mechanically adequate for typical masticatory loading, and further optimization, such as combining MCH with high-viscosity GIC may provide additional safety margins.

In addition to bulk mechanical strengthening, chitosan has been reported to enhance the shear bond strength of GIC to dentin,(38) which is critical for long-term clinical success. While bonding behavior was not evaluated in this study, it is plausible that the microchitosan used could confer similar benefits; this will be addressed in future work.

4.3 Biofunctional Attributes and Fluoride Release

Biofunctional attributes, including antimicrobial potential, remineralization support, and tissue compatibility, are highly relevant to restorative materials. The present study did not evaluate antibacterial activity, fluoride release, or pulp/tissue responses, and no biological claims are made based on our data. However, previous studies have shown that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation,(18, 39, 40) and that *Xylotrupes gideon*–derived nanochitosan can exhibit antibacterial effects and support sustained fluoride release in experimental restorative formulations.(19, 41)

Notably, prior work indicates that incorporating nanochitosan into GIC does not compromise fluoride release and may even enhance it, particularly under acidic conditions.(16, 25, 42) Chitosan's hydrogel-like network may modulate ion diffusion and support prolonged fluoride availability. Other reports have demonstrated that chitosan can promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammation when applied near pulp tissue.(43, 44) MCH's mucoadhesive behavior may also improve marginal sealing *in vivo*.(45) Together, these findings suggest that chitosan-modified GICs could offer multifunctional benefits, combining mechanical reinforcement with potential antibacterial and remineralizing effects; nevertheless, such biofunctional properties remain to be tested specifically for the present MCH-GIC formulation and should be addressed in future *in vitro* and *in vivo* studies.

4.4 Clinical Implications and Future Directions

Within the limitations of this *in vitro* study, *Xylotrupes gideon*–derived microchitosan appears to be a promising internal reinforcement for conventional GIC, particularly at a 1% loading. The observed

improvements in strength and hardness, together with the material's fluoride-releasing nature, support potential use in stress-bearing posterior restorations, atraumatic restorative treatment (ART), and liners or bases in deep cavities, provided that adequate adhesion and long-term behavior are confirmed. (1, 16, 27) However, the absence of flexural strength data represents an important limitation, as flexural performance is highly relevant to the clinical loading conditions of GIC restorations. To address this, future work will incorporate three-point or biaxial flexural strength testing as part of a broader mechanical characterization of *Xylotrupes gideon*-derived microchitosan–modified GICs.

Further work is required before clinical adoption. Priority areas include: (i) *in vivo* assessment of pulp and tissue responses, (ii) long-term durability under occlusal load, thermocycling, and pH cycling, (iii) evaluation of cytotoxicity and potential allergic reactions, particularly in pediatric and medically compromised patients, (iv) detailed studies of fluoride-release kinetics under cariogenic challenges, and (v) optimization of handling and setting characteristics for clinical workflow.

Finally, the use of *Xylotrupes gideon*–derived microchitosan highlights an environmentally conscious approach to dental biomaterials. Insect-based chitosan offers a locally available, potentially less allergenic alternative to marine sources and integrates effectively into GIC matrices.(1, 16, 18) Microchitosan thus represents a sustainable modifier that can enhance the mechanical performance of GIC while reducing reliance on traditional marine-derived additives. Such improvements in mechanical properties are critical for the biomechanical performance of GIC restorations in the oral cavity. Comprehensive biocompatibility testing, biofunctional evaluation, and clinical trials are necessary to confirm the safety and effectiveness of this approach and to support its broader clinical implementation.

Conclusion

Incorporating 1% microchitosan (MCH) from *Xylotrupes gideon* into glass ionomer cement (GIC) significantly enhanced its mechanical properties after 7 days of maturation in artificial saliva. This modification resulted in a 35% increase in compressive strength, a 51% improvement in tensile strength, and 46% increase in surface hardness. Although the 2% MCH group initially showed strong values, its performance declined over time, suggesting an upper threshold of reinforcement. This reduced performance was likely due to particle agglomeration, which undermined long-term stability. A 0.5% formulation produced inconclusive results, the optimized 1% formulation supports minimally invasive dentistry by prolonging restoration longevity and preserving tooth structure.

5 Ethics Approval

Ethical approval for this study was obtained from the Health Research Ethics Committee of the Faculty of Dentistry, Universitas Trisakti (Approval No: 006/Dosen/KEPK/FKG/06/2021).

6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

7 Author Contributions

AC: Writing – review and editing. CDM: Validation, Writing – original draft. DP: Investigation, Writing – original draft. EE: Supervision, Writing – original draft. FLK: Investigation, Methodology,

Writing – original draft. IG: Formal Analysis, Writing – original draft. KK: Resources, Writing – original draft. RT: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review and editing. SKW: Writing – review and editing. TSP: Visualization, Writing – original draft.

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530 **Table**

531 **Table 1.** Games-Howell significant value (*p*) for 24 hours (white) and 7 days (grey) compressive
532 strength, diametral tensile strength and Vickers microhardness.

Compressive strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.223	0.002*	0.032*	7 days
MCH 0.5%	0.707		0.020*	0.473	
MCH 1%	0.671	0.234		0.155	
MCH 2%	0.973	0.476	0.848		
		24 hours			
Diametral Tensile Strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.133	0.014*	0.025*	7 days
MCH 0.5%	0.012*		0.674	0.825	
MCH 1%	0.445	0.118		0.992	
MCH 2%	0.900	0.000*	0.040*		
		24 hours			
Vickers Microhardness	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		1.000	0.000*	0.341	7 days
MCH 0.5%	0.000*		0.038*	0.913	
MCH 1%	0.000*	0.719		0.003*	
MCH 2%	0.000*	0.882	0.999		
		24 hours			

533 * Significant difference between groups with $p < 0,05$

534

Figure captions

Figure 1. Scanning electron microscope images of microchitosan from *Xylotrupes gideon*. A. 10,000× magnification. B. 2,000× magnification.

Figure 2. Experimental workflow illustrating the synthesis of microchitosan from *Xylotrupes gideon* and its incorporation into glass ionomer cement for mechanical testing.

Figure 3. Compressive strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (**) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$).

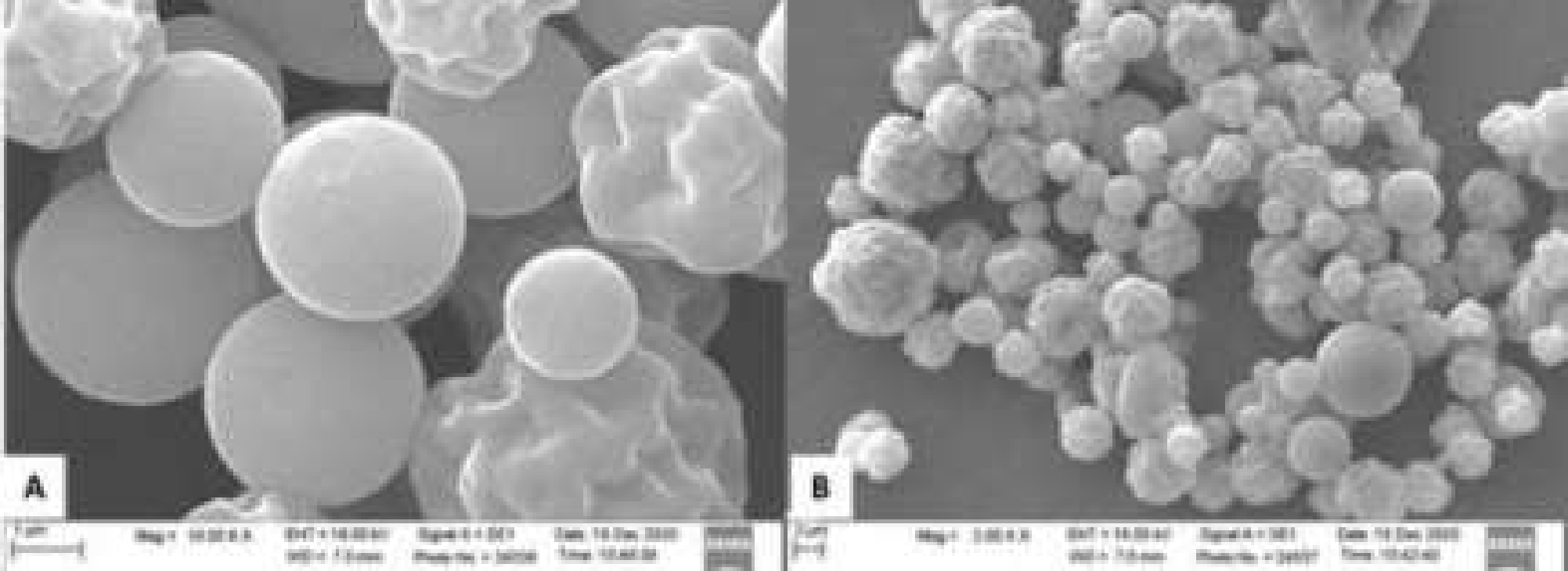
Figure 4. Diametral tensile strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (*,**) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$).

Figure 5. Representative load-displacement (stress–strain–type) curves for compressive strength (CS) and diametral tensile strength (DTS) tests of control and microchitosan-modified GICs (0.5%, 1%, and 2% MCH) at 24 hours and 7 days. All specimens were tested under monotonic loading to failure. The upward shift in the curves at 7 days, particularly in the 1% MCH group, reflects increased peak load and overall mechanical performance compared with the control.

Figure 6. Vickers surface hardness (VHN) of microchitosan-modified glass ionomer cement (GIC) formulations following 24-hour and 7-day immersion in artificial saliva. Asterisks (*,**) show significant differences among the groups with ANOVA and post hoc Games-Howell test ($p<0.05$). The data clearly show that the 1% microchitosan group exhibited the highest hardness values at both time points.

Data Availability Statement

Data may be shared on a reasonable request to the corresponding author.


A
B

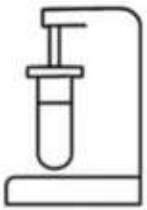
Exoskeleton drying

Demineralization
Deproteinization
Deacetylation

Chitosan

Ionic Gelation

Microchitosan
(MCH)



Samples of Modified
Glass Ionomer Cement

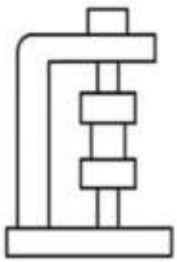
Control

0.5% (w/w) MCH

1 % (w/w) MCH

2 % (w/w) MCH

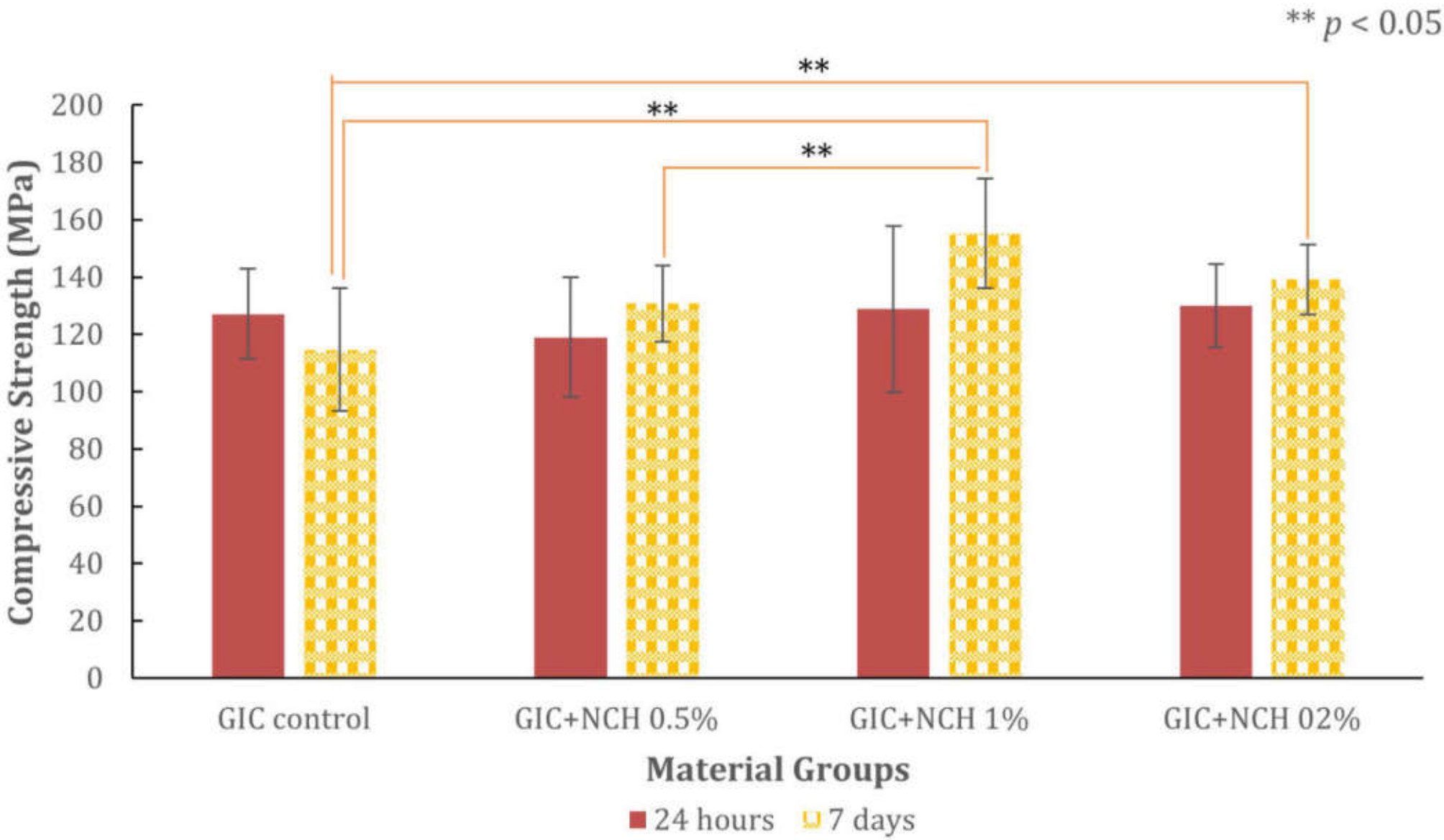
Incubation in Fusayama
Meyer Artificial Saliva
24 h and 7 d

