博 士 論 文

Effect of Chitin on the Physical and Mechanical Properties of Glass Ionomer Cements

(グラスアイオノマーセメントの物理的・機械的特性に対するキチンの影響)

令和7年3月申請

北海道大学
大学院歯学院口腔医学専攻

呉　楠
WUNAN

Effect of Chitin on the Physical and Mechanical Properties of Glass Ionomer Cements

グラスアイオノマーセメントの物理的・機械的特性に対するキチンの影響

WU NAN

THIS THESIS SUBMITTED IN PARTIAL

FULFILLMENT OF REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY IN THE

DEPARTMENT OF RESTORATIVE DENTISTRY

FACULTY OF DENTAL MEDICINE.

Hokkaido University

2025

This certificate hereby attests that the research work presented in the thesis titled "Effect of Chitin on the Physical and Mechanical Properties of Glass Ionomer Cements" was carried out by Wu Nan under the guidance and supervision of Professor Atsushi Tomokiyo. No portion of this thesis has been previously submitted for any other academic degree. This thesis is being submitted to the Department of Restorative Dentistry, Graduate School of Dental Medicine, Hokkaido University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the field of Dental Medicine.

Department of Restorative Dentistry, Graduate School of Dental Medicie,

Hokkaido University Kita 13, Nishi 7, Kita-ku, Sapporo 060-8586, Japan.

Date: _____

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Acknowledgments

First, I want to express my deepest gratitude to my parents for bringing me into this wonderful and vibrant world. Their love is as warm as sunshine, as gentle as a spring breeze, and as beautiful as blooming flowers. I feel their endless care and appreciate their selfless dedication.

Next, I am deeply grateful to Professor Atsushi Tomokiyo, a talented and accomplished scholar who has been extremely kind and supportive. When I encountered issues in my research, he went above and beyond to help me solve them. His assistance was critical in enabling me to complete my experiments. In moments of despair, he guided and encouraged me to keep moving forward. Words cannot fully express my gratitude for his guidance and support.

I would like to sincerely thank Professor Hidehiko Sano. His support made it possible for me to become a PhD student at Hokkaido University, fulfilling my long-cherished academic dream. He guided me through the transition from clinical dentistry to academic research, helping me bridge theory and practice. I aspire to make a mark in the research field, much like Professor Sano. As a world-renowned expert in adhesion, he is incredibly kind, knowledgeable, and always willing to help. He has provided immense support, patiently teaching me how to overcome the challenges of research.

Additionally, I would like to thank Clinical Associate Professor Monica Yamauti from the Department of Biomedical and Applied Sciences Indiana University School of Dentistry for her guidance throughout my PhD studies. Under her mentorship, I explored many new research directions, opening doors to new opportunities. The challenges I faced during this time have shaped me into a capable PhD student.

I am grateful to teacher Ushijima from the Support Section for Education and Research. Her patience and kindness have been invaluable to me; she guided me step-by-step in learning to use the Scanning Electron Microscopy and associated equipment. She also helped me meticulously select images, saving me a considerable amount of time.

I also extend my thanks to Associate Professor Kosuke Takahashi from the Division of Mechanical and Aerospace Engineering for providing me with lab resources and helping me resolve experimental challenges. Lastly, I am grateful to the teachers and students from the Department of Restorative Dentistry at Hokkaido University for their tremendous support in both my research and daily life.

I also want to express my sincere gratitude to Professor Fan Yang (Department of Stomatology, Zhejiang Provincial People's Hospital, Hangzhou, China). He was my mentor in clinical dentistry, and without his guidance, my clinical skills would not have improved so significantly. It was his influence that sparked my desire to pursue a

PhD. He fully supported my decision and helped me navigate difficult times, guiding me step by step toward a brighter future.

I am also thankful to Linhong Wang (Department of Stomatology, Zhejiang Provincial People's Hospital, Hangzhou, China) for her tremendous support, both academically and personally.

Finally, I am deeply grateful to my family and friends. They have provided both financial and emotional support throughout my PhD journey. When I faced financial difficulties, they were there to help, and during times of emotional exhaustion, they were like a lighthouse, guiding me forward. I am truly thankful for their presence, which has made my life journey all the more fulfilling.

ABSTRACT

Objective: This study aims to evaluate the effect of incorporating chitin (CH) on the physical and mechanical properties of three commercial glass ionomer cements at varying concentrations (0%, 2.5%, 5% and 10%).

Methods: GIC samples were prepared by incorporating three commercial GIC powders [Fuji IX GP (GP), Fuji IX GP Extra (EX) and Caredyne-Restore (CD)] with different CH concentrations (0%, 2.5%, 5% and 10%), followed by mixing with the liquid component in accordance with the manufacturer's recommended powder-to-liquid ratios. The morphology of GIC was analyzed using field-emission scanning electron microscope (FESEM). Setting time, water absorption and solubility, compression strength, and diameter tensile strength were evaluated. Setting times were analyzed with one-way ANOVA and Fisher's LSD post hoc test, along with Kruskal-Wallis and pairwise comparison tests. For other properties, one-way ANOVA followed by Tukey's HSD post hoc test was applied. Data are presented as mean ± standard deviation, with statistical significance set at $p < 0.05$.

Results: Adding CH significantly accelerated the setting time in both the EX 10% and CD 10% groups, reducing it by 31.2% and 37.5%, respectively ($p < 0.05$), while the GP 10% group showed a non-significant reduction of 12% ($p > 0.05$). Water absorption was markedly reduced in all CH-modified (GP, EX and CD) groups ($p < 0.05$); with 2.5% CH, reductions of 45%, 36% and 43% were observed in GP, EX and CD groups, respectively ($p < 0.001$). The EX (2.5%, 5% and 10%) group showed increased solubility compared to the control ($p < 0.001$). For compressive strength, no notable differences were observed between CH-modified (GP, EX and CD) and control groups after 24 hours of water storage. However, after 7 days, compressive strength in the EX group with 2.5% and 10% CH was significantly lower than in the control ($p < 0.05$). In the diameter tensile strength test, there was no significant difference between the EX and CD (2.5%, 5% and 10%) groups compared with the

control group, whereas the GP group with 2.5% CH demonstrated the highest diameter tensile strength at 24 hours (p = 0.036), though by day 7, differences in tensile strength across all groups had stabilized.

Conclusions: This study demonstrates the beneficial effects of incorporating CH into GIC, particularly at low concentrations. Incorporating CH at these levels does not significantly alter the setting time of GIC, but improves its mechanical strength and reduces water absorption. Notably, while an increase in solubility is observed, it remains within the negative value range, maintaining the material's integrity. This approach presents a promising strategy for optimizing GICs by utilizing natural polysaccharide fibers.

Keywords: glass ionomer cement, chitin, setting time, water absorption and solubility, compression strength, diameter tensile strength.

Chapter 1：Introduction

Dental caries, commonly known as cavities, arise from acidogenic bacterial activity on the tooth surface, leading to gradual pH reduction, demineralization, and ultimately cavity formation as the balance between demineralization and remineralization is disrupted[1]. Caries is one of the most widespread chronic diseases worldwide, posing a considerable healthcare burden on healthcare systems. In 2019, an estimated 3.5 billion people were affected by oral diseases, with untreated caries impacting over one-third of this population[2]. Expenditures for non-cancerous oral diseases reached approximately $387 billion globally in 2019, underscoring the urgency for effective interventions[2]. Beyond oral health, caries significantly affect chewing ability, aesthetics, growth, cognitive development, and quality of life[3, 4].

