

Effect of adding egg shell powder on the physicommechanical properties of Biodentine

Sanket Aras¹, Anamika Chetan Borkar¹, Rakesh Mishra², Prakriti Jaggi¹, Apeksha Gambhir¹, Dipti Khairajani¹

Abstract

Objective: This study evaluated the effect of incorporating 1 wt% egg shell powder (ESP) into Biodentine™ (BD) on its physicommechanical properties.

Methods: Chicken eggs were ground and sterilized. Then, one wt% ESP was added to BD powder and mixed with BD liquid. The mixture's pH and solubility were measured on 1, 3, 7, and 28 days. The setting time was determined using a Gillmore needle. Compressive strength was measured using a universal testing machine after 1 and 14 days. Surface chemistry was analyzed via FTIR spectroscopy. The findings were compared by independent samples t-test, the repeated measures ANOVA, and the Bonferroni post-hoc test ($\alpha = 0.05$).

Results: pH levels were comparable between the groups ($P > 0.05$). BD-ESP had a higher setting time than BD ($P < 0.001$). BD-ESP solubility was significantly higher than BD on day 1 ($P = 0.001$) but lower on days 7 ($P = 0.006$) and 28 ($P = 0.001$). On day 1, BD had higher compressive strength than BD-ESP ($P = 0.027$), whereas the compressive strength of both groups was comparable on day 14 ($P = 0.099$). FTIR analysis revealed an additional IR band associated with the phosphate band in the BD-ESP group, suggesting new apatite formation.

Conclusions: Incorporating 1% ESP into BD resulted in lower initial solubility but higher solubility after a week and a month, likely due to increased ion dissolution. ESP incorporation also promoted additional apatite formation but negatively impacted the setting time and initial strength of BD.

Keywords: Biodentine, Compressive strength, Dental cement, Egg shell, Endodontic, Fourier Transform Infrared Spectroscopy

Introduction

Vital pulp therapy (VPT) has long been recognized as an essential treatment for teeth diagnosed with reversible or irreversible pulpitis. Over the past two decades, VPT has been enhanced by the introduction of hydraulic calcium silicate cement, including mineral trioxide aggregate (MTA), calcium-enriched mixture cement (CEM), and Biodentine™ that stimulate the

healing of pulp tissues (1, 2). An ideal material for VPT should be biocompatible, impermeable, antibacterial, stimulate tissue regeneration, and be stable and easy to handle (3).

In 2010, Biodentine™ (BD), a tricalcium silicate-based material, was introduced as a superior alternative to mineral trioxide aggregate (MTA). It offers advantages such as a shorter setting time, improved handling, and more effective dentine bridge formation (4). However, BD also has limitations, including its relatively long setting time, higher solubility in varying pH environments, high cost, and the potential for color changes that may affect esthetics. There are also concerns about inconsistent clinical outcomes and limited long-term data about BD (5, 6).

Several materials have been introduced for incorporation into remineralizing agents to increase

¹ Department of Conservative Dentistry and Endodontics, Dr. D. Y. Patil Dental College and Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, India.

² Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, India.

*Corresponding Author: Sanket Aras
Email: sanket.aras@gmail.com

Accepted: 28 May 2024. Submitted: 3 March 2024.





Figure 1. Egg shell powder preparation and particle size analysis. A) Mortar and pestle; B) Egg shell powder; C) Egg shell powder passed through a sieve; D) measuring the weight of egg shell powder; E) A microscopic image showing the unprocessed microstructure of egg shell powder, highlighting the particle size and distribution; F) A microscopic image with cell count analysis, red circles indicating the particle size; G) The Cell Count/Size table summarizing the cell count based on the size of powder particles in micrometers (µm) and demonstrating the distribution of different cell sizes.

hard tissue remineralization and microhardness by enhancing hydroxyapatite crystal formation. These materials include bioactive glass, gallic acid, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), and egg shell powder (ESP) (7, 8). ESP, composed of 94% calcium carbonate, 1% calcium phosphate, 1% magnesium carbonate, and 4% organic materials, has been used in various clinical applications alone or combined with other materials, serving as a tissue engineering scaffold, bone graft material, or remineralizing agent. ESP may enhance the mechanical properties of dental materials by improving compressive strength, microhardness, and calcium ion release (9). Salem et al. (10) found that adding 1 wt.% ESP to GIC significantly increased its compressive strength from 4.58 MPa to 21.84 MPa. Furthermore, Ulku and Ünlü (11) demonstrated that adding 5% ESP to GIC increased the calcium ion release and remineralization potential of GIC. When combined with materials like MTA and BD, ESP improves mineral deposition and sealing at the dentine/material interface (12). Due to its low cost, wide availability, and regenerative potential, ESP shows promise as a filler material to enhance the mechanical properties of BD (13).