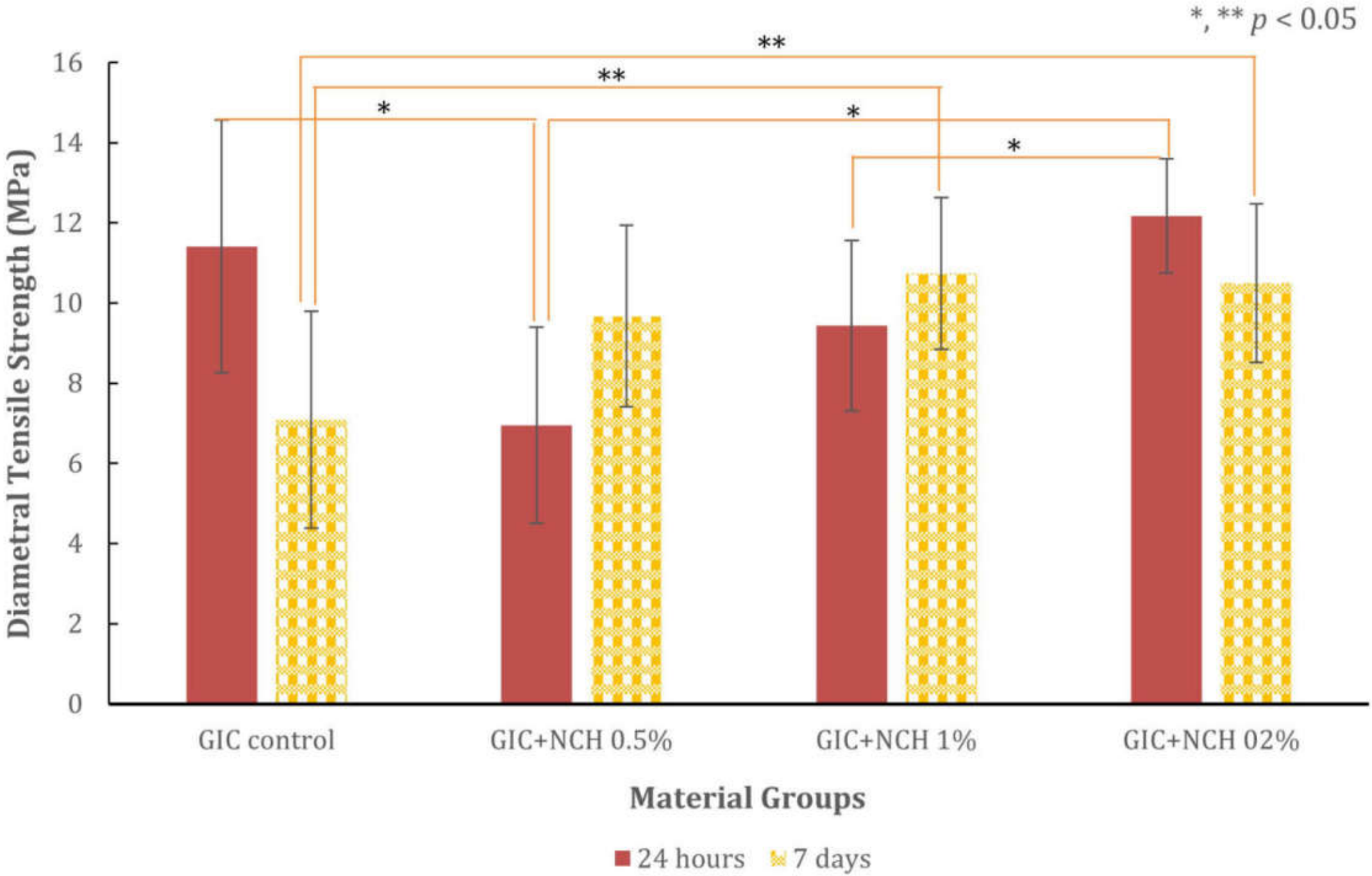


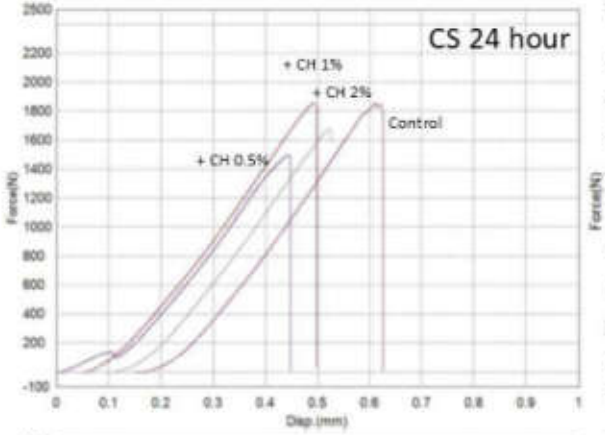
Compressive strength
Ø 3mm, t 6mm

Diametral tensile
strength
Ø 6 mm, t 4 mm

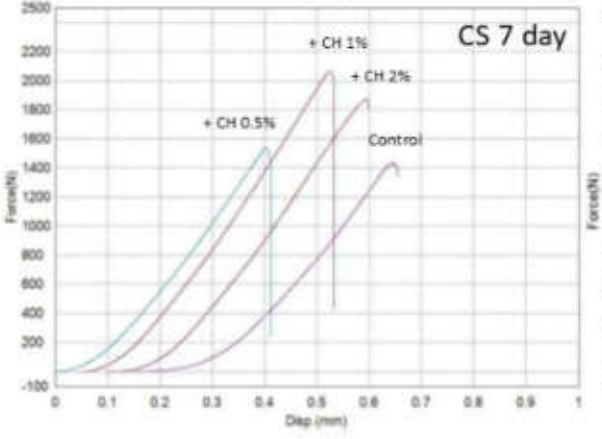
Vickers Micro Hardness
Ø 6 mm, t 4 mm

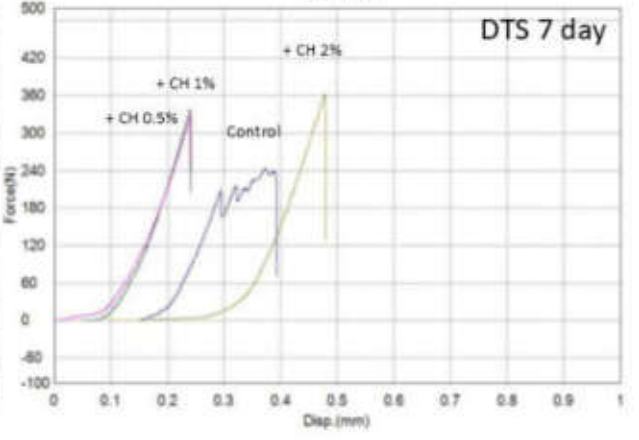

** $p < 0.05$
**
**
**
Compressive Strength (MPa)
200
180
160
140
120
100
80
60
40
20
0
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days

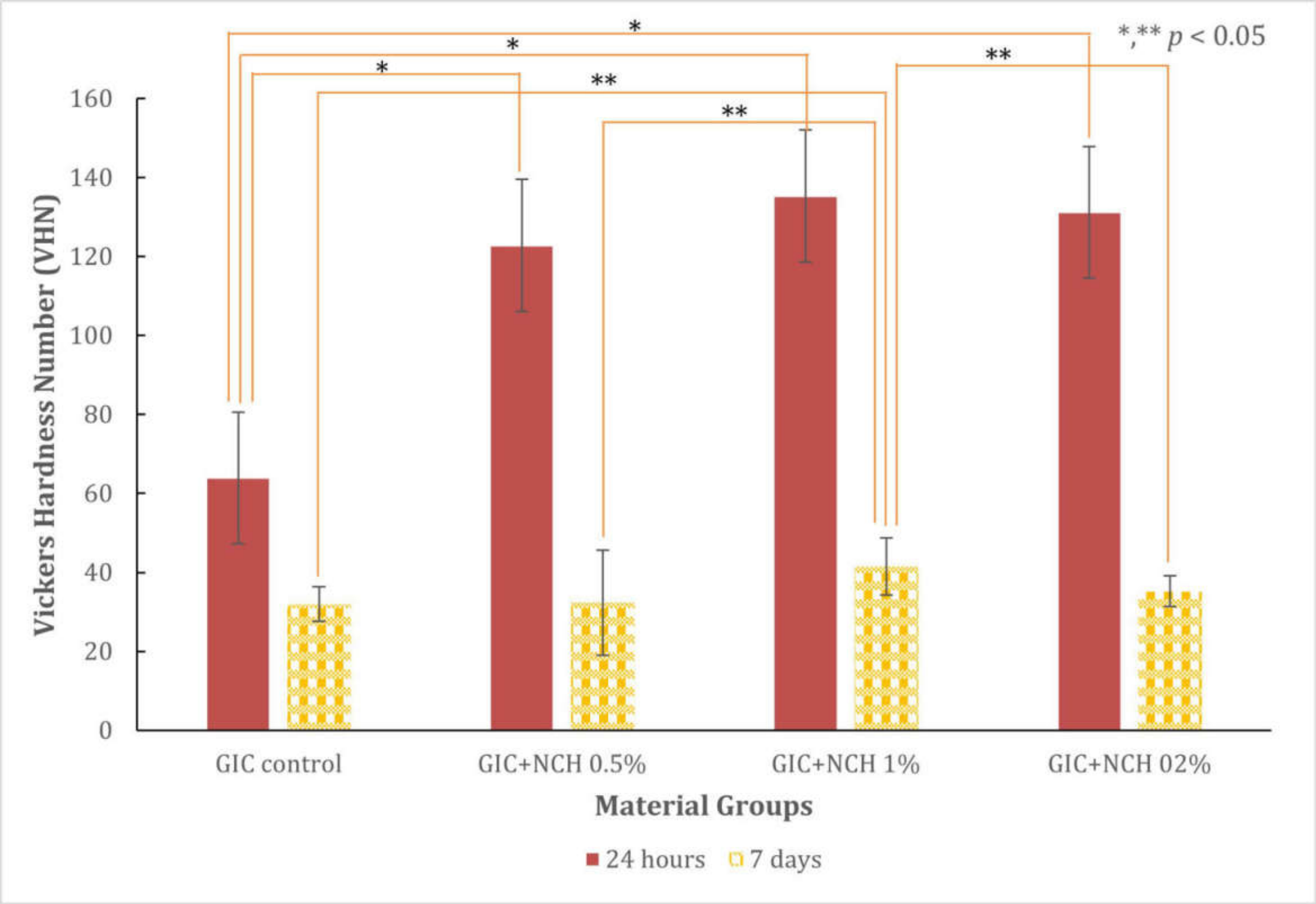

*, ** p < 0.05
**
**
*
*
*
16
14
12
10
8
6
4
2
0
Diametral Tensile Strength (MPa)
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days


CS 24 hour
+ CH 1%
+ CH 2%
Control
+ CH 0.5%
Force(N)
Disp.(mm)


DTS 24 hour
Control
+ CH 2%
+ CH 1%
+ CH 0.5%
Force(N)
Disp.(mm)


CS 7 day
+ CH 1%
+ CH 2%
+ CH 0.5%
Control
Force(N)
Disp.(mm)


DTS 7 day
+ CH 2%
+ CH 1%
+ CH 0.5%
Control
Force(N)
Disp.(mm)


*,** p < 0.05
*
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160
140
120
100
80
60
40
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0
Vickers Hardness Number (VHN)
GIC control
GIC+NCH 0.5%
GIC+NCH 1%
GIC+NCH 02%
Material Groups
24 hours
7 days

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	visible on your article. If you wish to make any changes, kindly provide the exact revised Alt-Text you would like to use, ensuring that the word-count remains at approximately 100 words for best accessibility results. **Further information on Alt-Text can be found** here. **Figure 1 Alt-Text** – Scanning electron microscope images showing polymer particles. Image A highlights large, smooth spherical particles at ten thousand times magnification. Image B depicts a mixture of smaller, rougher textured and smooth spherical particles at two thousand times magnification. **Figure 2 Alt-Text** – Flowchart illustrating the preparation and testing of modified glass ionomer cement. Starting with \"Xylotrupes gideon\" exoskeleton drying, it proceeds through demineralization, deproteinization, and deacetylation to obtain chitosan. Ionic gelation forms microchitosan (MCH). Samples of modified glass ionomer cement are tested with 0.5%, 1%, and 2% (w/w) MCH. They undergo incubation in Fusayama Meyer artificial saliva for twenty-four hours and seven days. Tests include compressive strength, diametral tensile strength, and Vickers microhardness, with specified diameters and thicknesses for each test. **Figure 3 Alt-Text** – Bar chart showing compressive strength in MPa of GIC control and GIC with varying NCH percentages at 24 hours and 7 days. GIC+NCH 1% shows the highest strength. Significant differences, denoted by asterisks, indicate $p < 0.05$. **Figure 4 Alt-Text** – Bar chart showing diametral tensile strength (in MPa) for various material groups: GIC control, GIC+NCH 0. 5%, 1%, and 2%. Strength measured at 24 hours (red bars) and 7 days (yellow patterned bars). Error bars indicate variability. Significant differences are marked with asterisks for p-values less than 0.05. **Figure 5 Alt-Text** – Four graphs compare the force versus displacement for different concentrations of CH additives over time. Top left: CS 24 hour shows increasing force with higher CH concentrations. Top right: DTS 24 hour shows similar trends with lower force values. Bottom left: CS 7 day continues the pattern with increased force after seven days. Bottom right: DTS 7 day also shows the increase but with less force compared to CS graphs. Each graph includes control and CH 0.5%, 1%, and 2% lines. **Figure 6 Alt-Text** – Bar chart comparing Vickers Hardness Number (VHN) across material groups: GIC control, GIC+NCH 0.5%, GIC+NCH 1%, and GIC+NCH 2%, measured at 24 hours and 7 days. Significant differences are indicated with asterisks for $p < 0.05$.	Figure 2: Flowchart illustrating the preparation and testing of modified glass ionomer cement (GIC). Starting with *Xylotrupes gideon* exoskeleton drying, it proceeds through demineralization, deproteinization, and deacetylation to obtain chitosan. Ionic gelation forms microchitosan (MCH). Samples of modified GIC are tested with 0.5%, 1%, and 2% (w/w) MCH addition. They undergo incubation in Fusayama Meyer artificial saliva for twenty-four hours and seven days. Tests include compressive strength, diametral tensile strength and Vickers microhardness, with specified diameters and thickness for each test. Figure 3: Please change as follows: Bar chart showing compressive strength in MPa of GIC control and GIC with varying MCH percentages at 24 hours and 7 days. GIC + MCH 1% shows the highest strength. Significant difference, denoted by asterisks (**) indicate p<0.05 with ANOVA and *post hoc* GamesHowell Figure 4: Bar chart showing diametral tensile strength (in MPa) for GIC control, GIC+MCH 0.5%, 1% and 2%. Strength measured at 24 hours (red bars) and 7 days (yellow patterned bars). Error bars indicate variability. Significant differences are marked with asterisks (*,**) for p-values less than 0.05 analyze with ANOVA and *post hoc* Games Howell. Figure 5: Four graphs compare the force versus displacement for different concentrations of CH additives over time. All specimens were tested under monotonic loading to failure. Top left: CS 24 hour shows increasing force with higher CH concentrations. Top right: DTS 24 hour shows similar trends with lower force values. Bottom left: CS 7 day continues the pattern with increased force after seven days. Bottom right: DTS 7 day also shows the increase but with less force compared to CS graphs. The upward shift in the curves at 7 days, particularly in the 1% MCH group, reflects increased peak load and overall mechanical performance compared with the control Figure 6: Bar chart comparing Vickers Hardness Number (VHN) across material groups: GIC Control, GIC + MCH 0,5%, GIC + MCH 1%, GIC + MCH 2%, measured at 24 hours and 7 days. Significant differences are indicated with asterisks for $p < 0.05$ using ANOVA and *post hoc* Games-Howell. The data clearly show that 1% microchitosan group exhibited the highest hardness values at both time points

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Xylotrupes gideon microchitosan-modified glass ionomer cement: *in vitro* assessment of mechanical properties

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Background: Glass ionomer cements (GIC) are valued as inherent fluoride-releasing dental restorative materials, while chitosan binds to negatively charged enamel surfaces, promoting mineral deposition and strengthening teeth. This study aimed to evaluate the mechanical properties of GIC modified with microchitosan derived from *Xylotrupes gideon*, using an *in vitro* experimental design. This study uniquely employs micro-scaled chitosan derived from the exoskeleton of Xylotrupes gideon, an insect-based, locally sourced, and environmentally sustainable alternative to conventional marine chitosan, to reinforce a conventional GIC.

Materials and methods: Microchitosan was extracted from *Xylotrupes gideon* and incorporated into conventional GIC at 0.5%, 1% and 2% (w/w). Compressive strength, diametral tensile strength, and surface microhardness were measured using standard testing equipment after immersion in artificial saliva for 24 h and 7 days. Statistical analysis was performed using one-way ANOVA followed by the Games-Howell *post hoc* test, with significance set at $p < 0.05$.

Results: The 1% microchitosan-modified GIC exhibited the most significant improvements compared to the unmodified control. After 7 days, compressive strength increased by 35.4%, diametral tensile strength by 51.3%, and surface hardness by 46.6% ($p < 0.05$). These enhancements are attributed to microscale reinforcement and chemical bonding between microchitosan and the GIC matrix.

Conclusion: The addition of 1% microchitosan derived from *Xylotrupes gideon* significantly improved the mechanical performance of GIC. This bioactive reinforcement shows promising potential for clinical restorative applications, though further investigation into its long-term biocompatibility and fluoride release is warranted. These findings highlight a novel combination of insect-derived micro-scale chitosan and conventional GIC, yielding mechanical gains comparable to those reported for nanochitosan-modified formulations while relying on a more sustainable chitosan source.

KEYWORDS

mechanical properties, compressive strength, diametral tensile strength, glassionomer cement, hardness, microchitosan, *Xylotrupes gideon*

1 Introduction

Dental composite materials, including glass ionomer cements (GICs), are widely recognized in restorative dentistry for their versatility, biocompatibility, and ability to bond chemically to dental hard tissues. GICs, in particular, are valued for their inherent fluoride release, which aids in the prevention of tooth decay and supports long-term tooth preservation (1, 2). These advantages make GICs ideal for pediatric dentistry and minimally invasive restorations where fluoride release and adhesion are critical; however, their adhesive performance varies significantly across formulations.

A major drawback of traditional GICs is their weak mechanical properties, including low compressive and tensile strength, poor wear resistance, and susceptibility to fracture under occlusal stress. These limitations preclude their use in stress-bearing restorations, particularly in posterior regions that are subjected to high occlusal loads (3). The other limitations are primarily attributed to the intrinsic chemistry of the GIC matrix, particularly the brittle nature of the glass phase and the sensitivity of the acid-base setting reaction to moisture and dehydration (4). Recent technology developments, particularly involving micro-sized chitosan and titanium dioxide, have demonstrated improved filler dispersion and particle–matrix bonding in glass ionomer cements, resulting in enhanced mechanical properties, matrix cohesion, fracture resistance, and bioactivity (5, 6) with chitosan enhancing particle-matrix bonding and decreasing microvoid formation (7).

