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Research Article

EFFECT OF EGGSHELL-DERIVED FILLER AND CHITOSAN INCORPORATION ON THE FLEXURAL STRENGTH OF RESIN-BASED COMPOSITE RESTORATIVE MATERIAL: AN IN VITRO STUDY

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Abstract: Resin-based composite restorative materials are widely used in dentistry; however, enhancing their mechanical properties remains a challenge. Natural biomaterials such as eggshell-derived filler and chitosan have shown potential due to their biocompatibility and reinforcing characteristics Objective: To evaluate the effect of eggshell-derived filler and chitosan incorporation on the flexural strength of a nano-hybrid resin-based composite restorative material. Methods: This in-vitro experimental study was conducted using a nano-hybrid composite resin (Filtek Z350, 3M ESPE). A total of 40 standardized rectangular specimens were fabricated and divided into four groups (n = 10): control, 3% eggshell, 1% chitosan, and a combination group. Eggshell powder was prepared through cleaning, drying, grinding, and sieving, while chitosan was dissolved in acetic acid for uniform mixing. Specimens (25 × 2 × 2 mm) were fabricated, light-cured, and stored in distilled water at 37°C for 24 hours. Flexural strength was measured using a three-point bending test on a universal testing machine. Data were analyzed using one-way ANOVA and Tukey post hoc test ($p \leq 0.05$) Results: All The highest mean flexural strength was observed in the eggshell group (112.4 ± 6.2 MPa), followed by the combined group (104.1 ± 6.0 MPa), control (95.2 ± 5.6 MPa), and chitosan group (88.7 ± 5.9 MPa). Statistical analysis showed significant differences among groups ($p < 0.001$). Conclusion: Eggshell-derived filler significantly improved flexural strength, while chitosan alone reduced it. Combined incorporation showed moderate improvement, indicating potential synergistic effects.

Keywords: antibacterial, chitosan, mechanical properties, resin composite.

INTRODUCTION

Resin-based composite materials are widely regarded as the material of choice in modern-day restorative dentistry because of their superior aesthetics, effective adhesion, and favorable handling characteristics. Their capacity to mimic natural tooth morphology and establish durable bonds with enamel and dentin makes them suitable for both anterior and posterior restorations (1,2). Advances in filler technology, resin chemistry, and polymerization systems have significantly improved the clinical performance of composite resins over the past decades (3). However, despite these improvements, mechanical limitations such as reduced flexural strength, polymerization shrinkage, and susceptibility to fracture under functional loading remain major concerns, particularly in stress-bearing areas (4,5).

Flexural strength is an important mechanical property of restorative materials, as it reflects their ability to resist deformation and fracture under masticatory forces. Composite restorations are subjected to repeated bending stresses during function, which may lead to crack initiation and propagation, ultimately resulting in restoration failure (6). Therefore, improving the flexural strength of composite resins is essential to enhance their longevity and clinical reliability

(7). Various strategies have been proposed to improve mechanical properties, including modification of filler type, size, and distribution, as well as incorporation of reinforcing agents (8,9).

In recent years, there has been growing interest in the use of natural and bio-derived materials as reinforcing fillers in dental composites. Eggshell is a readily available biowaste material primarily composed of calcium carbonate ($CaCO_3$), which can be converted into bioactive calcium phosphate or hydroxyapatite through chemical processing (10,11). Due to its high mineral content and structural similarity to natural hard tissues, eggshell-derived fillers have been investigated for use in dental and biomedical applications (11). Previous studies have demonstrated that incorporation of eggshell-derived fillers into polymer matrices can enhance mechanical properties such as compressive strength, hardness, and stiffness (11,12). Furthermore, the use of eggshell waste contributes to environmental sustainability and offers a cost-effective alternative to synthetic fillers (13).

Chitosan is a natural biopolymer derived from chitin, widely recognized for its biocompatibility, biodegradability, and antimicrobial properties. It has been extensively studied in various dental applications, including tissue engineering, drug delivery systems, and restorative materials (14,15). Chitosan has been shown to exhibit antibacterial activity against oral pathogens and promote tissue healing, making it a promising additive for dental biomaterials (16). However, due to its hydrophilic nature and limited mechanical strength, incorporation of chitosan into resin-based systems may influence polymerization and mechanical performance (17). Previous studies have reported that while

chitosan enhances biological properties, it may reduce mechanical strength when used alone, particularly at higher concentrations (18).

The combination of bioactive inorganic fillers and natural polymers has emerged as a novel approach to developing advanced dental materials with improved mechanical and biological properties. Studies involving hybrid systems combining hydroxyapatite and chitosan have demonstrated synergistic effects, including improved structural integrity, bioactivity, and antimicrobial performance (19,20). However, limited research has been conducted on the combined incorporation of eggshell-derived fillers and chitosan in resin-based composite restorative materials. Understanding the interaction between these components and their effect on mechanical properties is essential for optimizing material performance.