The physicochemical properties of materials must be thoroughly assessed before application in oral tissues. However, the properties of BD combined with ESP have not been evaluated according to the authors' knowledge. Several physicochemical properties should be assessed in pulp capping materials, such as pH,

solubility, setting time, compressive strength, and surface chemistry. pH values influence the materials' biological responses, such as reparative dentin formation and antibacterial effects (14). Solubility assessments ensure minimal dissolution in oral fluids, maintaining the material's structural integrity (15). Setting times is crucial as it affects clinical efficiency, mechanical properties, dimensional stability, and biocompatibility of dental materials (15). Compressive strength ensures that the material can withstand masticatory forces, contributing to the long-term durability of restoration (15). The present study aimed to add ESP to BD and evaluate its pH, setting time, solubility, compressive strength, and surface chemistry compared to BD alone. The null hypothesis was that adding ESP does not change BD's physicochemical properties.

Materials and methods

This study was approved by the research ethics committee of Dr. D. Y. Patil Dental College and Hospital, Pune, Maharashtra, India (DPU/489/25/2021).

ESP preparation

Chicken eggs were cleaned with sterile distilled water and boiled for 10 minutes at 100°C, and the internal white membrane was removed. The egg shells were ground into small pieces using a sterile mortar and pestle (Figure 1). The particles were sterilized in an autoclave at 121°C and 15 psi for 15-20 minutes. The particle sizes

were examined under a light microscope (SAGLO Research Equipments, India) at 10 X magnification, revealing sizes between 10-15 μm , similar to that of BD powder (Septodont, Saint-Maur-des-Fossés, France) (Figure 1). The prepared ESP was stored in airtight, sterile containers for further use (16).

ESP incorporation into BD

One wt% ESP was added to BD powder and mixed with five drops of BD liquid, according to the manufacturer's instructions. The following tests were then conducted:

pH measurement

Ten BD and ten BD-ESP specimens were prepared by placing the materials into polyethylene plastic tubes (1 cm long, 1 mm in diameter). These tubes were submerged in flasks containing 10 mL of distilled water and stored at 37°C in a hot air oven. After 24 hours, the pH of the water was measured using a digital pH meter (Elico-LI 120, India). The tubes were then placed in fresh flasks with 10 mL of deionized water, and the pH was recorded at intervals of 3, 7, and 28 days. Deionized water without immersed material served as the control.

Solubility

Solubility was measured according to ISO 4049:2019 standards. Ten BD and ten BD-ESP specimens were prepared in stainless steel ring molds (20 mm diameter, 1.5 mm height) and kept at 37°C for 24 hours. Then, the samples' initial dry weight (IDW) was measured.

Glass bottles were obtained to keep the samples during the test. The Initial bottle weight (IBW) was evaluated, and the samples were placed in bottles filled with 5 cc of distilled water and incubated at 37°C for 24 hours. Subsequently, the samples were washed with 15 cc of distilled water, and the remaining water was allowed to evaporate from the bottles in an oven at 105°C. The bottle's final dry weight (FDW) was then recorded.

The process was repeated to evaluate the material's solubility after 3, 7, and 28 days of incubation. Solubility was calculated using the following formula, expressed as a percentage of the IDW (17):

$$\text{Solubility} = \frac{\text{FDW} - \text{IBW}}{\text{IDW}} \times 100$$

Setting time measurement

Ten BD and ten BD-ESP specimens were packed into ring-shaped stainless-steel molds (10 mm diameter, 1 mm height). Setting time was measured using a Gillmore

needle weighing 100 ± 0.5 g with a 2.0 ± 0.1 mm diameter, per ISO 6876:2012 standards. The material was considered set when the needle no longer left marks on the surface. During testing, specimens were kept in a water bath at 37°C and 100% humidity.