Such reinforcement strategies yield structurally robust cement matrices characterized by minimized porosity and enhanced resistance to fracture propagation. Studies have shown that the incorporation of chitosan significantly enhances compressive and flexural strength, consistent with previous findings on quaternized chitosan-coated nanoparticles and carboxymethyl chitosan derivatives (8, 9) thereby improving matrix homogeneity and resistance to fracture propagation.

A previous study showed that the addition of chitosan to GIC has a positive effect on microhardness and improves surface roughness, making it a promising additive for dental restorative materials (10). This material reinforces the structural framework and enhances mechanical reliability, which in turn leads to improved fracture resistance, increased microhardness, and enhanced overall durability (11). The reinforcement effect demonstrated temporal dependence, with mechanical improvements more evident following extended maturation periods. Its ability to improve both the mechanical and biological properties of restorative materials makes it a compelling additive in dental biomaterials research. The molecular features of chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose], a versatile biopolymer, are chemically inert, non-toxic, and possess robust, broad antimicrobial activities due to its polycationic nature (12). Characterized by amino and hydroxyl functional groups, chitosan enables ionic and hydrogen bonding that strengthens adhesion to dental substrates, improves mechanical strength through particle–matrix cohesion, and supports tissue compatibility via biocompatibility and regenerative potential (13) across a wide range of dental applications.

Chitosan exhibits remarkable physicochemical properties and good biocompatibility, forming bonds with human proteins, cells, and organs (14). Chitosan exhibits multifunctionality in dental applications by contributing to mechanical reinforcement, promoting tissue regeneration, and acting as a bioactive agent in restorative formulations such as glass ionomer cements and adhesives. Recently, alternative sources of chitosan have emerged, including the exoskeletons of the *Xylotrupes gideon* beetle, which offer a sustainable and locally available biomaterial. Previous research indicates that this chitosan, derived from insects, enhances GIC performance in acidic oral environments (with critical pH levels), showing promise for clinical applications (15). These advantages support further investigation of its mechanical reinforcement potential in dental composites. Research indicates that this novel form of chitosan demonstrates superior mechanical enhancement capabilities when integrated into GICs, including increased compressive strength and Vickers hardness (16). Additionally, the microstructure improves particle dispersion and matrix interaction, which are critical for achieving homogeneity and minimizing microvoid formation during the setting process (7, 17).

In addition to mechanical benefits, chitosan may confer secondary biofunctional advantages, which warrant future exploration but were not addressed in the present study (18). These properties may enhance the clinical performance of restorations by reducing bacterial colonization, improving marginal integrity, and supporting the healing of the pulp-dentin interface, in line with

recent advancements in oral health technology (17). Notably, such modifications do not appear to compromise GIC's core biofunctional properties, such as fluoride ion release and adhesion to dentin (1, 11).

Therefore, this study aimed to investigate the effect of incorporating *Xylotrupes gideon*-derived microchitosan (on a submicron–micron scale) into conventional GIC on its mechanical properties, including compressive strength, diametral tensile strength, and surface hardness. By using an insect exoskeleton as a locally available and environmentally sustainable source of chitosan, this approach has potential for clinical feasibility, long-lasting nature, and biological functionality through the integration of eco-friendly materials in restorative dentistry (7).

2 Materials and methods

2.1 Synthesis and characterization of microchitosan from *xylotrupes gideon*

Microchitosan (MCH) was synthesized from the exoskeletons of *Xylotrupes gideon* beetles collected in Bogor, West Java, Indonesia. All body parts were detached and dried for 5 days, followed by extraction using established chitin-processing protocols: demineralization with 3N HCl, deproteinization with 3N NaOH, and deacetylation with 50% NaOH at 100°C to obtain chitosan.

The resulting chitosan was converted into microform via ionic gelation with sodium tripolyphosphate (TPP) in deionized water, stirred at 2,500 rpm for 4 h, intentionally omitting acetic acid to improve biocompatibility. A particle size analyzer (Horiba, Japan) revealed a spherical morphology with a size distribution of 200–4,000 nm, while SEM analysis also confirmed the morphology of the chitosan particles, as shown in Figure 1. The synthesized MCH was stored at 4°C in airtight containers until use (19).

The overall experimental workflow is illustrated in Figure 2, highlighting the synthesis of microchitosan from *Xylotrupes gideon* and its integration into glass ionomer cement for mechanical testing.

2.2 Preparation of modified glass ionomer cement

A commercially available Glass ionomer cement (Type IX Gold Label HS Posterior, GC, Tokyo, Japan) was used as the base material. The control group used unmodified GIC liquid, while the experimental groups consisted of 0.5%, 1%, and 2% (w/w) MCH added to the liquid component. Each modified liquid (0.5%, 1%, and 2% MCH) was vortex-mixed using the same standardized protocol immediately before mixing with the powder to improve homogeneity, acknowledging that achieving uniform dispersion becomes more challenging at higher MCH concentrations. Petroleum jelly was applied to the inner surfaces of the metal split mold. Then the mold was placed on a mylar strip, which was placed on a glass slab. The powder and liquid were dispensed on a paper pad and mixed manually according to the manufacturer's guidelines. After filling with the mixture, the mold was covered with another mylar strip, then covered with a second glass slab, and pressed for 30 s to extrude the excess material and obtain a uniformly smooth specimen surface. One hour after mixing, the specimens were removed from the mold, and any excess material was removed by dry grinding on both sides with 1,000-grit silicon carbide paper (1, 20, 21).

Compressive strength specimens were molded according to ISO 9917-1:2007: 3 mm diameter×6 mm height, while the specimens for diametrical tensile strength: 6 mm diameter × 4 mm thickness, and Vickers hardness: 6 mm diameter × 4 mm thickness. Specimen dimensions were confirmed using a digital caliper (Mitutoyo 500-196-30, Japan). After initial setting, all specimens were stored in 100% humidity at room temperature for 1 h. Compressive strength was calculated, in MPa, using the following equation: $C = (4p)/(\pi d2)$ where p is the maximum force applied, in Newton;

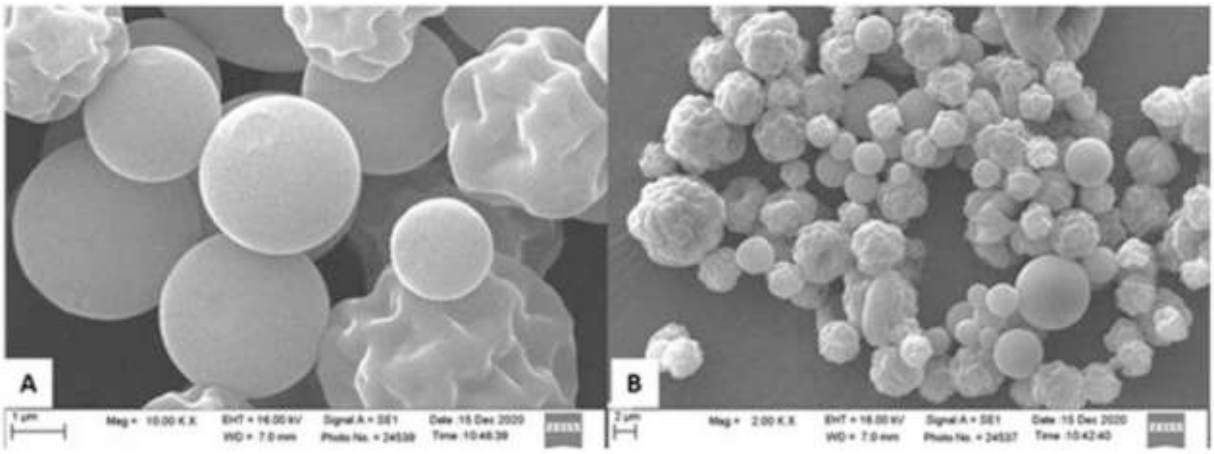

FIGURE 1
Scanning electron microscope images of microchitosan from *Xylotrupes gideon*. (A) 10,000× magnification. (B) 2,000× magnification.

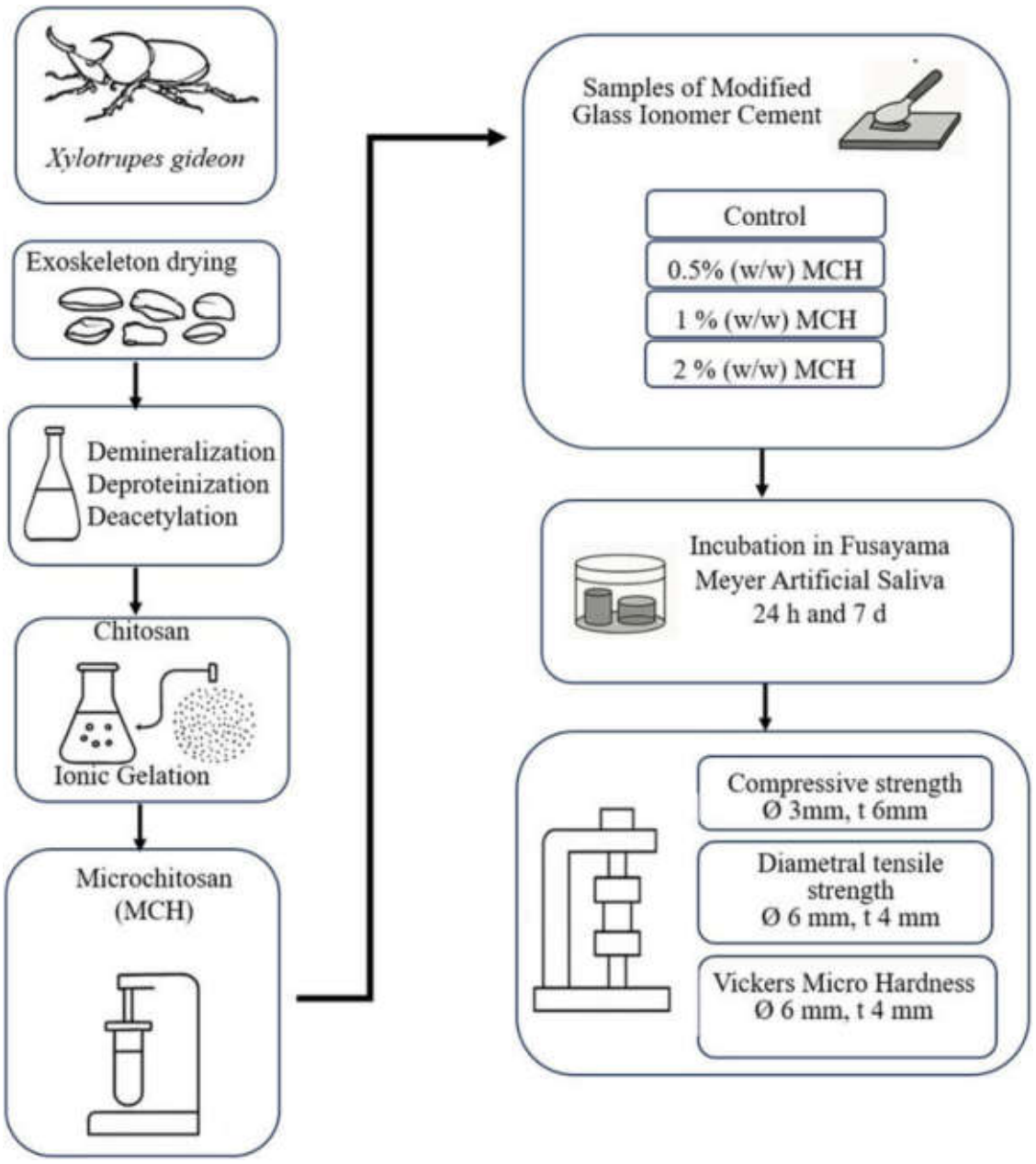

FIGURE 2
Experimental workflow illustrating the synthesis of microchitosan from *xylotrupes gideon* and its incorporation into glass ionomer cement for mechanical testing.

d is the average measured diameter of the specimen, in mm. The diametral tensile strength was also calculated in MPa, using the equation DTS = (2F)/(πdt), where F is the applied load, D is the diameter of the disk, and t is the thickness of the disk (22).

2.3 Conditioning and storage

A total of 80 specimens were prepared for each mechanical test and divided into four groups ($n = 20$ each), with each group further subdivided into two subgroups ($n = 10$) based on immersion time (24 h and 7 days). The minimum specimen size was estimated using the Lemeshow formula for comparing two independent means:

$$n = 2t^2(Z_{1-\alpha} + Z_{1-\beta})^2/(\mu_1 - \mu_2)^2$$

Where n is the minimum number of specimens per subgroup, μ_1 and μ_2 are the expected means from a previous study, τ is the common standard deviation, $Z_{1-\alpha}$ is the Z value for the selected significance level, and $Z_{1-\beta}$ is the Z value corresponding to the desired power.

A Fusayama-Meyer artificial saliva solution was prepared containing 0.4 g/L of NaCl, 0.4 g/L of KCl, 0.906 g/L of $CaCl_2 \cdot 2H_2O$, 0.690 g/L of $Na_2HPO_4 \cdot 2H_2O$, 0.005 g/L of $Na_2S \cdot 9H_2O$, and a pH of 6.75. Each specimen was immersed in 10 mL of artificial saliva in a sealed container and incubated at 37°C.

2.4 Mechanical testing

Mechanical properties were evaluated using a universal testing machine (Shimadzu, Japan) equipped with a 5 kN load cell, following validated protocols (23). Compressive and diametral tensile strength tests were conducted at a crosshead speed of 1 mm/min. Surface hardness was measured using a Shimadzu HMV-G microhardness tester (Kyoto, Japan), employing a micro Vickers diamond indenter with a 100 g load applied for 10 s (24). All mechanical testing adhered to ISO 9917-1:2007 guidelines and methodological frameworks.

2.5 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean and standard deviation, were calculated. Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was evaluated using Levene's test. When the assumption of homogeneity was violated, one-way ANOVA was followed by the Games–Howell *post hoc* test for pairwise comparisons. Pearson correlation coefficients were calculated to examine relationships among mechanical parameters. The minimum required specimen size per subgroup was determined *a priori* using the Lemeshow equation for comparing two means, yielding a specimen size of 7. To ensure adequate power and consistency with ISO 9917-1:2007 recommendations and previous GIC mechanical studies, which typically use 5–10 specimens per subgroup, we ultimately used $n = 10$ specimens per subgroup. A significance level of $p < 0.05$ was applied throughout.

3 Results

The compressive, diametral tensile strength, and hardness test results for all test groups are presented in Figures 3–6, while Table 1 presents the significance values for comparisons between groups. The data from 240 specimens (20 per group and 10 at different time points) were found to be normally distributed, as confirmed by the Kolmogorov–Smirnov test ($p = 0.200$). The addition of microchitosan (MCH) at all concentrations resulted in statistically significant increases in both compressive and diametral tensile strength compared to the control group ($p < 0.05$).

3.1 Compressive strength

The compressive strength results are presented in Figure 3. At 24 h, the control GIC group recorded a mean strength of 127.14 ± 15.82 MPa. Compared with the control, the 0.5% MCH group showed a slight reduction, whereas the 2% MCH group showed a numerically higher mean compressive

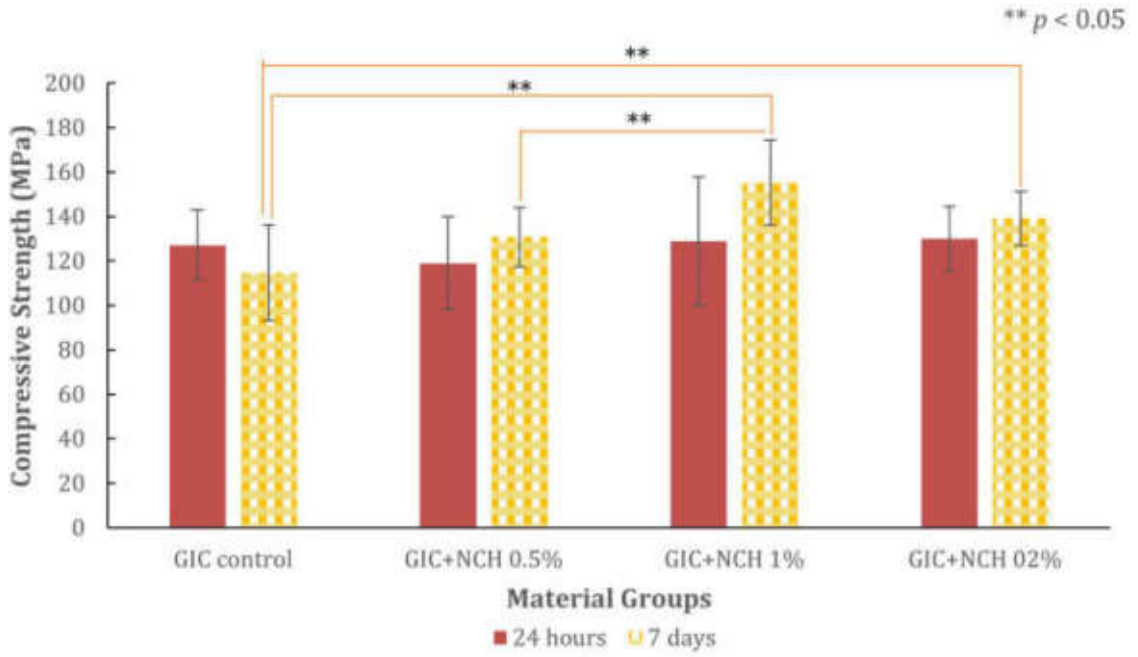

FIGURE 3
Compressive strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (**) show significant differences among the groups with ANOVA and *post hoc* Games-Howell test ($p < 0.05$).

