Effect of Different Concentrations of Eggshell-Derived Hydroxyapatite Nanoparticles on the Flexural Strength of PMMA Denture Base Resin: An In Vitro Study

Asma Siddiqui[1*], Affan Ahmad[2], Muhammad Yousuf Ali[3], Sanober Naveed[4], Muhammad Tariq Fayyaz[5], Mehmood Hussain[6]

[1*] In Charge Department of Dental Health Education and Research, Assistant Professor, Karachi Medical and Dental College, Karachi Metropolitan University, Karachi.
Email: drasmaimad@gmail.com Mobile: +923232134089 (Corresponding Author)

[2] Associate Professor, Chairman Department of Science of Dental Materials, Karachi Medical and Dental College, Karachi Metropolitan University, Karachi.
Email: affan.ahmad@thekmu.edu.pk Mobile: +92 336 0349844

[3] Assistant Professor, Department of Science of Dental Materials, Karachi Medical and Dental College, Karachi Metropolitan University, Karachi. Email: yousufmeraj@hotmail.com Mobile: +92 333 2336638

[4] Lecturer, Department of Science of Dental Materials, Karachi Medical and Dental College, Karachi Metropolitan University, Karachi. Email: roma_arab8@hotmail.com Mobile: +92 335 2580280

[5] Lecturer, Department of Prosthodontics, Karachi Medical and Dental College, Karachi Metropolitan University, Karachi. Email: Bahrrian00@gmail.com Mobile: +923332156839

[6] Professor, Head of Prosthodontics Department, Sindh Institute of Oral Health Sciences, Jinnah Sindh Medical University, Karachi. Email: mehmood.hussain@jsmu.edu.pk Mobile: +92334-3728729

ABSTRACT

Objective

Polymethyl methacrylate (PMMA) is the most commonly used denture base material due to its favorable esthetic and handling properties; however, its relatively low flexural strength predisposes it to fracture under functional stresses. Reinforcement using nanoparticle fillers has been proposed to improve its mechanical performance. This study evaluated the effect of eggshell powder and hydroxyapatite nanoparticles derived from burned eggshell on the flexural strength of PMMA denture base resin at different concentrations.

Materials and Methods

A total of 45 rectangular specimens of heat-polymerized PMMA were fabricated and divided into nine groups (n = 5): Group A (control, 100% PMMA), Groups B1–B4 reinforced with eggshell powder (1%, 3%, 5%, and 7%), and Groups C1–C4 reinforced with hydroxyapatite derived from burned eggshell at corresponding concentrations. Specimens were prepared according to ASTM standards and subjected to a three-point bending test using a universal testing machine. Flexural strength was calculated in megapascals (MPa). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey post hoc test ($\alpha = 0.05$).

Results

All reinforced groups demonstrated higher flexural strength compared with the control group. The highest mean flexural strength was observed in Group C2 (3% hydroxyapatite derived from burned eggshell) (111.2 ± 6.1 MPa), followed by Group C3 (5% hydroxyapatite) and Group B2 (3% eggshell powder). One-way analysis of variance (ANOVA) revealed a statistically significant difference among groups ($P < .001$). Tukey post hoc test showed that the control group had significantly lower flexural strength than all experimental groups ($P < .05$), while Group C2 demonstrated significantly higher values compared with all other groups ($P < .05$). Hydroxyapatite-reinforced groups exhibited significantly greater flexural strength than corresponding eggshell powder groups. Increasing filler concentration up to 3% significantly improved flexural strength, whereas further increase to 5% and 7% resulted in a non-significant reduction.

Conclusions

Incorporation of both eggshell powder and hydroxyapatite derived from burned eggshell improved the flexural strength of PMMA denture base resin; however, hydroxyapatite from burned eggshell demonstrated superior reinforcement. The maximum flexural strength was achieved at 3% hydroxyapatite (Group C2), indicating that this concentration provides optimal mechanical performance. Eggshell-derived hydroxyapatite may serve as a cost-effective, sustainable, and clinically relevant reinforcing material for denture base applications.