Initially developed in 1969 by Wilson and Kent of the British National Research Institute, traditional glass ionomer cements (GICs) consist of aluminosilicate glass and polyacrylic acid[5]. Calcium ions initially react with polyacrylic acid, leading to the formation of calcium polyacrylate and initiating cement setting, though with limited strength at this stage. Subsequently, aluminum ions form aluminum polyacrylate, significantly reinforcing the material in a process that continues for about 24 hours and is essential for achieving full cement maturation[5, 6].

Among materials for caries treatment, GICs stand out due to their ability to chemically bond with dental tissues, release fluoride, and demonstrate excellent biocompatibility[6]. These characteristics make them particularly valuable in managing caries in high-risk populations, providing both therapeutic and preventive advantages[7, 8]. In recognition of their significant utility, the World Health Organization included GICs in its List of Essential Medicines in 2021[7]. This inclusion underscores the importance of GICs as a versatile and cost-effective material in dental restorative care, especially in underserved regions where access to advanced dental treatments may be limited[7]. Despite widespread applications in dental restorations, sealants, and orthodontic adhesives, traditional GICs still exhibit low compressive strength, limited wear resistance, brittleness, and high susceptibility to cracking and dehydration during setting, leading to fractures and secondary caries over time[5, 6, 9, 10]. Indeed, fractures are a leading cause of restoration failure

within six years of placement[11]. To address these challenges, numerous studies have focused on enhancing GIC properties by incorporating various reinforcements such titanium dioxide, silver nanoparticles, nanosized bioactive glass, Al2O3 and SiO2 nanoparticles, glass fibers, chitosan, and cellulose have shown varying degrees of improvement in mechanical properties[12-17]. However, these modifications cannot fully resolve all inherent weaknesses, necessitating further research into enhancing GIC properties while preserving their favorable characteristics.

Chitin, a high-molecular-weight, linear polysaccharide, is the second most abundant natural polymer after cellulose and is widely sourced from crustaceans, mollusks, invertebrates, algae, insects and fungal cell walls[18-20]. Chitin has a unique structure composed of two monomer units: N-acetyl-glucosamine (NAG) and N-glucosamine. If the NAG level exceeds 50%, it is termed chitin, otherwise, it is referred to as chitosan[21]. Chitin contains abundant hydroxyl groups (C-6 has a primary hydroxyl and C-3 has a secondary hydroxyl), C-2 has an amino groups or their N-acetyl counterparts with the strong intermolecular and intramolecular hydrogen bonds contribute to the formation of linear aggregates with extended crystallinity[21, 22]. Chitin is highly hydrophobic and insoluble in most common solvents, including water and acidic or basic aqueous solutions, but it is soluble in strong concentrated inorganic acids[22]. Chitin's mechanical properties include tensile strength in the gigapascal range, and an axial modulus of approximately 41 GPa due to its honeycomb-like molecular structure, which efficiently dissipates various stresses[23]. Chitin's unique combination of biocompatibility and non-toxicity underpins its suitability for a wide range of biomedical applications[18]. These properties, coupled with its inherent antibacterial and antioxidant activity, as well as its thermal stability, make chitin an exceptional candidate for developing medical materials[18, 24]. Its physical and mechanical properties are source-dependent[25]. In dental applications, low concentrations of chitin have been reported to enhance resin adhesives' mechanical properties, microhardness, and resistance to hydrolytic degradation[19]. Whereas, the incorporation of 5% chitin in polylactic acid has shown substantial improvements in mechanical properties and water resistance[26]. Based on these characteristics, we hypothesize that chitin could enhance the mechanical and physical properties of GICs.

This study aims to develop chitin-enhanced GICs by incorporating nano-chitin into GIC powders and evaluating the resultant materials' setting time, water absorption and solubility, compressive strength, and diameter tensile strength. We null hypothesize that varying chitin concentrations (0%, 2.5%, 5% and 10%) across three commercial GIC formulations will not significantly affect these properties.

Material and methods

2.1. Material

Table 1. Materials tested, manufacturers, abbreviations, Powder / liquid ratio, compositions and batch numbers.

Material	Powder / liquid ratio (g/g)	Composition	Batch number
Fuji IX GP GC, Tokyo, Japan (GP)	3.6:1	Powder: fluoroaluminosilicate glass, polyacrylic acid Liquid: polyacrylic acid, polybasic carboxylic acid, Distilled water	231023
Fuji IX GP Extra GC, Tokyo, Japan (EX)	3.4:1	Powder: fluoroaluminosilicate glass, polyacrylic acid Liquid: polyacrylic acid, Polybasic carboxylic acid, Distilled water	230919
Caredyne-Restore GC, Tokyo, Japan (CD)	2.3:1	Powder: Fluorozincsilicate glass, Fluoroaluminosilicate glass Liquid: Polyacrylic acid, Polybasic carboxylic acid Distilled water	231024
Chitin, Koyo Chemical Co, Japan (CH)	—	Powder: N-acetyl-d-glucosamine group	BB760-KU

2.2. GIC-Chitin formulation preparation

To formulate GIC-CH powders, CH particles were incorporated at weight concentrations of 0%, 2.5%, 5% and 10%. Powders were precisely weighed using an electronic scale to ensure the manufacturer's specified powder-liquid ratios were met, with the powder dispensed prior to the liquid in each formulation to maintain accuracy across all GIC-CH preparations.

Powder measurement variability emerged as a notable factor in achieving consistent chitin-modified GIC preparation. To standardize measurement, we

explored four approaches to powder-liquid ratio consistency, summarized below (Table 2):

1. Traditional Method: We used a level scoop directly from the bottle (n = 10). The GP and EX brands showed the largest standard deviations, likely due to powder compression from scoop pressure, exceeding the optimal powder-liquid ratio before accounting for liquid variability. CD showed less variance, potentially due to its shallower scoop design, which minimized pressure effects. Although this method may inadvertently enhance GIC physical and mechanical properties—a potential clinical benefit—it does not provide the consistency required for reproducible research[27].
2. Alternative Scoop Method: We transferred the powder to a paper, allowing it to gently slide into the scoop until level (n = 10). This method reduced standard deviations for GP and EX but increased it for CD, possibly due to differences in scoop design. This method, however, was time-intensive and wasteful, and did not account for liquid variability.
3. Calculated Powder Measurement Method: To remove powder measurement variability, we measured the weight of each liquid drop (n = 10) and calculated the necessary powder weight based on the manufacturer's optimal ratio. This approach yielded the lowest standard deviation, as it removed the variability associated with powder measurement.
4. Individual Weight Calculation Method: Here, we measured the liquid weight each time, calculated the powder and chitin amounts based on the concentration, and used these values to achieve the exact powder-liquid ratio recommended by the manufacturer. Although precise, this method was time-consuming and could lead to liquid evaporation, potentially impacting results[28].

Based on this analysis, we adopted the third method for consistent powder measurement, as it offers a balance of fairness, repeatability, and uniformity in experimental results, which is essential for robust, evidence-based conclusions.

Table 2. provides the weights of GIC powder using each method, expressed as Mean ± SD (g).