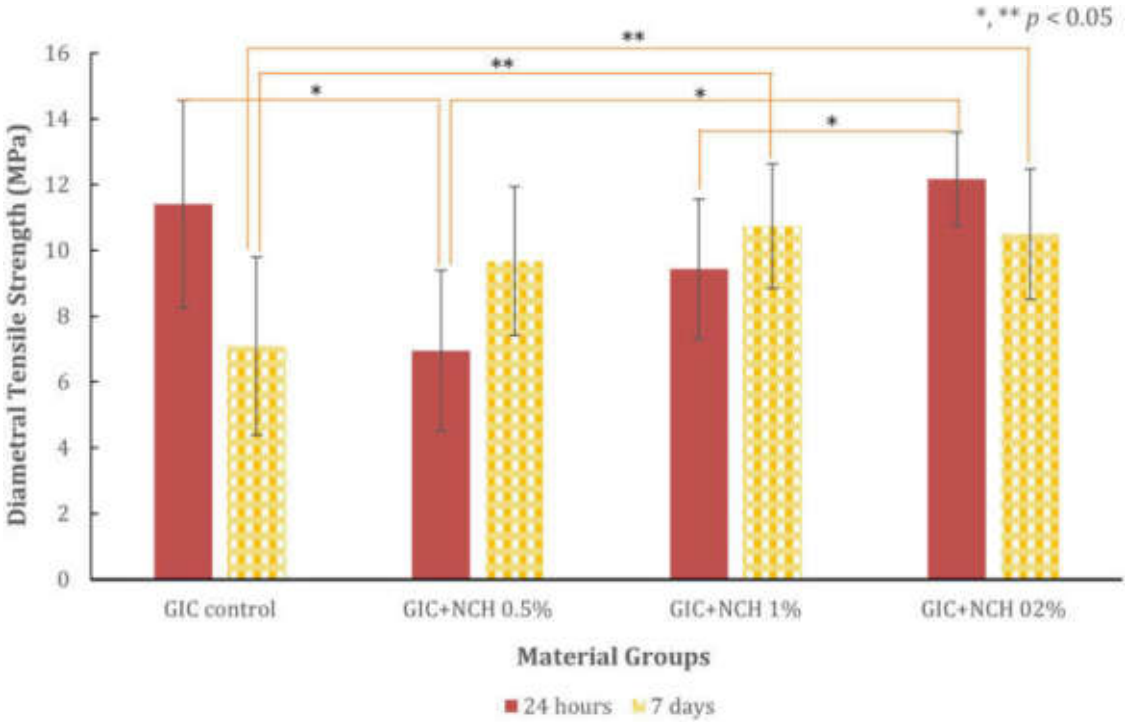

FIGURE 4
Diametral tensile strength (MPa) of microchitosan-modified glass ionomer cement (GIC) formulations after 24-hour and 7-day immersion in artificial saliva. Asterisks (*,**) show significant differences among the groups with ANOVA and *post hoc* Games-Howell test ($p < 0.05$).

strength (130.01 ± 14.40 MPa); however, this difference was not statistically significant ($p = 0.973$). The 1% MCH group was comparable to the control at this early time point.

After 7 days of immersion, the control group decreased to 114.63 ± 21.39 MPa. In contrast, all MCH groups showed higher values than the control. The highest compressive strength was observed in the 1% MCH group, which reached 155.16 ± 19.08 MPa ($p = 0.002$), representing a 35.4% increase over the 7-day control.

The corresponding compressive load-displacement curves (Figure 5, CS 24 h and CS 7 d) reveal predominantly linear elastic behavior, followed by abrupt failure in all groups, consistent with brittle, cement-like fracture. At 24 h, the slopes of the curves are broadly similar across groups, with only modest differences in peak load. After 7 days, the curves for all MCH groups shift towards higher peak loads compared with the control, with the 1% MCH group showing the highest peak load and largest displacement at failure, reflecting its superior strength and energy-absorbing capacity. The 0.5% MCH group exhibits the lowest peak load, while the 2% MCH and control curves lie between 0.5% and 1% MCH, mirroring the numerical ranking of compressive strength.

3.2 Diametral tensile strength

The diametral tensile strength results are presented in Figure 4. At 24 h, the control group showed a mean strength of 11.41 ± 3.16 MPa. Compared with the control, the 0.5% MCH group showed significantly lower strength, whereas the 1% and 2% MCH groups did not differ significantly from the control.

After 7 days, the control group decreased to 7.09 ± 2.70 MPa, while all MCH groups showed higher values. The 1% MCH group had the highest diametral tensile strength (10.73 ± 1.89 MPa, $p = 0.014$), corresponding to a 51% increase relative to the 7-day control.

The diametral tensile load-displacement curves (Figure 5, DTS 24 h and DTS 7 d) corroborate these trends. At 24 h, the control curve demonstrates the highest peak load, with the curves for the MCH groups clustered slightly below, reflecting the small or non-significant differences in DTS. By 7 days, the curves for all MCH-modified groups shift upward relative to the control, indicating higher peak loads at fracture. The 1% and 2% MCH groups in particular show steeper initial slopes and higher maxima than the control, whereas the 0.5% MCH curve occupies an intermediate position. This pattern is consistent with the 7-day DTS values, where 1% MCH provides the most pronounced reinforcement.

3.3 Vickers hardness

The Vickers microhardness test results at 24 h are given in Figure 6. The Control GIC had a microhardness mean value of 63.92 ± 15.17 VHN. The MCH-modified groups demonstrated

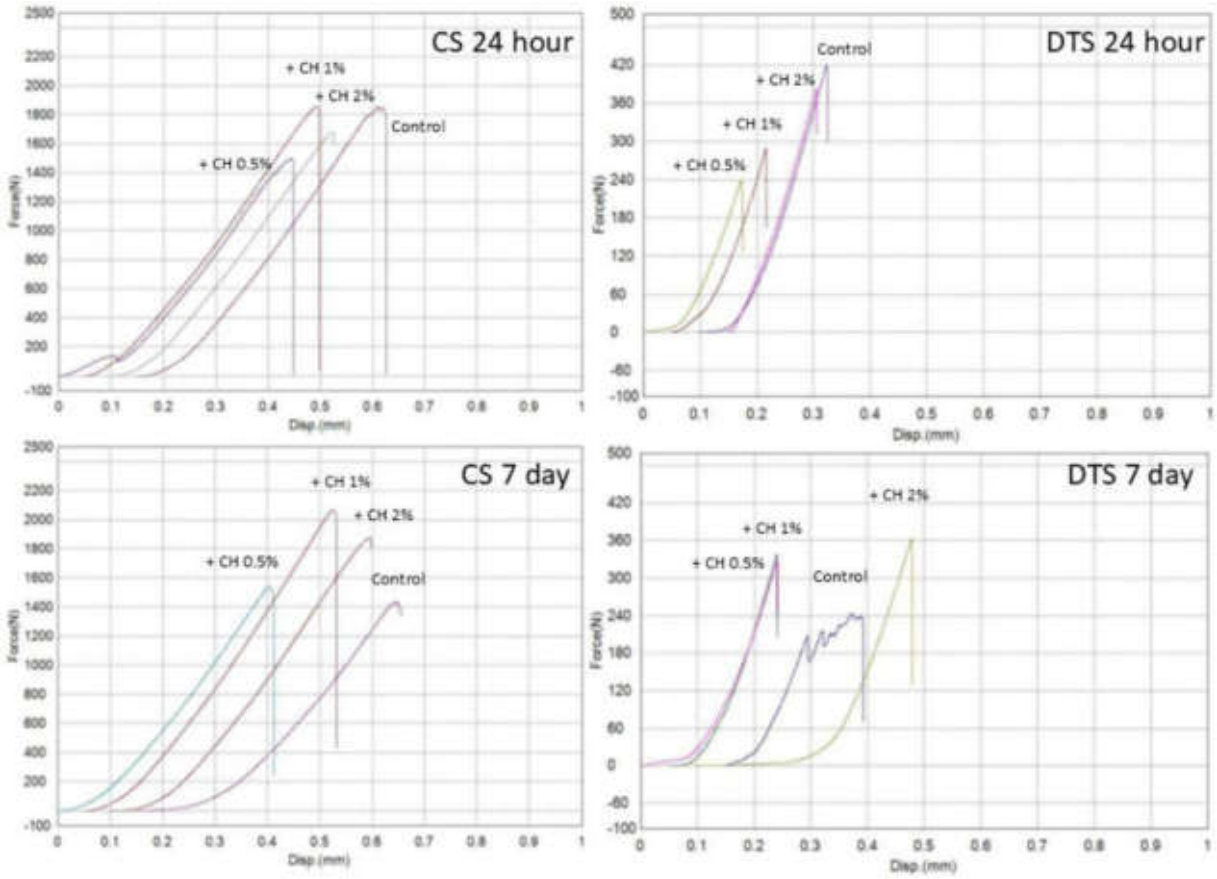

FIGURE 5
Representative load-displacement (stress–strain–type) curves for compressive strength (CS) and diametral tensile strength (DTS) tests of control and microchitosan-modified GICs (0.5%, 1%, and 2% MCH) at 24 h and 7 days. All specimens were tested under monotonic loading to failure. The upward shift in the curves at 7 days, particularly in the 1% MCH group, reflects increased peak load and overall mechanical performance compared with the control.

significantly increased hardness, with a peak at 1% MCH (135.30 ± 17.90 VHN; $p < 0.001$).

All groups experienced a decrease in hardness after 7 days, while the 1% MCH maintained the highest residual value (46.95 ± 7.32 VHN, $p < 0.001$).

3.4 Trends and correlation analysis

The inclusion of microchitosan resulted in a consistent increase in mechanical properties, particularly at 1%. While the 2% group showed strong initial values, its performance declined slightly over time, suggesting an upper threshold of reinforcement.

Pearson correlation analysis revealed strong positive correlations between microchitosan concentration and compressive strength ($r = 0.59$, $p = 0.002$), diametral tensile strength ($r = 0.57$, $p = 0.004$), and hardness ($r = 0.53$, $p = 0.011$). Seven-day specimens of compressive strength and diametral tensile strength showed a positive correlation ($r = 0.71$, $p = 0.001$). Additionally, compressive strength and hardness were positively correlated ($r = 0.64$, $p = 0.008$), indicating an interconnected strengthening effect.

4 Discussion

4.1 Summary of findings

This study demonstrated that incorporating *Xylotrupes gideon*–derived microchitosan (MCH) into a conventional GIC significantly improved its mechanical properties. The 1% MCH concentration produced the best results across all measurements compressive strength, diametral tensile strength, and surface hardness, particularly after 7 days of immersion in artificial saliva, indicating a time-dependent reinforcement effect (25). The 0.5% MCH group showed only modest improvements, whereas the 2% MCH group, despite some initial enhancement, was likely more prone to particle agglomeration, which compromised its long-term stability (6).

These findings align with previous research showing that chitosan can enhance the strength and durability of GICs and fit within the broader trend of nanomaterial-reinforced dental composites (16). The superior performance of 1% MCH is consistent with reports that nanochitosan outperforms other nanostructures (e.g., nano-silica, nano-zirconia) in GICs, largely because of its ability to form hydrogen bonds with the

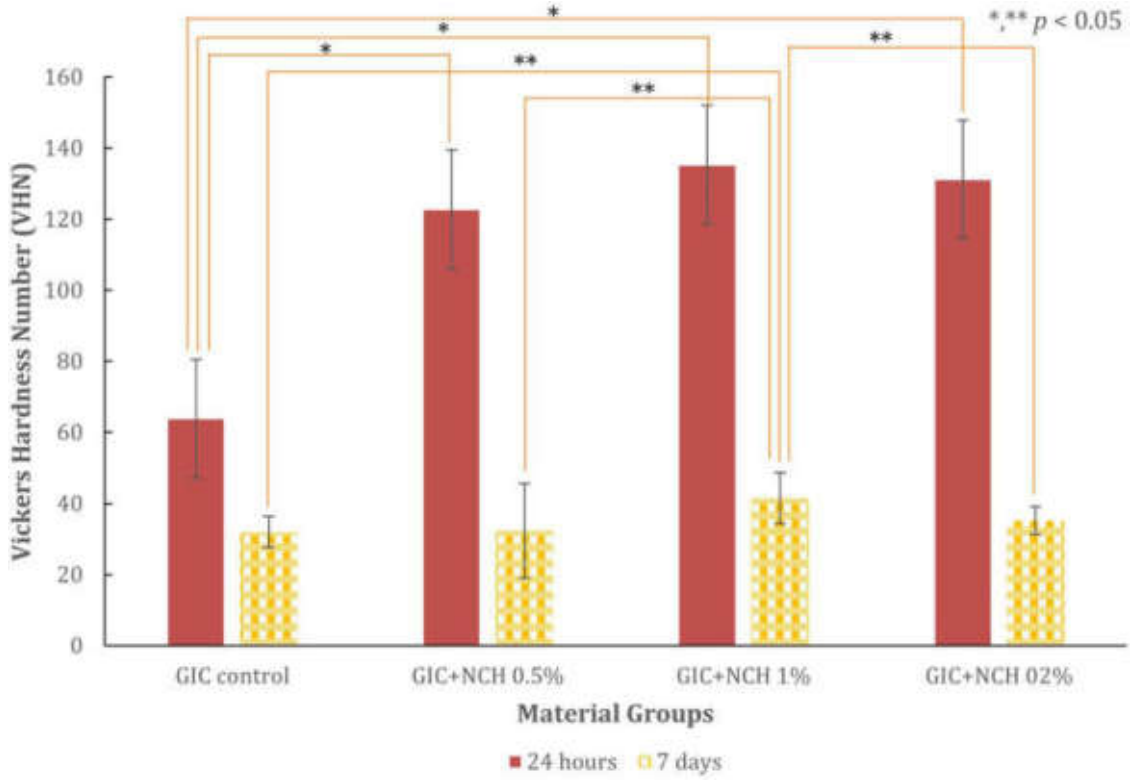

FIGURE 6
Vickers surface hardness (VHN) of microchitosan-modified glass ionomer cement (GIC) formulations following 24-hour and 7-day immersion in artificial saliva. Asterisks (*,**) show significant differences among the groups with ANOVA and *post hoc* Games-Howell test ($p < 0.05$). The data clearly show that the 1% microchitosan group exhibited the highest hardness values at both time points.

TABLE 1 Games-Howell significant value (p) for 24 h (white) and 7 days (grey) compressive strength, diametral tensile strength and vickers microhardness.

Compressive strength	Control	MCH 0.5%	MCH 1%	MCH 2%	
Control		0.223	0.002*	0.032*	7 days
MCH 0.5%	0.707		0.020*	0.473	
MCH 1%	0.671	0.234		0.155	
MCH 2%	0.973	0.476	0.848		
		24 h			
Diametral tensile strength	**Control**	**MCH 0.5%**	**MCH 1%**	**MCH 2%**	
Control		0.133	0.014*	0.025*	7 days
MCH 0.5%	0.012*		0.674	0.825	
MCH 1%	0.445	0.118		0.992	
MCH 2%	0.900	0.000*	0.040*		
		24 h			
Vickers microhardness	**Control**	**MCH 0.5%**	**MCH 1%**	**MCH 2%**	
Control		1.000	0.000*	0.341	7 days
MCH 0.5%	0.000*		0.038*	0.913	
MCH 1%	0.000*	0.719		0.003*	
MCH 2%	0.000*	0.882	0.999		
		24 h			

*Significant difference between groups with $p < 0,05$.

polyacrylic acid matrix, thereby improving cohesion and ion exchange (1). Our results confirm substantial gains in surface hardness and compressive strength compared with both unmodified GIC and GICs containing other nano-additives (26), supporting the concept that small-particle chitosan is a promising modifier for stress-bearing restorations, especially in Class I and V cavities where fluoride release and biocompatibility are important (27).