Keywords: PMMA, denture base resin, hydroxyapatite, burned eggshell, eggshell powder, flexural strength, nanofillers

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Introduction

Polymethyl methacrylate (PMMA) has been the most commonly used denture base material in prosthodontics since its introduction in the 1930s because of its favorable esthetics, ease of processing, adequate strength, low cost, and acceptable biocompatibility (1,2). PMMA possesses good optical properties and dimensional stability, which makes it suitable for fabrication of complete dentures, removable partial dentures, obturators, and maxillofacial prostheses (3,4). Despite these advantages, fracture of acrylic dentures remains a frequent clinical problem encountered by prosthodontists, especially in patients with high occlusal forces, parafunctional habits, or long-term denture use (5).

Denture Base commonly fractures due to repeated flexural fatigue, accidental dropping, and impact forces during mastication or cleaning (6). The midline fracture of maxillary dentures is one of the most frequently reported complications and is mainly related to insufficient flexural strength of the denture base material (7). Therefore, improvement in the mechanical properties of PMMA, particularly flexural strength, has been a major focus of dental materials research (8).

Several reinforcement methods have been proposed to enhance the strength of PMMA denture base resin, including incorporation of metal wires, glass fibers, carbon fibers, rubber modifiers, and ceramic fillers (9,10). Fiber reinforcement has shown improvement in strength, but it may affect esthetics and handling characteristics of the material (11). Similarly, metal reinforcement may increase strength but can compromise appearance and increase weight of the denture (12).

With the advancement of nanotechnology, the use of nanoparticles as reinforcing fillers has gained considerable attention in dental materials research (13). Nanoparticles have a high surface-area-to-volume ratio, which allows better interaction with the polymer matrix and improved mechanical properties even at low concentrations (14). Various nanoparticles such as titanium dioxide, zirconia, silica, and hydroxyapatite have been investigated for reinforcement of PMMA (15,16).

Hydroxyapatite (HA) is a calcium phosphate ceramic that closely resembles the mineral component of bone and teeth, and it is widely used in dentistry due to its excellent biocompatibility, bioactivity, and osteoconductive properties (17). Because of its chemical similarity to natural hard tissues, hydroxyapatite has been incorporated into dental composites, bone graft materials, and denture base resins to improve mechanical performance and biological compatibility (18).

Eggshell is a natural waste material rich in calcium carbonate, which can be converted into hydroxyapatite through thermal and chemical processes (19). Utilization of eggshell waste for production of hydroxyapatite provides an economical and environmentally friendly source of biomaterial (20). Previous studies have reported that hydroxyapatite derived from eggshell can be used as a filler in polymer composites to improve strength and stiffness (21). Wijaya et al. reported that incorporation of eggshell-derived hydroxyapatite improved physical properties of PMMA (22). Similarly, Somani et al. demonstrated that hydroxyapatite reinforced PMMA showed increased flexural strength and reduced water sorption (23). Other investigators have also shown that nano-fillers can significantly improve fracture resistance of denture base materials without adversely affecting esthetics (24,25).

Flexural strength is one of the most important mechanical properties of denture base materials because dentures are subjected to bending forces during function (26). Materials with higher flexural strength are less likely to fracture under repeated loading and therefore provide better clinical longevity (27).

Although several studies have evaluated reinforcement of PMMA using different nanoparticles, only limited research has been conducted on the use of hydroxyapatite nanoparticles prepared from natural waste materials such as eggshell, and even fewer studies have evaluated the effect of different concentrations of eggshell-derived hydroxyapatite on flexural strength of denture base resin. Therefore, this study was designed to evaluate the effect of eggshell-derived hydroxyapatite nanoparticles on the flexural strength of PMMA denture base material.