Method	GP（g）	EX（g）	CD（g）
1	0.23241（51.35）	0.2349(72.47）	0.139028（22.72）
2	0.16538（36.92）	0.17295（58.74）	0.10833（58.93）
3	0.18914（15.1）	0.17836（21.92）	0.12466（11.76）
4	Control	Control	Control
	GP（g）	EX（g）	CD（g）
Liquid	0.05254（15.1）	0.05246（21.92）	0.0542（11.76）

2.3. Setting time evaluation using Gilmore needle

Initial and final setting times were determined using a Gillmore needle (Humboldt MFG, USA) following ADA and ISO 9917-1:2007 standards. To simulate oral conditions, we developed a custom distillation unit with controlled temperature (37°C ± 1°C) and relative humidity (>90%) that featured a boiling pot to simulate breathing, an operational table to mimic intraoral teeth positioning, and a curtain to replicate lip motion. This novel setup—applied for the first time in such testing—provides an accurate simulation of the oral environment. Experimental trials (N=30) indicated consistent maintenance of temperature (mean 90.7°C, SD 0.57) and humidity (mean 37.15%, SD 0.16), with fluctuations well within ±1°C and ±1%, respectively.

The cement was prepared in accordance with ISO 9917-1:2007 standards and the manufacturer's instructions. After mixing, the material was placed into a rectangular aluminum foil mold measuring 8.00 mm × 10.00 mm × 5.00 mm. Setting time was assessed using a Gilmore needle (1.06 mm in diameter, 453.6 g in weight), applied vertically to the cement surface for 5 seconds. Indentations were made every 30 seconds at different points, with the needle cleaned between applications. The process continued until no visible circular indentation was observed. The setting time was recorded as the duration from the start of mixing to the moment when the needle failed to leave a mark on the surface.

2.4. Water Absorption and Solubility evaluation

Following ISO 4049:2019 standards, water absorption and solubility were assessed. Material was placed in an acrylic cavity mold (15 mm × 1 mm) and compressed between acrylic plates to eliminate excess, then incubated at 37°C until a stable mass (m_1) was obtained. Each specimen's diameter and thickness were measured to calculate volume (V) as $V=\pi\times r^2\times h$. Samples were stored in distilled water at 37°C for 7 days, weighed to obtain mass (m_2), and then dried until reaching a final constant mass (m_3). Water absorption (W_{sp}) and solubility (W_{sl}) were calculated using: $W_{sp}=(m_2-m_3)/V$ and $W_{sl} = (m_1-m_3) / V$

2.5. Compressive strength evaluation

In accordance with ISO 9917-1:2007 standards, compressive strength (CS) tests utilized cylinder samples (4 mm diameter, 6 mm height) prepared in a split steel mold, and with petroleum jelly was applied to facilitate sample removal. Samples were then stored at 37°C and a minimum 30% humidity for one hour. Further, samples were stored in distilled water at 37°C for 23 hours and 7 days. CS was determined using a universal testing machine (Instron Corp, Canton, MA， US) at a crosshead speed of 0.75 mm/min until fracture, calculated as: $CS=4P/\pi d^2$

where P is the maximum applied load in Newtons (N), and d is the sample diameter in (mm).

2.6. Diameter tensile strength evaluation

DTS was tested according to ADA Standard 27 and BS 6039:1981 using cylindrical samples (6 mm diameter, 3 mm height). Samples were incubated at 37°C and >30% humidity, then submerged in distilled water for 23 hours and 7 days. During testing, damp filter paper ensured contact between the sample and testing machine

plates. DTS was determined using a universal testing machine (Instron Corp, Canton, MA， US) at a crosshead speed of 0.75 mm/min, applying stress along the specimen's diameter until fracture. DTS was calculated as: $DTS=2P/\pi dh$

where P is the maximum applied load in Newtons (N), d is the sample diameter in millimeters (mm), and h is the sample height in millimeters (mm).

2.7. Morphological analysis

After compression testing, the sample surfaces were coated for 120 seconds using a Pt-Pd ion sputtering technique (E-1030 Ion Sputter Coater; Hitachi High-Technologies, Tokyo, Japan). Subsequently, a field-emission scanning electron microscope (FE-SEM; JSM-4800 F, JEOL Ltd, Tokyo, Japan) was used to examine the morphology of the coated fracture at an accelerating voltage of 10 kV.

2.8. Statistical Analysis

Statistical analysis of setting times was performed using one-way ANOVA with Fisher's LSD post hoc test, along with by Kruskal-Wallis and pairwise comparison tests (SPSS v29). For water absorption, solubility, compressive strength, and diameter tensile strength, one-way ANOVA followed by Tukey's HSD post hoc test was applied (SPSS v29). Data are presented as mean ± standard deviation, with statistical significance set at $p < 0.05$.

Chapter 3: Results

3.1. Setting time

Table 3 and Figure 1 showed significant differences in setting times among the tested materials. Low chitin concentrations exhibited no significant effect on setting time, while a 10% addition reduced setting times significantly in the EX and CD groups ($p < 0.001$), by 31.1% and 37.4% respectively, relative to controls. No statistically significant differences were observed within the GP group ($p > 0.05$). In the CD group, the setting times for the control, 2.5% and 5% chitin concentrations exceeded the range specified by ISO 9917-1:2007.

Table 3. Setting time expressed as Mean ± SD (seconds) according to chitin concentration (% wt) added to each material.

Groups	Control	2.5%	5%	10%
Fuji IX GP	$251 \pm 13.4^{A\,a}$	$257 \pm 16.4^{A\,a}$	$251 \pm 13.4^{A\,a}$	$221 \pm 13.4^{A\,a}$
Fuji IX GP Extra	$323 \pm 16.4^{A\,b}$	$281 \pm 13.4^{AB\,a}$	$323 \pm 16.4^{A\,b}$	$222.5 \pm 15.0^{B\,a}$
Caredyne-Restore	$545 \pm 0.0^{A\,c}$	$533 \pm 26.8^{A\,b}$	$485 \pm 0.0^{AB\,c}$	$341 \pm 13.4^{B\,b}$

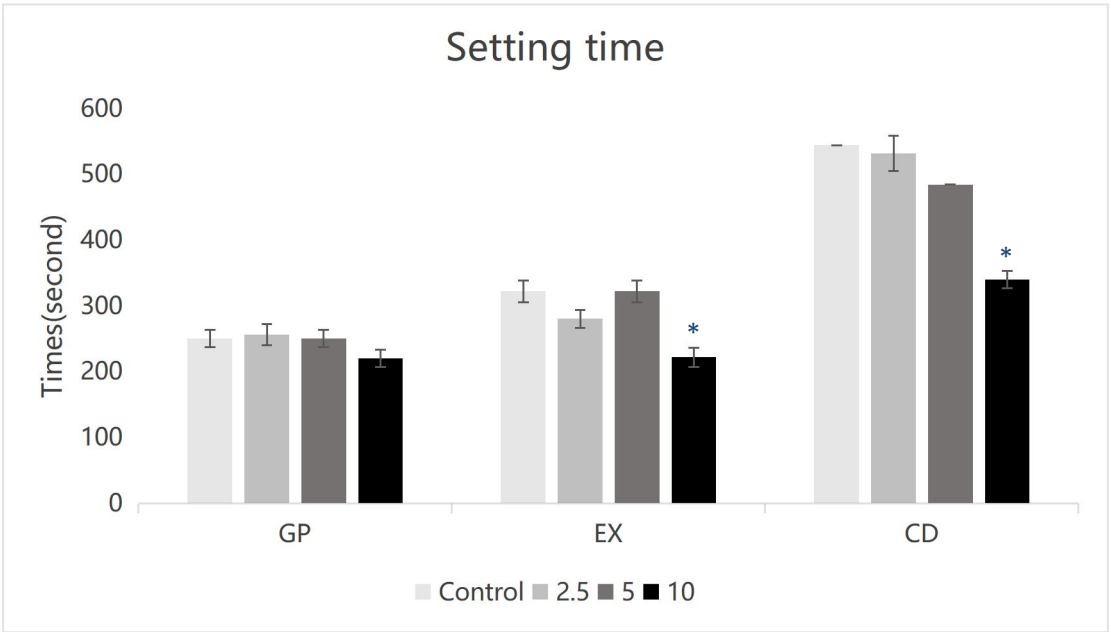


Figure 1. Setting time expressed as Mean ± SD (seconds) according to chitin concentration (% wt) added to each material. * indicates significant difference compared to control.