Although several studies have investigated the effect of individual fillers such as hydroxyapatite, silica, and zirconia on composite properties, there is a lack of evidence regarding the combined effect of eggshell-derived fillers and chitosan on flexural strength of resin-based composites. The present study aims to address this gap by evaluating the influence of these natural biomaterials on the mechanical performance of a nano-hybrid composite resin.

Materials and Methods

This in-vitro experimental study was conducted in the Dental Materials Laboratory. Karachi Medical and Dental College and the Flexural Strength testing was performed at Pakistan Council for Scientific and Industrial Research (P.C.S.I.R.), Karachi.

The study was carried out over a period of eight months, beginning with approval from the Ethical Review Board of Karachi Medical and Dental College and concluding with the completion of the write-up.

The sample size for this in-vitro study was calculated based on previously published studies evaluating the flexural strength of modified resin-based composites. The calculation was performed using the formula for comparing means among multiple groups:

$n = 2\sigma^2 (Z\ \alpha/2+Z\ \beta\ )^2\ d2$

Where: n = sample size per group σ = expected standard deviation from previous studies

d = minimum detectable difference between group means

$Z\alpha/2$ = standard normal deviate at 95% confidence level (1.96) $Z\beta$ = standard normal deviate at 80% power (0.84)

Based on prior literature, the standard deviation of flexural strength for composite materials was estimated to be approximately 5–7 MPa, and the minimum clinically significant difference was considered to be 10 MPa. Substituting these values into the formula yielded a minimum sample size of approximately 8 specimens per group (9,19,20).

To improve the statistical reliability and account for potential experimental errors, the sample size was increased to **10 specimens per group**, resulting in a total of 40 specimens for the study.

Grouping

A total of 40 specimens were prepared and divided into four groups (n = 10) as mentioned in Table 1.

Preparation of Eggshell Powder

Eggshells were collected, washed with distilled water, and the inner membrane was removed. The shells were dried in a hot air oven at 100°C for 24 hours to eliminate moisture. The dried shells were ground into fine powder using a mechanical grinder and sieved through a mesh (≤75 µm) to obtain uniform particle size.

To improve purity, the powder was calcined at 800–900°C for 2 hours to remove organic residues. The prepared eggshell powder was stored in airtight containers until use.

Preparation of Chitosan

Commercially available Chitosan (iHerb) was dissolved in 1% (v/v) acetic acid and stirred for 2–3 hours to obtain a homogeneous solution. The solution was filtered to remove undissolved particles and used in a uniform form for incorporation into the composite. Care was taken to avoid agglomeration.

Specimen Preparation

A nano-hybrid composite (**Filtek Z350**) was used as the base material. Eggshell powder and/or chitosan were incorporated into the composite according to group allocation using an analytical balance. The mixture was blended thoroughly to ensure uniform dispersion.

The modified composite was placed into stainless steel molds (25 mm × 2 mm × 2 mm) following ISO 4049 standards (21). A Mylar strip and glass slide were applied to obtain a smooth surface. Specimens were light-cured using an LED curing unit (1000–1200 mW/cm²) for 20–40 seconds.

After curing, specimens were removed, inspected for defects, and stored in distilled water at 37°C for 24 hours before testing.

Flexural Strength Testing

Flexural strength was measured using a three-point bending test on a Universal Testing Machine (**Instron, Binghamton, NY, USA**). Specimens were placed on supports with a span length of 20 mm, and load was applied at the center at a crosshead speed of 0.5–1 mm/min until fracture.

The maximum load at fracture was recorded, and flexural strength was calculated using:

$$Q = 3FL / 2BH^2$$

Were

- Q = flexural strength (MPa)
- F = maximum load at fracture (N)
- L = distance between supports (mm)
- B = specimen width (mm)
- H = specimen thickness (mm)