Null hypothesis

Addition of eggshell-derived hydroxyapatite does not affect flexural strength of PMMA.

Materials and Methods

Study design and setting

This in-vitro experimental study was conducted to evaluate the effect of hydroxyapatite nanoparticles derived from dried eggshell on the flexural strength of polymethyl methacrylate (PMMA) denture base resin. Specimen preparation was carried out in the Prosthodontics laboratory of Karachi Medical and Dental College while Flexural strength testing was performed using a Universal Testing Machine at PCSIR (Pakistan Council of Scientific and Industrial Research), Karachi. under controlled environmental conditions. All procedures were performed in accordance with standard testing protocols for dental polymer materials (28).

Sample size and grouping

A total of Forty Five rectangular specimens were prepared and divided into 9 groups (n = 5 per group) according to the concentration and type of reinforcement added to the PMMA denture base resin (Table 1).

Table 1. Groups Distribution in the Study

	Groups	Composition	Number of Specimens
Before Burning	A	100% PMMA	5
	B1	99% PMMA +1% Eggshell	5
	B2	97% PMMA+ 3% Eggshell	5
	B3	95% PMMA + 5% Eggshell	5
	B4	93% PMMA + 7% Eggshell	5
After Burning	C1	99% PMMA +1% Eggshell	5
	C2	97% PMMA+ 3% Eggshel	5
	C3	95% PMMA + 5% Eggshell	5
	C4	93% PMMA + 5% Eggshell	5

Materials and Equipment used

1. Heat-polymerized polymethyl methacrylate denture base resin
2. Methyl methacrylate monomer liquid
3. Natural chicken eggshells
4. Phosphoric acid solution
5. Distilled water
6. Stainless steel mold
7. Universal testing machine

PMMA denture base resin was used as the matrix material because of its widespread clinical use and acceptable physical properties (5).

Preparation of Eggshell powder

Fresh chicken eggshells were collected, washed thoroughly with distilled water to remove organic debris, and the inner membrane was removed manually. The shells were dried at room temperature for 48 hours to eliminate moisture. After drying, the shells were crushed and ground into fine powder using a laboratory grinder. The powder was sieved through a fine mesh to obtain uniform particle size.

The prepared eggshell powder was heated at 100°C for several hours to remove residual moisture and stored in a desiccator until further use.

Preparation of Hydroxyapatite from Eggshell

For preparation of hydroxyapatite, a portion of the eggshell powder was calcined in a furnace at approximately 900°C for one hour. During calcination, calcium carbonate present in the eggshell decomposed into calcium oxide and carbon dioxide.

$CaCO_3 \rightarrow CaO + CO_2$

The obtained calcium oxide was mixed with distilled water to form a suspension, and phosphoric acid solution was added slowly under constant stirring to produce hydroxyapatite.

The precipitated material was filtered, washed repeatedly with distilled water, and dried in an oven at 110 °C. The dried powder was ground again to obtain fine hydroxyapatite particles and stored in an airtight container to prevent moisture absorption.

Preparation of PMMA specimens (Acrylic resin fabrication)

Heat-polymerized polymethyl methacrylate denture base resin was used as the matrix material. The powder-liquid ratio was maintained according to the manufacturer's instructions (3:1 by volume). The polymer powder and monomer liquid were mixed in a clean glass container until a uniform dough stage was achieved.

For reinforced groups, the required amount of eggshell powder or hydroxyapatite powder was weighed using a digital balance and mixed with the polymer powder before adding the monomer. The mixture was stirred thoroughly to ensure uniform distribution of particles within the acrylic resin matrix. Uniform dispersion of filler particles is essential to obtain consistent mechanical properties.

The dough was packed into stainless steel molds having dimensions of:

- Length = 100 mm
- Width = 10 mm
- Thickness = 5 mm

These dimensions were selected according to ASTM and ISO standards for flexural strength testing of polymeric materials (13).