3.2. Water Absorption and Solubility

Figures 2 and Tables 4 and 5 reveal significant differences in water absorption of GIC with added chitin ($p < 0.05$). Particularly, in the GP, EX and CD groups, the 2.5% chitin group had the lowest water absorption compared to the control ($p < 0.001$), with reductions of 45%, 36% and 43%, respectively. Water absorption increased as chitin concentration increased. In the solubility tests, solubility was significantly higher in the EX (2.5%, 5% and 10%) group than in the control ($p < 0.001$), but there were no significant differences in the GP and CD groups compared to their controls ($p \geq 0.257$ and $p \geq 0.062$, respectively). Additionally, we observed that in high powder-liquid ratio GP and EX groups, solubility increased with chitin concentration up to a critical point, after which it declined. In the low powder-liquid ratio CD group, solubility increased consistently with higher chitin concentrations. The water absorption of all three materials mostly exceeded the ISO standard (<40 μg/mm³), while solubility generally met the ISO standard (<7.5 μg/mm³).

Table 4. Water absorption expressed as Mean ± SD (seconds) according to chitin concentration (% wt) added to each material.

Groups	Control	2.5%	5%	10%
Fuji IX GP	$134.43 \pm 7.55^{A\,c}$	$74.42 \pm 16.41^{C\,b}$	$88.92 \pm 15.43^{BC\,b}$	$101.56 \pm 7.86^{B\,c}$
Fuji IX GP Extra	$161.53 \pm 17.22^{A\,b}$	$102.97 \pm 32.38^{B\,a}$	$119.66 \pm 4.76^{B\,a}$	$122.91 \pm 6.65^{B\,b}$
Caredyne-Restore	$185.62 \pm 13.79^{A\,a}$	$105.97 \pm 9.52^{C\,a}$	$112.86 \pm 21.97^{C\,a}$	$161.31 \pm 16.13^{B\,a}$

Table 5. Water solubility expressed as Mean ± SD (seconds) according to chitin concentration (% wt) added to each material.

Groups	Control	2.5%	5%	10%
Fuji IX GP	$-36.75 \pm 5.58^{A\,a}$	$-38.75 \pm 6.11^{A\,a}$	$-27.49 \pm 15.81^{A\,a}$	$-35.04 \pm 5.21^{A\,a}$
Fuji IX GP Extra	$-37.82 \pm 5.74^{A\,a}$	$-17.31 \pm 6.80^{B\,c}$	$-4.70 \pm 6.65^{C\,b}$	$-12.69 \pm 3.82^{B\,b}$
Caredyne-Restoe	$-12.69 \pm 7.06^{A\,b}$	$-26.16 \pm 3.22^{A\,b}$	$-19.61 \pm 14.83^{A\,ab}$	$-14.78 \pm 12.78^{A\,b}$

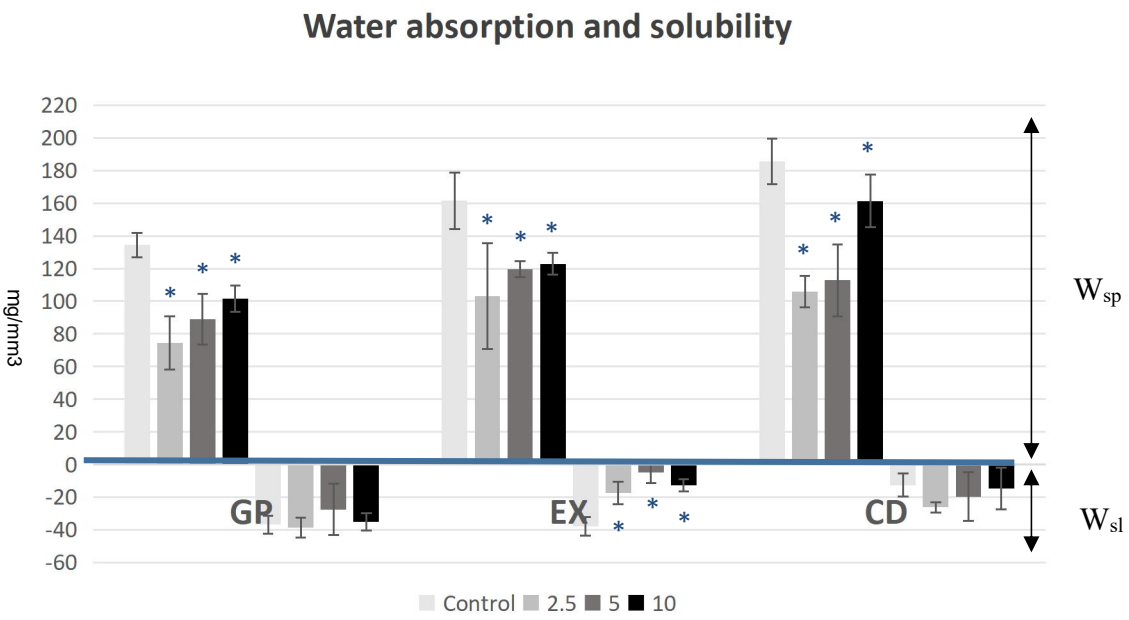


Figure 2. Water absorption and solubility expressed as Mean ± SD (seconds) according to chitin concentration (% wt) added to each material. * indicates significant difference compared to control.

3.3. compressive strength

Figures 3 and Tables 6 and 7 reveal distinct differences in compressive strength across the modified GIC materials. Following 24 hours of water storage, there were no statistically significant differences in compressive strength between experimental and control groups within GP, EX and CD formulations ($p > 0.05$). Similarly, after seven-day water immersion, compressive strength in GP and CD groups remained comparable to controls. Notably, however, in the EX group, samples with 2.5% and 10% chitin exhibited significantly reduced compressive strength relative to controls ($p < 0.05$).

Table 6. Compressive strength is expressed as Mean ± SD (MPa) according to chitin concentration (% wt) added to each material after 24 hours of storage in distilled water.

Groups	Control	2.5%	5%	10%
Fuji IX GP	62.49 ± 35.67^{Aa}	58.83 ± 27.17^{Aa}	40.40 ± 20.00^{Aa}	44.69 ± 10.00^{Aa}
Fuji IX GP Extra	66.52 ± 21.53^{Aa}	48.51 ± 16.40^{Aa}	54.42 ± 21.60^{Aa}	37.45 ± 6.10^{Aa}
Caredyne-Restore	43.65 ± 27.15^{Aa}	35.81 ± 8.14^{Aa}	36.45 ± 0.07^{Aa}	35.92 ± 5.14^{Aa}

Table 7. Compressive strength is expressed as Mean ± SD (MPa) according to chitin concentration (% wt) added to each material after 7 day of storage in distilled water.