Compressive Strength

According to the ISO 9917-1:2007 standard, the compressive strength values were measured. Ten BD and ten BD-ESP specimens were prepared using stainless steel cylindrical molds (4 mm in diameter and 6 mm in height) and left to dry for 15 minutes. Then, they were incubated for 1 hour at 37°C in 100% humidity. The compressive strength was measured using the universal testing machine (UTM; Instron, High Wycombe, UK) with a 1 mm/min crosshead speed after 1 and 14 days. The compressive strength was calculated using the following formula, in which F is the force per unit area (kg), 9.807 represents the gravitational constant, and A is the base area (18):

$$\text{CS} = \frac{F \times 9.807}{A}$$

Surface Chemistry

Ten BD and ten BD-ESP specimens were prepared by condensing the material into Teflon molds (5 mm x 2 mm). The disk weighed approximately 0.15 grams. The discs were immersed in phosphate-buffered saline (PBS) in a plastic container at 37°C for 7 days. Surface chemistry, including surface components and elemental distribution, was analyzed in a dry environment using Fourier-transform infrared (FTIR) spectroscopy (Shimadzu FTIR-8400S, Japan).

Statistical Analysis

Data were analyzed using SPSS 22.0 (IBM Corp., Armonk, N.Y., USA). The independent samples t-test was employed to compare pH, solubility, and compressive strength between the groups at each time interval. The repeated measures ANOVA and Bonferroni post-hoc tests were used to assess differences within each group at various time intervals. Statistical significance was set at $P < 0.05$.

Results

Table 1 presents the pH levels of the study groups at different time intervals. Based on the independent samples t-test, the pH levels were comparable between the groups at each time point ($P > 0.05$; Table 1).

Table 1. Mean $\pm$ standard deviation of pH in the study groups at different time intervals

Groups	Time intervals				P value
	Day 1	Day 3	Day 7	Day 28	
Biodentine	11.41 $\pm$ 0.26 ^A	9.83 $\pm$ 0.11 ^B	9.42 $\pm$ 0.13 ^C	8.34 $\pm$ 0.22 ^D	0<0.001*
Biodentine with egg shell powder	11.60 $\pm$ 0.19 ^A	9.93 $\pm$ 0.23 ^B	9.48 $\pm$ 0.32 ^C	8.25 $\pm$ 0.15 ^D	0<0.001*
P value	0.139	0.396	0.704	0.380	

*P < 0.05 indicates a significant difference in pH values between the time intervals in each group according to the repeated measures ANOVA. In each row, different uppercase letters represent a significant difference among the time intervals according to the Bonferroni post-hoc test.

However, the repeated measures ANOVA test revealed significant differences in pH levels within each group across the time intervals ($p < 0.05$; Table 1). Pairwise The Bonferroni post-hoc test comparisons showed that BD and BS-ESP pH values significantly decreased from day 1 to day 28, with a significant difference between all time intervals ($P < 0.05$; Table 1).

The mean setting time of BD-ESP (43.01 $\pm$ 0.35 minutes) was significantly longer than that of BD (40.21 $\pm$ 0.50 minutes; $P < 0.001$).

Table 2 shows the solubility of the study groups at different time intervals. The independent samples t-test revealed that BD had significantly higher solubility than BD-ESP on day 1 ($P = 0.007$; Table 2). However, BD-ESP had a higher solubility on days 7 ($P = 0.006$) and 28 ($P = 0.001$). According to the ANOVA test, the solubility within each group differed significantly across the time intervals ($P < 0.001$; Table 2). Pairwise comparisons using the Bonferroni post-hoc test indicated that BD's solubility at 24 hours was significantly lower than on days 7 and 28 ($P < 0.05$). On day 3, solubility was lower than on day 28, and on day 7, it was lower than on day 28 ($P < 0.05$; Table 2). For BD-ESP, the solubility values increased significantly from day 1 to 28 with significant differences between all time points ($P < 0.001$; Table 2)

Table 3 provides the groups' compressive strength values on days 1 and 14. The independent samples t-test showed that on day 1, BD had a significantly higher mean compressive strength than BD-ESP ($P = 0.027$; Table 3). However, on day 14, the compressive strength was comparable between the two groups ($P = 0.099$; Table 3).