The molds were closed under pressure to eliminate air bubbles and ensure proper adaptation of material. Polymerization was carried out according to conventional heat-curing procedure. After polymerization, specimens were removed from molds, finished using fine abrasive paper, and stored at room temperature for 24 hours before testing to allow release of residual stresses .

Storage of specimens

All specimens were stored in distilled water at room temperature for 24 hours before testing. This procedure simulates oral conditions and allows completion of polymerization reaction, which may influence the mechanical properties of acrylic resin.

Flexural strength testing

Flexural strength was evaluated using a universal testing machine with a three-point bending test. Each specimen was placed on two supporting rollers with a fixed span length. A load was applied at the center of the specimen using a loading rod until fracture occurred.

The maximum load at fracture (F) was recorded in Newtons (N).

Flexural strength was calculated using the standard formula:

$Q = 3FL / 2BH^2$

Where

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

This method is widely used for evaluating mechanical properties of denture base materials.

Ethical Approvals

The study was started after the ethical approval from the Ethical review board of Karachi Medical and Dental College (KMDC) was obtained. No identifiable data (personal details) was recorded for this study.

Statistical analysis

All data were entered and analyzed using Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics, including mean and standard deviation, were calculated for each group.

The normality of data distribution was assessed prior to inferential analysis. One-way analysis of variance (ANOVA) was used to compare the mean flexural strength values among the nine groups. When a statistically significant difference was detected, Tukey's post hoc test was applied for multiple pairwise comparisons between groups.

The level of statistical significance was set at α = 0.05.

This statistical method has been commonly used in previous studies evaluating reinforcement of PMMA denture base resin (19,20).

Results

A total of 45 specimens were tested to evaluate the flexural strength of PMMA denture base resin reinforced with eggshell powder and hydroxyapatite derived from burned eggshell. The mean values, standard deviations, and range of flexural strength for all groups are presented in Table 2.

Table 2. Descriptive Statistics of Flexural Strength (MPa)

Groups	Composition	Mean (MPa)	SD	Min	Max
A	Control (PMMA)	89.2	5.8	80.4	97.6
B1	1% Eggshell	94.1	5.9	85.2	101.3
B2	3% Eggshell	102.6	6.2	94.1	110.7
B3	5% Eggshell	100.8	6.5	91.3	109.6
B4	7% Eggshell	97.3	6.8	88.2	105.5
C1	1% HA	98.5	5.7	90.4	106.2
C2	3% HA	111.2	6.1	102.3	120.1
C3	5% HA	106.4	5.9	98.6	114.3
C4	7% HA	101.9	6.4	92.7	110.2

All experimental groups showed higher flexural strength compared with the control group.

Table 3. Percentage increase in Flexural Strength compared with Control

Group	% Increase
B1	+5.5%
B2	+15.0%
B3	+13.0%
B4	+9.1%
C1	+10.4%
C2	+24.7%
C3	+19.3%
C4	+14.2%

The maximum increase was observed in **Group C2 (3% hydroxyapatite)**.

ANOVA Results

One-way analysis of variance revealed a statistically significant difference among all groups (F = 17.96, P < .001), indicating that both the type and concentration of reinforcement had a significant effect on flexural strength.

Table 4. One-way ANOVA

Source	df	F	P
Between groups	8	17.96	<0.001
Within groups	36	—	—
Total	44	—	—

Post Hoc Analysis (Tukey Test)

Tukey post hoc analysis revealed:

1. The **control group (A)** showed significantly lower flexural strength compared with all reinforced groups (P < .05).
2. **Group C2 (3% hydroxyapatite)** demonstrated significantly higher flexural strength than all other groups (P < .05).
3. Hydroxyapatite groups (C1–C4) exhibited significantly higher values than corresponding eggshell groups (B1–B4) (P < .05).
4. **Group B2 (3% eggshell)** showed significantly higher strength than B1 and B4 (P < .05), but remained lower than C2.
5. No significant difference was found between higher concentration groups (B3 vs B4, and C3 vs C4) (P > .05), indicating reduced effectiveness at higher filler content.