Groups	Control	2.5	5	10
Fuji IX GP	$90.65 \pm 58.99^{A\,a}$	$87.98 \pm 27.17^{A\,a}$	$80.39 \pm 13.56^{A\,a}$	$69.36 \pm 12.77^{A\,a}$
Fuji IX GP Extra	$102.80 \pm 11.94^{A\,a}$	$65.29 \pm 18.81^{BC\,ab}$	$85.37 \pm 19.53^{AB\,a}$	$52.64 \pm 7.29^{C\,b}$
Caredyne-Restore	$50.75 \pm 11.10^{A\,b}$	$36.80 \pm 14.71^{A\,b}$	$44.78 \pm 7.90^{A\,b}$	$45.24 \pm 4.83^{A\,b}$

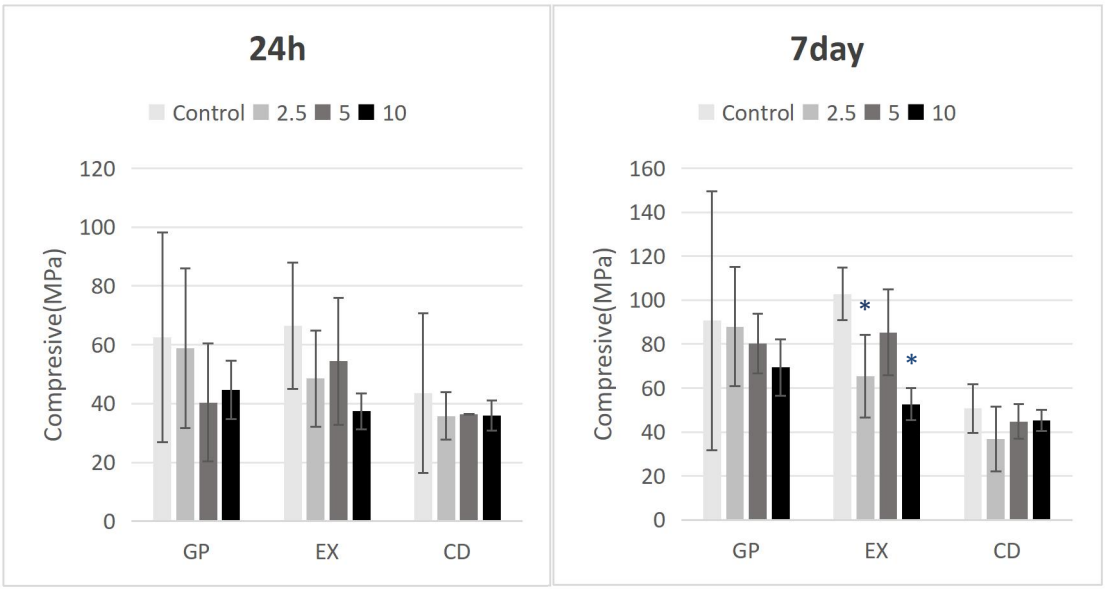


Figure 3. Compressive strength is expressed as Mean ± SD (MPa) according to chitin concentration (% wt) added to each material after 24 hour and 7 day of storage in distilled water. * indicates significant difference compared to control.

3.4. Diameter tensile strength

Figures 4 along with Tables 8 and 9, illustrate significant differences in the diameter tensile strength of GIC materials modified with chitin. The addition of chitin demonstrated enhancements in mechanical performance, with the GP 2.5 group showing the highest diameter tensile strength after 24 hours of water storage compared to controls ($p = 0.036$). In contrast, no statistically significant changes were observed in the EX and CD groups ($p > 0.05$). After seven days of water immersion, no significant differences in diameter tensile strength were noted between experimental groups and controls across GP, EX and CD formulations ($p > 0.05$).

Within each group, however, the 2.5% chitin concentration consistently achieved the highest strength. Noteworthy is the variation in diameter tensile strength between GIC brands when low chitin concentrations were used, highlighting the influence of brand ingredient compositions.

Table 8. Diameter tensile strength is expressed as Mean ± SD (MPa) according to chitin concentration (% wt) added to each material after 24 hours of storage in distilled water.

Groups	Control	2.5	5	10
Fuji IX GP	12.60 ± 0.86^{Aa}	15.05 ± 1.54^{Ba}	14.26 ± 1.52^{Aa}	14.25 ± 1.03^{Aa}
Fuji IX GP Extra	10.14 ± 1.65^{Ab}	12.27 ± 1.51^{Ab}	11.27 ± 1.53^{Ab}	10.83 ± 1.15^{Ab}
Caredyne-Restore	8.02 ± 0.79^{Ac}	8.77 ± 1.22^{Ac}	8.60 ± 1.18^{Ac}	9.90 ± 1.04^{Ab}

Table 9. Diameter tensile strength is expressed as Mean ± SD (MPa) according to chitin concentration (% wt) added to each material after 7 day of storage in distilled water.

Groups	Control	2.5	5	10
Fuji IX GP	15.13 ± 1.93^{Aa}	17.43 ± 0.98^{Aa}	16.08 ± 1.32^{Aa}	15.04 ± 1.69^{Aa}
Fuji IX GP Extra	10.93 ± 2.17^{Ab}	13.57 ± 2.08^{Ab}	12.04 ± 0.49^{Ab}	11.56 ± 1.55^{Ab}
Caredyne-Restore	9.25 ± 1.23^{Ab}	10.15 ± 0.59^{Ac}	9.58 ± 0.44^{Ac}	9.91 ± 0.86^{Ab}