FTIR analysis (Figure 2) revealed that BD and BD-ESP surfaces contained carbonate and phosphate apatite bands with varying peak intensities. As summarized in Table 4, peaks in the 1150-1080 cm^{-1} range (phosphate groups (P-O)) suggested the presence of calcium phosphate-based fillers, commonly found in bioactive dental materials. An IR band in the 1200 cm^{-1} region, associated with phosphate, indicates new apatite formation in the BD-ESP group.

Discussion

The present study evaluated the effect of adding ESP on the pH, setting time, solubility, CS, and surface chemistry of Biodentine (BD). The null hypothesis was partially rejected, as except for pH, all tested properties were statistically different between BD and BD-ESP.

In the present study, both groups showed high pH values after 24 hours of setting. An alkaline environment

Table 2. Mean $\pm$ standard deviation of study groups' solubility percentage at different time intervals

Groups	Time intervals				P value
	Day 1	Day 3	Day 7	Day 28	
Biodentine	2.07 $\pm$ 0.14 ^A	2.37 $\pm$ 0.22 ^{A,B}	2.67 $\pm$ 0.20 ^B	3.56 $\pm$ 0.25 ^C	<0.001*
Biodentine with egg shell powder	1.88 $\pm$ 0.08 ^A	2.41 $\pm$ 0.15 ^B	2.95 $\pm$ 0.13 ^C	4.03 $\pm$ 0.15 ^D	<0.001*
P value	0.007**	0.688	0.006**	0.001**	

*P < 0.05 indicated a significant difference in solubility values between the time intervals in each group according to the repeated measures ANOVA.

In each row, different uppercase letters represent a significant difference among the time intervals, according to the Bonferroni post-hoc test.

** P < 0.05 indicated significant differences in solubility between groups according to the independent samples t-test.

Table 3. Mean $\pm$ standard deviation of study groups compressive strength (MPa) at different time intervals

Groups	Time intervals	
	Day 1	Day 14
Biodentine	13.56 $\pm$ 0.76	19.39 $\pm$ 0.76
Biodentine with egg shell powder	10.80 $\pm$ 2.37	21.26 $\pm$ 2.26
P value	0.027*	.099

*Values less than 0.05 represent significantly different compressive strength in each time interval between the groups according to independent samples t-test.