All groups exhibited a decrease in hardness after 7 days, consistent with previous work reporting a 40%–45% decline in microhardness of conventional GICs following thermocycling (28, 29). Notably, the 1% MCH group retained the highest residual hardness (46.95 VHN), consistent with the notion that reinforced GICs resist degradation better than unmodified formulations.

4.2 Mechanical reinforcement

The mechanical enhancements observed with MCH-modified GIC can be explained by microscale dispersion and interfacial interactions between MCH and the GIC matrix. MCH particles (200–4,000 nm) can occupy microvoids and irregularities, similarly to the homogenizing effect reported for nano-hydroxyapatite/chitosan composites (30) and microhybrid composites, resulting in a denser, more uniform structure that reduces crack initiation and propagation. In addition, the amino and hydroxyl groups of chitosan form hydrogen and ionic bonds with polyacrylic acid chains, increasing cross-link density and internal cohesion (4, 17). In this way, microchitosan addresses microstructural weaknesses inherent to the acid–base setting mechanism and may support greater restoration durability (4).

At early maturation (24 h), however, MCH particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disturbing optimal ionic cross-linking within the GIC matrix. This competition, together with the introduction of additional interfaces, may reduce initial network rigidity and create localized microvoids, which is consistent with the modest reductions or limited improvements in compressive and diametral tensile strength observed at 24 h for some concentrations. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1% group.

Beyond reporting the overall particle size range of 200–4,000 nm, more detailed particle size descriptors, such as the mean particle size, full size distribution profile, and polydispersity index, were not determined in this study. This represents a limitation of the current work, as such parameters would enable a more precise interpretation of how particle size, dispersion quality, and potential agglomeration contribute to the mechanical response of the different MCH concentrations. Future investigations will therefore include comprehensive physicochemical characterization of *Xylotrupes gideon*–derived microchitosan, including mean size, polydispersity, and surface charge, to better correlate these properties with the observed mechanical reinforcement of the GIC matrix.

The 1% concentration aligns with findings from previous studies, which have shown that 1%–1.5% chitosan maximizes mechanical gains in GICs without agglomeration, underscoring the critical role of nanoparticle concentration in dental composites (27). The good performance of 1% MCH is consistent with the general role of colloidal stability in such systems. Although zeta potential was not measured here, previous studies have shown that a high surface charge in chitosan-modified GICs improves nanoparticle dispersion, mechanical strength, and fluoride release (25). In contrast, quaternized chitosan-coated nanoparticles have demonstrated superior interfacial bonding and more uniform filler distribution, supporting improved flexural strength and fracture resistance in reinforced GIC systems (8). Together with reports on other nano-enhanced restorative formulations (3), these findings suggest that colloidal stability is a key determinant of functional integration and mechanical performance.

Our findings on the increase in Vickers surface hardness with MCH addition are in agreement with earlier studies, which show that chitosan positively influences GIC microhardness (10). Previous work comparing chitosan with hydroxyapatite revealed that chitosan not only preserved microhardness but was particularly effective in reducing surface roughness (31), supporting our conclusion that incorporating micro-sized chitin derivatives from *Xylotrupes gideon* contributes to a more durable and homogeneous GIC matrix. Furthermore, the incorporation of carboxymethyl chitosan into the liquid phase of GIC has been shown to significantly enhance flexural strength, fracture toughness, and compressive strength at low concentrations, reinforcing the view that chitosan derivatives strengthen the GIC network via hydrogen bonding and improved interfacial cohesion (9).

The decline in mechanical performance observed at 2% MCH is consistent with the tendency of higher nanoparticle concentrations to promote agglomeration and disrupt uniform dispersion (17). Conversely, very low concentrations (e.g., below 0.5%) may be insufficient to achieve effective reinforcement. In the present work, the interpretation that 2% MCH leads to increased agglomeration is therefore inferred from the mechanical trends and the known behavior of over-loaded particle-reinforced systems, rather than from direct microstructural evidence. Enhanced powder–liquid interactions during setting, driven by the high surface energy of MCH, likely contributed to favorable gelation and final structure at 1%, in line with observations that optimized powder–liquid interactions support improved mechanical outcomes (32). MCH thus appears to improve hardness and mechanical durability while preserving essential GIC properties, including adhesion (33). At the same time, the absence of fractographic SEM analysis of fractured GIC–MCH specimens in this study is a further limitation that prevents direct visualization of crack paths, particle distribution, and agglomerate formation at different MCH concentrations. A dedicated follow-up investigation, including fracture-surface SEM for all MCH loadings is planned to elucidate these microstructural–mechanical correlations more clearly.

Previous work has shown that resin coatings can significantly enhance the viscoelastic behavior and environmental resistance of highly viscous GICs (34). While such strategies focus on external surface protection, our results suggest that internal reinforcement with chitosan may offer comparable benefits by increasing matrix cohesion and long-term mechanical stability without necessitating additional coating layers. In another study, shrimp shell–derived chitosan combined with hydrophilic fibers yielded a composite with compressive strength of 32.67 MPa and tensile strength of 17.18 MPa (35). Although their tensile strength values were higher, our 1% MCH–GIC formulation achieved a much higher compressive strength (155.16 MPa),

When compared with other reinforced GICs, the 1% MCH-GIC formulation (compressive strength 155.16 MPa) approaches the values reported for zirconia-reinforced GIC (160.91 MPa) and silver-alloy GIC (151.47 MPa) (21). These results exceed the ISO 9917-1:2016 minimum compressive strength threshold of 100 MPa for conventional restorative GICs (36). Although the compressive strength remains below that of enamel (262 MPa) and dentin (234 MPa), and below values for some high-viscosity GICs (301 MPa), it is still well above the estimated stress generated by maximum bite forces (≈21.2 MPa, assuming a 20 mm^2 contact area) (22, 37). Thus, the present MCH-GIC appears mechanically adequate for typical masticatory loading, and further optimization, such as combining MCH with high-viscosity GIC may provide additional safety margins.

In addition to bulk mechanical strengthening, chitosan has been reported to enhance the shear bond strength of GIC to dentin (38), which is critical for long-term clinical success. While bonding behavior was not evaluated in this study, it is plausible that the microchitosan used could confer similar benefits; this will be addressed in future work.

4.3 Biofunctional attributes and fluoride release

Biofunctional attributes, including antimicrobial potential, remineralization support, and tissue compatibility, are highly relevant to restorative materials. The present study did not evaluate antibacterial activity, fluoride release, or pulp/tissue responses, and no biological claims are made based on our data. However, previous studies have shown that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation (18, 39, 40), and that *Xylotrupes gideon*–derived nanochitosan can exhibit antibacterial effects and support sustained fluoride release in experimental restorative formulations (19, 41).

Notably, prior work indicates that incorporating nanochitosan into GIC does not compromise fluoride release and may even enhance it, particularly under acidic conditions (16, 25, 42). Chitosan's hydrogel-like network may modulate ion diffusion and support prolonged fluoride availability. Other reports have demonstrated that chitosan can promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammation when applied near pulp tissue (43, 44). MCH's mucoadhesive behavior may also improve marginal sealing *in vivo* (45). Together, these findings suggest that chitosan-modified GICs could offer multifunctional benefits, combining mechanical reinforcement with potential antibacterial and remineralizing effects; nevertheless, such biofunctional properties remain to be tested specifically for the present MCH-GIC formulation and should be addressed in future *in vitro* and *in vivo* studies.

4.4 Clinical implications and future directions

Within the limitations of this *in vitro* study, *Xylotrupes gideon*–derived microchitosan appears to be a promising internal reinforcement for conventional GIC, particularly at a 1% loading. The observed improvements in strength and hardness, together with the material's fluoride-releasing nature, support potential use in stress-bearing posterior restorations, atraumatic restorative treatment (ART), and liners or bases in deep cavities, provided that adequate adhesion and long-term behavior are confirmed (1, 16, 27). However, the absence of flexural strength data represents an important limitation, as flexural performance is highly relevant to the clinical loading conditions of GIC restorations. To address this, future work will incorporate three-point or biaxial flexural strength testing as part of a broader mechanical characterization of *Xylotrupes gideon*-derived microchitosan–modified GICs.

Further work is required before clinical adoption. Priority areas include: (i) *in vivo* assessment of pulp and tissue responses, (ii) long-term durability under occlusal load, thermocycling, and pH cycling, (iii) evaluation of cytotoxicity and potential allergic reactions, particularly in pediatric and medically compromised patients, (iv) detailed studies of fluoride-release kinetics under cariogenic challenges, and (v) optimization of handling and setting characteristics for clinical workflow.

Finally, the use of *Xylotrupes gideon*–derived microchitosan highlights an environmentally conscious approach to dental biomaterials. Insect-based chitosan offers a locally available, potentially less allergenic alternative to marine sources and integrates effectively into GIC matrices (1, 16, 18). Microchitosan thus represents a sustainable modifier that can enhance the mechanical performance of GIC while reducing reliance on traditional marine-derived additives. Such improvements in mechanical properties are critical for the biomechanical performance of GIC restorations in the oral cavity. Comprehensive biocompatibility testing, biofunctional evaluation, and clinical trials are necessary to confirm the safety and effectiveness of this approach and to support its broader clinical implementation.

5 Conclusion

Incorporating 1% microchitosan (MCH) from *Xylotrupes gideon* into glass ionomer cement (GIC) significantly enhanced

its mechanical properties after 7 days of maturation in artificial saliva. This modification resulted in a 35% increase in compressive strength, a 51% improvement in tensile strength, and 46% increase in surface hardness. Although the 2% MCH group initially showed strong values, its performance declined over time, suggesting an upper threshold of reinforcement. This reduced performance was likely due to particle agglomeration, which undermined long-term stability. A 0.5% formulation produced inconclusive results, the optimized 1% formulation supports minimally invasive dentistry by prolonging restoration longevity and preserving tooth structure.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Ethical approval for this study was obtained from the Health Research Ethics Committee of the Faculty of Dentistry, Universitas Trisakti (Approval No: 006/Dosen/KEPK/FKG/06/2021). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RT: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. FK: Investigation, Methodology, Writing – original draft. CM: Validation, Writing – original draft. DP: Investigation, Writing – original draft. EE: Supervision, Writing – original draft. TP: Visualization, Writing – original draft. KK: Resources, Writing – original draft. IG: Formal analysis, Writing – original draft. SW: Writing – review & editing. AC: Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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There are illustrative elements in figure 2 that seem like they could come from an image database (like Shutterstock, Pexels, Biorender, Figdraw).

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Dear Rosa,

Thank you for your response. We look forward to receiving the updated version so we can proceed with publication of your article.

Best regards,
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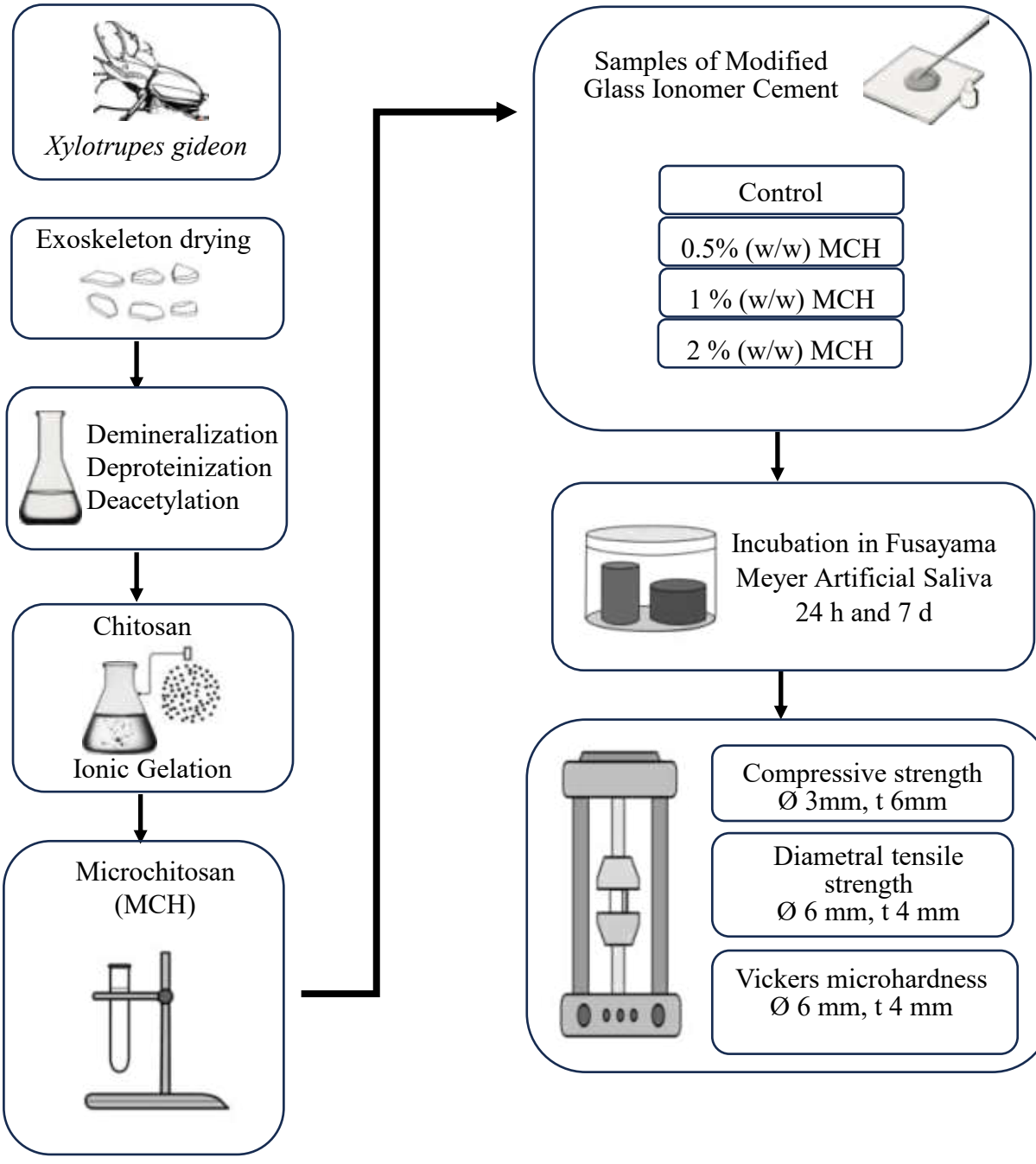
Xylotrupes gideon
Exoskeleton drying
Demineralization
Deproteinization
Deacetylation
Chitosan
Ionic Gelation
Microchitosan
(MCH)
Samples of Modified
Glass Ionomer Cement
Control
0.5% (w/w) MCH
1 % (w/w) MCH
2 % (w/w) MCH
Incubation in Fusayama
Meyer Artificial Saliva
24 h and 7 d
Compressive strength
Ø 3mm, t 6mm
Diametral tensile
strength
Ø 6 mm, t 4 mm
Vickers microhardness
Ø 6 mm, t 4 mm

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Xylotrupes gideon microchitosan-modified glass ionomer cement: *in vitro* assessment of mechanical properties

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Background: Glass ionomer cements (GIC) are valued as inherent fluoride-releasing dental restorative materials, while chitosan binds to negatively charged enamel surfaces, promoting mineral deposition and strengthening teeth. This study aimed to evaluate the mechanical properties of GIC modified with microchitosan derived from *Xylotrupes gideon*, using an *in vitro* experimental design. This study uniquely employs micro-scaled chitosan derived from the exoskeleton of Xylotrupes gideon, an insect-based, locally sourced, and environmentally sustainable alternative to conventional marine chitosan, to reinforce a conventional GIC.

Materials and methods: Microchitosan was extracted from *Xylotrupes gideon* and incorporated into conventional GIC at 0.5%, 1% and 2% (w/w). Compressive strength, diametral tensile strength, and surface microhardness were measured using standard testing equipment after immersion in artificial saliva for 24 h and 7 days. Statistical analysis was performed using one-way ANOVA followed by the Games-Howell *post hoc* test, with significance set at $p < 0.05$.

Results: The 1% microchitosan-modified GIC exhibited the most significant improvements compared to the unmodified control. After 7 days, compressive strength increased by 35.4%, diametral tensile strength by 51.3%, and surface hardness by 46.6% ($p < 0.05$). These enhancements are attributed to microscale reinforcement and chemical bonding between microchitosan and the GIC matrix.