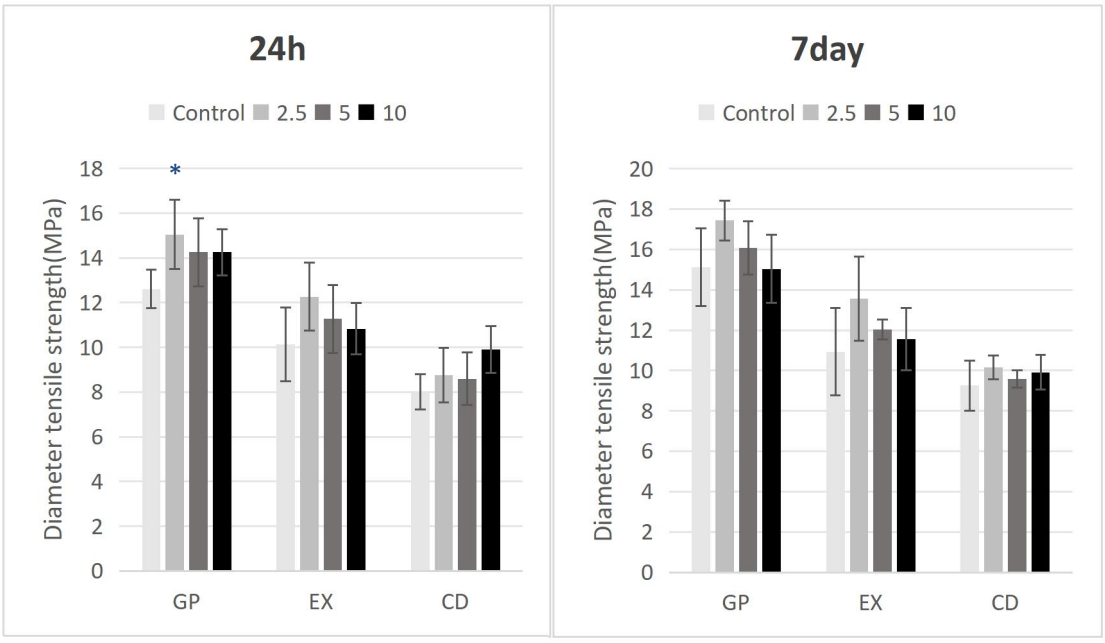
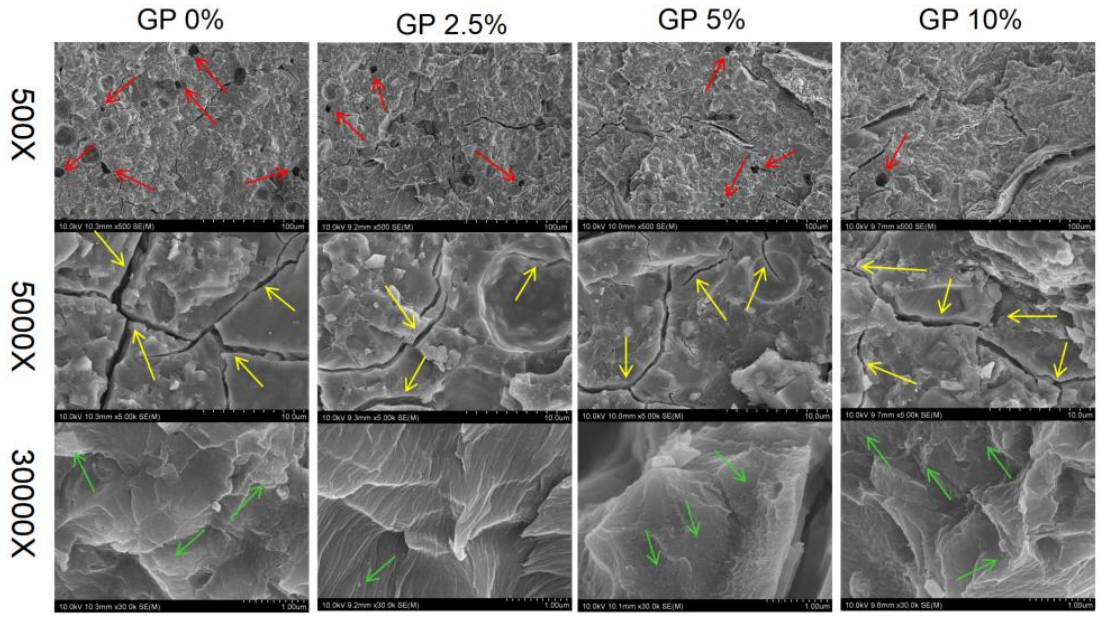


Figure 4. Diameter tensile strength is expressed as Mean ± SD (MPa) according to chitin concentration (% wt) added to each material after 24 hours and 7 day of storage in distilled water. * indicates significant difference compared to control.

3.5. Morphological analysis

Scanning electron microscopy analysis revealed concentration-dependent morphological changes in the fracture surfaces of GIC matrices modified with chitin across GP, EX and CD formulations. At a chitin concentration of 2.5%, all groups demonstrated a reduction in void size and crack width, coupled with a smoother, denser microstructure characterized by a uniform distribution of chitin. These structural refinements likely enhanced interfacial adhesion by minimizing tension and improving matrix cohesion. In contrast, the control samples exhibited a distinctly granular texture. However, increasing chitin concentrations led to progressive granularity and surface roughness, alongside fewer but larger voids and wider cracks, indicative of microstructural degradation. The observed correlations between chitin concentration, powder-to-liquid ratios, and crack dimensions underscore the pivotal role of composition and preparation parameters in determining GIC matrix integrity and performance.



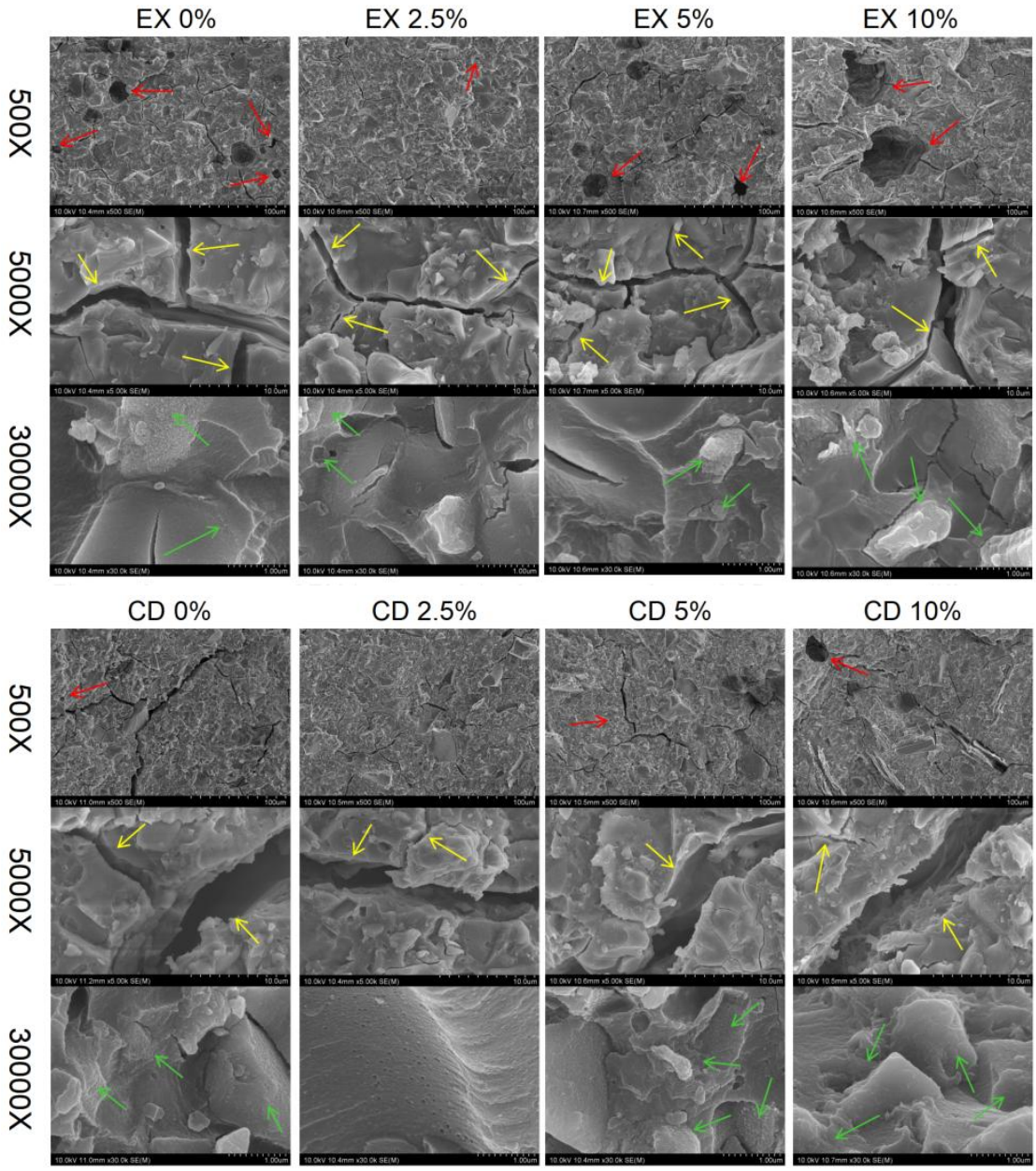


Figure 5. represents SEM images of the fracture surface of GIC under different magnification. The red arrows represent voids, the yellow arrows represent cracks, and the green arrows represent particles.