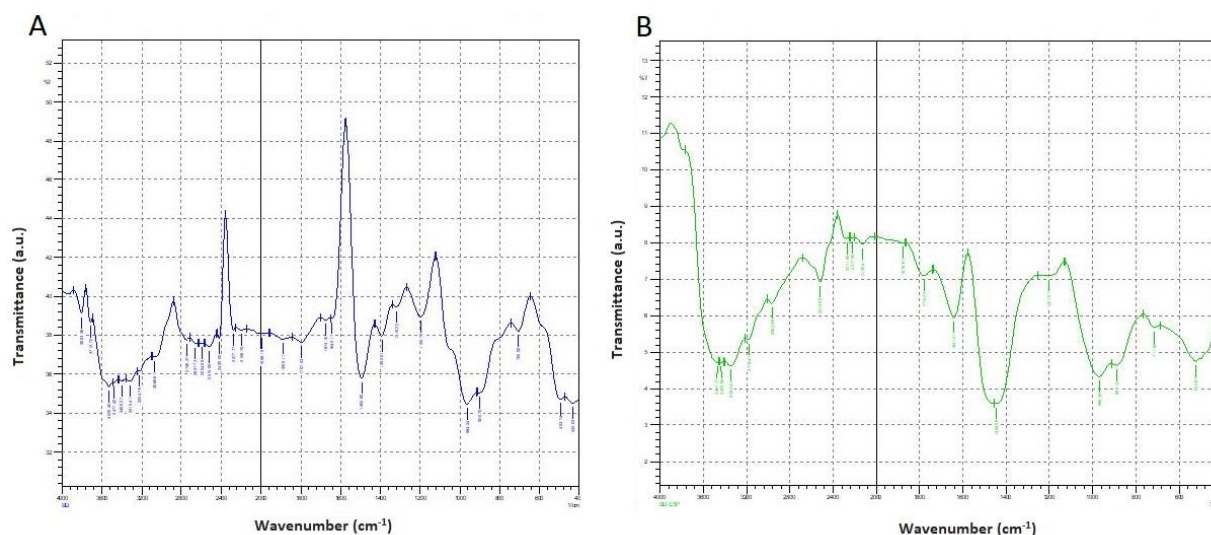


Figure 2 A) FTIR Analysis of BD: The band at 3529 cm^{-1} is associated with N-H groups. The band represents the methylene groups (CH_2) at 2925 cm^{-1} . The carboxylic acid group ($\text{C}=\text{O}$) is assigned to the band at 1797 cm^{-1} , and the bands further corroborate this at 1674 and 1419 cm^{-1} , which correspond to the COO^- group of carboxylic acids. The band at 1645 cm^{-1} can be attributed to either an aromatic ring or a secondary amine. The band confirms the amine group at 795 cm^{-1} , while bands between 1410 and 1500 cm^{-1} support the presence of an aromatic ring. The band represents the CN group at 1168 cm^{-1} . B) FTIR Analysis of BD-ESP: BD-ESP showed carbonate (CO_3^-) groups between 1240.14 and 2331.78 cm^{-1} , $\text{C}=\text{C}$ stretching at 1641 cm^{-1} , and $\text{C}-\text{H}$ stretching at 2962.46 cm^{-1}

enhances the materials' osteogenic potential and antibacterial activity. The ability of calcium silicate-based cements to alkalinize the medium is associated with the hydration process. After contact with water, calcium silicate hydrate ($\text{C}-\text{S}-\text{H}$) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) is produced. $\text{Ca}(\text{OH})_2$ dissociation raises the pH while releasing calcium ions into the medium (19, 20). This process is responsible for the prolonged alkaline pH observed in BD (21). In an alkaline environment, dentinal collagen fibers denature, facilitating mineral exchange and the penetration of Ca^{2+} (22).

The pH of BD-ESP was measured as 11.6 after 24 hours, consistent with the findings of Helal et al. (14). According to a previous study, ESP has a moderately alkaline pH, which explains why the pH of BD did not increase significantly after adding the ESP (23).

However, combining these materials with a high calcium and phosphate content may create favorable conditions for remineralization (24). A study by Salah et al. (25) found that when used as a direct pulp-capping agent, egg shell powder induced less inflammation than calcium hydroxide. The calcium carbonate in the egg shell powder dissociates in water and releases hydroxyl and Ca^{2+} ions, increasing the environment's pH. This process is most active at 24 hours (20).

The pH of the samples decreased from 11.41 on day 1 to 8.34 on day 28 for BD and from 11.60 to 8.25 for BD-ESP. The pH of BD decreases over time due to the progressive hydration of its calcium silicate particles and the gradual consumption of $\text{Ca}(\text{OH})_2$ (26). Therefore, the hydroxyl ion (OH^-) concentration reduces and decreases the pH as the cement matures and stabilizes (26).

Table 4. FTIR Interpretation

Wavenumber (cm^{-1})	Functional Group/Component	Type of Vibration
3600-3200	O-H (hydroxyl groups in fillers, water content)	Stretching
3500-3300	N-H (amines in resin matrix)	Stretching
3000-2850	C-H (alkyl chains in polymers, resins)	Stretching
1750-1700	C=O (carbonyl groups in ester/urethane)	Stretching
1650-1580	C=C (aromatic rings in bis-GMA, UDMA)	Stretching
1450-1400	C-H (alkanes, CH_2 , CH_3)	Bending (scissoring)
1250-1150	C-O-C (ether linkages in monomers)	Stretching
1150-1080	P-O (phosphate groups in fillers)	Stretching
1050-950	Si-O (silica-based fillers)	Stretching
900-700	C-H (out-of-plane bending in aromatic rings)	Bending
650-500	M-O (metal-oxygen in fillers)	Stretching

A faster setting time helps reduce bacterial contamination and material loss during the final processing stages. Calcium carbonate (CaCO_3) has been shown to accelerate the setting reaction and shorten the setting time in calcium silicate cement (27). In the current study, the setting time of BD-ESP was significantly longer than BD (43.01 minutes versus 40.21 minutes). However, in a previous study, adding 3% and 5% ESP decreased the setting time of CEM cement from 63.33 min to 56.67 min in the CEM group with 3% ESP and to 51.67 min in the CEM group with 5% ESP (28). The difference in results of this study and that of Sedigh-Shams et al. (28) might be explained by the lower percentage of ESP in the present study (1%). Shiferaw et al. suggested that the higher the egg shell content, the faster the setting time rate (29).