RESULTS

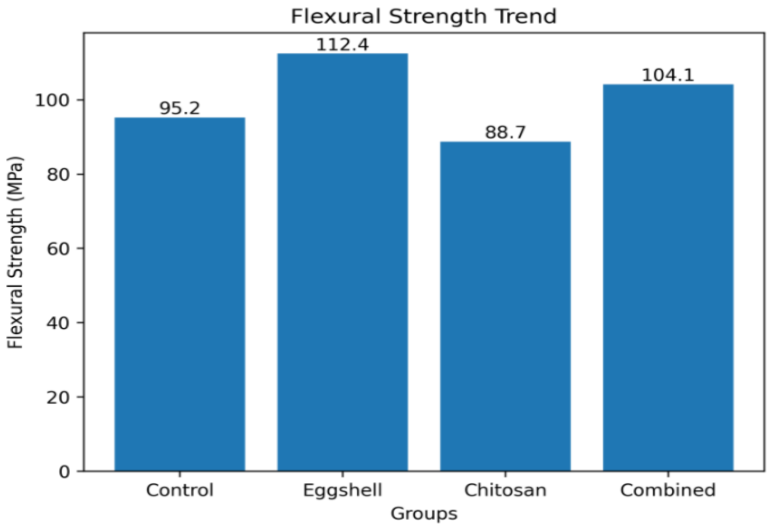
Table 1. Groups in the Study

Group	Composition	*n*
A	Control (Filtek Z 350 only)	10
B	Filtek Z 350 + 3% Eggshell	10
C	Filtek Z 350 + 1% Chitosan	10
D	Filtek Z350 + 3% Eggshell + 1% Chitosan	10

Table 2. Mean Flexural Strength of Groups (MPa)

Group	Composition	Mean (MPa)	SD	Min
A	Control (Filtek-Z350)	95.2	5.6	87.1
B	Filtek-Z350 + 3% Eggshell	112.4	6.2	102.5
C	Filtek-Z350 + 1% Chitosan	88.7	5.9	79.8
D	Filtek-Z350 + Eggshell + Chitosan	104.1	6.0	95.2

Figure 1: Bar Chart (Flexural Strength Comparison)



The descriptive statistics presented in **Table 2** indicate a distinct variation in flexural strength among the tested groups. Group B (3% eggshell) demonstrated the highest mean flexural strength, whereas Group C (1% chitosan) exhibited the lowest values relative to the control. The combined group (Group D) showed intermediate values, suggesting partial improvement in mechanical performance.

Statistical Analysis

The collected data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) software (version 23.0; IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean values and standard deviations, were calculated for flexural strength in each group.

To compare the mean flexural strength among the four groups, one-way analysis of variance (ANOVA) was applied. When a statistically significant difference was identified, post hoc multiple comparisons were performed using Tukey's Honestly Significant Difference (HSD) test to determine intergroup differences.
A p-value of ≤ 0.05 was considered statistically significant for all analyses. The results were presented in the form of tables and graphs for clarity and comparison.

This trend is corroborated by **Figure 1** , where the graphical representation clearly illustrates the superior performance of the eggshell-modified composite and the reduction in strength associated with chitosan incorporation. The findings suggest that eggshell-derived filler enhances structural integrity, whereas chitosan alone may compromise mechanical properties.

Table 3. Percentage Change Compared to Control

Group	% Change
B (Eggshell)	+18.1%
C (Chitosan)	−6.8%
D (Combined)	+9.3%

Table 4. One-Way ANOVA

Source	df	F-value	P-value
Between groups	3	18.72	<0.001
Within groups	36	—	—

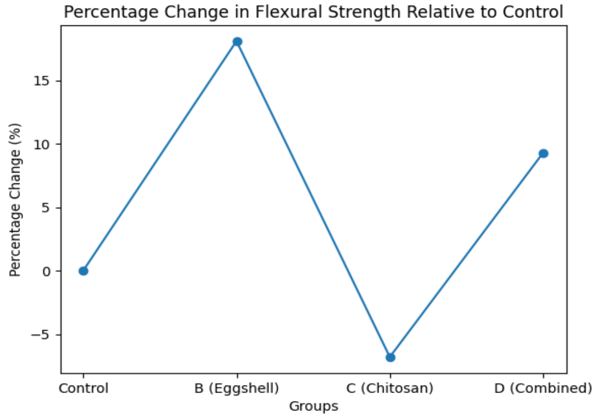
Table 5. Tukey Post Hoc Analysis

Comparison	Significance
A vs B	Significant
A vs C	Significant
A vs D	Significant
B vs D	Significant
C vs D	Significant

The percentage variation relative to the control, as shown in **Table 3** , demonstrates that eggshell incorporation resulted in a marked increase in flexural strength (+18.1%), while chitosan incorporation led to a reduction (−6.8%). The combined formulation exhibited a moderate improvement (+9.3%), indicating a partial compensatory effect. Inferential statistical analysis using one-way ANOVA (**Table 4**) revealed a statistically significant difference among the groups ($p < 0.001$), confirming that the observed variations were not due to random chance.

Post hoc multiple comparisons using Tukey's test (**Table 5**) further demonstrated that all intergroup differences were statistically significant. Notably, Group B showed significantly higher flexural strength than all other groups, while Group C exhibited significantly lower values compared to both the control and combined groups. These findings substantiate the differential influence of the tested additives on composite performance.

Figure 2: Percentage Change Graph



The graphical representation in **Figure 2** highlights the relative percentage changes in flexural strength across the experimental groups. The eggshell group demonstrated the greatest positive deviation, whereas the chitosan group showed a negative trend. The combined group exhibited a moderate increase, reinforcing the observation that eggshell incorporation partially offsets the detrimental effect of chitosan on mechanical strength.