Chapter 4: Discussion

Dental caries treatment remains a significant challenge for healthcare providers and researchers, with over one-third of the global population affected by untreated caries[2]. The World Health Organization has recognized the importance of GIC for its affordability and ease of use, listing it on the Essential Medicines List to address the oral healthcare needs of low-income populations[7]. However, GIC has various drawbacks, which prompt the need to enhance its physical and mechanical properties [5]. Chitin is abundant in nature, biocompatible[19], and costs approximately $140 per kilogram—around 28% of the cost of cellulose—which would increase the cost of each GIC set by about 1 cent[17], offering patients a cost-effective product. The main goal of our study was to improve the clinical performance of GIC by enhancing its physical and mechanical properties through chitin modification.

The results of our study indicated that the addition of 10% chitin reduced the setting time of the EX and CD groups by 31.2% and 37.5%, respectively, compared to the control group ($p < 0.001$). The water absorption test revealed significant differences in water absorption of GIC with added chitin ($p < 0.05$). In solubility testing, solubility was significantly higher in the EX (2.5%, 5% and 10%) group compared to the control ($p < 0.001$). CS results showed that after 7 days of water storage, in the EX group, the samples with 2.5% and 10% chitin concentrations exhibited significantly lower compressive strength than the control ($p < 0.05$). DTS testing results showed that after 24 hours of water storage, the GP 2.5 group demonstrated the highest DTS compared to the control group ($p = 0.036$). Therefore, incorporating 2.5% chitin into GIC does not significantly affect its setting time but enhances its mechanical strength and reduces water absorption. However, the observed increase in solubility, while remaining within a negative value range, maintaining the materials integrity. These characteristics position chitin-modified GIC as a promising high-performance restorative material for caries treatment, particularly in managing caries in at-risk populations.

4.1 *Setting time*

This study aimed to provide accurate insights into the effects of modified GICs on setting time, serving as a reference for future researchers. Chitin is highly hydrophobic, insoluble in common solvents, including water and acidic or basic aqueous solutions, but dissolves in concentrated inorganic acids, demonstrating robust chemical resistance[22]. When incorporated into GICs, chitin primarily acts as a filler. However, as shown in Fig. 5, chitin may interact with GICs through surface adhesion. The chitin chains are rich in hydroxyl and acetamide groups, enabling hydrogen bonding with the hydroxyl groups on GIC particles and the carboxyl groups of polyacrylic acid[29].

According to the manufacturer's specifications, nano-chitin, with a density of 1.37 g/cm³ (solid), is less dense than GIC powder (2.40 – 4.0 g/cm³)[30]. Thus, at equivalent weights, incorporating chitin increases the powder-liquid ratio, as chitin's smaller particle size and larger surface area enhance acid-base reaction potential[31]. As depicted in Fig. 5, chitin is uniformly distributed in the GIC matrix, reducing setting time. However, increasing chitin concentration increased the mixture viscosity, particularly at 10%, where mixing uniformity became challenging, leading to incomplete setting reactions. Experimental results showed that adding 10% chitin reduced the setting time by 31.2% and 37.5% in EX and CD groups, respectively (both statistically significant, $p < 0.001$). In contrast, the GP group exhibited a 12% reduction, though without statistical significance ($p > 0.05$). Prior studies suggest smaller particle sizes and increased surface area contribute to shorter setting times[14]. However, our findings indicate that low chitin concentrations had no significant impact. Notably, the control groups of GP, EX and CD differed significantly in setting time, with GP being the shortest and CD the longest. This aligns with previous reports emphasizing the role of powder-to-liquid ratio in reducing setting time[27]. Our materials had powder-to-liquid ratio of GP (3.6:1), EX (3.4:1) and CD (2.3:1), suggesting CD longer setting time is attributable to its lower powder-to-liquid ratio. The introduction of chitin increased the powder-to-liquid ratio, particularly at high

concentrations, which explains the reduced setting time with 10% chitin. While particle size and surface area are often considered, density—a key factor in altering the powder-to-liquid ratio—must not be overlooked. Interestingly, the setting time of CD at 0%, 2.5% and 5% exceeded ISO 9917-1:2007 standards, posing clinical challenges. However, the significant reduction in setting time with 10% chitin may benefit both clinicians and patients.

4.2 Water Absorption and Solubility

The durability of cements is influenced by numerous factors, with water absorption and solubility playing critical roles. These parameters significantly affect the material's integrity, as water infiltration and dissolution can degrade the cement matrix, leading to restoration fractures and marginal breakdown[32]. Such compromised regions promote bacterial colonization, increasing the risk of secondary caries and eventual failure of restorations[32]. Chitin, known for its high hydrophobicity and insolubility in conventional solvents (e.g., water, acidic, or alkaline solutions), only dissolves in concentrated inorganic acids, which weakens its adhesion to GIC[22]. This hydrophobicity potentially limits water penetration into the material, thereby reducing W_{sp} and W_{sl}. Our study demonstrates that incorporating chitin significantly reduces water absorption, particularly at low concentrations, markedly decreases both W_{sp} ($p < 0.001$). As shown in Fig. 5, chitin may actively engage with GIC via surface adhesion, indicating its potential role in modifying the matrix microstructure. This interplay underscores chitin's dual role, both as a structural filler and as an interactive component enhancing the material's properties[15].

According to GIC water absorption theories[33], two mechanisms are proposed: free-volume theory, where water diffuses into the matrix through cracks and voids as “loosely bound water,” and interaction theory, where water penetrates by binding with hydrophilic groups as “bound water.” Similar to previous findings with 5% chitin in polylactic acid, which showed enhanced physical and mechanical properties,

particularly lower water absorption[26], However, Elevated concentrations of chitin weaken its adhesion to polyacrylic acid, resulting in reduced interfacial bonding. Figure 5 illustrates that this increase in chitin content exacerbates the formation of voids and cracks at the chitin-GIC matrix interface, promoting water ingress. Consequently, higher chitin concentrations are associated with increased water absorption. This phenomenon highlights the importance of balancing chitin content to optimize the material's microstructural integrity and moisture resistance. Research indicates that high fiber concentrations in GIC can lead to structural defects and voids, as well as poor adhesion between polyacrylic acid and fibers, resulting in gaps that facilitate water absorption[16, 34]. In our study, the CD group, with the lowest powder-to-liquid ratio, exhibited the highest water absorption, likely due to fewer ionic bonds within the matrix, increasing material solubility[33, 35]. Water absorption values for the three materials mostly exceeded the ISO standard (less than 40 $\mu g/mm^3$), whereas solubility values were generally within the ISO limit (less than 7.5 $\mu g/mm^3$).