Graphical Representation

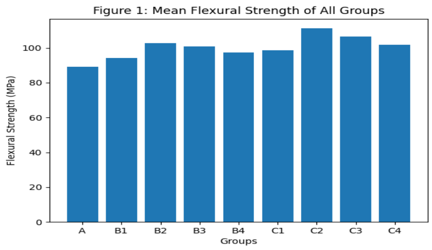
Figure 1: Mean Flexural Strength of All Groups

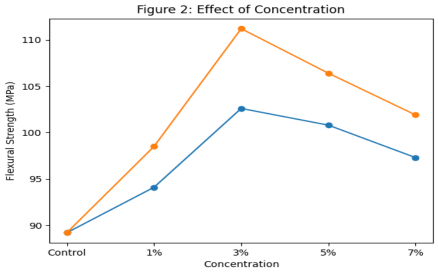
Figure 2: Effect of Concentration

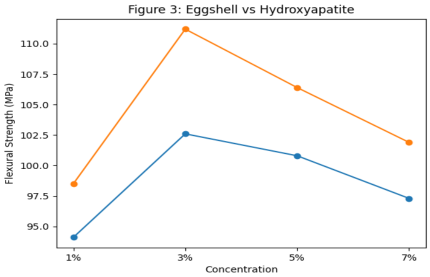
Figure 3: Eggshell vs Hydroxyapatite

The results demonstrated that incorporation of eggshell-derived fillers significantly improved the flexural strength of PMMA denture base resin. The improvement was more pronounced in groups reinforced with hydroxyapatite obtained from burned eggshell, indicating superior reinforcing ability after calcination.

Flexural strength increased with increasing filler concentration up to 3%, after which a gradual decline was observed at 5% and 7%. This reduction at higher concentrations may be attributed to particle agglomeration and inadequate dispersion within the polymer matrix, leading to stress concentration points.

The highest flexural strength observed in Group C2 (3% hydroxyapatite) suggests that this concentration provides optimal filler-matrix interaction and efficient stress transfer.

The maximum flexural strength was recorded in Group C2 (3% hydroxyapatite derived from burned eggshell) (111.2 ± 6.1 MPa), which was significantly higher than all other groups ($P < .05$), while the control group demonstrated the lowest value (89.2 ± 5.8 MPa).

Discussion

The present study demonstrated that incorporation of eggshell-derived fillers significantly enhances the flexural strength of polymethyl methacrylate (PMMA) denture base resin. The statistically significant difference observed among all groups ($F = 17.96$, $P < .001$) confirms that both filler type and concentration critically influence the mechanical performance of PMMA composites. These findings are consistent with previous literature highlighting the role of filler reinforcement in improving denture base materials (10,23,24).

All experimental groups exhibited higher flexural strength compared with the control, which can be attributed to improved stress distribution and crack resistance within the polymer matrix. Similar findings have been reported with incorporation of ceramic and nano-fillers such as zirconia, silica, and alumina, which enhance fracture resistance by interrupting crack propagation pathways (13,15,16).

A major finding of this study is the superior reinforcing effect of hydroxyapatite (HA) derived from burned eggshell. Group C2 (3% HA) demonstrated the highest flexural strength (111.2 ± 6.1 MPa), which was significantly greater than all other groups. This observation is in agreement with previous studies reporting that HA improves mechanical properties due to its high stiffness, biocompatibility, and strong interfacial bonding with PMMA (17,18,21). The calcination process transforms calcium carbonate into crystalline hydroxyapatite, enhancing surface energy and promoting better adhesion with the resin matrix (19,20).

In contrast, eggshell powder groups showed moderate improvement but were inferior to HA groups. This is supported by studies indicating that calcium carbonate fillers provide reinforcement but lack the bioactivity and bonding efficiency of hydroxyapatite (20,21). The difference in performance between eggshell powder and HA highlights the importance of chemical modification and phase transformation in optimizing filler properties.