Conclusion: The addition of 1% microchitosan derived from *Xylotrupes gideon* significantly improved the mechanical performance of GIC. This bioactive reinforcement shows promising potential for clinical restorative applications, though further investigation into its long-term biocompatibility and fluoride release is warranted. These findings highlight a novel combination of insect-derived micro-scale chitosan and conventional GIC, yielding mechanical gains comparable to those reported for nanochitosan-modified formulations while relying on a more sustainable chitosan source.

KEYWORDS

mechanical properties, compressive strength, diametral tensile strength, glassionomer cement, hardness, microchitosan, *Xylotrupes gideon*

1 Introduction

Dental composite materials, including glass ionomer cements (GICs), are widely recognized in restorative dentistry for their versatility, biocompatibility, and ability to bond chemically to dental hard tissues. GICs, in particular, are valued for their inherent fluoride release, which aids in the prevention of tooth decay and supports long-term tooth preservation (1, 2). These advantages make GICs ideal for pediatric dentistry and minimally invasive restorations where fluoride release and adhesion are critical; however, their adhesive performance varies significantly across formulations.

A major drawback of traditional GICs is their weak mechanical properties, including low compressive and tensile strength, poor wear resistance, and susceptibility to fracture under occlusal stress. These limitations preclude their use in stress-bearing restorations, particularly in posterior regions that are subjected to high occlusal loads (3). The other limitations are primarily attributed to the intrinsic chemistry of the GIC matrix, particularly the brittle nature of the glass phase and the sensitivity of the acid-base setting reaction to moisture and dehydration (4). Recent technology developments, particularly involving micro-sized chitosan and titanium dioxide, have demonstrated improved filler dispersion and particle–matrix bonding in glass ionomer cements, resulting in enhanced mechanical properties, matrix cohesion, fracture resistance, and bioactivity (5, 6) with chitosan enhancing particle-matrix bonding and decreasing microvoid formation (7).

Such reinforcement strategies yield structurally robust cement matrices characterized by minimized porosity and enhanced resistance to fracture propagation. Studies have shown that the incorporation of chitosan significantly enhances compressive and flexural strength, consistent with previous findings on quaternized chitosan-coated nanoparticles and carboxymethyl chitosan derivatives (8, 9) thereby improving matrix homogeneity and resistance to fracture propagation.

A previous study showed that the addition of chitosan to GIC has a positive effect on microhardness and improves surface roughness, making it a promising additive for dental restorative materials (10). This material reinforces the structural framework and enhances mechanical reliability, which in turn leads to improved fracture resistance, increased microhardness, and enhanced overall durability (11). The reinforcement effect demonstrated temporal dependence, with mechanical improvements more evident following extended maturation periods. Its ability to improve both the mechanical and biological properties of restorative materials makes it a compelling additive in dental biomaterials research. The molecular features of chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose], a versatile biopolymer, are chemically inert, non-toxic, and possess robust, broad antimicrobial activities due to its polycationic nature (12). Characterized by amino and hydroxyl functional groups, chitosan enables ionic and hydrogen bonding that strengthens adhesion to dental substrates, improves mechanical strength through particle–matrix cohesion, and supports tissue compatibility via biocompatibility and regenerative potential (13) across a wide range of dental applications.

Chitosan exhibits remarkable physicochemical properties and good biocompatibility, forming bonds with human proteins, cells, and organs (14). Chitosan exhibits multifunctionality in dental applications by contributing to mechanical reinforcement, promoting tissue regeneration, and acting as a bioactive agent in restorative formulations such as glass ionomer cements and adhesives. Recently, alternative sources of chitosan have emerged, including the exoskeletons of the *Xylotrupes gideon* beetle, which offer a sustainable and locally available biomaterial. Previous research indicates that this chitosan, derived from insects, enhances GIC performance in acidic oral environments (with critical pH levels), showing promise for clinical applications (15). These advantages support further investigation of its mechanical reinforcement potential in dental composites. Research indicates that this novel form of chitosan demonstrates superior mechanical enhancement capabilities when integrated into GICs, including increased compressive strength and Vickers hardness (16). Additionally, the microstructure improves particle dispersion and matrix interaction, which are critical for achieving homogeneity and minimizing microvoid formation during the setting process (7, 17).

In addition to mechanical benefits, chitosan may confer secondary biofunctional advantages, which warrant future exploration but were not addressed in the present study (18). These properties may enhance the clinical performance of restorations by reducing bacterial colonization, improving marginal integrity, and supporting the healing of the pulp-dentin interface, in line with recent advancements in oral health technology (17). Notably, such modifications do not appear to compromise GIC's core

biofunctional properties, such as fluoride ion release and adhesion to dentin (1, 11).

Therefore, this study aimed to investigate the effect of incorporating *Xylotrupes gideon*-derived microchitosan (on a submicron–micron scale) into conventional GIC on its mechanical properties, including compressive strength, diametral tensile strength, and surface hardness. By using an insect exoskeleton as a locally available and environmentally sustainable source of chitosan, this approach has potential for clinical feasibility, long-lasting nature, and biological functionality through the integration of eco-friendly materials in restorative dentistry (7).

2 Materials and methods

2.1 Synthesis and characterization of microchitosan from *xylotrupes gideon*

Microchitosan (MCH) was synthesized from the exoskeletons of *Xylotrupes gideon* beetles collected in Bogor, West Java, Indonesia. All body parts were detached and dried for 5 days, followed by extraction using established chitin-processing protocols: demineralization with 3N HCl, deproteinization with 3N NaOH, and deacetylation with 50% NaOH at 100°C to obtain chitosan.

The resulting chitosan was converted into microform via ionic gelation with sodium tripolyphosphate (TPP) in deionized water, stirred at 2,500 rpm for 4 h, intentionally omitting acetic acid to improve biocompatibility. A particle size analyzer (Horiba, Japan) revealed a spherical morphology with a size distribution of 200–4,000 nm, while SEM analysis also confirmed the morphology of the chitosan particles, as shown in Figure 1. The synthesized MCH was stored at 4°C in airtight containers until use (19).

The overall experimental workflow is illustrated in Figure 2, highlighting the synthesis of microchitosan from *Xylotrupes gideon* and its integration into glass ionomer cement for mechanical testing.

2.2 Preparation of modified glass ionomer cement

A commercially available Glass ionomer cement (Type IX Gold Label HS Posterior, GC, Tokyo, Japan) was used as the base material. The control group used unmodified GIC liquid, while the experimental groups consisted of 0.5%, 1%, and 2% (w/w) MCH added to the liquid component. Each modified liquid (0.5%, 1%, and 2% MCH) was vortex-mixed using the same standardized protocol immediately before mixing with the powder to improve homogeneity, acknowledging that achieving uniform dispersion becomes more challenging at higher MCH concentrations. Petroleum jelly was applied to the inner surfaces of the metal split mold. Then the mold was placed on a mylar strip, which was placed on a glass slab. The powder and liquid were dispensed on a paper pad and mixed manually according to the manufacturer's guidelines. After filling with the mixture, the mold was covered with another mylar strip, then covered with a second glass slab, and pressed for 30 s to extrude the excess material and obtain a uniformly smooth specimen surface. One hour after mixing, the specimens were removed from the mold, and any excess material was removed by dry grinding on both sides with 1,000-grit silicon carbide paper (1, 20, 21).

Compressive strength specimens were molded according to ISO 9917-1:2007: 3 mm diameter×6 mm height, while the specimens for diametrical tensile strength: 6 mm diameter × 4 mm thickness, and Vickers hardness: 6 mm diameter × 4 mm thickness. Specimen dimensions were confirmed using a digital caliper (Mitutoyo 500-196-30, Japan). After initial setting, all specimens were stored in 100% humidity at room temperature for 1 h. Compressive strength was calculated, in MPa, using the following equation: $C = (4p)/(\pi d2)$ where p is the maximum force applied, in Newton; d is the average measured diameter of the specimen, in mm. The diametral tensile strength was also calculated in MPa, using the

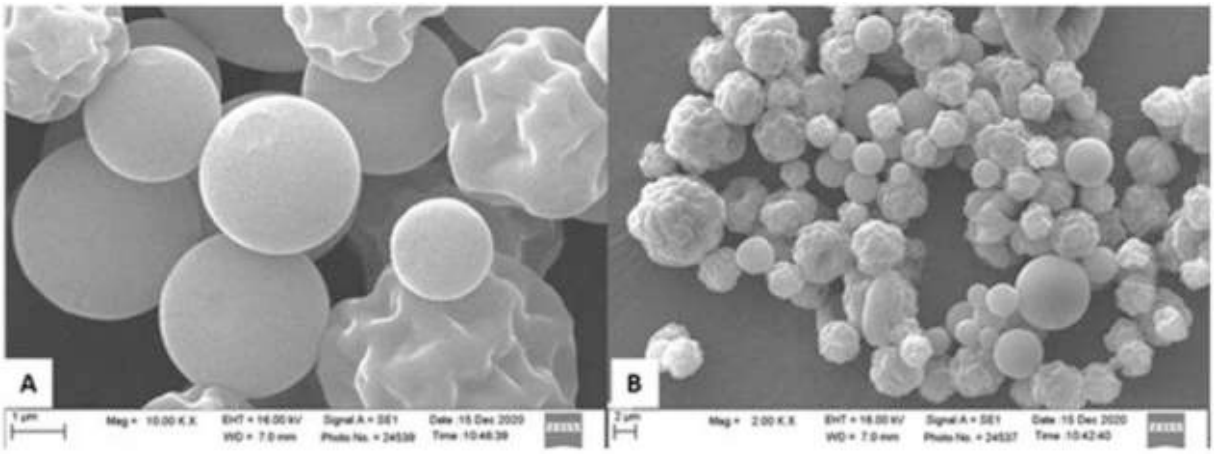

FIGURE 1
Scanning electron microscope images showing particles of microchitosan from *Xylotrupes gideon*. Image **(A)** highlights large, smooth spherical particles at 10,000 times magnification. Image **(B)** depicts a mixture of smaller, cougher textured and smooth spherical particles at 2,000 times magnification.

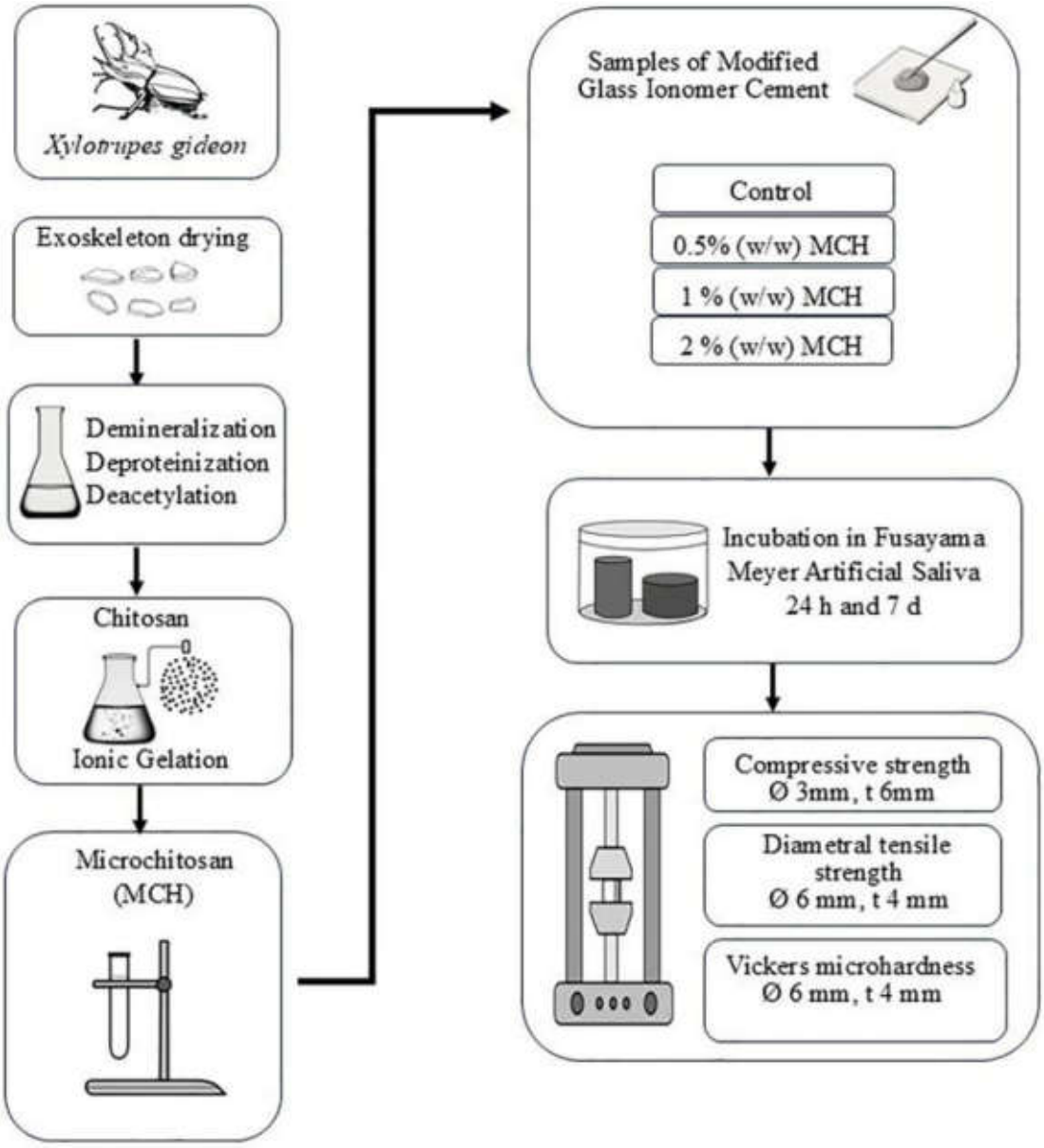

FIGURE 2
Flowchart illustrating the preparation and testing of modified glass ionomer cement (GIC). Starting with *Xylotrupes gideon* exoskeleton drying, it proceeds through demineralization, deproteinization, and deacetylation to obtain chitosan. Ionic gelation forms microchitosan (MCH). Samples of modified GIC are tested with 0.5%, 1%, and 2% (w/w) MCH addition. They undergo incubation in Fusayama Meyer artificial saliva for twenty-four hours and seven days. Tests include compressive strength, diametral tensile strength and Vickers microhardness, with specified diameters and thickness for each test.

equation DTS = (2F)/(πdt), where F is the applied load, D is the diameter of the disk, and t is the thickness of the disk (22).

2.3 Conditioning and storage

A total of 80 specimens were prepared for each mechanical test and divided into four groups ($n = 20$ each), with each group further subdivided into two subgroups ($n = 10$) based on immersion time (24 h and 7 days). The minimum specimen size was estimated using the Lemeshow formula for comparing two independent means:

$$n = 2\tau^2(Z_{1-\alpha} + Z_{1-\beta})^2/(\mu_1 - \mu_2)^2$$

Where n is the minimum number of specimens per subgroup, μ_1 and μ_2 are the expected means from a previous study, τ is the common standard deviation, $Z_{1-\alpha}$ is the Z value for the selected significance level, and $Z_{1-\beta}$ is the Z value corresponding to the desired power.

A Fusayama-Meyer artificial saliva solution was prepared containing 0.4 g/L of NaCl, 0.4 g/L of KCl, 0.906 g/L of $CaCl_2 \cdot 2H_2O$, 0.690 g/L of $Na_2HPO_4 \cdot 2H_2O$, 0.005 g/L of $Na_2S \cdot 9H_2O$, and a pH of 6.75. Each specimen was immersed in 10 mL of artificial saliva in a sealed container and incubated at 37°C.

2.4 Mechanical testing

Mechanical properties were evaluated using a universal testing machine (Shimadzu, Japan) equipped with a 5 kN load cell, following validated protocols (23). Compressive and diametral tensile strength tests were conducted at a crosshead speed of 1 mm/min. Surface hardness was measured using a Shimadzu HMV-G microhardness tester (Kyoto, Japan), employing a micro Vickers diamond indenter with a 100 g load applied for 10 s (24). All mechanical testing adhered to ISO 9917-1:2007 guidelines and methodological frameworks.

2.5 Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including the mean and standard deviation, were calculated. Data normality was assessed using the Kolmogorov–Smirnov test, and homogeneity of variances was evaluated using Levene's test. When the assumption of homogeneity was violated, one-way ANOVA was followed by the Games–Howell *post hoc* test for pairwise comparisons. Pearson correlation coefficients were calculated to examine relationships among mechanical parameters. The minimum required specimen size per subgroup was determined *a priori* using the Lemeshow equation for comparing two means, yielding a specimen size of 7. To ensure adequate power and consistency with ISO 9917-1:2007 recommendations and previous GIC mechanical studies, which typically use 5–10 specimens per subgroup, we ultimately used $n = 10$ specimens per subgroup. A significance level of $p < 0.05$ was applied throughout.