The solubility of a root-end filling or perforation repair material influences its sealing ability, biocompatibility, and interactions with the surrounding environment. Since BD is also used as a root-end filling material, the solubility tests conducted in the present study adhered to the International Standard for root canal sealers (ISO 6876:2001) (30). According to this standard, after a 24-hour immersion of the specimens in water, the solubility must not exceed 3% of the total mass (ISO 6876:2012). Indeed, root canal sealers should have a low void percentage to avoid bacterial contamination. BD has a lower solubility than MTA, which results in a slower release of calcium ions (31).

In this study, BD-ESP's solubility was lower than BD on day 1, comparable to BD on day 3, and higher than BD on days 7 and 28. These findings partially align with Sedigh-Shams et al. (28), who reported that adding 3% and 5% ESP decreased the solubility of CEM cement after 1, 7, and 14 days. Singh et al. (17) attributed the increased solubility of BD-ESP to its prolonged ion dissolution compared to BD. This sustained ion release creates a favorable environment for tissue healing (32). The high solubility observed in the current study might be concerning due to the potentially poor sealing and void formation. However, Beshr et al. (12) demonstrated that adding 1% ESP by weight to MTA and BD improved mineral deposition at the dentine/material interface and the sealing ability by promoting the formation of an interfacial layer.

In the present study, the compressive strength of BD-ESP was lower than that of BD on day 1, likely due to the longer setting time. After 14 days, the compressive strength of BD-ESP surpassed that of BD, although the difference was not statistically significant. BD typically shows a gradual increase in compressive strength over

time, with consistent improvement during its setting process. According to Kaur et al. (33), the compressive strength of BD eventually surpasses that of MTA. The addition of ESP has been reported to increase compressive strength in materials such as GI (10, 16), CEM cement (28), and BD (10). Furthermore, Jaber et al. (34) found that adding ESP to Portland cement enhanced its physical properties across all tested weight ratios (5%, 10%, 15%, and 20%). In their study, the addition of 15% ESP resulted in a 29% increase in compressive strength values compared to the control group. The improvement in mechanical strength seen in these dental materials may be attributed to the main component of ESP, CaCO_3 . This compound accelerates the hydration process in Portland cement, contributing to increased strength in the early stages (28). However, compared to the present study, higher ESP percentages were added to the materials in the mentioned study (34).

The characteristic vibrations of atomic groups such as silicate, carbonate, hydroxyl, and phosphate in the investigated molecule can be identified using FTIR spectroscopy. BD primarily consists of tri-calcium silicate (80.1%), with calcium chloride acting as an accelerator. The composition of cements, including BD and BD-ESP, can influence the amount and rate of apatite deposition. In the present study, BD-ESP exhibited an additional IR band associated with phosphate in the 1200 cm^{-1} region. This indicates the formation of new apatite, closely resembling the spectra of sound dentin (35).

The present study has some limitations, the most prominent being its *in vitro* setting. The effects of BD-ESP should be confirmed with clinical trials. The purity of ESP can vary due to factors such as egg source and processing methods. The chicken's diet also influences egg shell composition. These parameters, however, were not controlled in the present study. Further research is needed to explore the effect of incorporating higher ESP concentrations on the properties of BD and its potential for inducing odontogenic differentiation of pulpal stem cells.

Conclusions

Adding ESP to BD did not alter its pH but prolonged the setting time and decreased the initial compressive strength, which is unfavorable. BD-ESP showed lower initial solubility than BD. However, higher solubility was observed after a week and a month, possibly indicating an increased ion dissolution rate and bioactivity. Moreover, adding one wt% ESP improved the formation

of additional apatite, closely resembling the spectra of sound dentin.

Acknowledgments

We thank Dr. D. Y. Patil Dental College and Hospital and Dr. D. Y. Patil Vidyapeeth for their support.