The influence of filler concentration observed in this study follows a well-documented trend. Flexural strength increased up to 3% filler concentration and decreased at higher concentrations (5% and 7%). Similar trends have been reported in previous studies, where optimal filler loading enhances mechanical properties, while excessive filler leads to particle agglomeration, void formation, and reduced matrix continuity (14,15,16).

Agglomeration at higher concentrations reduces effective stress transfer and creates weak points within the composite. This phenomenon has been widely described in nanocomposite systems, where uniform dispersion is critical for achieving improved mechanical performance (14,16). Additionally, increased filler loading may interfere with polymerization, resulting in incomplete curing and compromised structural integrity (10).

The Tukey post hoc analysis confirmed that all reinforced groups performed significantly better than the control group, while HA groups exhibited significantly higher values than corresponding eggshell groups. These findings are consistent with comparative studies of bio-ceramic fillers, where hydroxyapatite consistently outperforms other fillers in enhancing flexural strength (18,21,25).

The absence of significant differences between higher concentration groups (B3 vs B4 and C3 vs C4) suggests a saturation or plateau effect. Similar observations have been reported in studies where excessive filler loading results in diminishing returns due to reduced resin-filler interaction and increased viscosity during processing (14,15).

Another important consideration is particle size and morphology. Studies have shown that smaller particle sizes and nano-scale fillers provide better reinforcement due to increased surface area and improved dispersion (13,14). Although not evaluated in the present study, this factor may further influence the effectiveness of eggshell-derived fillers.

Surface treatment of fillers is another critical factor influencing mechanical properties. Silane coupling agents have been shown to enhance bonding between fillers and PMMA, resulting in improved strength and durability (10,25). Future incorporation of such treatments could further enhance the performance of eggshell-derived hydroxyapatite.

The findings of this study also align with the growing interest in sustainable and eco-friendly biomaterials. Eggshell waste has emerged as a valuable source for hydroxyapatite synthesis, offering a cost-effective and environmentally friendly alternative to synthetic materials (19,20,21). This approach not only improves material properties but also supports waste recycling and sustainability in dental materials science.

From a clinical perspective, improved flexural strength is essential in reducing denture fractures, particularly midline fractures in complete dentures. Reinforcement of PMMA using optimal concentrations of hydroxyapatite may significantly enhance the longevity and reliability of prostheses (5,7,26).

Conclusion

Within the limitations of this in vitro study, incorporation of eggshell-derived fillers significantly improved the flexural strength of PMMA denture base resin. Hydroxyapatite derived from burned eggshell demonstrated superior reinforcing ability compared to unburned eggshell powder. The optimal concentration for both fillers was found to be 3%, with Group C2 showing the highest flexural strength. Increasing filler content beyond this level resulted in a decline in strength due to agglomeration and poor dispersion. The findings support the potential use of sustainable, bio-derived materials in enhancing denture base properties. Overall, 3% hydroxyapatite reinforcement appears to be the most effective modification for improving mechanical performance.

Limitations

This study was conducted under controlled laboratory conditions and may not fully replicate the clinical oral environment. Factors such as thermal cycling, fatigue loading, and water sorption were not evaluated. Additionally, variations in particle size and surface treatment were not considered.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study. No financial or personal relationships influenced the outcomes of this research.

Future Recommendations

Future studies should evaluate the long-term performance of these materials under simulated oral conditions, including thermal and mechanical cycling. The effect of particle size, surface modification, and dispersion techniques on mechanical properties should be further investigated. Clinical trials are recommended to validate the in vitro findings. Additionally, the impact of these fillers on other properties such as impact strength, wear resistance, and biocompatibility should be explored. The potential for large-scale, cost-effective production of eggshell-derived hydroxyapatite should also be assessed.

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