3 Results

The compressive, diametral tensile strength, and hardness test results for all test groups are presented in Figures 3–6, while Table 1 presents the significance values for comparisons between groups. The data from 240 specimens (20 per group and 10 at different time points) were found to be normally distributed, as confirmed by the Kolmogorov–Smirnov test ($p = 0.200$). The addition of microchitosan (MCH) at all concentrations resulted in statistically significant increases in both compressive and diametral tensile strength compared to the control group ($p < 0.05$).

3.1 Compressive strength

The compressive strength results are presented in Figure 3. At 24 h, the control GIC group recorded a mean strength of 127.14 ± 15.82 MPa. Compared with the control, the 0.5% MCH group showed a slight reduction, whereas the 2% MCH group showed a numerically higher mean compressive

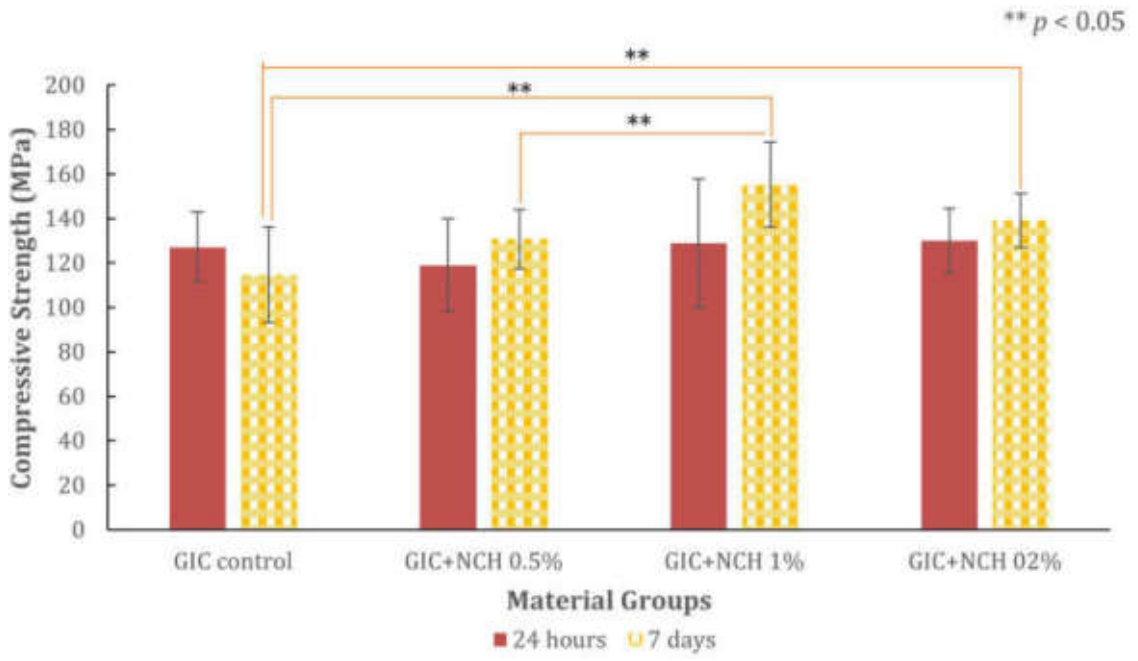

FIGURE 3
Bar chart showing compressive strength in MPa of GIC control and GIC with varying MCH percentages at 24 hours and 7 days. GIC + MCH 1% shows the highest strength. Significant difference, denoted by asterisks (**) indicate $p<0.05$ with ANOVA and *post hoc* Games-Howell.

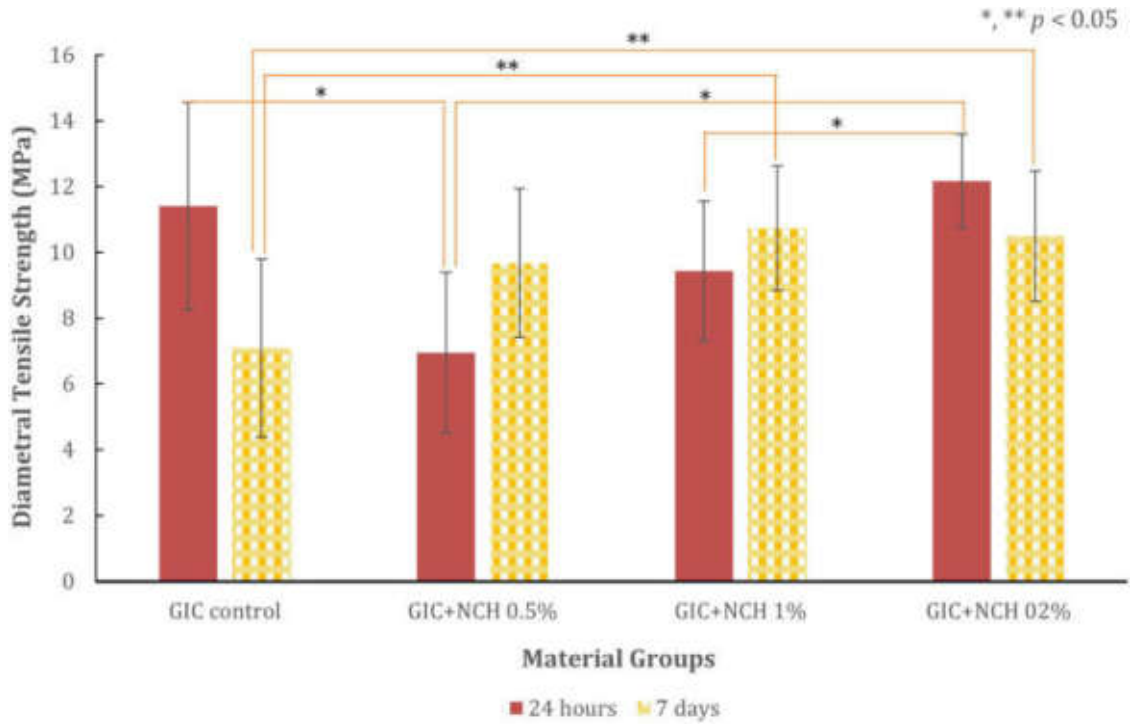

FIGURE 4
Bar chart showing diametral tensile strength (in MPa) for GIC control, GIC+MCH 0.5%, 1% and 2%. Strength measured at 24 hours (red bars) and 7 days (yellow patterned bars). Error bars indicate variability. Significant differences are marked with asterisks (*,**) for p-values less than 0.05 analyze with ANOVA and *post hoc* Games Howell.

strength (130.01 ± 14.40 MPa); however, this difference was not statistically significant ($p = 0.973$). The 1% MCH group was comparable to the control at this early time point.

After 7 days of immersion, the control group decreased to 114.63 ± 21.39 MPa. In contrast, all MCH groups showed higher values than the control. The highest compressive strength was observed in the 1% MCH group, which reached 155.16 ± 19.08 MPa ($p = 0.002$), representing a 35.4% increase over the 7-day control.

The corresponding compressive load-displacement curves (Figure 5, CS 24 h and CS 7 d) reveal predominantly linear elastic behavior, followed by abrupt failure in all groups, consistent with brittle, cement-like fracture. At 24 h, the slopes of the curves are broadly similar across groups, with only modest differences in peak load. After 7 days, the curves for all MCH groups shift towards higher peak loads compared with the control, with the 1% MCH group showing the highest peak load and largest displacement at failure, reflecting its superior strength and energy-absorbing capacity. The 0.5% MCH group exhibits the lowest peak load, while the 2% MCH and control curves lie between 0.5% and 1% MCH, mirroring the numerical ranking of compressive strength.

3.2 Diametral tensile strength

The diametral tensile strength results are presented in Figure 4. At 24 h, the control group showed a mean strength of 11.41 ± 3.16 MPa. Compared with the control, the 0.5% MCH group showed significantly lower strength, whereas the 1% and 2% MCH groups did not differ significantly from the control.

After 7 days, the control group decreased to 7.09 ± 2.70 MPa, while all MCH groups showed higher values. The 1% MCH group had the highest diametral tensile strength (10.73 ± 1.89 MPa, $p = 0.014$), corresponding to a 51% increase relative to the 7-day control.

The diametral tensile load-displacement curves (Figure 5, DTS 24 h and DTS 7 d) corroborate these trends. At 24 h, the control curve demonstrates the highest peak load, with the curves for the MCH groups clustered slightly below, reflecting the small or non-significant differences in DTS. By 7 days, the curves for all MCH-modified groups shift upward relative to the control, indicating higher peak loads at fracture. The 1% and 2% MCH groups in particular show steeper initial slopes and higher maxima than the control, whereas the 0.5% MCH curve occupies an intermediate position. This pattern is consistent with the 7-day DTS values, where 1% MCH provides the most pronounced reinforcement.

3.3 Vickers hardness

The Vickers microhardness test results at 24 h are given in Figure 6. The Control GIC had a microhardness mean value of 63.92 ± 15.17 VHN. The MCH-modified groups demonstrated

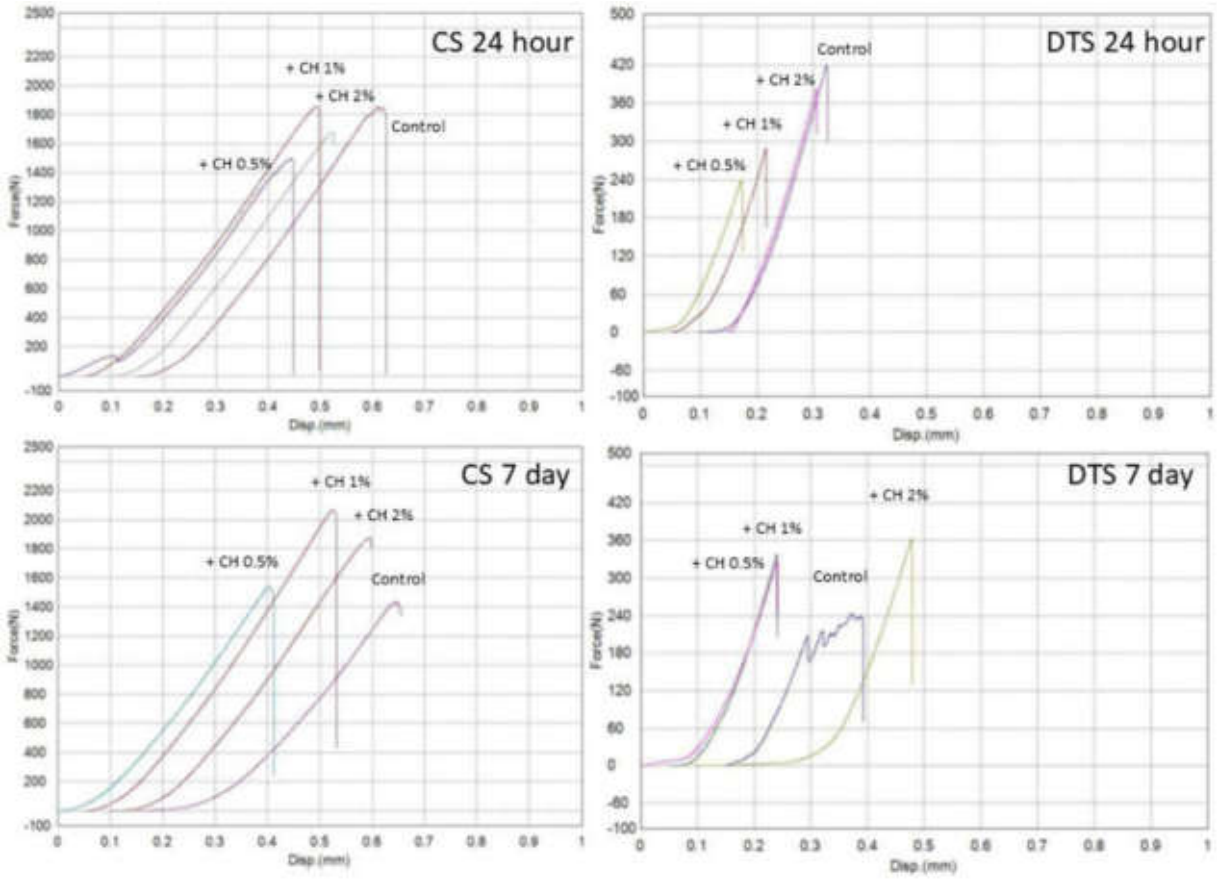

FIGURE 5
Four graphs compare the force versus displacement for different concentration of CH additives over time. All specimens were tested under monotonic loading to failure. Top left: CS 24 hour shows increasing force with higher CH concentrations. Top right: DTS 24 hour shows similar trends with lower force values. Bottom left: CS 7 day continues the pattern with increased force after seven days. Bottom right: DTS 7 day also shows the increase but with left force compared to CS graphs. The upward shift in the curves at 7 days, particularly in the 1% MCH group, reflects increased peak load and overall mechanical performance compared with the control.

significantly increased hardness, with a peak at 1% MCH (135.30 ± 17.90 VHN; $p < 0.001$).

All groups experienced a decrease in hardness after 7 days, while the 1% MCH maintained the highest residual value (46.95 ± 7.32 VHN, $p < 0.001$).

3.4 Trends and correlation analysis

The inclusion of microchitosan resulted in a consistent increase in mechanical properties, particularly at 1%. While the 2% group showed strong initial values, its performance declined slightly over time, suggesting an upper threshold of reinforcement.

Pearson correlation analysis revealed strong positive correlations between microchitosan concentration and compressive strength ($r = 0.59$, $p = 0.002$), diametral tensile strength ($r = 0.57$, $p = 0.004$), and hardness ($r = 0.53$, $p = 0.011$). Seven-day specimens of compressive strength and diametral tensile strength showed a positive correlation ($r = 0.71$, $p = 0.001$). Additionally, compressive strength and hardness were positively correlated ($r = 0.64$, $p = 0.008$), indicating an interconnected strengthening effect.

4 Discussion

4.1 Summary of findings

This study demonstrated that incorporating *Xylotrupes gideon*–derived microchitosan (MCH) into a conventional GIC significantly improved its mechanical properties. The 1% MCH concentration produced the best results across all measurements compressive strength, diametral tensile strength, and surface hardness, particularly after 7 days of immersion in artificial saliva, indicating a time-dependent reinforcement effect (25). The 0.5% MCH group showed only modest improvements, whereas the 2% MCH group, despite some initial enhancement, was likely more prone to particle agglomeration, which compromised its long-term stability (6).

These findings align with previous research showing that chitosan can enhance the strength and durability of GICs and fit within the broader trend of nanomaterial-reinforced dental composites (16). The superior performance of 1% MCH is consistent with reports that nanochitosan outperforms other nanostructures (e.g., nano-silica, nano-zirconia) in GICs, largely

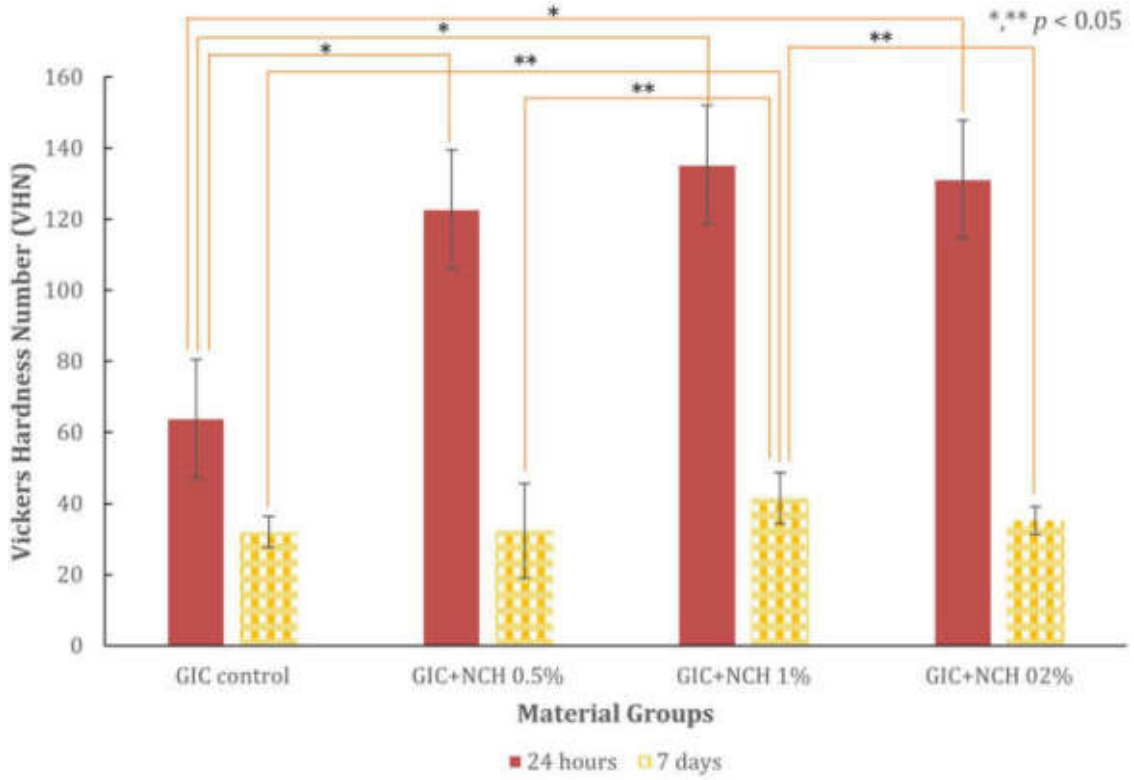

FIGURE 6
Bar chart comparing Vickers Hardness Number (VHN) across material groups: GIC Control, GIC + MCH 0,5%, GIC + MCH 1%, GIC + MCH 2%, measured at 24 hours and 7 days. Significant differences are indicated with asterisks for $p < 0.05$ using ANOVA and *post hoc* Games-Howell. The data clearly show that 1% microchitosan group exhibited the highest hardness values at both time points.