Conflicts Of Interest

The authors declared that they have no conflict of interest.

Authors' Contribution Statement

SA and ACB contributed to the project conception, study management, supervision, data collection, and analysis. RM contributed to data collection, chemical analysis, and interpretation. PJ, AG, and DK contributed to gathering data and writing and editing the manuscript. All the authors read and approved the final manuscript.

Ethical approval

All experimental procedures were performed in accordance with the study's ethical guidelines and were approved by the Institutional Ethical Committee (DPU/489/25/ 2021), Dr. D. Y. Patil Dental College and Hospital, Pimpri, Pune, India.

Funding

There was no source of funding to declare.

References

1. Santos JM, Pereira JF, Marques A, Sequeira DB, Friedman S. Vital Pulp Therapy in Permanent Mature Posterior Teeth with Symptomatic Irreversible Pulpitis: A Systematic Review of Treatment Outcomes. *Medicina* 2021;57(6):573.
2. Parisay I, Ghoddusi J, Forghani M. A review on vital pulp therapy in primary teeth. *Iranian Endod J* 2015;10(1):6-12.
3. Hanna SN, Perez Alfayate R, Prichard J. Vital Pulp Therapy an Insight Over the Available Literature and Future Expectations. *Eur Endod J* 2020;5(1):46-53.
4. Küçükkaya Eren S. Clinical applications of calcium silicate-based materials: a narrative review. *Aust Dent J* 2023;68:S96-S109.
5. Pushpa S, Maheshwari C, Maheshwari G, Sridevi N, Duggal P, Ahuja P. Effect of pH on solubility of white Mineral Trioxide Aggregate and Biodentine: An in vitro study. *J Dent Res Dent Clin Dent Prospects* 2018;12(3):201-207.
6. Slaboseviciute M, Vasiliauskaite N, Drukteinis S, Martens L, Rajasekharan S. Discoloration Potential of Biodentine: A Systematic Review. *Materials (Basel)* 2021;14(22).
7. Asadi M, Majidinia S, Bagheri H, Hoseinzadeh M. The Effect of Formulated Dentin Remineralizing Gel Containing Hydroxyapatite, Fluoride, and Bioactive Glass on Dentin Microhardness: An In Vitro Study. *Int J Dent* 2024;2024(1):4788668.
8. Parisay I, Boskabady M, Bagheri H, Babazadeh S, Hoseinzadeh M, Esmaeilzadeh F. Investigating the efficacy of a varnish containing gallic acid on remineralization of enamel lesions: an in vitro study. *BMC Oral Health* 2024;24(1):175.
9. Punj S, Singh J, Singh K. Ceramic biomaterials: Properties, state of the art and future perspectives. *Ceram Int* 2021;47(20):28059-28074.
10. Salem AM, Ali MS, Abdul Rahim RA. Evaluation of compressive strength and setting time of some types of calcium silicate based materials modified with chicken egg shell powder. *Al-Azhar J Dent Sci* 2023;26(4):477-483.
11. Ülkü SG, Ünlü N. Could Chicken Eggshell Powder Be The Solution in Dentistry? *Dent & Med J - R* 2023;5(3):1-10.
12. Beshir KA, Abdelrahim RA. Enhanced bioactivity and interfacial marginal adaptation of mineral trioxide aggregate and Biodentine materials modified by eggshell powder at root dentine. *Tanta Dent J* 2019;16(3):136-141.
13. Opris H, Baciut M, Bran S, Dinu C, Armencea G, Opris D, et al. Characterization of eggshell as a bio-regeneration material. *Med Pharm Rep* 2023;96(1):93-100.
14. Helal MB, Sheta MS, Alghonemy WY. Comparing the remineralization potential of undemineralized dentin powder versus chicken eggshell powder on artificially induced initial enamel carious lesions: an in-vitro investigation. *BMC Oral Health* 2024;24(1):1048.
15. Hamdy TM. Evaluation of compressive strength, surface microhardness, solubility and antimicrobial effect of glass ionomer dental cement reinforced with silver doped carbon nanotube fillers. *BMC Oral Health* 2023;23(1):777.
16. Allam G, Abd El-Geleel O. Evaluating the mechanical properties, and calcium and fluoride release of glass-ionomer cement modified with chicken eggshell powder. *Dent J* 2018;6(3):40.
17. Singh S, Podar R, Dadu S, Kulkarni G, Purba R. Solubility of a new calcium silicate-based root-end filling material. *J Conserv Dent Endod* 2015;18(2):149-153.
18. Hambire U, Tripathi V, Mapari A. Improvement in the compressive strength and flexural strength of dental composite. *ARPN J Eng Appl Scie* 2012;7(8):1-4.
19. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. *J Endod* 2005;31(2):97-100.
20. Zhou HM, Shen Y, Zheng W, Li L, Zheng YF, Haapasalo M. Physical properties of 5 root canal sealers. *J Endod* 2013;39(10):1281-1286.
21. Camilleri J, Sorrentino F, Damidot D. Investigation of the hydration and bioactivity of radiopacified tricalcium silicate cement, Biodentine and MTA Angelus. *Dent Mater* 2013;29(5):580-593.
22. Koubi S, Elmerini H, Koubi G, Tassery H, Camps J. Quantitative evaluation by glucose diffusion of microleakage in aged calcium silicate-based open-sandwich restorations. *Int J Dent* 2012;2012:105863.
23. Eliwa ME, Riad D, Hamoda MA, Farouk H. A comparative study of the biological and histological response of eggshell nanoparticles, hesperidin, and calcium hydroxide as direct pulp capping materials: An in vivo study on rat molars. *Adv Dent J* 2023;5(4):768-784.
24. Liu L, Hou S, Xu G, Gao J, Mu J, Gao M, et al. Evaluation of osteogenic properties of a novel injectable bone-repair material containing strontium in vitro and in vivo. *Front Bioeng Biotechnol* 2024;12:1390337.