Discussion

The present in-vitro study evaluated the effect of incorporating eggshell-derived filler and chitosan into a nano-hybrid resin-based composite, specifically Filtek Z350, on its flexural strength. The results demonstrated statistically significant differences among all groups ($p < 0.001$), indicating that modification of composite composition directly influences mechanical performance.

The control group exhibited a mean flexural strength of 95.2 ± 5.6 MPa, which is consistent with previously reported values for nano-hybrid composites (22,23). Although such materials are widely used due to their favorable esthetics and handling, their mechanical limitations—particularly susceptibility to fracture under functional loading—remain a concern (22, 23). Flexural strength is a key determinant of clinical durability, especially in stress-bearing areas (24, 25). The incorporation of 3% eggshell-derived filler resulted in the highest flexural strength (112.4 ± 6.2 MPa), representing an 18.1% increase compared to the control. This improvement can be attributed to the high calcium carbonate content of eggshell, which enhances stiffness and promotes efficient stress transfer within the resin matrix . Additionally, the fine particle size and calcination process likely improved filler dispersion and interfacial bonding, reducing stress concentration sites. Thesefindings are consistent with previous studies demonstrating that mineral-based fillers significantly improve the mechanical properties of composite materials (26, 27). In contrast, the incorporation of 1% chitosan (Chitosan (iHerb)) resulted in a reduction in flexural strength (88.7 ± 5.9 MPa; −6.8%). This decrease may be attributed to the hydrophilic nature of chitosan and its potential interference with polymerization, leading to reduced cross-linking density andweaker mechanical integrity. Furthermore, inadequate compatibility between chitosan and the hydrophobic resin matrix may result in poor interfacial adhesion and microstructural defects. Similar findings have been reported in studies where chitosan-containing composites exhibited compromised mechanical strength despite improved biological properties (28, 29).

The combined incorporation of eggshell and chitosan (Group D) resulted in a moderate improvement in flexural strength (104.1 ± 6.0 MPa; +9.3%) compared to the control. Although this value was lower than the eggshell-only group, it remained significantly higher than both the control and chitosan-only groups. This suggests a partial synergistic effect, where eggshell-derived filler compensates for the negative impact of chitosan. Comparable results have been reported in hydroxyapatite–chitosan composite systems, where the combination of inorganic and organic components improved overall structural performance (30, 31). The statistical analysis supports these observations. One-way ANOVA confirmed significant intergroup differences, while Tukey post hoc analysis demonstrated that the eggshell group was superior to all others, and the chitosan group showed significantly lower values . The graphical trends further reinforce these findings, with the eggshell group showing the highest positive deviation, chitosan demonstrating a negative effect, and the combined group exhibiting moderate improvement.

From a clinical perspective, the enhancement in flexural strength observed with eggshell incorporation suggests improved resistance to fracture and potentially greater longevity of restorations in load-bearing areas. Conversely, while chitosan offers antimicrobial and biocompatible advantages, its use as a sole reinforcing agent may not be suitable where high mechanical strength is required. The combined formulation may offer a balance between mechanical reinforcement and biological functionality, although further optimization is needed.

Despite these promising findings, the limitations of this study must be acknowledged. The in-vitro design does not fully replicate oral conditions, and factors such as thermal cycling, water sorption, and long-term fatigue were not evaluated. Additionally, only a single concentration of each additive was tested, limiting the ability to determine optimal formulations.

Conclusion

Within the limitations of this in-vitro study, modification of Filtek Z350 with eggshell-derived filler significantly enhanced flexural strength, demonstrating its effectiveness as a reinforcing biomaterial. The observed improvement is likely due to the high calcium carbonate content and improved stress distribution within the resin matrix.

In contrast, chitosan incorporation alone resulted in reduced flexural strength, suggesting compromised polymer network integrity due to its hydrophilic nature and limited compatibility with the resin system. However, the combined incorporation of eggshell and chitosan produced a moderate improvement compared to the control, indicating a partial compensatory or synergistic effect.

Overall, eggshell-derived filler shows strong potential as a sustainable reinforcing agent, while hybrid formulations with chitosan may offer a balance between mechanical and biological properties, warranting further optimization and long-term evaluation.

Limitations of the Study

This in-vitro study does not fully replicate the complex oral environment, including thermal fluctuations, saliva, and masticatory forces. Only a single
concentration of eggshell and chitosan was evaluated, limiting assessment of dose-dependent effects. Long-term properties such as water sorption, wear resistance, and fatigue behavior were not investigated. Additionally, the sample size was relatively small, which may affect the generalizability of the findings.

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Conflict of Interest

The authors declare that there are no conflicts of interest related to this study. No financial or personal relationships influenced the design, conduct, or reporting of this research.

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