TABLE 1 Games-Howell significant value (p) for 24 hours (white) and 7 days (grey) compressive strength, diametral tensile strength and vickers microhardness.

Compressive strength	Control	MCH 0.5%	MCH 1%	MCH 2%	Time
Control		0.223	0.002*	0.032*	7 days
MCH 0.5%	0.707		0.020*	0.473	
MCH 1%	0.671	0.234		0.155	
MCH 2%	0.973	0.476	0.848		
Time	24 hours				
Diametral tensile strength	**Control**	**MCH 0.5%**	**MCH 1%**	**MCH 2%**	**Time**
Control		0.133	0.014*	0.025*	7 days
MCH 0.5%	0.012*		0.674	0.825	
MCH 1%	0.445	0.118		0.992	
MCH 2%	0.900	<0.001*	0.040*		
Time	24 hours				
Vickers microhardness	**Control**	**MCH 0.5%**	**MCH 1%**	**MCH 2%**	**Time**
Control		1.000	<0.001*	0.341	7 days
MCH 0.5%	<0.001*		0.038*	0.913	
MCH 1%	<0.001*	0.719		0.003*	
MCH 2%	<0.001*	0.882	0.999		
Time	24 hours				

*Significant difference between groups with $p < 0.05$.

because of its ability to form hydrogen bonds with the polyacrylic acid matrix, thereby improving cohesion and ion exchange (1). Our results confirm substantial gains in surface hardness and compressive strength compared with both unmodified GIC and GICs containing other nano-additives (26), supporting the concept that small-particle chitosan is a promising modifier for stress-bearing restorations, especially in Class I and V cavities where fluoride release and biocompatibility are important (27).

All groups exhibited a decrease in hardness after 7 days, consistent with previous work reporting a 40%–45% decline in microhardness of conventional GICs following thermocycling (28, 29). Notably, the 1% MCH group retained the highest residual hardness (46.95 VHN), consistent with the notion that reinforced GICs resist degradation better than unmodified formulations.

4.2 Mechanical reinforcement

The mechanical enhancements observed with MCH-modified GIC can be explained by microscale dispersion and interfacial interactions between MCH and the GIC matrix. MCH particles (200–4,000 nm) can occupy microvoids and irregularities, similarly to the homogenizing effect reported for nano-hydroxyapatite/chitosan composites (30) and microhybrid composites, resulting in a denser, more uniform structure that reduces crack initiation and propagation. In addition, the amino and hydroxyl groups of chitosan form hydrogen and ionic bonds with polyacrylic acid chains, increasing cross-link density and internal cohesion (4, 17). In this way, microchitosan addresses microstructural weaknesses inherent to the acid–base setting mechanism and may support greater restoration durability (4).

At early maturation (24 h), however, MCH particles and their positively charged amino groups may interact competitively with polyacrylic acid and multivalent cations (Ca^{2+}/Al^{3+}), temporarily disturbing optimal ionic cross-linking within the GIC matrix. This competition, together with the introduction of additional interfaces, may reduce initial network rigidity and create localized microvoids, which is consistent with the modest reductions or limited improvements in compressive and diametral tensile strength observed at 24 h for some concentrations. With extended maturation (7 days) in artificial saliva, ongoing ion exchange and secondary cross-linking between the GIC matrix and microchitosan (via ionic and hydrogen bonding) likely produce a more cohesive, denser matrix, which explains the substantial strength gains at 7 days, particularly for the 1% group.

Beyond reporting the overall particle size range of 200–4,000 nm, more detailed particle size descriptors, such as the mean particle size, full size distribution profile, and polydispersity index, were not determined in this study. This represents a limitation of the current work, as such parameters would enable a more precise interpretation of how particle size, dispersion quality, and potential agglomeration contribute to the mechanical response of the different MCH concentrations. Future investigations will therefore include comprehensive physicochemical characterization of *Xylotrupes gideon*–derived microchitosan, including mean size, polydispersity, and surface charge, to better correlate these properties with the observed mechanical reinforcement of the GIC matrix.

The 1% concentration aligns with findings from previous studies, which have shown that 1%–1.5% chitosan maximizes mechanical gains in GICs without agglomeration, underscoring the critical role of nanoparticle concentration in dental composites (27). The good performance of 1% MCH is consistent with the general role of colloidal stability in such systems. Although zeta potential was not measured here, previous studies have shown that a high surface charge in chitosan-modified GICs improves nanoparticle dispersion, mechanical strength, and fluoride release (25). In contrast, quaternized chitosan-coated nanoparticles have demonstrated superior interfacial bonding and more uniform filler distribution, supporting improved flexural strength and fracture resistance in reinforced GIC systems (8). Together with reports on other nano-enhanced restorative formulations (3), these findings suggest that colloidal stability is a key determinant of functional integration and mechanical performance.

Our findings on the increase in Vickers surface hardness with MCH addition are in agreement with earlier studies, which show that chitosan positively influences GIC microhardness (10). Previous work comparing chitosan with hydroxyapatite revealed that chitosan not only preserved microhardness but was particularly effective in reducing surface roughness (31), supporting our conclusion that incorporating micro-sized chitin derivatives from *Xylotrupes gideon* contributes to a more durable and homogeneous GIC matrix. Furthermore, the incorporation of carboxymethyl chitosan into the liquid phase of GIC has been shown to significantly enhance flexural strength, fracture toughness, and compressive strength at low concentrations, reinforcing the view that chitosan derivatives strengthen the GIC network via hydrogen bonding and improved interfacial cohesion (9).

The decline in mechanical performance observed at 2% MCH is consistent with the tendency of higher nanoparticle concentrations to promote agglomeration and disrupt uniform dispersion (17). Conversely, very low concentrations (e.g., below 0.5%) may be insufficient to achieve effective reinforcement. In the present work, the interpretation that 2% MCH leads to increased agglomeration is therefore inferred from the mechanical trends and the known behavior of over-loaded particle-reinforced systems, rather than from direct microstructural evidence. Enhanced powder–liquid interactions during setting, driven by the high surface energy of MCH, likely contributed to favorable gelation and final structure at 1%, in line with observations that optimized powder–liquid interactions support improved mechanical outcomes (32). MCH thus appears to improve hardness and mechanical durability while preserving essential GIC properties, including adhesion (33). At the same time, the absence of fractographic SEM analysis of fractured GIC–MCH specimens in this study is a further limitation that prevents direct visualization of crack paths, particle distribution, and agglomerate formation at different MCH concentrations. A dedicated follow-up investigation, including fracture-surface SEM for all MCH loadings is planned to elucidate these microstructural–mechanical correlations more clearly.

Previous work has shown that resin coatings can significantly enhance the viscoelastic behavior and environmental resistance of highly viscous GICs (34). While such strategies focus on external surface protection, our results suggest that internal reinforcement with chitosan may offer comparable benefits by increasing matrix cohesion and long-term mechanical stability without necessitating additional coating layers. In another study, shrimp shell–derived chitosan combined with hydrophilic fibers yielded a composite with compressive strength of 32.67 MPa and tensile strength of 17.18 MPa (35). Although their tensile strength values were higher, our 1% MCH–GIC formulation achieved a much higher compressive strength (155.16 MPa),

When compared with other reinforced GICs, the 1% MCH-GIC formulation (compressive strength 155.16 MPa) approaches the values reported for zirconia-reinforced GIC (160.91 MPa) and silver-alloy GIC (151.47 MPa) (21). These results exceed the ISO 9917-1:2016 minimum compressive strength threshold of 100 MPa for conventional restorative GICs (36). Although the compressive strength remains below that of enamel (262 MPa) and dentin (234 MPa), and below values for some high-viscosity GICs (301 MPa), it is still well above the estimated stress generated by maximum bite forces (≈21.2 MPa, assuming a 20 mm^2 contact area) (22, 37). Thus, the present MCH-GIC appears mechanically adequate for typical masticatory loading, and further optimization, such as combining MCH with high-viscosity GIC may provide additional safety margins.

In addition to bulk mechanical strengthening, chitosan has been reported to enhance the shear bond strength of GIC to dentin (38), which is critical for long-term clinical success. While bonding behavior was not evaluated in this study, it is plausible that the microchitosan used could confer similar benefits; this will be addressed in future work.

4.3 Biofunctional attributes and fluoride release

Biofunctional attributes, including antimicrobial potential, remineralization support, and tissue compatibility, are highly relevant to restorative materials. The present study did not evaluate antibacterial activity, fluoride release, or pulp/tissue responses, and no biological claims are made based on our data. However, previous studies have shown that chitosan's positively charged surface can disrupt bacterial membranes and inhibit biofilm formation (18, 39, 40), and that *Xylotrupes gideon*–derived nanochitosan can exhibit antibacterial effects and support sustained fluoride release in experimental restorative formulations (19, 41).

Notably, prior work indicates that incorporating nanochitosan into GIC does not compromise fluoride release and may even enhance it, particularly under acidic conditions (16, 25, 42). Chitosan's hydrogel-like network may modulate ion diffusion and support prolonged fluoride availability. Other reports have demonstrated that chitosan can promote the proliferation of fibroblasts and odontoblasts, enhance pulp healing, and reduce inflammation when applied near pulp tissue (43, 44). MCH's mucoadhesive behavior may also improve marginal sealing *in vivo* (45). Together, these findings suggest that chitosan-modified GICs could offer multifunctional benefits, combining mechanical reinforcement with potential antibacterial and remineralizing effects; nevertheless, such biofunctional properties remain to be tested specifically for the present MCH-GIC formulation and should be addressed in future *in vitro* and *in vivo* studies.

4.4 Clinical implications and future directions

Within the limitations of this *in vitro* study, *Xylotrupes gideon*–derived microchitosan appears to be a promising internal reinforcement for conventional GIC, particularly at a 1% loading. The observed improvements in strength and hardness, together with the material's fluoride-releasing nature, support potential use in stress-bearing posterior restorations, atraumatic restorative treatment (ART), and liners or bases in deep cavities, provided that adequate adhesion and long-term behavior are confirmed (1, 16, 27). However, the absence of flexural strength data represents an important limitation, as flexural performance is highly relevant to the clinical loading conditions of GIC restorations. To address this, future work will incorporate three-point or biaxial flexural strength testing as part of a broader mechanical characterization of *Xylotrupes gideon*-derived microchitosan–modified GICs.

Further work is required before clinical adoption. Priority areas include: (i) *in vivo* assessment of pulp and tissue responses, (ii) long-term durability under occlusal load, thermocycling, and pH cycling, (iii) evaluation of cytotoxicity and potential allergic reactions, particularly in pediatric and medically compromised patients, (iv) detailed studies of fluoride-release kinetics under cariogenic challenges, and (v) optimization of handling and setting characteristics for clinical workflow.

Finally, the use of *Xylotrupes gideon*–derived microchitosan highlights an environmentally conscious approach to dental biomaterials. Insect-based chitosan offers a locally available, potentially less allergenic alternative to marine sources and integrates effectively into GIC matrices (1, 16, 18). Microchitosan thus represents a sustainable modifier that can enhance the mechanical performance of GIC while reducing reliance on traditional marine-derived additives. Such improvements in mechanical properties are critical for the biomechanical performance of GIC restorations in the oral cavity. Comprehensive biocompatibility testing, biofunctional evaluation, and clinical trials are necessary to confirm the safety and effectiveness of this approach and to support its broader clinical implementation.

5 Conclusion

Incorporating 1% microchitosan (MCH) from *Xylotrupes gideon* into glass ionomer cement (GIC) significantly enhanced

its mechanical properties after 7 days of maturation in artificial saliva. This modification resulted in a 35% increase in compressive strength, a 51% improvement in tensile strength, and 46% increase in surface hardness. Although the 2% MCH group initially showed strong values, its performance declined over time, suggesting an upper threshold of reinforcement. This reduced performance was likely due to particle agglomeration, which undermined long-term stability. A 0.5% formulation produced inconclusive results, the optimized 1% formulation supports minimally invasive dentistry by prolonging restoration longevity and preserving tooth structure.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by Ethical approval for this study was obtained from the Health Research Ethics Committee of the Faculty of Dentistry, Universitas Trisakti (Approval No: 006/Dosen/KEPK/FKG/06/2021). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

RT: Conceptualization, Data curation, Methodology, Project administration, Writing – original draft, Writing – review & editing. FK: Investigation, Methodology, Writing – original draft. CM: Validation, Writing – original draft. DP: Investigation, Writing – original draft. EE: Supervision, Writing – original draft. TP: Visualization, Writing – original draft. KK: Resources, Writing – original draft. IG: Formal analysis, Writing – original draft. SW: Writing – review & editing. AC: Writing – review & editing.

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Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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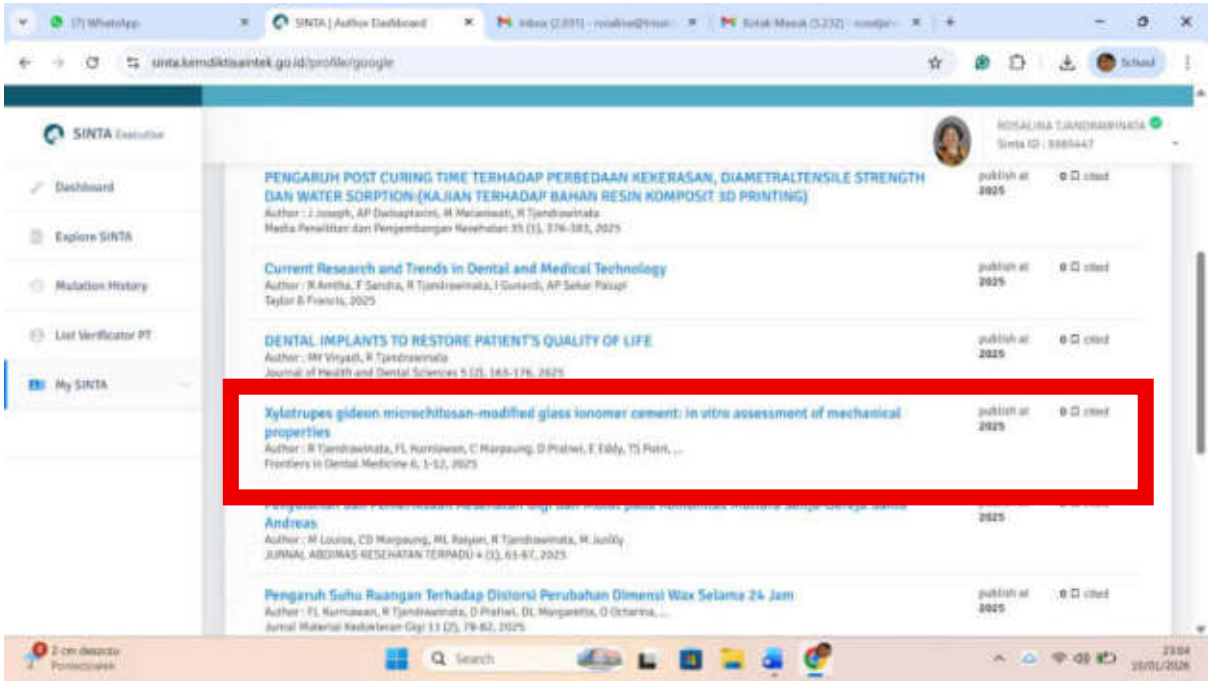
7. Bukti konfirmasi artikel tercatat di SINTA



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