We observed negative solubility values, which may be explained as follows. First, internal microcracks generated upon GIC dehydration can heal upon water exposure, a phenomenon known as GIC “self-healing”[36]. Secondly, prolonged water storage promotes salt bridge formation within GIC, increasing the proportion of bound water and material weight[6]. Finally, negative solubility does not imply the absence of dissolution but rather reflects incomplete material dehydration[37]. In the solubility experiment, we observed a significant increase in the solubility of the EX (2.5%, 5% and 10%) group ($p < 0.001$). However, it is noteworthy that the solubility values remained negative, indicating that the material retained its integrity despite the increase. The observed solubility increase may be related to the specific formulation and brand composition of the GIC used, which could contribute to variability in the dissolution behavior. Such findings underscore the importance of considering brand-specific components when evaluating the solubility and durability of modified GICs. Flores Ledesma studies suggest a correlation between setting time and

solubility across cement types. We observed a similar trend in the CD group, where longer setting times were correlated with higher water solubility. In particular, CD was developed for root surface caries, where reducing water absorption and solubility is crucial due to frequent submersion in gingival fluid.

A limitation of the water absorption test is that the weight increase reflects water gain alone, though it actually represents a balance between water absorption and the dissolution of low-molecular-weight organics. Therefore, the actual water absorption rate may be slightly higher than the experimental value[38].

4.3 Compressive strength

Chewing is a frequent and essential function in daily eating, and compression during chewing is particularly important in food breakdown. Consequently, the compressive strength of restorative materials in resisting food-induced compressive forces is critical[39]. In fact, compressive strength testing is the sole strength metric specified by ISO 9917-1:2003, the international standard for powder/liquid acid-base cements and restorative materials[40].

Chitin's unique molecular structure consists of N-acetylglucosamine and N-glucosamine units in varying proportions[21]. The hydroxyl and acetamide groups in chitin interact with the polyacrylic acid matrix of GIC through hydrogen bonding rather than direct chemical reactions, acting as a physical enhancer of mechanical properties[29]. However, hydrogen bonds are weaker than covalent or ionic bonds, which limits their ability to strengthen bonding interfaces.

In our study, GIC compressive strength after seven days of water storage significantly decreased with the addition of 10 wt.% chitin ($p < 0.05$), potentially due to chitin's high hydrophobicity and lack of solubility in typical solvents like water or acidic and basic aqueous solutions[22]. This hydrophobicity may reduce chitin’s bonding capacity with GIC, the high chitin concentration caused noticeable increases in voids and cracks within the matrix, as evident in the widened crack zones of the 10% chitin group (Figure 5). likely to create microstructural defects and gaps between

the chitin and matrix that may act as stress concentration sites, thereby compromising the cement's mechanical properties[41]. Additionally, the high concentration of chitin disrupts the powder-to-liquid ratio, further compromising the material's performance[27]. Chitin's relatively low density (1.37 g/cm^3) compared to GIC powders (2.40 – 4.0 g/cm^3) increases the powder volume per unit mass[30]. This alteration complicates the mixing process, reduces the efficiency of the acid-base reactions, and subsequently limits polymer cross-linking[42]. Additional, Because Some chitin chains separate and interact with each other rather than engaging with PAA or the surface of GIC particles[29]. Such factors collectively contribute to the diminished mechanical strength observed in the high-chitin GIC formulations. Similarly, one study reported that increasing chitosan from 0.0044 wt.% to 0.012 wt.% in GIC significantly degraded mechanical properties[29]. According to the manufacturer, adding 10% chitin reduced the glass powder-liquid ratio, lowering GP from 3.6:1 to 3.2:1, EX from 3.4:1 to 3.0:1 and CD from 2.3:1 to 2.0:1. Prior studies report that reduced powder-to-liquid ratio can decrease mechanical strength in GICs[27]. Thus, these factors collectively explain the reduction in mechanical strength in high-chitin GIC formulations. Notably, in solubility tests, the EX group exhibited a significant increase, possibly contributing to its reduced compressive strength[13]. Moreover, a significant drop in compressive strength was observed in the EX group at a 2.5% chitin concentration ($p < 0.05$). This reduction could be attributed to insufficient control of testing parameters during manual mixing, where uncontrolled variables such as laboratory environmental conditions, operator experience and fatigue may have contributed to the variability. These inconsistencies align with previously reported findings in similar studies[43, 44]. Such inconsistencies highlight the critical importance of precise preparation methods in ensuring reliable performance outcomes. In developing dental materials, optimizing mechanical properties should be balanced with other critical performance factors to meet clinical requirements.

4.4 Diameter tensile strength

Although not specified in ISO standards for aqueous cements, DTS is often used to assess brittle materials. Unlike compressive loading, brittle materials commonly fail under tensile stress due to crack propagation, which is more likely under tensile rather than compressive loads[45]. Since brittle materials like GIC cannot undergo direct tensile strength measurements, the British Standards Institute adopted DTS testing, which better simulates material failure by crack expansion[46]. In this method, compressive force is applied across the cylinder's diameter, with evidence indicating that compressive components inhibit tensile crack propagation. Localized shear stress below the contact area may also contribute to shear failure prior to tensile failure at the sample's center[46].

Results (Table 7 and 8) show that GIC with added chitin exhibited increased DTS, especially in the GP group with 2.5% chitin, which had significantly higher DTS than the control group (p = 0.036). Similar increases, though not statistically significant, were observed across GP (5% and 10%) groups with added chitin. We hypothesize that the fine chitin particles fill voids between unreacted GIC glass particles, providing more bonding sites for PAA, reducing voids and cracks, limiting water infiltration, and thus reducing microstructural defects and stress concentration risk[12, 15, 41]. Notably, as chitin concentration increased, DTS declined in GP, EX and CD groups, potentially due to some chitin chains separate and interact with each other rather than engaging with PAA or the surface of GIC particles[29]. Chitin's hydroxyl and acetamide groups likely bond with PAA, forming a network around inorganic particles and potentially reducing interfacial tension between GIC components, thereby enhancing mechanical properties[29]. Over time, further acid-base reactions in water may strengthen mechanical performance. Seven-day samples in Tables 7 and 8 showed higher DTS values than those at 24 hours, likely due to ongoing maturation of the setting reaction, which can continue for up to a year, further enhancing GIC strength[5, 47].

Limitation

This study has certain limitations. The setting dynamics of GIC-chitin remain unexplored, leaving the underlying chemical reactions partially uncharacterized. The water absorption analysis is further limited by its inability to differentiate between pure water uptake and the concurrent dissolution of low-molecular-weight organics, suggesting that the true water absorption rate may slightly exceed the measured values[38]. Additionally, while mechanical strength provides insight into the restorative performance under controlled conditions, it does not encompass the complexity of the oral environment.

Conclusion

In conclusion, this study demonstrates incorporating of chitin with GICs, which offers potential benefits, particularly at low concentrations that do not significantly alter the setting time of GIC but improves its mechanical strength and reduces water absorption. Notably, while an increase in solubility is observed, it remains within the negative value range, maintaining the material's integrity. This approach provides a promising pathway for modifying the physical and mechanical properties of GICs through natural polysaccharide fibers. Future research should continue to evaluate the optimal chitin concentration for GICs by assessing physical, chemical, biological, and adhesive properties to provide comprehensive data for clinical application.

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