25. Salah M, Kataia MM, Kataia EM, El Din EA, Essa ME. Evaluation of eggshell powder as an experimental direct pulp capping material. *Futur Dent J* 2018;4(2):160-164.
26. Islam R, Islam MRR, Tanaka T, Alam MK, Ahmed HMA, Sano H. Direct pulp capping procedures - Evidence and practice. *Jpn Dent Sci Rev* 2023;59:48-61.
27. Kim D, Lee H, Chung M, Kim S, Song M, Kim E. Effects of fast- and slow-setting calcium silicate-based root-end filling materials on the outcome of endodontic microsurgery: a retrospective study up to 6 years. *Clin Oral Investig* 2020;24(1):247-255.
28. Sedigh-Shams M, Nabavizadeh M, Jafari E. Effects of White Chicken Eggshell Powder on Compressive Strength, Water Solubility, and Setting Time of Calcium-Enriched Mixture. *Iran Endod J* 2023;18(3):152-158.
29. Shiferaw N, Habte L, Thenepalli T, Ahn JW. Effect of Eggshell Powder on the Hydration of Cement Paste. *Materials* 2019;12(15):2483.
30. Cavenago BC, Pereira TC, Duarte MA, Ordinola-Zapata R, Marciano MA, Bramante CM, Bernardineli N. Influence of powder-to-water ratio on radiopacity, setting time, pH, calcium ion release and a micro-CT volumetric solubility of white mineral trioxide aggregate. *Int Endod J* 2014;47(2):120-126.
31. Grech L, Mallia B, Camilleri J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. *Dent Mater* 2013;29(2):e20-28.
32. Almutairi B. Solubility, Water Sorption, and Shear Bond Strength of Contemporary Pulp Capping Agents Biodentine, TheraCal LC, and Bioactive Glass Nanoparticles to the Composite Resin Restoration. *J Biomater Tissue Eng* 2023;13(12):1126-1132.
33. Kaur M, Singh H, Dhillon JS, Batra M, Saini M. MTA versus Biodentine: Review of Literature with a Comparative Analysis. *J Clin Diagn Res* 2017;11(8):Zg01-zg05.
34. Jaber HA, Mahdi RS, Hassan AK. Influence of eggshell powder on the Portland cement mortar properties. *Mater Today* 2020;20:391-396.
35. Xie J, Gowen A, Xu W, Xu J. Analysing micro- and nanoplastics with cutting-edge infrared spectroscopy techniques: a critical review. *Anal Methods* 2024;16(15):2177-2197.